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Distribution and epidemiological relatedness of methicillin-resistant *Staphylococcus aureus* isolated from companion dogs, owners, and environments

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ABSTRACT. This study aimed to investigate the distribution and epidemiological relatedness of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from companion dogs, owners, and residential environments of 72 households. Sampling was performed twice from January to June 2018 and a total of 2,592 specimens were collected. The specimens collected from each household were streaked on CHROM agar *S. aureus* and the colonies grown on the medium were further identified using a mass spectrometry microbial identification system. Antimicrobial susceptibility testing, Panton-Valentine-Leukocidin (*PVL*) gene PCR, staphylococcal cassette chromosome *mec* (SCC*mec*) typing, *Staphylococcus aureus* Protein A (*spa*) typing, pulsed-field gel electrophoresis (PFGE), and multi-locus sequence typing (MLST) were conducted to evaluate the phenotypic and genotypic characteristics of the MRSA isolates. A total of 65 *S. aureus* strains (2.5%) were isolated and 49 (1.9%) of 65 strains were MRSA displaying cefoxitin-resistance with *mecA* carriage. MRSA strains were isolated from dogs (n=6, 9.2%), owners (n=27, 41.5%), and residential environments (n=16, 24.6%), respectively. Overall prevalence of non-duplicated MRSA was 16.7% (12/72 households) at household level. ST72-SCC*mec* IVc MRSA clones predominantly appeared in MRSA-positive families. Furthermore, PFGE analyses showed that ST72-SCC*mec* IVc-t324 is shared between dog owners and dogs. To our knowledge, this is the first study to report the sharing of ST72 MRSA between dogs and their owners.

KEY WORDS: dog, epidemiological relatedness, methicillin-resistant *Staphylococcus aureus* (MRSA), owner, ST72-SCC*mec* IVc-t324

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According to a previous study [15], *Staphylococcus aureus* (*S. aureus*) is a typical member of the microbiota of the human body, frequently found in the upper respiratory tract and on the skin. However, they can become an opportunistic pathogen, causing skin infections including respiratory infections. As previously described [2], methicillin-resistant *S. aureus* (MRSA) is a public health problem throughout the world. An estimated 20% to 30% of the human populations are known as long-term carriers of *S. aureus*, which can be found as normal skin flora and in the nasal cavity [11, 22]. They are most commonly distributed in nosocomial infections and also cause the wound infections after surgery.

Since the mid-1990s, MRSA has been known as an important cause of human infections in the general population, which has been mostly designated as community-associated (CA) MRSA [13]. Since 2000, studies in the United States and Canada have reported that community-acquired MRSA strains of staphylococcal cassette chromosome *mec* (SCC*mec*) type IV cause serious infections in healthy people in the community [17]. In South Korea, panton-valentine-leukocidin (PVL)-negative multilocus sequence type 72 (ST72)-SCC*mec* type IV has been reported as the predominant CA-MRSA strain in human medicine [10]. Furthermore, it is reported that MRSA ST72-SCC*mec* IV clones in both the community-onset health care infection and hospital-

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onset infection were increasing; this frequency was higher in Korea in 2007–2009 [9]. In addition, PVL-positive CA-MRSA should be considered in the cases of severe community-acquired pneumonia, such as necrotizing pneumonia, and skin and soft tissue infections [8].

Since 2000, MRSA has emerged as a multidrug resistant or an opportunistic pathogen in companion dogs, pet owners, veterinary hospitals, and veterinarians [21]. It can be transmitted from humans to animals, moving to the veterinary hospital setting and circulating in the community [6, 12, 14, 26, 27]. As described earlier [7], the MRSA could be present in the veterinary practice and this opportunistic pathogen can also be identically distributed among companion dogs and human contact surfaces. Furthermore, veterinary hospital environments contaminated with MRSA needs to be recognized as a top priority to monitor the presence and spread of veterinary nosocomial and zoonotic pathogens, as well as when designing and executing infection control programs in veterinary hospitals. Ultimately, these previous reports suggest that the dogs with MRSA may act as zoonotic carriers, spreading MRSA to their surroundings.

According to the Ministry of Food, Agriculture, Forestry and Livestock Food Statistics in 2015, the number of companion animals was 9,179,000 [20]. The number of households with companion animals was 28.1% (5.93 million households) out of 21.1 million households in South Korea. To date, although the demand for companion animals increases, the presence of resistant strains such as MRSA has not been evaluated in residential environments where humans and animals live together. As described in previous report [7], MRSA strains are present on humans, animals, and environmental surfaces in the veterinary hospital setting and that they might be transmitted between them. In the same context, our study aimed to identify the epidemiological association in an environment living with companion dogs and their owners that promotes the presence and transmission of MRSA in the community.

MATERIALS AND METHODS

Experimental design

Sampling was carried out to investigate the distribution and clonal spread of MRSA in the family members and residential environment living with companion dogs in the community. The sampling range consisted of a total of 72 households, including companion dogs, two family members, and residential environments per household. Sampling was performed twice in four provinces (Seoul, Gyeonggi, Chungnam and Jeonnam) from January to June 2018. The specimens for each subject are as follows: 3 specimens from family members (nose, hands, and feces), 4 specimens from dogs (nose, eyes, inguinal part, and rectum), and 8 specimens from residential environments (sofa, TV screen, keyboard, pillow cover, toilet seat, pet seat, pet toys, and pet feeding bowl). These sampling sites were used based on a previous study [3]. In addition, the owner selection for sample collection was included the animal hospital workers and their families who living with dogs. Moreover, the cases without previous history of illness and antibiotic use within 6 months were selected when sampled from dogs and people. The samplings were conducted by each veterinarian in their home. For sampling, sterile transport swab (COPAN, Brescia, Italy), sterilized saline, sampling guide manual, name pen, and stool exam paper (Better Life, L&S Co., Seoul, South Korea) were used. Based on a previous study [24], to swab the nose of human and dogs, the cotton swabs wetted in normal saline was gently introduced into the anterior nostril and rubbed on the entire nostril several times. Human fecal sampling was carried out according to the instructions of the stool exam paper. Briefly, the stool exam paper was attached to both sides of the toilet seat and the feces on the paper were collected using a sterile swab. To collect the environmental specimens, the cotton swabs were soaked in sterilized saline and swabbed the surface of each object at intervals of 1–2 cm. The sample collection from people, pet, and environment in each household was performed simultaneously. The swabbed samples collected from each household were stored at 4°C and subjected to the bacterial culture experiment.

Bacterial culture and identification

All swab samples were cultured on BD BBL™ CHROMagar™ Staph aureus medium (Beckton, Dickinson and Co., Sparks, MD, USA) for 48 hr at 37°C and a pink colony was subcultured on mannitol salt agar. The isolates were identified as *Staphylococcus aureus* by Gram staining (Gram Stain Set, Remel, Dartford, UK), catalase test (Hydrogen peroxide solution 3%, Sigma-Aldrich, Co., St. Louis, MO, USA) and mass spectrometry microbial identification system (VITEK® MS, bioMérieux, Marcy l'Etoile, France).

Antimicrobial susceptibility testing

The disc diffusion assay was based on the criteria published by Clinical Laboratory of Standards Institute [1]. The antimicrobial susceptibility discs (Oxoid Limited, Basingstoke, UK) used in this study are as follows: cefoxitin (FOX, 30 μg), erythromycin (ERY, 15 μg), clindamycin (DA, 2 μg), linezolid (LZD, 30 μg), trimethoprim/sulfamethoxazole (SXT, 25 μg), and quinupristin/dalfopristin (SYN, 15 μg). The MIC values for the vancomycin and teicoplanin were evaluated using Oxoid M.I.C.Evaluator Strips (Oxoid, UK) according to the manufacturer's instructions. *Staphylococcus aureus* ATCC 29213 and MRSA ATCC 43300 were used as the quality control strains.

DNA isolation

Genomic DNA was prepared using the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany), according to the manufacturer's instruction, but an additional step was included for *S. aureus* by adding lysostaphin (Sigma-Aldrich) at 0.5 mg/m*l* at the cell lysis step. The extracted DNA was used as a template in PCR experiments for SCC*mec* typing, *spaA* typing, *PVL* detection, and multi-locus sequence typing (MLST).

SCCmec typing

Before performing the staphylococcal chromosome cassette (SCC) *mec* typing, all MRSA isolates were confirmed by PCR amplification of the *mecA* gene and SCC*mec* typing was determined by a previously described method [18]. The results were interpreted according to the guidelines of the IWG-SCC (www.sccmec.org). To confirm the subtype of MRSA SCC*mec* type IV strains, the J1 region of SCC*mec* IV element was investigated by using a previously described multiplex PCR method [17].

MLST

MLST for *S. aureus* was performed by a previously described method [4]. Severn housekeeping genes were used in the MLST scheme and the fragments were amplified by using the corresponding primers. The PCR products were directly sequenced in an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster city, CA, USA). Allele numbers and sequence type (ST) were determined with the database accessible via https://pubmlst.org/saureus/.

spa typing

The *spa* typing was performed as described previously [5]. Primers for the amplification and sequencing of the X region of the *spaA* gene were purchased (Cogenbiotech, Seoul, Korea); the sequences are as follows: Spa-F, 5′-AGACGATCCTTCGGTGAGC-3′ and Spa-R, 5′-GCT TTT GCA ATG TCA TTT ACT G-3′. The purified PCR fragments were directly sequenced in an ABI Prism 3100 genetic analyzer (Applied Biosystems). Each *spa* type was determined with the RidomStaph Type software (http://spa.ridom.de/spatypes.shtml).

Detection of PVL gene

Primers used for the detection of *PVL* genes were Luk-PV-1 (5′-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A-3′) and Luk-PV-2 (5′-GCA TCA AGT GTA TTG GAT AGC AAA AGC-3′), which amplify a 433 base-pair fragment specific for *lukS/F–PV* genes, encoding the PVL S/F bicomponent proteins as previously described [16]. The MRSA isolates were screened for the genes (*lukF*-PV and *lukS*-PV) encoding the components of the PVL toxin by PCR amplification.

PFGE

Pulsed-field gel electrophoresis (PFGE) was conducted according to the oxacillin-resistant *S. aureus* on PulseNet procedure (https://www.cdc.gov/mrsa/pdf/ar_mras_PFGE_s_aureus.pdf) for the molecular typing of *S. aureus* using the CHEF Mapper apparatus (Bio-Rad Laboratories, Hercules, CA, USA). Genomic DNA was digested with *Sma*I (Roche Diagnostics GmbH, Mannheim, Germany). *Xba*I-digested DNA from *Salmonella* Braenderup H9812 was used as the standard size. Briefly, the prepared agarose plugs were transferred to EC lysis buffer (6 mM Tris HCl, 1 M NaCl, 100 mM EDTA, 0.5% Brij-58, 0.2% sodium deoxycholate, and 0.5% sodium lauroyl sarcosine) and incubated at 37°C for 5 hr in an orbital rocker. After rinsing 4 times in TE buffer for 30 min at room temperature, the equilibrated plug slices were digested with 30 U of *Sma*I at 25°C for 3 hr for *S. aureus* and 40 U of *Xba*I (Roche Diagnostics GmbH) at 37°C for 3 hr for *Salmonella* Braenderup H9812, respectively. The parameters for running the CHEF Mapper were as follows: 0.5X Tris-borate-EDTA buffer at 14°C and 6 V/cm for 21 hr, 5 sec for initial switch and 40 sec for final switch. The gel images were converted to TIFF files and the band profiles were analyzed using the BioNumerics software version 4.0 (Applied Maths, Kortrijk, Belgium) with the Dice coefficient and the unweighted pair-group method with arithmetic mean.

RESULTS

Distribution of MRSA in households

A total of 2,592 samples were collected through two samplings in 72 households comprising dogs, owners, and the residential environment from January through June 2018 (Table 1). Overall, sixty-five (2.5%, 65/2,592) *S. aureus* strains were isolated and subsequently, 49 (75.4%) of 65 strains were identified as MRSA based on cefoxitin resistance and *mecA* gene detection. Thirty-five

Table 1. Number of samples collected from the 72 households including dogs, owners, and the residential environment by region from January to June 2018 in South Korea

Province (no. of household)	No. of target subjects[a)]		No. of collected specimens for[b)]			
	Dogs (n=72)	Owners (n=144)	Dogs (n=576)	Owners (n=864)	Environment (n=1,152)	Total (n=2,592)
Seoul (n=40)	40	80	320	480	640	1,440
Gyeonggi (n=19)	19	38	152	228	304	684
Chungnam (n=8)	8	16	64	96	128	288
Jeonnam (n=5)	5	10	40	60	80	180

a) Sampling was supported by veterinarians and health workers at domestic animal hospitals and a total of 72 households were selected based on their families and dogs. b) Sampling from animals, humans and residential environments was performed twice. Sample collection proceeded as follows. Family members: nose, skin (hands), and feces (3 sites); dogs: nose, eyes, and skin (inguinal area) (3 sites); environment: pillow cover, sofa, TV screen, keyboard, toilet seat cover, pet toys, pet feeding bowl, and pet seats (8 sites).

Table 2. Distribution of methicillin-susceptible *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* strains among dogs, their owners, and residential environment in the 72 households

No. of household	Region	No. (%) of strains isolated from the collected specimens									
		Dog (n=10)		Owner A (vet staff) (n=25)		Owner B (family) (n=10)		Environment (n=20)		Total (n=65)	
		MSSA (n=4)	MRSA (n=6)	MSSA (n=6)	MRSA (n=19)	MSSA (n=2)	MRSA (n=8)	MSSA (n=4)	MRSA (n=16)	MSSA (n=16)	MRSA (n=49)
01	Seoul	1 (1.5)	-	-	-	-	-	-	-	1 (1.5)	-
02	Seoul	-	1 (1.5)	-	2 (3.1)	-	1 (1.5)	-	5 (7.7)	-	9 (13.8)
03	Seoul	-	-	-	-	-	1 (1.5)	-	1 (1.5)	-	2 (3.1)
04	Seoul	-	-	1 (1.5)	-	1 (1.5)	-	-	-	2 (3.1)	-
07	Seoul	-	-	-	3 (4.6)	-	-	-	-	-	3 (4.6)
11	Seoul	-	-	1 (1.5)	-	1 (1.5)	-	2 (3.1)	-	4 (6.2)	-
13	Jeonnam	1 (1.5)	-	-	-	-	-	-	-	1 (1.5)	-
14	Jeonnam	-	-	1 (1.5)	-	-	-	1 (1.5)	-	2 (3.1)	-
15	Jeonnam	2 (3.1)	-	2 (3.1)	-	-	-	-	-	4 (6.2)	-
19	Gyeonggi	-	-	-	1 (1.5)	-	-	-	-	-	1 (1.5)
22	Seoul	-	-	-	1 (1.5)	-	1 (1.5)	-	-	-	2 (3.1)
26	Seoul	-	3 (4.6)	-	2 (0.5)	-	4 (6.2)	-	3 (4.6)	-	12 (18.4)
28	Seoul	-	-	-	-	-	-	1 (1.5)	-	1 (1.5)	-
29	Seoul	-	-	-	3 (4.6)	-	1 (1.5)	-	-	-	4 (6.2)
35	Seoul	-	-	1 (1.5)	-	-	-	-	-	1 (1.5)	-
40	Gyeonggi	-	1 (1.5)	-	3 (4.6)	-	-	-	4 (6.2)	-	8 (12.3)
46	Seoul	-	-	-	1 (1.5)	-	-	-	1 (1.5)	-	2 (3.1)
47	Seoul	-	1 (1.5)	-	1 (1.5)	-	-	-	2 (3.1)	-	4 (6.2)
48	Seoul	-	-	-	1 (1.5)	-	-	-	-	-	1 (1.5)
62	Gyeonggi	-	-	-	1 (1.5)	-	-	-	-	-	1 (1.5)

MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*. The number in parentheses represents the percentage of isolates among all *S. aureus* isolates.

(53.8%) of them were detected in healthy human (owner A and owner B) and 27 (41.5%) of them were identified as MRSA. Ten isolates (15.3%, 10/65) were isolated from dogs and 6 (9.2%) of them were MRSA. Twenty (30.8%, 20/65) strains were found in residential environment samples and 16 (24.6%) of them were MRSA (Table 2).

In this study, MRSA positivity defined the presence of more than one MRSA in each household and the results were summarized for each sample (Tables 3 and 4). It was positive in 12 (16.7%, 12/72) out of 72 households. Twelve MRSA-positive household codes were 2, 3, 7, 19, 22, 26, 29, 40, 46, 47, 48, and 62. Among them, MRSA was present in various samples (human, dog, and residential environment) in four households (2, 26, 40, and 47) (5.6%, 5/72). It was present only in humans in six households (7, 19, 22, 29, 48, and 62) and in both humans and the residential environment in two households (3 and 46), respectively.

Three MRSA strains from dogs in three households were isolated from the inguinal part, nose, and eyes, respectively. MRSA from the residential environment was positive in 6 households (8.3%, 6/72). It was isolated from pillow cover (n=6), followed by sofa (n=3), toilet seat cover (n=3), keyboards (n=2), and pet toys (n=1).

Antimicrobial resistance

Forty-nine (75.4%) of the total *S. aureus* isolates were resistant to cefoxitin (Table 3). Out of the 10 antibiotics tested, only 2 MRSA isolates were resistant to the antibiotics, erythromycin, clindamycin, and trimethoprim/sulfamethoxazole. Of the MRSA isolates, erythromycin resistance was only found in the two households (22 and 40).

Molecular epidemiological characterization

The epidemiological relatedness among the MRSA strains which were isolated from the different households in the region, was examined using representative molecular epidemiological tools for *S. aureus*, SCC*mec* typing, *spa* typing, MLST, and PFGE (Table 3). The MRSA strains were identified as SCC*mec* type IV (95.9%, 47/49) in 47 isolates from 11 households and type III (4.1%, 2/49) in 2 isolates from one household. Furthermore, MRSA strains belonging to SCC*mec* type IV were identified as IVc by multiplex PCR. However, seven different *spa* types were identified (t037, t148, t324, t664, t8074, t11970, and t12699) in the MRSA isolates. The t324 type was the most prevalent in five households (02, 26, 40, 46, and 48), in which MRSA was identified from almost all samples, including companion dogs, owners, and residential environments. Two STs, ST72, and ST239 were divided by MLST. Almost all MRSA strains from 11 households were identified as ST72 with SCC*mec* IVc except for a single household (ST239, SCC*mec* III). In addition, *PVL* gene was not detected in all MRSA strains.

In this study, the clonal relatedness among ST72-IVc MRSA isolates from dogs, owners, and residential environments in four households (02, 26, 40, and 47) had >96% similarity in PFGE dendrogram analysis (Fig. 1). In only one household (Code 47), the

Table 3. Antimicrobial susceptibilities and molecular characteristics of 49 methicillin-resistant *Staphylococcus aureus* strains isolated from dogs, owners, and residential environments

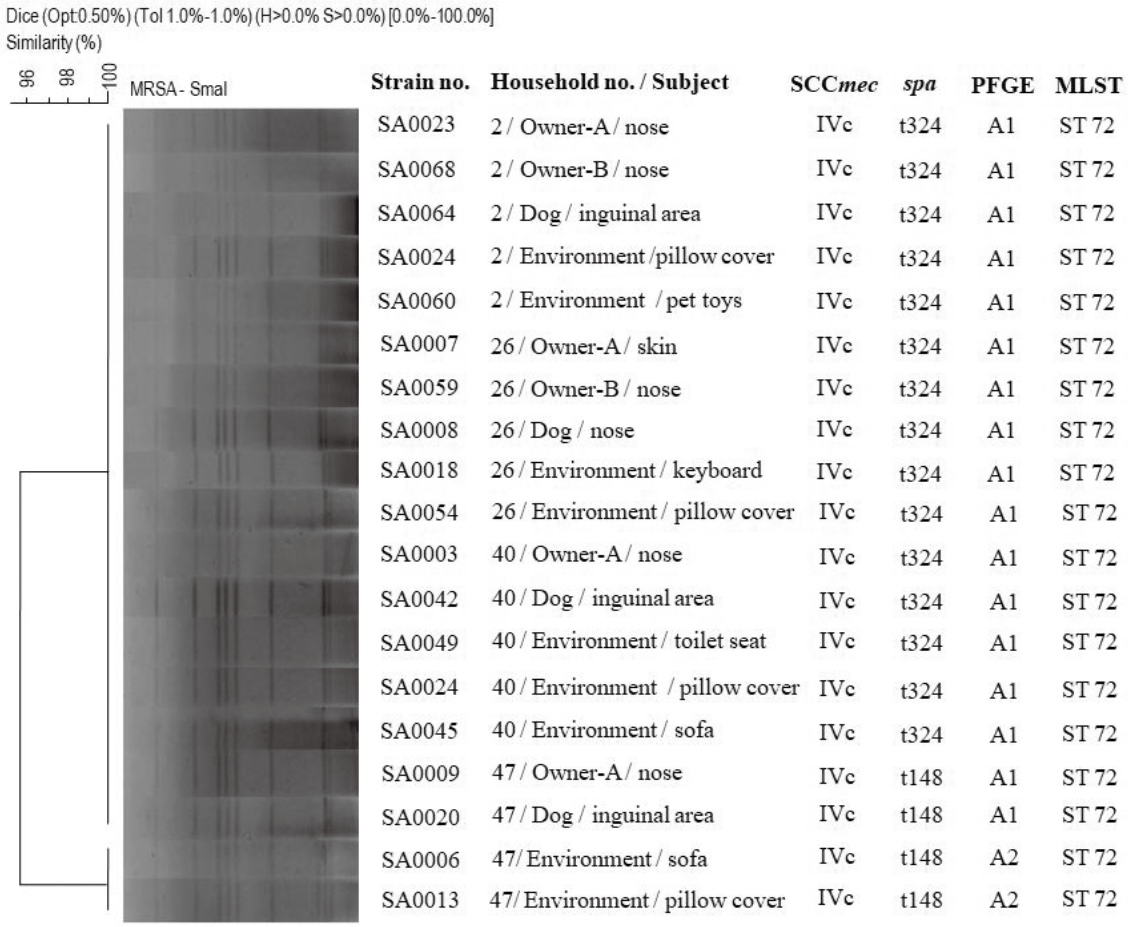
No. of household	Region	Sampling date	Stain no.	Origin[a)]	Age/Sex/Species	Specimen	Antibiogram	SCC*mec* type	*Spa* type	MLST type
02	Seoul	March 14	Z0118SA0023,	Owner-A	27/F	Nose	FOX	IVc	t324	72
02	Seoul	June 4	Z0118SA0067	Owner-A	27/F	Nose	FOX	IVc	t324	72
02	Seoul	June 4	Z0118SA0068	Owner-B	31/F	Nose	FOX	IVc	t324	72
02	Seoul	June 4	Z0118SA0064	Dog	9/IF/ Yorkshireterrier	Inguinal area	FOX	IVc	t324	72
02	Seoul	March 14	Z0118SA0024	Environment		Pillow cover	FOX	IVc	t324	72
02	Seoul	June 4	Z0118SA0062	Environment		Keyboard	FOX	IVc	t324	72
02	Seoul	June 4	Z0118SA0061	Environment		Toilet seat	FOX	IVc	t324	72
02	Seoul	June 4	Z0118SA0060	Environment		Pet toy	FOX	IVc	t324	72
02	Seoul	June 4	Z0118SA0065	Environment		Sofa	FOX	IVc	t12699	72
03	Seoul	June 7	Z0118SA0063	Owner-B	5/M	Nose	FOX	IVc	t148	72
03	Seoul	June 7	Z0118SA0066	Environment		Pillow cover	FOX	IVc	t148	72
07	Seoul	March 19	Z0118SA0029,	Owner-A (vet)	33/M	Nose	FOX	IVc	t8074	72
07	Seoul	March 19	Z0118SA0030	Owner-A (vet)	33/M	Skin	FOX	IVc	t8074	72
07	Seoul	June 10	Z0118SA0069	Owner-A (vet)	33/M	Nose	FOX	IVc	t8074	72
19	Gyeonggi	May 2	Z0118SA0046	Owner-A (vet)	28/F	Nose	FOX	IVc	t664	72
22	Seoul	April 20	Z0118SA0043	Owner-A (vet)	30/F	Nose	FOX, ERY, DA, SXT	III	t037	239
22	Seoul	April 20	Z0118SA0044	Owner-B	18/F	Nose	FOX, ERY, DA, SXT	III	t037	239
26	Seoul	February 27	Z0118SA0007	Owner-A (vet)	47/M	Skin	FOX	IVc	t324	72
26	Seoul	May 24	Z0118SA0055	Owner-A (vet)	47/M	Skin	FOX	IVc	t324	72
26	Seoul	February 27	Z0118SA0015	Owner-B	15/F	Skin	FOX	IVc	t324	72
26	Seoul	February 27	Z0118SA0016	Owner-B	15/F	Skin	FOX	IVc	t324	72
26	Seoul	May 24	Z0118SA0059	Owner-B	15/F	Nose	FOX	IVc	t324	72
26	Seoul	May 24	Z0118SA0057	Owner-B	15/F	Skin	FOX	IVc	t324	72
26	Seoul	February 27	Z0118SA0008,	Dog	7/CM/Shetland Sheepdog	Nose	FOX	IVc	t324	72
26	Seoul	February 27	Z0118SA0011	Dog		Eye	FOX	IVc	t324	72
26	Seoul	May 24	Z0118SA0056	Dog	7/CM/Shetland Sheepdog	Nose	FOX	IVc	t324	72
26	Seoul	February 27	Z0118SA0018	Environment		Keyboard	FOX	IVc	t324	72
26	Seoul	May 24	Z0118SA0052	Environment		Keyboard	FOX	IVc	t324	72
26	Seoul	May 24	Z0118SA0054	Environment		Pillow cover	FOX	IVc	t324	72
29	Seoul	February 28	Z0118SA0017,	Owner-A	40/M	Nose	FOX	IVc	t11970	72
29	Seoul	May 25	Z0118SA0053	Owner-A	40/M	Nose	FOX	IVc	t11970	72
29	Seoul	February 28	Z0118SA0014	Owner-B	39/M	Nose	FOX	IVc	t11970	72
29	Seoul	May 25	Z0118SA0058	Owner-A	40/M	Skin	FOX	IVc	t11970	72
40	Gyeonggi	January 19	Z0118SA0003	Owner-A	34/F	Nose	FOX, ERY	IVc	t324	72
40	Gyeonggi	May 2	Z0118SA0047,	Owner-A	34/F	Skin	FOX, ERY	IVc	t324	72
40	Gyeonggi	May 2	Z0118SA0048	Owner-A	34/F	Nose	FOX, ERY	IVc	t324	72
40	Gyeonggi	May 2	Z0118SA0042	Dog	3/CM/Pekingese	Inguinal area	FOX, ERY	IVc	t324	72
40	Gyeonggi	January 19	Z0118SA0002,	Environment		Toilet seat	FOX, ERY	IVc	t324	72
40	Gyeonggi	May 2	Z0118SA0049	Environment		Toilet seat	FOX, ERY	IVc	t324	72
40	Gyeonggi	May 2	Z0118SA0050	Environment		Pillow cover	FOX, ERY	IVc	t324	72
40	Gyeonggi	May 2	Z0118SA0045	Environment		Sofa	FOX, ERY	IVc	t324	72
46	Seoul	February 23	Z0118SA0012	Owner-A	46/M	Skin	FOX	IVc	t324	72
46	Seoul	February 23	Z0118SA0004	Environment		Pillow cover	FOX	IVc	t324	72
47	Seoul	February 26	Z0118SA0009	Owner-A	44/F	Nose	FOX	IVc	t148	72
47	Seoul	February 26	Z0118SA0020	Dog	5/CM/ Yorkshireterrier	Inguinal area	FOX	IVc	t148	72
47	Seoul	February 26	Z0118SA0013	Environment		Pillow cover	FOX	IVc	t148	72
47	Seoul	February 26	Z0118SA0006	Environment		Sofa	FOX	IVc	t148	72
48	Seoul	February 23	Z0118SA0005	Owner-A	50/M	Skin	FOX	IVc	t324	72
62	Gyeonggi	March 7	Z0118SA0021	Owner-A	43/F	Skin	FOX	IVc	t148	72

a) Owner A, veterinarian; Owner B, family member; Environment: residential environment. F, female; M, male; FOX, cefoxitin; ERY, erythromycin; DA, clindamycin; SXT, trimethoprim/sulfamethoxazole; SCC*mec*, staphylococcal cassette chromosome *mec*; *spa*, staphylococcal protein A; MLST, multilocus sequence type.

Table 4. Positive rate of methicillin-resistant *Staphylococcus aureus* isolated from dogs, owners, and residential environments of 72 households

Subject[a)]	No (%). of MRSA-positive household (N=72)
Owner-A	4 (5.6)
Owner-A and owner-B (both)	2 (2.8)
Owner-A and residential environment	2 (2.8)
Owner-A, dog, and residential environment	2 (2.8)
Both people, dog, and residential environment	2 (2.8)
No. (%) of total MRSA household	12 (16.7)

a) Owner-A: veterinary hospital worker; owner-B: veterinarian family. Methicillin-resistant *Staphylococcus aureus* (MRSA) positivity was found in the following residential environments: pillow cover (6 households, 8.3%), keyboard (2, 2.8%), toilet seat (2, 2.8%), sofa (3, 4.2%), and pet toys (1, 1.4%).



Strain no.	Household no. / Subject	SCC*mec*	*spa*	PFGE	MLST
SA0023	2 / Owner-A / nose	IVc	t324	A1	ST 72
SA0068	2 / Owner-B / nose	IVc	t324	A1	ST 72
SA0064	2 / Dog / inguinal area	IVc	t324	A1	ST 72
SA0024	2 / Environment /pillow cover	IVc	t324	A1	ST 72
SA0060	2 / Environment / pet toys	IVc	t324	A1	ST 72
SA0007	26 / Owner-A / skin	IVc	t324	A1	ST 72
SA0059	26 / Owner-B / nose	IVc	t324	A1	ST 72
SA0008	26 / Dog / nose	IVc	t324	A1	ST 72
SA0018	26 / Environment / keyboard	IVc	t324	A1	ST 72
SA0054	26 / Environment / pillow cover	IVc	t324	A1	ST 72
SA0003	40 / Owner-A / nose	IVc	t324	A1	ST 72
SA0042	40 / Dog / inguinal area	IVc	t324	A1	ST 72
SA0049	40 / Environment / toilet seat	IVc	t324	A1	ST 72
SA0024	40 / Environment / pillow cover	IVc	t324	A1	ST 72
SA0045	40 / Environment / sofa	IVc	t324	A1	ST 72
SA0009	47 / Owner-A / nose	IVc	t148	A1	ST 72
SA0020	47 / Dog / inguinal area	IVc	t148	A1	ST 72
SA0006	47/ Environment / sofa	IVc	t148	A2	ST 72
SA0013	47/ Environment / pillow cover	IVc	t148	A2	ST 72

Fig. 1. Pulsed-field gel electrophoresis patterns, staphylococcal cassette chromosome *mec* (SCC*mec*) types, staphylococcus protein (*spa*) types, and STs (sequence types) against 19 methicillin-resistant *Staphylococcus aureus* strains isolated from dogs, dog owners, and residential environments in four households (02, 26, 40, and 47).

PFGE cluster and STs were almost identical to the other 3 households, but the *spa* type was identified as t148.

DISCUSSION

Until recently, MRSA has been known as an opportunistic pathogen that emerges not only in the hospital environment but also in the community. Companion animals have become an important model for monitoring antibiotic-resistant bacteria, such as MRSA through public health risks, which can directly transmit resistant bacteria to other individual or the surrounding environment as MRSA carriers in the community [23, 27]. Besides, studies for the transmission of MRSA from humans to animals have been limited. A 31-year-old nurse working in a Dutch nursing home was identified as an MRSA carrier and the same clone was detected

from her daughter and healthy dog [23]. A 76-year-old man with a chronic illness was infected with MRSA after receiving hospital treatment, and soon after, bacteria isolated from his dog infected with skin disease were found to be the same MRSA clone identified in the elderly owner [19]. As a result, the previous reports suggest that MRSA can become colonized by direct contact with companion animals living with humans.

Results of this study demonstrated that MRSA was positive in 12 (16.7%) of the surveyed 72 households in the four provinces. In twice sampling for 6 months, MRSA was repeatedly isolated from humans, dogs, and residential environments of several surveyed households. In particular, in one household (code 26), same MRSA clone was found consistently in two family members and a companion dog. The overall MRSA detection rate was predominant in the surroundings, which are in contact with humans and dogs. In this study and the previous reports, there is no direct evidence that MRSA has been transmitted from human to dog. However, we carefully suggest that, as in humans, MRSA could adapt to companion dogs as bacterial flora as they are consistently present for several months on the skin of the nose. The presence of MRSA in home indicates that they could spread to the surrounding environment of household when any family member or companion dog is infected or contaminated from elsewhere.

Recently, the risk of MRSA as a cause of skin infection in companion animals and equine species in the veterinary practices has been reported in several countries [6, 26, 27]. Furthermore, the occurrence of MRSA in dogs and cats is being discussed as the result of a “dissemination or spill-over” from human medicine or human invasive clones [25] since the genetic relatedness and composition of human and animal MRSA are indistinguishable. Therefore, companion animals can be affected by the spread of human invasive clones. Our study demonstrated that the predominant MRSA clone originated from humans and has been found in companion dogs of the community. As a result, the presence of MRSA in residential settings suggests that it might be a source of infection for residents and pets.

Molecular genotyping analysis showed that MRSA strains in this study were classified as ST72-SCC*mec* IV, including *spa* types t324 (07-23-12-12-17-20-17-12-12-17) and t148 (07-23-12-21-12-17-20-17-12-12-17). The ST72-IV MRSA strains were identified in 11 different households to have the same genotype. In particular, MRSA clones belonging to ST72-IVc showed almost the same pattern in the PFGE dendrogram analysis. Several studies have reported that PVL-negative ST72-SCC*mec* IV MRSA in both the community and nosocomial infection is the most common clone in Korea [9, 10], but it is unclear whether these MRSA strains are phylogenetically identical clones. However, in a narrow range of studies, our study showed that the MRSA strains were distributed as the same clones in each region and thus these may be endemic clones in the community. Besides, the colonization of MRSA in the nasal mucosa of companion dogs was confirmed through different sampling times. Thus, companion dogs harboring ST72-IV MRSA also may act as the potential mediators of MRSA infection by contaminating the surrounding environment, including humans. This study analyzed the epidemiological relatedness of MRSA in the animal hospital personnel, their families, living companion dogs, and the residential environment. It can be concluded that the MRSA clones isolated from humans, dogs, and environments in our study are not notably different from the clones that are prevalent in Korea. In one study, a human MRSA carrier was treated at the hospital and confirmed as MRSA negative by the end of treatment, but MRSA was detected again shortly after. The MRSA from his family and dog were identified with the same clone by PFGE, SCC*mec* typing and *spa* typing. The authors suggested that the antimicrobial therapy against MRSA carriers, including healthcare workers and any infected family member or companion animal, is important to prevent the recurrence of MRSA carriage, as previously described [23].

In conclusion, ST72-IV MRSA was predominantly distributed and spread in households where dogs and family members live together. As mentioned above, dogs and their owners carried genetically identical MRSA strains, suggesting transmission of MRSA between dogs and their owners. The transmission of MRSA ST72-SCC*mec* IV clonal lineage is of concern and appropriate public health controls should be provided. Further investigation on interspecies transmission of MRSA in different companion animals (i.e. cats and exotic species) is necessary in future research.

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