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mecA Gene Transferrability and Antibiogram of Zoonotic *Staphylococcus intermedius* from Animals, Staff, and the Environment in Animal Hospitals in Korea

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***Staphylococcus intermedius* is a common cause of otitis externa, pyoderma, and wound infections in companion animals. Although *S. intermedius* infections are rare in humans, it is zoonotic, with several case reports describing fatal human infections. Presently, we sought to isolate *S. intermedius* strains from various sources at animal hospitals nationwide in Korea, examine their antibiotic susceptibilities, and determine the possibility of horizontal transmission between animals and humans. Pulsed-field gel electrophoresis (PFGE) was used to compare the *mecA* gene in *S. intermedius* strains from humans, animals, and the environment in animal hospitals. A total of 119 *S. intermedius* strains were isolated from 529 samples. Using the disk diffusion method, over 90% of the isolates were found to be susceptible to cephalothin, amoxicillin–clavulanic acid, vancomycin, imipenem, nitrofurantoin, and amikacin, whereas 97.5% and 98.3% of the isolates were resistant to penicillin and ampicillin, respectively. Among the 39 *S. intermedius* strains harboring *mecA*, similar PFGE patterns were observed between seven isolates from an animal, two isolates from veterinary staff, and the environment in one animal hospital, and single isolates from an animal and a veterinarian at another hospital. This result suggests the possibility of horizontal transmission of *S. intermedius* containing *mecA* between humans, animals, and the environment in animal hospitals and also emphasizes on the importance of *S. intermedius* with *mecA* as a possible emerging threat to public health.**

Keywords: *Staphylococcus intermedius*, methicillin-resistant *Staphylococcus intermedius* (MRSI), multidrug resistant, animal hospital, transferrability of *mecA* gene

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Staphylococcus intermedius is the most isolated Gram-positive and coagulase-positive staphylococci in both healthy and diseased dogs. Human infections are rare, but *S. intermedius* has been implicated as a zoonotic organism in invasive as well as noninvasive infections [9] arising from dog-bite wounds [23], infectious endocarditis in those infected with the Human Immunodeficiency Virus [24], food poisoning outbreak [20], otitis externa [29], and catheter-related bacteremia [31].

Methicillin resistance in staphylococci is usually caused by the acquisition of a mobile gene element termed a staphylococcal cassette chromosome *mec* (SCC*mec*) [1, 7], which is one of the major public health concerns because of the limited therapeutic options [6]. Much less is known of the SCC*mec* typing of methicillin-resistant *S. intermedius* (MRSI), which can cause pneumonia after a coronary bypass surgery, brain abscess, and empyema in humans [2, 12, 33]. In The Netherlands, the same MRSI strains were detected in infected surgical wounds from pets that had undergone surgery at the same animal hospital. The MRSI strain was also found from a healthy dog, a surgeon, and nurses/technicians who were directly involved in the surgeries, and even from the environmental samples [30], suggesting a transmission of MRSI between humans and animals. More precisely, transmission may be one-way - from dogs to humans – because only one case of *S.*

intermedius infection was observed in an examination of the nasopharyngeal flora of 144 healthy veterinary staff members [30]. *S. intermedius* is rarely isolated from the normal flora of humans, even from those who are regularly exposed to animals [21].

Compared with animal hospitals, *S. intermedius* nosocomial infections such as those occurring in surgical wounds, urinary tract, and respiratory tract represent a problem that must not be overlooked in human hospitals [5]. However, there are also many similarities between human and animal hospitals that include widespread use and overuse of antibiotics, complex treatments, prolonged hospitalization of critically ill patients, development of emergency medicine, and the appearance of large intensive care units in veterinary clinics [5]. As veterinarians and staff in animal hospitals are frequently exposed to dogs, they may have a high risk of *S. intermedius* or MRSI infections, and may also play a carrier role. In particular, MRSI and methicillin-resistant *S. pseudointermedius* (MRSP) are growing concerns in veterinary clinics around the world [3]. Transmission may occur not only between animal, human, and environment groups, but also between these groups (*i.e.*, human-to-human and animal-to-animal). Thus, *S. intermedius*, especially MRSI, represents a potential serious public health concern.

Transmission of *S. intermedius* between animals and veterinarians [13, 15] may also be present in Korea, and might be an emerging problem for public health. Presently, we sought to determine whether horizontal transmission of *S. intermedius* strains was present among various sources using samples obtained from five different animal referral hospitals in four different regions of Korea (Seoul, Gyeongi, Chungbuk, and Jeju) and to determine the antibiotic susceptibility of *S. intermedius* isolates.

Materials and Methods

Sampling and Bacterial Identification

A total of 529 samples were obtained of which 271 were from 54 dogs; 170 from 77 hospital staff including veterinarians, veterinary technicians, and receptionists; and 88 from the hospital environment of five animal hospitals (four teaching hospitals and one private referral hospital) in Seoul, Gyeonggi-do, Chungcheong-do, and Jeju-do. The 271 canine samples were taken from the anus (n=52), horizontal ear canal (n=83), nasal mucosa (n=61), skin (n=56), wound infection area (n=8), pad (n=4), and urine (n=7). Human samples were taken from the hand (palm and the skin between fingers; n=93) and nasal cavity (n=77). All samples from animals and humans were taken using BD BBL Culture Swabs (Becton-Dickinson, Sparks, MD, U.S.A.). Environment samples were taken using a meat/turkey carcass sampling kit (Nasco, Fort Atkinson, WI, U.S.A.). In a university animal teaching hospital and a private referral animal hospital, testing was done twice with an interval of 3 months and 19 months, respectively. *S. intermedius* was isolated and identified using various biochemical tests, with additional confirmation being done using a Vitek 2 GPI card (bioMerieux, Lyon, France) and polymerase chain reaction (PCR) with previously described *S. intermedius*-specific primers [4, 26] (Table 1).

Antimicrobial Susceptibility Tests

Antimicrobial susceptibilities of *S. intermedius* isolates to 16 different antimicrobials from 11 classes were tested by a disk diffusion test according to the Clinical and Laboratory Standards Institute (CLSI, M2-A9) guideline [8]. The tested antibiotics and their concentrations were ampicillin (10 μg); amoxicillin–clavulanic acid (30 μg); amikacin (30 μg); chloramphenicol (30 μg); clindamycin (2 μg); cephalothin (30 μg); ciprofloxacin (5 μg); cefotaxime (30 μg); erythromycin (15 μg); nitrofurantoin (300 μg); gentamicin (10 μg); imipenem (10 μg); penicillin (10 units); trimethoprim–sulfamethoxazole (23.75 μg, 1.25 μg); tetracycline (30 μg), and vancomycin (30 μg) (BD BBL). In addition, the minimal inhibitory concentrations (MICs) to oxacillin (Sigma–Aldrich, St. Louis, MO, U.S.A.), vancomycin (Sigma–Aldrich), and orbifloxacin (Riedel–deHaen, Seelze, Germany) were determined by a broth microdilution method according to the CLSI guideline (M2-A9) [8]. Isolates with vancomycin MIC≥16 mg/l and orbifloxacin MIC≥8 mg/l were defined as being vancomycin and orbifloxacin resistant, respectively. Isolates with oxacillin MIC≥4 mg/l were classified as being methicillin resistant, and were further confirmed by PCR using primer sets targeting the *mecA* gene as MRSI (Table 1). *S. intermedius* ATCC 29663 and *S. aureus* ATCC 29213 were used as reference strains.

DNA Preparation and PCR Amplification

S. intermedius isolates were cultured overnight on 5% sheep blood agar plates (Promed, Ansan, Korea) at 37°C. The chromosomal DNA was extracted using the DNeasy tissue kit (Qiagen, Valencia, CA, U.S.A.) according to the manufacturer's instructions for Gram-positive bacteria, with a modification of the cell lysis step using 50 U/ml lysostaphin (Sigma–Aldrich), and amplified following a program described previously [4, 33] using *S. intermedius* ATCC 29663 and *S. aureus* ATCC 29213 as reference strains.

Screening of Methicillin-Resistant *S. intermedius* SCC*mec* Types

For the classification of the methicillin-resistant SCC*mec* group of MRSI, a recently described new multiplex PCR method using 4 primer sets (β and α3, 937 bp; ccrCF and ccrCR, 518 bp; 1272F1 and 1272R, 415 bp; 15RmecA and 5R431, 359 bp) [6] was used.

Pulsed-Field Gel Electrophoresis Analysis (PFGE) of *S. intermedius*

For the analysis of possible transmission of MRSI or *mecA* gene between human, animals, and the environment of hospitals, PFGE was carried out using previous methods [22] with a few modifications using PFGE settings as follows: initial pulse time of 10 s and final pulse time of 35 s.

Results

Identification of *S. intermedius* from Animal, Hospital Staff, and Hospital Environment Sources

One hundred nineteen (22.5%) strains of *S. intermedius* were isolated from 529 samples. We could obtain only one strain per *S. intermedius*-positive sample. Of the 119

Table 1. Description of oligonucleotide primers and their PCR amplicons size.

Amplicon and primer	Primer sequence (5'–3')	Amplicon size (bp)	Reference
nuc			
SInuc1	CAA TGG AGA TGG CCC TTT TA	125	[4]
SInuc2	AGC GTA CAC GTT CAT CTT G		
16S rDNA			
Primer-1	CCG TAT TAG CTA GTT GGT GG	901	[33]
Primer-2	GAA TGA TGG CAA CTA AGT TC		
mecA			
mecA1	AAA ATC GAT GGT AAA GGT TGG C	532	[29]
mecA2	AGT TCT GCA GTA CCG GAT TTG C		
blaZ			
blaZ1	ACT TCA ACA CCT GCT GCT TTC	173	[26]
blaZ2	TGA CCA CTT TTA TCA GCA ACC		
ccrA2-B			
β	ATT GCC TTG ATA ATA GCC YTC T	937	[16]
α	TAA AGG CAT CAA TGC ACA AAC ACT		
ccrC			
ccrCF	CGT CTA TTA CAA GAT GTT AAG GAT AA	518	[17]
ccrCR	CCT TTA TAG ACT GGA TTA TTC AAA ATA T		
IS1272			
1272F1	GCC ACT CAT AAC ATA TGG AA	415	[6]
1272R1	CAT CCG AGT GAA ACC CAA A		
mecA-IS431			
5RmecA	TAT ACC AAA CCC GAC AAC TAC	359	[6]
5R431	CGG CTA CAG TGA TAA CAT CC		

strains, 73 (61.3%) were from animals, 34 (28.6%) were from hospital staff, and 12 (10.1%) were from the hospital environment. The description of the animal isolates was as follows: 19/52 (36.5%) from anus, 20/83 (24.1%) from horizontal ear canal, 17/61 (27.9%) from nasal mucosa, 11/56 from skin (19.6%), 3/8 (37.5%) from wound infection area, 3/4 (75%) from pad, and 0/7 (0%) from urine specimen. With regards hospital staff, 20/93 (21.5%) and 14/77 (18.8%) were isolated from the nasal cavity and hand, respectively. A survey of the hospital staff showed that none previously or presently displayed similar symptoms that could be attributed to *S. intermedius* in animals. The 12 *S. intermedius* hospital environment isolates were obtained from the floor (n=5), cages (n=2), examination desk (n=1), doorknob (n=2), examination room computer (n=1), and toilet seat cover (n=1).

Antibiotic Resistance of *S. intermedius* Strains

The result of disk diffusion test is shown in Fig. 1A. Resistance to more than three antimicrobial classes represented multidrug resistance (MDR); MDR was exhibited by 70 (58.8%) of the 119 *S. intermedius* isolates. The MDR rate of *S. intermedius* was 58.9% (43/73) in animals, 58.8% (20/34) in hospital staff, and 58.3% (7/12) in the environment. Resistance to five antimicrobial classes was at least 50% in all three group; 53.4% (39/73) in animal isolates, 50% (17/34) in hospital staff isolates, and 50% (6/12) in hospital environment isolates. Oxacillin MIC testing revealed 49 *S. intermedius* isolates (41.2%: 27 animal strains, 14 human strains, and 8 environmental strains) to be resistant, which included 11 strains with the *mecA* gene that did not display oxacillin resistance in the MIC test. Seventy isolates (58.8%) (46 animal strains, 20 human strains, and 4 environmental strains) were susceptible to oxacillin. Forty-two isolates (35.3%) were resistant, 5 isolates (4.2%) intermediate, and 72 isolates (60.5%) susceptible to orbifloxacin. All the 119 *S. intermedius* isolates were susceptible to vancomycin according to the MIC test, whereas the disk diffusion method showed one animal and human isolate each to be resistant. The MIC test result to the three antibiotics oxacillin, vancomycin, and orbifloxacin are shown in Fig. 1B and Tables 2–4.

Identification of MRSI Using *mecA* Gene PCR

PCR was performed to detect the *mecA* gene in all 119 *S. intermedius* isolates. The *mecA* gene was detected in 39 isolates; of these, 17 exhibited MIC-determined oxacillin resistance. Five *S. intermedius* strains were obtained from five animals (24 samples) having skin lesions or a history of skin infections. None of these carried the *mecA* gene.

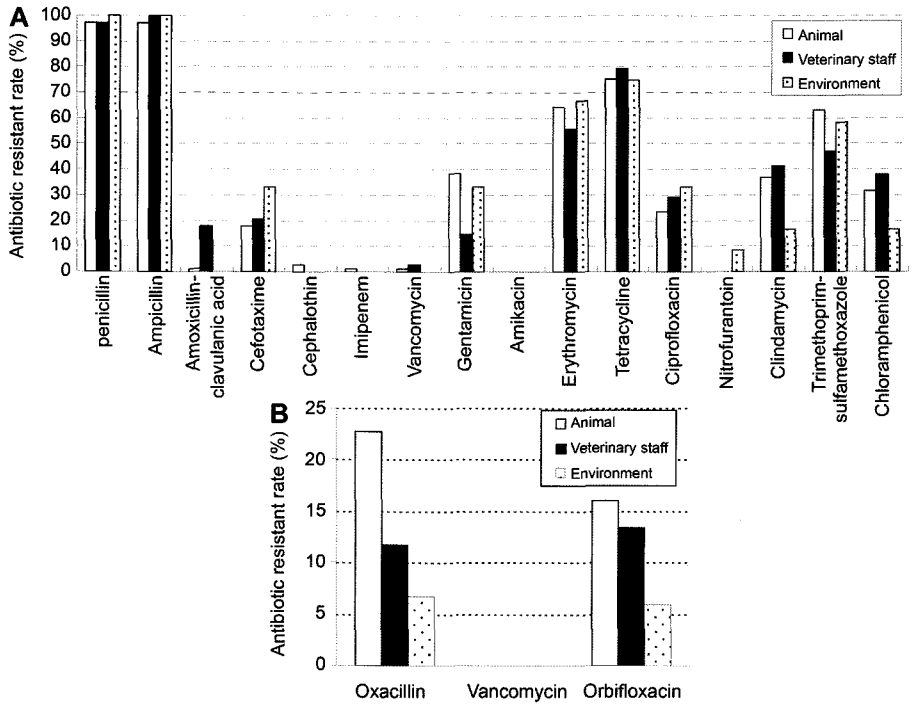

Fig. 1. Antibiogram of *Staphylococcus intermedius* from animals, veterinary staff, and hospital environment to 16 different antimicrobials from 12 different antimicrobial classes examined by the disk diffusion method (**A**) and antibiogram of those isolates to oxacillin, vancomycin, and orbifloxacin examined by the broth microdilution method (**B**).

SCC*mec* Typing of *S. intermedius* Isolates

Out of 39 *mecA* gene-containing *S intermedius* strains, the SCC*mec* types of 20 isolates (51.28%) could be classified using multiplex PCR for SCC*mec* typing [6] [Fig. 2]. The most common SCC*mec* type was V (19 of 20), which was detected in 10 animal isolates (47.6%), 6 hospital staff isolates (50%), and three hospital environment isolates (50%). The remaining hospital isolate contained type IV SCC*mec*. All 20 strains belonged to community-acquired MRSI (CA-MRSI) [6].

Table 2. Genetic and phenotypic characteristics of the 12 methicillin-resistant *Staphylococcus intermedius* isolates from veterinary staff.

Strain number	Region	Year	Origin	Isolation site	MIC (mg/l)[a]			PCR results		SCC*mec* type
					Oxacillin	Vancomycin	Orbifloxacin	*mecA*	*blaZ*	
K6-1	Seoul	2008/4	Veterinary staff #1	Nasal mucosa	64 (R)	0.5 (S)	32 (R)	+	+	NT
K6-1A	Seoul	2008/4	Veterinary staff #1	Nasal mucosa	>128 (R)	1 (S)	32 (R)	+	+	NT
K7[c]	Seoul	2008/4	Veterinary staff #2	Hand	0.125 (R)	2 (S)	128 (R)	+	+	V
K17	Seoul	2008/4	Veterinary staff #3	Hand	128 (R)	0.5 (S)	32 (R)	+	+	NT
K18	Seoul	2008/4	Veterinary staff #3	Nasal mucosa	0.125 (R)[b]	0.5 (S)	64 (R)	+	+	V
K21[c]	Seoul	2008/4	Veterinary staff #4	Hand	0.125 (R)[b]	2 (S)	128 (R)	+	+	V
K21-1	Seoul	2008/4	Veterinary staff #4	Hand	64 (R)	0.5 (S)	32 (R)	+	+	NT
K22	Seoul	2008/4	Veterinary staff #4	Nasal mucosa	8 (R)	1 (S)	32 (R)	+	+	NT
H2 19-2	Gyeonggi	2008/4	Veterinary staff #5	Hand	2 (R)	0.5 (S)	128 (R)	+	+	V
H2 27-1	Gyeonggi	2008/4	Veterinary staff #6	Nasal mucosa	0.125 (R)[b]	0.5 (S)	1 (S)	+	+	NT
H2 34-1[c]	Gyeonggi	2008/4	Veterinary staff #7	Nasal mucosa	0.125 (R)[b]	2 (S)	128 (R)	+	+	V
H2 40-1	Gyeonggi	2008/4	Veterinary staff #8	Nasal mucosa	0.25 (R)[b]	1 (S)	128 (R)	+	+	V

[a](R) Resistant; (I) Intermediate; (S) Susceptible.

[b]According to Clinical and Laboratory Standards Institute [8], these isolates must be oxacillin resistant despite the minimum inhibitory concentration <4 mg/l, because they possess the *mecA* gene.

[c]All isolates had the identical test results for MIC (OXA, VAN, ORB), disk diffusion test (P, AM, AmC, CTX, CF, IPM, Va, GM, AN, E, Te, CIP, F/M, CC, SXT, C), detection of antibiotic-resistant gene *blaZ*, *mecA*, and SCC*mec* typing.

Table 3. Genetic and phenotypic characteristics of the six environmental methicillin-resistant *Staphylococcus intermedius* isolates.

Strain number	Region	Year	Origin	Isolation site	MIC (mg/l)[a]			PCR results		SCC*mec* type
					Oxacillin	Vancomycin	Orbifloxacin	*mecA*	*blaZ*	
K42[c]	Seoul	2008/4	Environment #1	Doorknob	0.125 (R)[b]	2 (S)	128 (R)	+	+	V
K57	Seoul	2008/4	Environment #2	Toilet stool	8 (R)	1 (S)	128 (R)	+	+	IV
H2 43	Gyeonggi	2008/4	Environment #3	Cage	1 (R)[b]	1 (S)	128 (R)	+	+	NT
H2 50A	Gyeonggi	2008/4	Environment #4	Computer (Diagnosis room)	64 (R)	1 (S)	32 (R)	+	+	V
H2 53A	Gyeonggi	2008/4	Environment #5	Chair (Waiting room)	0.125 (R)[b]	0.5 (S)	64 (R)	+	+	NT
H2 64A	Gyeonggi	2008/4	Environment #6	Floor	0.125 (R)[b]	1 (S)	64 (R)	+	+	V

[a](R) Resistant; (I) Intermediate; (S) Susceptible.
[b]According to Clinical and Laboratory Standards Institute [8], these isolates must be oxacillin resistant despite the minimum inhibitory concentration <4 mg/l, because they possess the *mecA* gene.
[c]All isolates had the identical test results for MIC (OXA, VAN, ORB), disk diffusion test (P, AM, AmC, CTX, CF, IPM, Va, GM, AN, E, Te, CIP, F/M, CC, SXT, C), detection of antibiotic-resistant gene *blaZ*, *mecA*, and SCC*mec* typing.

PFGE Analysis

A total of seven MRSI strains (four isolates from one animal, two hospital staff, and one hospital environment sample, all of which were taken from the same hospital) showed similar PFGE patterns (Fig. 3). In addition, one MRSI isolate from a veterinarian's nasal mucosa and one MRSI isolate from the anus of a dog sampled in the private referral animal hospital showed the same PFGE pattern. There were no similar patterns between MRSI isolates from the different animal hospitals.

Table 4. Genetic and phenotypic characteristics of the 21 methicillin-resistant *Staphylococcus intermedius* isolates from animals.

Strain number	Region	Year	Origin	Isolation site	MIC (mg/l)[a]			PCR results		SCC*mec* type
					Oxacillin	Vancomycin	Orbifloxacin	*mecA*	*blaZ*	
S1 37	Seoul	2006/11	Animal #1	Skin	32 (R)	1 (S)	1 (S)	+	+	NT
S2 19-2	Seoul	2007/1	Animal #2	Horizontal ear canal	8 (R)	1 (S)	1 (S)	+	+	NT
S2 40	Seoul	2007/1	Animal #3	Skin	16 (R)	1 (S)	1 (S)	+	+	NT
S2 64	Seoul	2007/1	Animal #4	Wound region	16 (R)	1 (S)	0.5 (S)	+	+	NT
S2 67-2	Seoul	2007/1	Animal #5	Nasal mucosa	8 (R)	1 (S)	0.5 (S)	+	+	NT
S2 69-2	Seoul	2007/1	Animal #5	Wound region	4 (R)	1 (S)	1 (S)	+	+	NT
C1	Chungbuk	2007/6	Animal #6	Anus	0.5 (R)[b]	1 (S)	1 (S)	+	+	NT
C2	Chungbuk	2007/6	Animal #6	Nasal mucosa	16 (R)	1 (S)	0.5 (S)	+	+	NT
C5	Chungbuk	2007/6	Animal #6	Horizontal ear canal	4 (R)	1 (S)	1 (S)	+	+	NT
C17	Chungbuk	2007/6	Animal #7	Nasal mucosa	8 (R)	1 (S)	0.5 (S)	+	+	NT
C18	Chungbuk	2007/6	Animal #7	Horizontal ear canal	64 (R)	1 (S)	1 (S)	+	+	V
C27	Chungbuk	2007/6	Animal #8	Horizontal ear canal	0.25 (R)[b]	1 (S)	1 (S)	+	+	V
C29	Chungbuk	2007/6	Animal #8	Nasal mucosa	0.5 (R)[b]	1 (S)	1 (S)	+	+	V
C30	Chungbuk	2007/6	Animal #8	Skin	0.5 (R)[b]	1 (S)	1 (S)	+	+	V
K31[c]	Seoul	2008/4	Animal #9	Skin	0.125 (R)[b]	2 (S)	128 (R)	+	+	V
K32[c]	Seoul	2008/4	Animal #9	Horizontal ear canal	0.125 (R)[b]	2 (S)	128 (R)	+	+	V
K33-1[c]	Seoul	2008/4	Animal #9	Pad	0.125 (R)[b]	2 (S)	128 (R)	+	+	V
K34	Seoul	2008/4	Animal #9	Anus	0.125 (R)[b]	1 (S)	128 (R)	+	+	V
K35[c]	Seoul	2008/4	Animal #9	Wound region	0.125 (R)[b]	2 (S)	128 (R)	+	+	V
H2 2-1A[c]	Gyeonggi	2008/4	Animal #10	Anus	0.125 (R)[b]	2 (S)	128 (R)	+	+	V
H2 2-2	Gyeonggi	2008/4	Animal #10	Horizontal ear canal	0.125 (R)[b]	1 (S)	>128 (R)	+	+	NT

[a](R) Resistant; (I) Intermediate; (S) Susceptible.
[b]According to Clinical and Laboratory Standards Institute [8], these isolates must be oxacillin resistant despite the minimum inhibitory concentration <4 mg/l, because they possess the *mecA* gene.
[c]All isolates had the identical test results for MIC (OXA, VAN, ORB), disk diffusion test (P, AM, AmC, CTX, CF, IPM, Va, GM, AN, E, Te, CIP, F/M, CC, SXT, C), detection of antibiotic-resistant gene *blaZ*, *mecA*, and SCC*mec* typing.

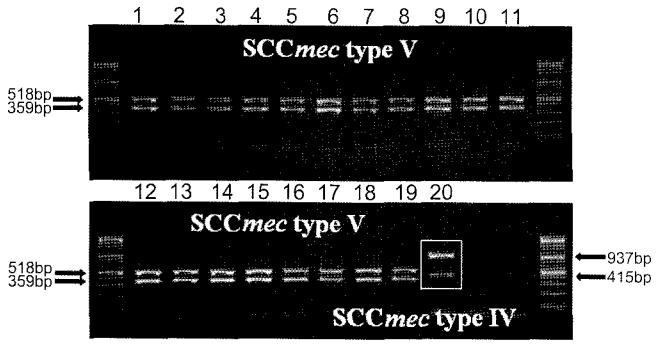

Fig. 2. Multiplex PCR assay for identification of SCC*mec* types of methicillin-resistant *Staphylococcus intermedius*.
Lanes 1–5 and 14–18, animal isolates; Lanes 6–8 and 11–13, hospital staff isolates; Lanes 9, 10, 19, and 20, hospital environment isolates.

Discussion

To our knowledge, this study is the first published analysis of the antibiograms and possible transferability of *S. intermedius* isolated from animals, veterinary staff, and animal hospital environments in Korea. During the first collecting phase in 2006/2007, 60 *S. intermedius* isolates (51 animal, 5 hospital staff, and 4 hospital environment) from four different animal hospitals were isolated. The *mecA* gene was detected in 14 *S. intermedius* animal isolates.

The low number of *S. intermedius* isolates in hospital staff and hospital environment (n=9) was insufficient to conclude that there is obviously no horizontal transmission between animals, staff, and the hospital environment. Therefore, additional samples were obtained from two animal hospitals (201 samples); 59 *S. intermedius* isolates (animal, n=22; hospital staff, n=29; hospital environment, n=8) were obtained, and 7, 12, and 6 *S. intermedius* isolates from animal, hospital staff, and hospital environment, respectively, harbored the *mecA* gene. A total of 119 *S. intermedius* were isolated from 529 samples (22.5%), which is a higher isolation rate than that reported in a previous study [27], in which only 462 *S. intermedius* isolates were evident from 28,392 samples (1.63%). Although *S. intermedius* is a temporary bacterium on the skin and hair coat of dogs [16], the isolation rate was the lowest (19.6%) compared with the other sampling area. The reason for the low rate of *S. intermedius* from the skins may be due to random and spotwise sampling of the animal's skin.

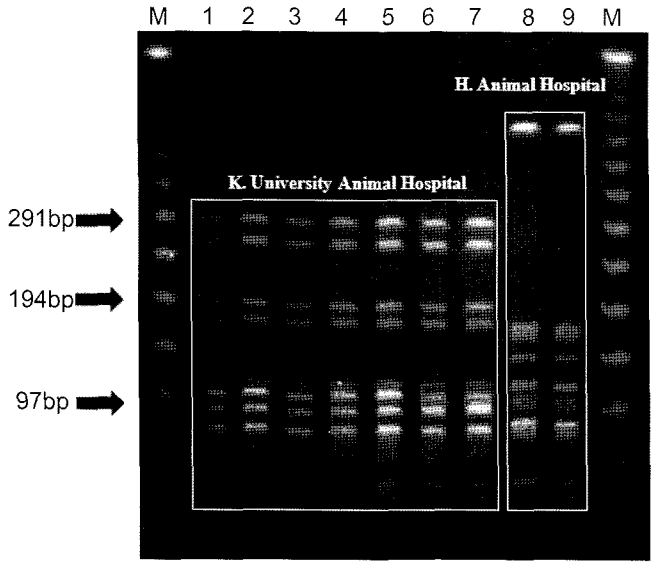

Fig. 3. Pulsed-field gel electrophoresis for the analysis of possible transmission of methicillin-resistant *Staphylococcus intermedius* or *mecA* gene.
Lane 1, Veterinary staff 1; Lane 2, Veterinary staff 2; Lanes 3–6 from a single animal (dog); Lane 7, Environment; Lane 8, Animal; Lane 9, Veterinary staff.

The high antibiotic resistance of *S. intermedius* among the animal isolates against penicillin and ampicillin (Fig. 1A) might be due to the frequent use of penicillin G (54 kg in 2004 and 87 kg in 2005) and ampicillin (57 kg in 2004 and 61 kg in 2005) in small animal hospitals in the past few years in Korea. Comparison of the disk diffusion test results of animal *S. intermedius* isolates with eight different antimicrobial agents from the present study with two previous studies [5, 25] are shown in Supplemental Table 1. All three groups showed high resistant rates against penicillin, whereas the *S. intermedius* isolates displayed 100% resistance against amoxicillin–clavulanic acid and cephalothin [25], which is markedly higher compared with resistance rates of 1.37% determined presently and the rate of 2.74% determined in the other study [5]. This difference might be due to the variation in the sampling source (wound area versus wound-free area), diverse selective pressure exhibited by the use of different antibiotics in different regions (Germany, Switzerland, and Korea), and sampling sources.

For the treatment of Gram-negative and Gram-positive bacterial infections including pyoderma and otitis, a new third-generation fluoroquinolone antimicrobial agent called orbifloxacin has been developed exclusively for companion animals [17]. Presently, oxacillin MIC results indicated a resistance rate of 41.18%, which is markedly different from the rate of 0% reported by five other studies. An extremely higher orbifloxacin resistance rate was shown in the present study compared with one reported in another study [10] (35.29% vs. 0.42%) (Supplemental Table 2).

A total of 39 isolates (32.8%) possessed the *mecA* gene and among them only 20 (51.28 %) isolates could be classified according to the method of Boye *et al.* [6], where the identification rate was described as 98%. Additionally, in the latter study, the SCC*mec* type IV was shown in 84% of the isolates, followed by type V (6%), type I (4%), and type II (3%). Presently, the SCC*mec* type V was identified in 95% of the typeable isolates, followed by type IV (5%)

(Fig. 2). A considerable difference in numbers of typeable isolates and SCC*mec* type was evident. The different sources (human hospital vs. animal hospital) and bacterial species of the samples (*S. aureus* vs. *S. intermedius*), along with the regional differences (Denmark vs. Korea) may be the reason for the low identification rate and the different distribution of SCC*mec* types.

The MRSI isolates were compared using PFGE to see if the isolates in the three groups were related to one another. Seven isolates (hospital staff n=2; animals n=4; environment n=1) from university animal hospital K and two isolates (one each from an animal and a hospital staff person) from the private hospital H displayed the same PFGE pattern (Fig. 3). These isolates were also compared by their antibiograms (Tables 2–4). Isolates 2–5 were obtained from a single dog in a hospital and from the two veterinarians in charge of that animal. It is not clear how the MRSI strains were transmitted, but the results of this study indicate that horizontal transmission of MRSI or the *mecA* gene from hospital staff, animal, and the environment may be operative. To confirm the horizontal transmission, a chronological isolation of MRSI is necessary, which should be done as a separate research in further studies. The two human isolates (K1 and K7) with the same PFGE pattern from hospital K also suggest a possible horizontal transmission from human-to-human. The PFGE patterns of *mecA* bore no similarity between the five animal hospitals. Therefore, transmission of the *mecA* gene and/or MRSI between the animal hospitals may not be operative.

Hospital staff with *S. intermedius* showed no clinical signs of disease, based on a survey completed by the individuals. If true, this highlights the importance for vigilance by hospital staff, who may serve as carriers for the pathogen. To prevent transmission and avoid the outbreak of disease caused by *S. intermedius* and MRSI, prudent use of antibiotics and strict infection control practices in animal hospitals should be enforced. In addition, continuous monitoring and molecular epidemiological studies should be followed. As the animals spend the main time with their owner and in their homes, continuous sampling of these two groups will give us more information about the spread and antibiograms of *S. intermedius*.

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