수의학석사학위논문

Methicillin-Resistant Staphylococci Isolated from Companion Animals and Humans: Antibiograms and Genetic Comparison

반려동물과 사람 유래 메티실린 내성 포도상구균: 항생제 내성과 유전적 특성 비교

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By

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(Major: Veterinary Microbiology)

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Abstract

Community-associated methicillin-resistant staphylococci (CA-MRS) are considered an important problem in many countries. CA-MRS can disseminate not only within human communities, but also between companion animals and their owners as they share environments and living conditions. In this study, 592 staphylococci were isolated from February to April 2012 by taking swab samples from companion animals, owners, the general public who did not have contact with companion animals, and the veterinary hospital staff in Seoul, Korea. Prevalence of the isolates, their antimicrobial resistance patterns, and genetic relationships were subsequently investigated.

The most prevalent species isolated from companion animals was *Staphylococcus intermedius* (55.5%). In addition, all 3 human groups—animal owners, the general public, and veterinary hospital staff—carried the common predominant species *Staphylococcus epidermidis* (51.7%, 43.3%, and 56.1%, respectively). All 4 groups showed the highest resistance rate against the

antimicrobial agent ampicillin, and all were susceptible to amikacin. Isolates from companion animal showed higher resistance rates against chloramphenicol, enrofloxacin, and sulfamethoxazole than that shown by the human isolates ($P < 0.05$). Antimicrobial resistance patterns among the general public group were similar to that of the owner group. These results suggest that antimicrobial resistance patterns in humans may not be influenced by physical contact of humans with companion animals. In addition, the resistance patterns shown by the general public group were different from those shown by the veterinary hospital group. Among 15 antimicrobial agents, 8 (ampicillin, cefoxitin, ciprofloxacin, enrofloxacin, erythromycin, oxacillin, sulfamethoxazole, and tetracycline) were more resistible in the veterinary hospital staff group; individuals in this group are more likely to be exposed to bacteria and antimicrobial agents ($P < 0.05$). Moreover, several isolates with a genetic similarity of 99%, as measured using Random amplification of polymorphic DNA-polymerase chain reaction (RAPD-PCR) were detected from companion animals and their owner in the same household, suggesting the possibility of contact transmission of bacteria between the 2 groups. However, results of comparisons of *ccr* gene sequences associated with methicillin resistance in staphylococci suggested that horizontal transfer of *ccr* genes between companion animals and owners did not occur.

In conclusion, transmission of staphylococci may occur in households between companion animals and owners; however, the possibility of horizontal transfer of

methicillin resistance-associated genes between 2 groups can be ruled out in the present study.

Keywords: Companion animal, owner, methicillin-resistant staphylococci (MRS), transmission, antimicrobial resistance

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Methicillin-resistant staphylococci (MRS) have been a serious problem in humans and animals because they can induce severe and potentially fatal infections. In particular, community-associated (CA)-MRS has been significant research focus in many countries. As CA-MRS prevalence has increased, households have become potential primary locations of transmission (Davis *et al.*, 2012). Accordingly, companion animals living indoors have also been suspected as a source of antimicrobial resistant microorganisms (Lloyd, 2007, Guardabassi *et al*., 2004). In Europe and North America, 4 major clonal methicillin-resistant *Staphylcoccus pseudintermedius* (MRSP) lineages are dominant; moreover, the isolates displayed resistance to the major classes of antibiotics, which could cause a serious therapeutic problem (Perrenten *et al*., 2010). Researchers have suggested a possible role for companion animals in the transfer of antimicrobial resistance (Barber *et al*., 2003). In particular, reports about the carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) in companion animals have increased significantly (Bramble *et al*., 2011). Evidence, albeit indirect, suggests that companion animals can be affected by the MRSA infection and colonization in humans under home environments (Loeffler *et al*., 2010). In Germany, MRSA isolates from pets closely resembled MRSA isolates that were widely disseminated in German hospitals. Researchers concluded that transmission of

MRSA between pet animals and humans might have occurred (Strommenger *et al.*, 2006). In addition to transmission from companion animals to owners, researchers have demonstrated that transmission can take place from humans to companion animals (Broens *et al*., 2008). This evidence was detected in the case of an MRSA-infected patient and his dog (Bronwyn *et al*., 2009). MRSA was found in the bacterial cultures from a skin biopsy sample obtained from the patient, and his companion dog was revealed to have been infected by indistinguishable MRSA strains. Although transmission between owners and their companion animals has not been proved definitely, fact remains that several cases are being reported in which companion animals and owners share indistinguishable strains that can cause infections in both. In order to understand bacterial interspecies transmission and make effective strategies to prevent any further spreading of pathogenic strains in households, close investigation should be conducted beforehand. In this study, firstly, MRS prevalence through companion animals, owners, and the general public who did not have contact with companion animals, and veterinary hospital staffs were inspected. Secondly, overall antimicrobial resistance tests were performed and analyzed statistically to compare resistance rates and trends among each group. In addition, genetic relatedness of MRS isolates was examined to speculate possibilities of bacterial transmission between companion animals and owners. In addition to transmission of bacterial strains, transfer of gene elements was examined. Partial sequences of the *ccr* gene complex were analyzed

for a part of the staphylococcal cassette chromosome (SCC) element that contains *mecA*, which confers methicillin resistance in staphylococci.

MATERIALS AND METHODS

Bacterial isolates

From February to April 2012, swab samples were collected from companion animals including dogs (n = 45) and cats (n = 2), their owners (n = 57), the general public (n = 37) who did not keep companion animals, and veterinary hospital staff (n = 20). Sample collection from companion animals, owners, and veterinary hospital staff was carried out at 7 local veterinary hospitals in Seoul, Korea. All samples were taken simultaneously when owners visited veterinary hospital with their companion animals, with the cooperation of the veterinary medical staff. For the general public, the samples were collected from volunteer university students. Swabs were collected from the external auditory meatus (n = 92), medial canthus (n = 32), interdigital cleft (n = 93), nasal cavity (n = 83), and anus (n = 41) and then cultured on blood agar plates (Komed, Korea). Thereafter, *Staphylococcus* spp. were identified using a Bruker Biotyper matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometer (MS) 3.0 (Bruker Daltonics, Bremen, Germany) and a VITEK II system (Biomerieux, France).

Antimicrobial resistance test

Antimicrobial resistance patterns were tested by using the disc diffusion method according to Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2008). Bacterial isolates were cultured in tryptic soy broth (TSB, Becton, Dickinson and Company, USA) at 37°C overnight. Appropriate TSB cultures were taken and subsequently added into 3 mL of 0.85% saline to equivalent turbidity of a 0.5 McFarland standard by DensiCHEK-plus (Biomerieux, France). Then 0.5 McF 0.85% saline was spread evenly on Mueller-Hinton agar (MHA, Becton, Dickinson and Company, USA) using an autoclaved swab. Within 15 min after spreading saline, antimicrobial agent discs were dispensed using a Sensi-Disc dispenser (Becton, Dickinson and company, USA). In this study, 15 antimicrobials were tested: ampicillin (AM), oxacillin (OXA), amoxicillin (AMC), ciprofloxacin (CIP), enrofloxacin (ENR), erythromycin (E), gentamicin(GM), chloramphenicol (C), tetracycline (TET), vancomycin (VA), sulfamethoxazole (SXT), amikacin (AN), cefazolin (CZ), cefoxitin (FOX), and cefotaxime (CTX). Plates containing antimicrobial agent discs were incubated at 37℃ for 16–18 h. The zone of inhibition was then measured to investigate if the isolates were susceptible to the tested antimicrobial agents or resistant according to CLSI criteria.

Random amplification of polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis

The 2 most prevalent species, *S. intermedius* and *S. epidermidis*, were analyzed using RAPD-PCR to examine their genetic relationships. The partially degenerated oligonucleotide 5′-GGTCGAC*Y*TT*N*GY*N*GG*R*TC-3′ (N: A, T, C, or G; Y: C or T; and R: A or G) was used as a primer (Limansky *et al*., 1998). The PCR was performed in a C1000 thermal cycler (Bio-Rad, Hercules, California). The final 50-μl reaction mixture contained 2.5 U of *h-Taq* DNA polymerase (Solgent, Korea), 2 μM oligonucleotide primer, 200 μM each dATP, dGTP, dGCT, and dTTP, and 10× *h-Taq* reaction buffer. PCR was then performed under the following conditions: denaturation at 95℃ for 5 min, 3 cycles at 95℃ for 1 min, 37℃ for 1 min, and 72℃ for 30 s, followed by 32 cycles at 95℃ for 1 min, 55℃ for 1 min, and 72℃ for 30 s, and final extenstion at 72℃ for 10 min. Then, 20 μL of the PCR product mixture dyed with ethidium bromide was analyzed using electrophoresis with 100 V for 50 min in 1.2% agarose gels. RAPD-PCR band patterns were analyzed using GelComparII (Applied Maths, Belgium) with 99% similarity.

PCR for *mecA* and *ccr* gene

Isolates carrying *mecA* genes were sorted out via PCR using primers mA1 (5′-TGCTATCCACCCTCAAACAGG-3′) and mA2 (5′-AACGTTGTAACCACCCCAAGA-3′) as described in an earlier study (Kondo *et al*., 2007). A C1000 thermal cycler (Bio-Rad, USA) was used for amplification with an initial denaturation at 94℃ for 2 min, followed by 30 cycles of denaturation at 94℃ for 2 min, annealing at 57℃ for 1 min, extension at 72℃ for 2 min, and a final elongation at 72℃ for 2 min. Amplified PCR product mixtures were analyzed using electrophoresis at 120 V for 40 min in 1.2% agarose gels. In addition, the *ccr* gene sequence was analyzed as describe previously (Lina *et al*., 2006). The same C1000 Thermal cycler (Bio-rad, USA) was used for amplification at 94℃ for 5 min, followed by 35 cycles at 94℃ for 30 s, at 55℃ for 30 s and at 72℃ for 45 s followed by 72℃ for 10 min. Amplified PCR products were analyzed using electrophoresis on 1.2% agarose gels.

Statistical analysis

Antimicrobial resistant patterns were analyzed statistically by using the Chi-square test to investigate differences among phenotypes in all 4 groups.

RESULTS

Bacterial isolates

A total of 592 *Staphylococcus* isolates, including 146 isolates from companion animals, 178 from owners, 120 from the general public who did not have contact with companion animals, and 148 from veterinary hospital staffs, were isolated (Table 1). *S. epidermidis* was the most prevalent species in 3 groups except the companion animal group—51.7% in owner isolates, 43.3% in public isolates, and 56.1% in veterinary hospital staff isolates. The most prevalent species in the companion animal isolates was *S. intermedius* (55.5%). Twenty-two *Staphylococcus aureus* isolates were detected—2 from companion animals, 3 from owners, 11 from the public, and 6 from veterinary hospital staff. *S. hominis* was distributed evenly in all groups, with 11.0% in companion animal isolates, 12.9% in owner isolates, 10.8% in public isolates, and 8.8% in veterinary hospital staff isolates.

Among the 5 different areas from where the sample swabs were collected, the external auditory meatus was the source for 177 (29.9%) staphylococcal isolates (Table 2). The most dominant species from external auditory meatus isolates was *Staphylococcus capitis* with 53 (29.9%) isolates. Other dominant species were *S. intermedius* (57.8%) and *S. hominis* (26.4%) from the medial canthus and interdigital cleft, respectively. *S. epidermidis* was the predominant species in

samples from both the nasal cavity and the anus, accounting for 74.2% and 37.3% of the total microbial profile, respectively. The highest number of *S. intermedius* samples were isolated from the interdigital cleft, whereas the highest number of *S. epidermidis* were isolated from the nasal cavity.

Antimicrobial resistance patterns

Among the 15 antimicrobials evaluated, the highest resistance rate was observed to AM in all 4 groups. Seventy-six percent of companion animal isolates was resistant to AM; 64.6% of owner isolates, 60.0% of public isolates, and 75.7% of veterinary hospital staff isolates were also resistant to AM. Interestingly, all 592 isolates were susceptible to AN. A comparatively low resistance rate (0.5%) was shown against VA in all 592 isolates. In addition, the isolates were mostly susceptible (resistance rates < 5%) to AMC, CZ, and CTX. The overall resistance rates are summarized in Table 3.

Companion animal isolates showed significantly higher resistance rates against C, ENR, SXT, and TET than those from all human groups (owners, general public, and veterinary hospital staff) isolates ($P < 0.05$). Against AM, CIP, and E, companion animal isolates had statistically similar resistance rates relative to that of veterinary hospital staff isolates and significantly higher resistance rates relative to that of human owner isolates and public isolates ($P < 0.05$). Interestingly, all human isolates, including hospital staff isolates, were more

resistant against GM than companion animal isolates were ($P < 0.05$). However, relative to animal samples, owner isolates and veterinary hospital staff isolates were more resistant to FOX and OXA, but public isolates were not.

Among the 4 groups, owner isolates and public isolates showed the most similar resistance patterns, yet owner isolates had higher resistance rates against GM and OXA compared to public isolates ($P < 0.05$). On the other hand, veterinary hospital staff isolates showed a significantly higher resistance rate to AM, E, OXA, and TET than owner isolates did ($P < 0.05$). Furthermore, public isolates and veterinary hospital staff isolates, which are the least connected, showed the most different resistance patterns to 8 antimicrobial agents (AM, FOX, CIP, ENR, E, OXA, SXT, and TET). All 8 antimicrobial agents were more resistible in veterinary hospital staff isolates ($P < 0.05$).

No significant differences were found in the resistance to AN, AMC, CZ, CTX, and VA across all 4 groups.

RAPD-PCR analysis

RAPD-PCR analysis was performed partially on 2 prevalent species—*S. intermedius* and *S. epidermidis*—among companion animal isolates and owner isolates. Fifty-eight *S. intermedius* isolates and 49 *S. epidermidis* isolates were sorted according to the households of origin where the companion animals and owners were living together.

The 58 isolates of *S. intermedius* showed 5 notable groups of identical isolates with 99% genetic similarity (Figure 1). Isolates in group A and group B were acquired from one individual companion animal—JG and ORO, respectively. Two companion animals MG and MS in group C live in the same household. Furthermore, all 4 isolates—A3-1, A7-1, A11-1, and A1-2 in group D—were acquired from 2 companion animals (MG, MS) and the owner (J) who were living together. In addition, group E isolates were acquired from 2 companion animals (CN, RM) that lived in the same household.

In case of the 49 isolates of *S. epidermidis*, 4 groups appeared with 99% similarity (Figure 2). Two owners from different households in group A once visited the same veterinary hospital K. Two isolates A17-3 and A15-2 in group B were isolated from 1 person. However, isolates in groups C and D were not related to each other in each group.

There was no close genetic relation among isolates in both species regardless of whether it carried the *mecA* gene or not.

Detection of *mecA* gene and *ccr* gene complex sequence typing

Two hundred twenty-six (38.2%) isolates were detected possessing the *mecA* gene, including 48 (32.9%) companion animal isolates, 74 (41.6%) owner isolates, 18 (15%) public isolates, and 86 (58.1%) veterinary hospital staff isolates. Methicillin-resistant *S. aureus* (MRSA), which can be determined by *mecA* gene

expression, was not found among the 22 *S. aureus* isolates in this study. Table 4 shows isolates possessing the *mecA* gene. Although 1 staphylococcal isolate possessed *mecA*, other isolates acquired from the same individual (companion animal or owner) did not always carry the *mecA* gene, even if they had originated from the same species.

Accordingly, we performed *ccr* gene complex sequence typing on those 2 species, and then generated phylogenetic trees (Figure 3). We assumed each approximate *ccr* gene type by running the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI) with sequence results. Four groups of identical *ccrA* gene samples were detected (Figure 3[A]). Among them, 1 group with 2 *S. intermedius* isolates A5-1 and A5-2 was acquired from 1 companion animal MS. Other isolates included in 3 different groups did not have any common aspects with respect to the households or the hospitals. Moreover, 4 groups of identical *ccrB* gene complexes were detected (Figure 3[B]). Three *S. intermedius* isolates K324, K323, and K324 were acquired from 1 companion animal ORO, and they showed identical *ccrB* gene complex. In the same group, companion animal CJ had an experience to visit the same veterinary hospital K with the companion animal ORO. Other isolates in 3 different groups had no common aspects between them. Interestingly, analysis of phylogenetic trees showed that both *ccrA* and *ccrB* gene complex types were separated according to the respective species—*S. intermedius* and *S. epidermidis*

(Figure 3).

DISCUSSION

A total of 592 staphylococcal isolates were obtained from companion animals, owners, the general public who did not keep any companion animals, and veterinary hospital staff. The most prevalent species isolated from companion animals was *S. intermedius* (55.5%), whereas in the 3 other human groups—the owners, the general public, and the veterinary hospital staff—the predominant species was *S. epidermidis* (51.7%, 43.3%, and 56.1%, respectively). Both the prevalent species and the antimicrobial resistance patterns differed according to the host species. The antimicrobial resistance patterns of isolates from owners and the general public were more similar than those from any other groups, despite their different frequency of physical contact with the companion animals. These results suggested that physical contact of humans with companion animals was not a factor that affected the antimicrobial resistance patterns of staphylococci. However, as expected, isolates from the general public and veterinary hospital staff showed dissimilar resistance patterns. Resistance rates against 8 antimicrobial agents were significantly higher in the veterinary hospital staff than in the general public. This may be because the veterinary hospital staffs are at a higher risk for exposure to bacteria and antimicrobial agents in veterinary hospitals. Resistance rates against 5 antimicrobial agents (AN, AMC, CZ, CTX,

and VA) were very low in all 4 groups, and were not significantly different among any groups.

RAPD-PCR results revealed that some isolates from companion animals and owners who had been exposed to the same environments, such as households and veterinary hospitals, had over 99% genetic similarity. Several studies have been conducted to investigate the spread of staphylococci among humans and their companion animals. Simoons-Smit *et al.* also reported genetic evidence suggesting that patients with *S. aureus* infections, their families, and companion animals in the same households shared similar clonal origins of *S. aureus* (Simoons-Smit *et al.,* 2000). Transmission of methicillin-resistant *S. aureus* (MRSA) strains among an MRSA infected patient, or a hospital staff member, their family members, and companion dogs were reported in some studies (van Duijkeren *et al.,* 2005, van Duijkeren *et al.,* 2004). In the United Kingdom, pulsed-field gel electrophoresis patterns of MRSA strains acquired from wound infections of 210 companion animals were indistinguishable from those of current predominant strains among humans (Rich *et al.,* 2005). Along with the results of the previous studies, present results demonstrated that transmission of the MRS strain occurred between animals and humans in households, which indicates that MRS could be an emerging problem in communities. In addition, the directivity and the mechanism of bacterial transmission between companion animals and

humans should be investigated in order to predict further issues of pathogenic bacterial transmission.

Following the analysis of the *ccr* gene sequence types, this study showed that *ccr* gene transfer between staphylococci from companion animals and their owners did not occur. The types of staphylococci *ccr* gene complexes analyzed in this study differed according to their origin—human owners or companion animals. In other words, the *ccr* gene complex types analyzed in this study were separated into 2 groups according to each predominant staphylococcal species of origin, *S. epidermidis* and *S. intermedius.* Interestingly, among the isolates from owners, 3 *S. epidermidis* types—A17-2, A12-4, and K75-1—were revealed to carry the *ccrA2B2* allotype in this study. In a previous study, staphylococci with the *ccrA2B2* allotype were described as SCC*mec* type IV; this type has a high transmissibility (Ito *et al.,* 2004). Moreover, *S. epidermidis* with SCC*mec* type IV isolated from healthy human individuals was considered to cause conversion of commensal *S. aureus* to MRSA (Hiramatsu *et al.,* 2001).

Most studies on the transmission of staphylococci have focused on MRSA, and not on methicillin-resistant coagulase-negative staphylococci (MR-CNS), which has a variety of species and genetic characteristics. Although this study could not prove the transfer of the *ccr* gene complex between companion animals and owners through the shared environment, this study results suggested the possibility of transmission of staphylococci, including MR-CNS, between

companion animals and their owners. Accordingly, owners as well as veterinary medical staff need to be aware of possible pathogenic bacterial transmission, and appropriate control strategies should be implemented to prevent zoonotic infections that can occur among interspecies and within the related community including households.

Table 1. *Staphylococcus* spp. isolated from companion animals[*] and humans[§] from February to April 2012

Species	No. of isolates				
	Companion animals	Owners	Public	Veterinary hospital staff	Total
S. aureus	2	3	11	6	22
S. auricularis	0	0	0	1	1
S. capitis	7	23	15	24	69
S. caprae	5	5	3	3	16
S. cohnii	1	0	0	1	2
S. epidermidis	9	92	52	83	236
S. haemolyticus	6	7	6	12	31
S. hom. hominis	0	3	5	0	8
S. hominis	16	23	13	13	65
S. intermedius	81	8	0	0	89
S. lugdunensis	1	1	2	1	5
S. nepalensis	1	0	0	0	1
S. pasteuri	1	2	0	0	3
S. pseudintermedius	0	0	0	1	1
S. saprophyticus	1	3	2	0	6
S. schleiferi	6	2	0	2	10
S. sciuri	1	0	1	0	2
S. simulans	1	1	0	0	2
S. warneri	6	5	10	1	22
S. xylosus	1	0	0	0	1
Total	146	178	120	148	592

[*] Companion animal samples were obtained from dogs and cats at the veterinary hospital.

[§] Samples from human owners and veterinary hospital staff were collected at the veterinary hospital. Samples from the general public were collected from volunteer students from the veterinary school.

Table 2. *Staphylococcus* spp. isolated from companion animals and humans from February to April 2012, according to isolation sites

Species	No. of isolates					
	External auditory meatus	Medial canthus	Interdigital cleft	Nasal cavity	Anus	Total
S. aureus	3	0	2	16	1	22
S. auricularis	1	0	0	0	0	1
S. capitis	53	3	11	2	0	69
S. caprae	11	1	3	0	1	16
S. cohnii	0*	0	0	1	1	2
S. epidermidis	50	7	36	121	22	236
S. haemolyticus	7	1	12	6	5	31
S. hom. hominis	0	0	1	0	7	8
S. hominis	15	3	39	5	3	65
S. intermedius	18	26	28	3	14	89
S. lugdunensis	0	1	1	1	2	5
S. nepalensis	0	0	1	0	0	1
S. pasteuri	2	1	0	0	0	3
S. pseudintermedius	0	0	0	1	0	1
S. saprophyticus	1	0	3	0	2	6
S. schleiferi	4	0	3	3	0	10
S. sciuri	0	0	2	0	0	2
S. simulans	0	0	2	0	0	2
S. warneri	12	2	3	4	1	22
S. xylosus	0	0	1	0	0	1
Total	177	45	148	163	59	592

Table 3. Antimicrobial resistance patterns of staphylococcal isolates from companion animals and humans from February to April 2012

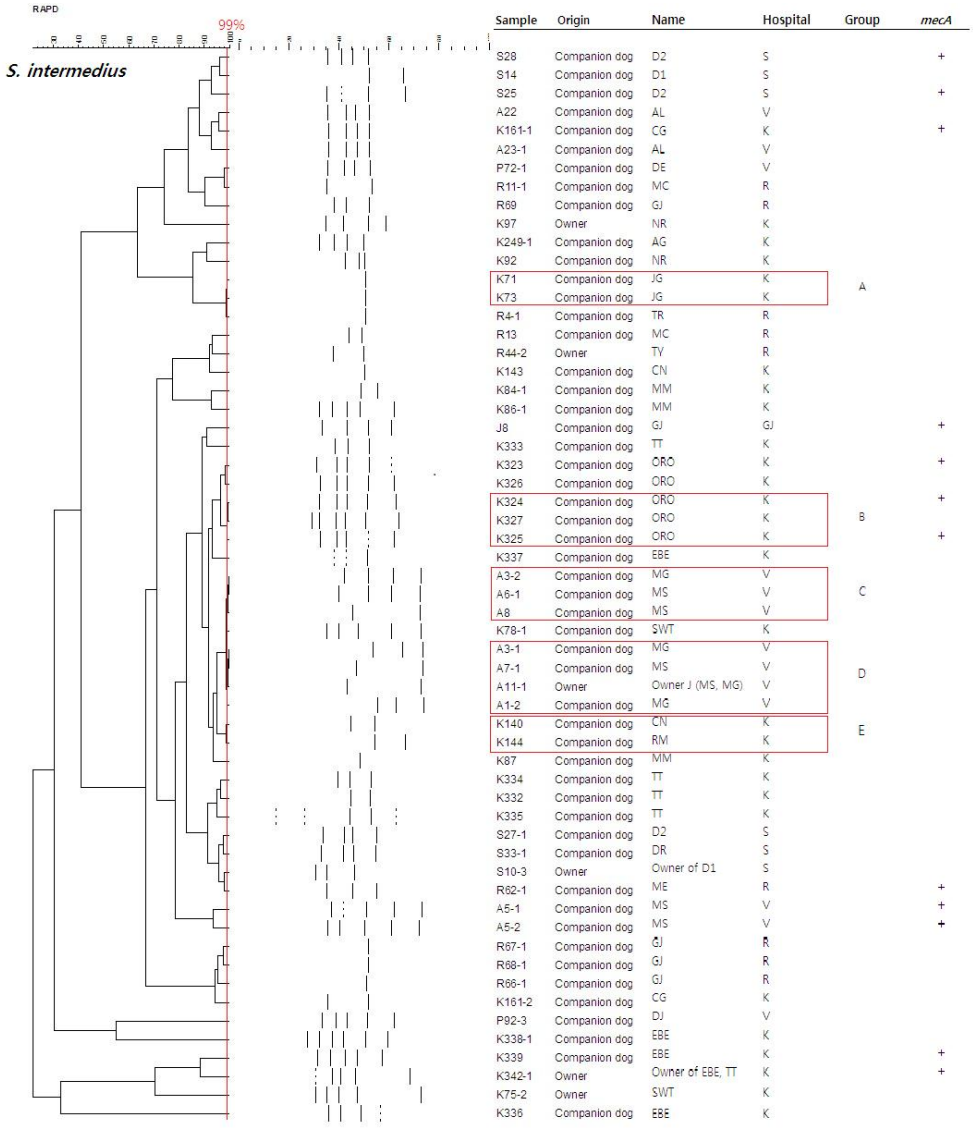
Antimicrobial agent	No. of resistant staphylococcal isolates				
	Companion animals	Owners	Public	Veterinary hospital staff	Total
	(n = 146)	(n = 178)	(n = 120)	(n = 148)	(n = 592)
Amikacin	0 (0)*	0 (0)	0 (0)	0 (0)	0 (0)
Amoxicillin	3 (2.1)	7 (3.8)	2 (1.7)	3 (2.0)	15 (2.5)
Ampicillin	111 (76.0)	115 (64.6)	72 (60.0)	112 (75.7)	410 (69.3)
Cefazolin	2 (1.4)	6 (3.4)	0 (0)	2 (1.4)	10 (1.7)
Cefotaxime	4 (2.7)	8 (4.5)	0 (0)	7 (4.7)	19 (3.2)
Cefoxitin	4 (2.7)	15 (8.4)	5 (4.2)	18 (12.2)	42 (7.1)
Chloramphenicol	22 (15.1)	7 (3.9)	8 (6.7)	11 (7.4)	48 (8.1)
Ciprofloxacin	26 (17.8)	12 (6.7)	3 (2.5)	15 (10.1)	56 (9.5)
Enrofloxacin	27 (18.5)	8 (4.5)	1 (0.8)	11 (7.4)	47 (7.9)
Erythromycin	66 (45.2)	47 (26.4)	28 (23.3)	59 (39.9)	200 (33.8)
Gentamicin	6 (4.1)	41 (23.0)	14 (11.7)	27 (18.2)	88 (14.9)
Oxacillin	19 (13.0)	54 (30.3)	17 (14.2)	70 (47.3)	160 (27.0)
Sulfamethoxazole	56 (38.4)	19 (10.7)	7 (5.8)	24 (16.2)	106 (17.9)
Tetracycline	70 (47.9)	38 (21.3)	17 (14.2)	50 (33.8)	175 (29.6)
Vancomycin	1 (0.7)	1 (0.6)	0 (0)	1 (0.7)	3 (0.5)

* No. of isolates (%)

−Antimicrobial resistance patterns were determined using the disc diffusion method according to Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2008).

Table 4. Staphylococcal isolates possessing *mecA* obtained from companion animals and humans from February to April 2012

Species	No. of isolates				
	Companion animals	Owners	Public	Veterinary hospital staff	Total
S. aureus	0	0	0	0	0
S. auricularis	0	0	0	0	0
S. capitis	2	2	0	1	5
S. caprae	1	0	0	0	1
S. cohnii	0	0	0	0	0
S. epidermidis	3	54	15	69	141
S. haemolyticus	5	5	1	8	19
S. hom. hominis	0	0	0	0	0
S. hominis	10	11	2	4	27
S. intermedius	23	2	0	0	25
S. lugdunensis	0	0	0	0	0
S. nepalensis	0	0	0	0	0
S. pasteuri	1	0	0	0	1
S. pseudintermedius	0	0	0	1	1
S. saprophyticus	0	0	0	0	0
S. schleiferi	1	0	0	2	3
S. sciuri	0	0	0	0	0
S. simulans	0	0	0	0	0
S. warneri	2	0	0	1	3
S. xylosus	0	0	0	0	0
Total	48	74	18	86	226



Sample	Origin	Name	Hospital	Group	*mecA*
S28	Companion dog	D2	S		+
S14	Companion dog	D1	S		
S25	Companion dog	D2	S		+
A22	Companion dog	AL	V		
K161-1	Companion dog	CG	K		+
A23-1	Companion dog	AL	V		
P72-1	Companion dog	DE	V		
R11-1	Companion dog	MC	R		
R69	Companion dog	GJ	R		
K97	Owner	NR	K		
K249-1	Companion dog	AG	K		
K92	Companion dog	NR	K		
K71	Companion dog	JG	K	A	
K73	Companion dog	JG	K		
R4-1	Companion dog	TR	R		
R13	Companion dog	MC	R		
R44-2	Owner	TV	R		
K143	Companion dog	CN	K		
K84-1	Companion dog	MM	K		
K86-1	Companion dog	MM	K		
J8	Companion dog	GJ	GJ		+
K333	Companion dog	TT	K		
K323	Companion dog	ORO	K		+
K326	Companion dog	ORO	K		
K324	Companion dog	ORO	K		+
K327	Companion dog	ORO	K	B	
K325	Companion dog	ORO	K		+
K337	Companion dog	EBE	K		
A3-2	Companion dog	MG	V		
A6-1	Companion dog	MS	V	C	
A8	Companion dog	MS	V		
K78-1	Companion dog	SWT	K		
A3-1	Companion dog	MG	V		
A7-1	Companion dog	MS	V	D	
A11-1	Owner	Owner J (MS, MG)	V		
A1-2	Companion dog	MG	V		
K140	Companion dog	CN	K	E	
K144	Companion dog	RM	K		
K87	Companion dog	MM	K		
K334	Companion dog	TT	K		
K332	Companion dog	TT	K		
K335	Companion dog	TT	K		
S27-1	Companion dog	D2	S		
S33-1	Companion dog	DR	S		
S10-3	Owner	Owner of D1	S		
R62-1	Companion dog	ME	R		+
A5-1	Companion dog	MS	V		+
A5-2	Companion dog	MS	V		+
R67-1	Companion dog	GJ	R		
R68-1	Companion dog	GJ	R		
R66-1	Companion dog	GJ	R		
K161-2	Companion dog	CG	K		
P92-3	Companion dog	DJ	V		
K338-1	Companion dog	EBE	K		
K339	Companion dog	EBE	K		+
K342-1	Owner	Owner of EBE, TT	K		+
K75-2	Owner	SWT	K		
K336	Companion dog	EBE	K		

Figure 1. Dendrogram of RAPD-PCR patterns of 58 *Staphylococcus intermedius* isolated from companion animals and owners

Five groups of isolates with 99% genetic similarity are described as A, B, C, D, and E.

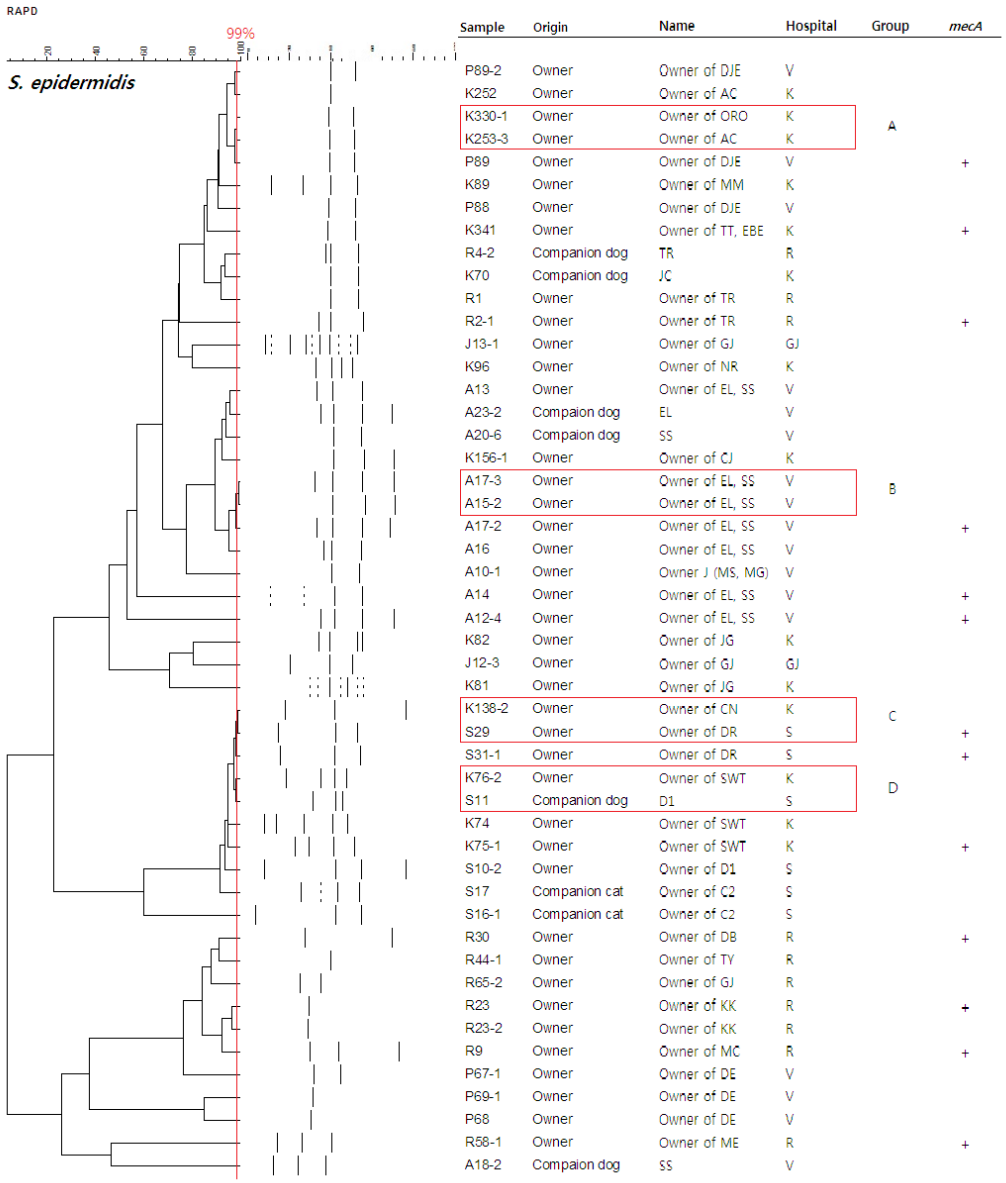
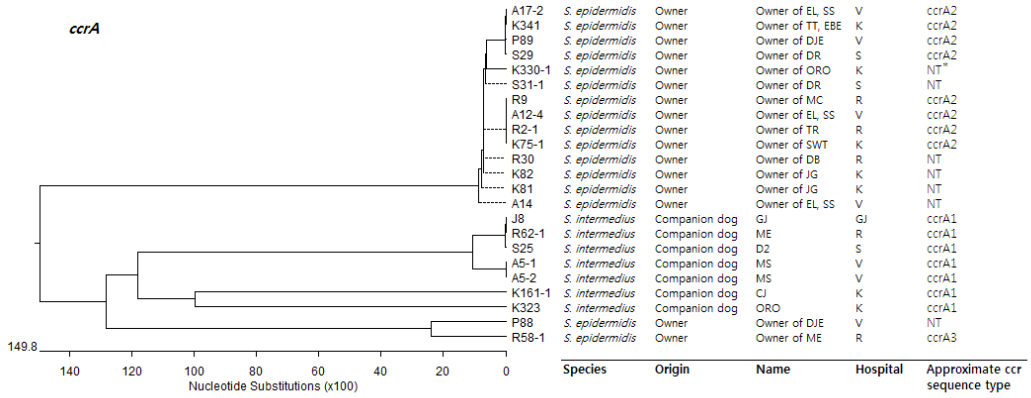


Figure 2. Dendrogram of RAPD-PCR patterns of 49 *Staphylococcus epidermidis* isolated from companion animals and owners

Four groups of isolates with 99% genetic similarity are described as A, B, C, and D.

(A)



(B)

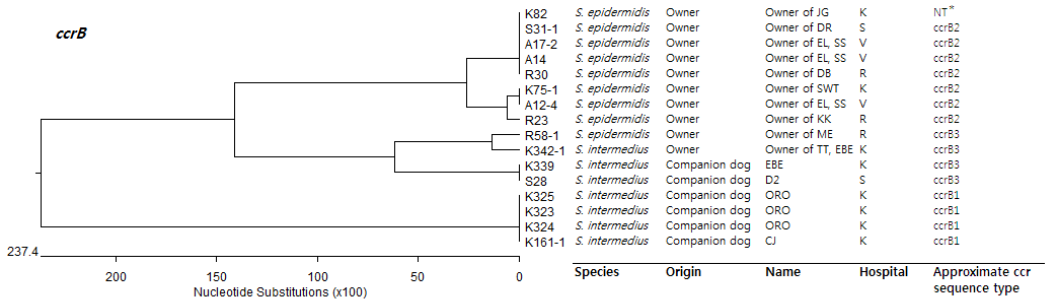


Figure 3. Dendrograms of *ccr* gene complex sequence types

(A) *ccrA* gene complex of *Staphylococcus intermedius* and *Staphylococcus epidermidis*; (B) *ccrB* gene complex of *S. intermedius* and *S. epidermidis*

* NT, nontypable

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국문초록

반려동물과 사람 유래
메티실린 내성 포도상구균: 항생제 내성과 유전적 특성 비교

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지역사회를 통해 확산되고 있는 메티실린 내성 포도상구균, 일명 community-associated methicillin-resistant staphylococci (CA-MRS)는 전세계적으로 문제가 되고 있다. 특히, 같은 주거환경을 공유하며 많은 신체적 접촉이 이루어지는 반려동물과 보호자 사이의 MRS 교환 및 전파 여부를 밝히기 위한 많은 연구가 이루어 지고 있으며, 대부분의 연구결과들은 반려동물과 보호자가 같은 MRS 균주를 공유하며 간혹 반려동물과 보호자 모두에서 감염증의 원인이 될 수도 있음이 보고되었다. 본 연구에서는 2012 년 2 월부터 2 개월 간 서울시 내 7 개 동물병원에 내원하는 반려동물과 보호자들, 병원관계자들과 반려동물을 키우지 않는 일반인으로부터 샘플링을 통해 총 592 개의 *Staphylococcus* spp. 를 분리하여 균종 별 분포를 살펴보고, 이에 대해 항생제 감수성 검사를 실시하여 각 그룹별 항생제 내성 경향과 내성률

($P < 0.05$), 그리고 유전적 상동성을 비교하였다. *mecA* 유전자를 지니고 있는 균주들을 확인하였고, *S. inermedius* 와 *S. epidermidis* 의 *ccr* 유전자를 sequencing 하여 그 상관관계를 분석하였다.

그 결과 반려동물에서는 *S. intermedius* (55.5%) 가 가장 많이 분리되었고, 반면 사람 즉, 보호자와 동물병원 관계자 그리고 일반인에서는 각각 51.7%, 43.3%, 56.1%로 *S. epidermidis* 가 가장 많이 분리되었다. 네 그룹 모두에서 ampicillin 에 대한 항생제 내성률이 가장 높았고, amikacin 에 대해서는 모든 분리주가 감수성을 나타냈다. 반려동물 분리주는 chloramphenicol, enrofloxacin, 그리고 sulfamethoxazole 에서 사람 분리주보다 유의하게 높은 수준의 내성률을 보였다 ($P < 0.05$). 사람 그룹 사이에서는, 반려동물 보호자와 일반인 분리주가 가장 유사한 내성률 분포 양상을 보였으며, 이로써 반려동물을 키우는지 여부가 항생제 내성 분포에는 그다지 영향을 미치지 않는 것으로 추측할 수 있다. 반면, 가장 관련이 없고 서로 접촉 가능성이 매우 적은 동물병원 관계자와 일반인 분리주가 가장 상이한 양상을 보였는데 8 개의 항생제에 대해 동물병원 관계자가 모두 유의적으로 높은 내성률을 나타냈다. 이는 동물병원 관계자가 치료 환경에서 균과 항생제 치료에 노출이 많기 때문인 것으로 사료된다. 99%

이상의 상동성을 나타낸 균주들의 대부분은 주로 같은 가정에서 거주하고 있는 반려동물과 보호자로부터 분리되었다. *ccr* 유전자의 염기서열을 비교, 분석한 결과, *ccr* 유전자는 반려동물과 보호자 간 상이한 type 이 분포하고 있었고, 같은 sequence 의 *ccr* 유전자를 지니고 있는 균주들의 대부분은 서로 연관성이 없는 개체에서 분리된 균주들이었다.

본 연구 결과 반려동물과 보호자는 한 가정 내에서 주거환경을 공유하며 높은 수준의 신체적 접촉을 통해 병원균 자체의 교환이 일어날 가능성이 있으나, 포도상구균 상호간에 메티실린 내성 유전자 전달이 일어날 가능성은 매우 낮은 것으로 나타났다.

주요어: 반려동물, 사람, 메티실린 내성 포도상구균, 전달, 항생제 내성

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