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Carriage of *Staphylococcus schleiferi* from canine otitis externa: antimicrobial resistance profiles and virulence factors associated with skin infection

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ABSTRACT

The recent emergence of *Staphylococcus schleiferi* in dogs with otitis externa or skin and soft tissue infections has become a significant zoonotic issues. In the current study, we investigated 1) the carriage rates of *S. schleiferi* among major staphylococci in healthy dogs and dogs with otitis externa, 2) antibiotic susceptibility profiles of *S. schleiferi,* particularly methicillin resistance (MR), and 3) virulence factors associated with skin and soft tissue infections such as ability to form biofilm, resistance to cationic antimicrobial peptides (CAMPs), and carriage of staphylococcal enterotoxin genes. Among the 21 *S. schleiferi* isolates, 5 isolates (24%) were determined to be methicillin-resistant (MRSS). Staphylococcal cassette chromosome *mec* (SCC*mec*) typing revealed the presence of SCC*mec* type V in 4 MRSS isolates and type VII in one MRSS. Higher levels of antibiotic resistance, especially multidrug resistance, were observed in MRSS isolates compared to the methicillin-susceptible *S. schleiferi* (MSSS) isolates. In addition, MRSS isolates exhibited enhanced ability to form biofilm under static condition and all the 5 MRSS isolates carried three or more enterotoxin genes. However, there were no significant differences in resistance to CAMPs between MRSS and MSSS isolates. These findings suggest that coagulase-negative *S. schleiferi* is becoming more prevalent in canine otitis externa cases. Our results also highlight the presence of multidrug-resistant MRSS isolates with enhanced biofilm production and carriage of multiple enterotoxins.

Keywords: Staphylococcal otitis externa; dogs; methicillin resistance; virulence factors

INTRODUCTION

Since it was first isolated from human clinical specimens by Freney et al. (1988) [1], *Staphylococcus schleiferi* subsp. *schleiferi* (*S. schleiferi*) has been implicated in a number of infections in humans and companion animals [1-5]. Although coagulase-positive *Staphylococcus pseudintermedius* has most frequently been recognized as a causative pathogen

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for canine pyoderma and otitis [6-8], the pathogenic potential of *S. schleiferi* and other coagulase-negative staphylococci (CoNS) in dogs has been underestimated. Furthermore, the recent emergence of *S. schleiferi* among clinically healthy dogs and dogs with otitis externa and/or pyoderma has become a significant global issues in veterinary medicine due its high antimicrobial resistance and variety of virulence factors [2,3,9].

In Korea, several reports on staphylococcal colonization in healthy and diseased dogs have been published over the last decade, mainly focusing on *S. pseudintermedius* isolates [8,10,11]. However, to the best of the authors' knowledge, no study has investigated the prevalence of *S. schleiferi* and other CoNS staphylococci in healthy dogs and dogs with otitis externa that have not received antimicrobial treatment in Korea. Thus, the aim of the current study was to investigate 1) the carriage rates of *S. schleiferi* among major staphylococcal species in clinically healthy dogs and dogs with otitis externa, 2) the genotypic and phenotypic correlates associated with antimicrobial resistance in *S. schleiferi*, including the detection of the *mecA* gene, staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and antimicrobial resistance profiles, and 3) important virulence factors associated with skin and soft tissue infections such as biofilm formation, susceptibilities to two prototypical antimicrobial peptides (K9CATH and LL-37) of canine and human origins, and carriage of staphylococcal enterotoxins (SEs) genes.

MATERIALS AND METHODS

Bacterial isolation and identification

A total of 69 staphylococcal strains were isolated from swab samples collected from 80 dogs (42 healthy dogs and 38 dogs with otitis externa) that were referred to three different tertiary veterinary hospitals located in the cities of Seoul, Seongnam, and Yongin from 2017 to 2018. None of the dogs had prior exposure to any antimicrobial agent within the previous 30 days. Two samples were obtained from each dog (> 12 months old of both sexes) from both ears using separate sterile cotton swabs, and these were inoculated onto Baird-Parker agar plates (Difco, USA) and blood agar plates at 37°C for 24-48 h.

All staphylococcal strains were identified by using both a Vitek 2 system (BioMérieux, France) and 16S rRNA sequencing (Cosmogenetech, Korea). Sequencing of *tuf* gene was also performed to confirm *S. schleiferi* isolates [12]. Coagulase production was confirmed as described in previous research [13].

Antimicrobial susceptibility assays

Susceptibility assays were performed using the disc diffusion methods according to the 2017 Clinical and Laboratory Standards Institute guidelines [14]. The antimicrobial agents used were ampicillin (AMP, 10 μg), chloramphenicol (CHL, 30 μg), erythromycin (ERY, 15 μg), enrofloxacin (ENR, 5 μg), gentamicin (GEN, 10 μg), kanamycin (KAN, 30 μg), oxacillin (OXA, 1 μg), rifampicin (RIF, 5 μg), trimethoprim-sulfamethoxazole (SXT, 23.75–1.25 μg), and tetracycline (TET, 30 μg). In addition to the disc diffusion tests, OXA minimal inhibitory concentrations (MICs) were determined for all *S. schleiferi* isolates by using 2-fold broth microdilution method at concentrations ranging from 0.015 μg/mL to 32 μg/mL [14,15]. *S. schleiferi* strains exhibiting OXA MIC of ≥ 0.5 μg/mL were determined as methicillin-resistant *S. schleiferi* (MRSS) [15].

Detection of *mecA* gene and SCC*mec* typing

Because OXA-susceptible staphylococci may harbor the *mecA* gene [16], polymerase chain reaction (PCR) assays for the detection of *mecA* gene was performed on all staphylococcal isolates independent of antimicrobial susceptibility assays, as described before with minor modifications [17]. Briefly, the primers used to amplify *mecA* were *mecA*-F (5′-TGCTATCCACCCTCAAACAGG-3′) and *mecA*-R (5′-AACGTTGTAACCACCCCAA GA-3′). PCR amplification was carried out with an initial denaturation at 95°C for 2 min followed by 30 cycles of amplification (denaturation at 95°C for 30 sec, annealing at 57°C for 30 sec, and extension at 72°C for 2 min) and final extension at 72°C for 5 min.

SCC*mec* types were determined by using multiplex PCR analysis as described in previous research with minor modifications [17]. Briefly, PCR was performed to amplify cassette chromosome recombinase genes (*ccrA1*, *ccrB1*, *ccrA2, ccrB2, ccrA3, ccrB3*, and *ccrC*) and *mec* regulatory genes. The combinations of *ccr* types and classes of *mec* gene complexes were used to determine the SCC*mec* types of the *S. schleiferi* isolates.

Detection of SEs

Detection of 19 different SEs in *S. schleiferi* isolates was performed by using a multiplex PCR method as described previously [18,19] with minor modification. Briefly, four separate multiplex PCR reactions were set for the amplification of 19 SE genes: set 1 for *sea*, *seb*, *sec*, *sed*, *see*; set 2 for *seg, seh, sei, selj, selp*; set 3 for *selk, selm, selo, tst1*; and set 4 for *sell, seln, selq, selr, selu*. Mixture of genomic DNA samples from reference *Staphylococcus aureus* strains were used for positive controls for each multiplex PCR (FRI472: *sed, seg, selj, sell, selm, seln, selo, selr, selu* [20]; MW2: *seh* [21]; FRI913: *sea, sec, see, selk, selq, tst1* [22]; COL: *seb* [23]; and N135: *sei, selp* [24]).

Biofilm formation assays

Biofilm formation under static conditions was determined on all the *S. schleiferi* isolates as described previously [25]. Briefly, *S. schleiferi* cells from overnight culture were adjusted to a density of 0.5 McFarland standard and diluted 1:100 into brain heart infusion broth (Becton Dickinson, France) supplemented with 0.5% glucose. Then, 200 μL of the staphylococcal cell suspension was transferred to 96-flat well polystyrene cell culture plates (SPL life sciences, Korea) and incubated for 48 h at 37°C as described before [25]. After 48 h incubation, wells were washed twice with phosphate-buffered saline, air dried, and stained with 5% safranin for 5 min (Sigma, USA). The dying agent adherent on the cells was dissolved by 30% acetic acid (Sigma) and the absorbance was measured at OD_{492nm} [25]. The extent of biofilm formation in *S. aureus* Newman strain was normalized to 100%. A minimum of three independent biofilm assays was performed for each *S. schleiferi* isolate.

In vitro susceptibilities to cationic antimicrobial peptides (CAMPs)

Cathelicidins represent prototypical CAMPs and are characterized the key elements in the host defense mechanisms within epithelial cells, intestinal mucosa, and skin. Human cathelicidin (LL-37) [26] and canine cathelicidin (K9CATH) [27] were synthesized at GL Biochen (China) with purity > 95%.

Standard MIC testing in nutrient broth, such as Mueller Hinton broth, may inhibit the bactericidal activity of CAMPs [28]. Thus, *in vitro* susceptibility assays with LL-37 and K9CATH were carried out as previously described using the 2 h microdilution method in RPMI-1640 medium (Sigma) supplemented with 5% Luria-Bertani broth. Briefly, the assays were performed with LL-37 (1 μg/mL) and K9CATH (3 μg/mL) using an initial *S. schleiferi*

cell inoculum of 5×10^3 CFUs. These LL-37 and K9CATH concentrations were determined through extensive preliminary assays displaying their inability to completely kill the initial inoculum of the *S. schleiferi* cells over the 2-h assay duration. Data represent the relative percentage of surviving CFUs (± standard deviation [SD]) of CAMP-treated versus untreated cells. At least three independent experiments were performed for each cathelicidin peptides.

Statistical analysis

The Mann-Whitney *U* test was used for statistical analysis of all quantitative assay data using IBM SPSS Statistics 23 software (SPSS, USA). Significance was determined at *p* value of < 0.05.

RESULTS

Profiles of staphylococci among dogs with otitis externa and healthy dogs

As shown in **Table 1**, 42 coagulase-positive staphylococci (CoPS) and 27 CoNS were isolated from ear swab samples from 80 dogs. The most common otitis externa-associated staphylococci observed in this study were *S. pseudintermedius* (26 isolates) and *S. schleiferi* (19 isolates), which were present in 50% and 39% of the dogs with otitis externa, respectively. All the 41 *S. pseudintermedius* and 21 *S. schleiferi* strains were isolates from each individual dog except for the four diseased dogs, which were positive for both *S. pseudintermedius* and *S. schleiferi*.

Fifteen of the 42 healthy dogs (31%) were also positive for *S. pseudintermedius*. In contrast, only two *S. schleiferi* strains were isolated from two healthy dogs, indicating a lower carriage rate of *S. schleiferi* among healthy dogs compared to the *S. pseudintermedius*.

MRSS and SCC*mec* types

MRSS were identified by the detection of *mecA* and OXA resistance (≤ 17 mm of zone diameter). Overall, 5/21 *S. schleiferi* (24%) isolates were determined as MRSS. We then determined the SCC*mec* types of the 5 MRSS isolates. Interestingly, 4 of the 5 MRSS isolates were SCC*mec* type V and the other one MRSS isolate was SCC*mec* type VII. These data indicate that the methicillin resistance (MR) observed in the *S. schleiferi* isolates was mainly conferred by SCC*mec* type V.

Multidrug resistance (MDR) among *S. schleiferi* isolates

The MRSS isolates exhibited increased levels of resistance to AMP, ENR, and KAN compared to the methicillin-susceptible *S. schleiferi* (MSSS) isolates (**Fig. 1A**). Regardless of MR and disease

Table 1. Profiles of *Staphylococcus* spp. isolated from dogs with otitis externa and healthy dogs

Staphylococcal strains	No. (%) of staphylococcal isolates from	
	Dogs with otitis externa (n = 38)	Healthy dogs (n = 42)
CoPS		
S. pseudintermedius	26 (50)	15 (31)
S. aureus	1 (3)	0
CoNS		
S. schleiferi	19 (39)	2 (5)
S. capitis	2 (5)	0
S. epidermidis	1 (3)	0
S. haemolyticus	0	1 (2)
S. saprophyticus	0	1 (2)
S. equorum	0	1 (2)

CoPS, coagulase-positive staphylococci; CoNS, coagulase-negative staphylococci.
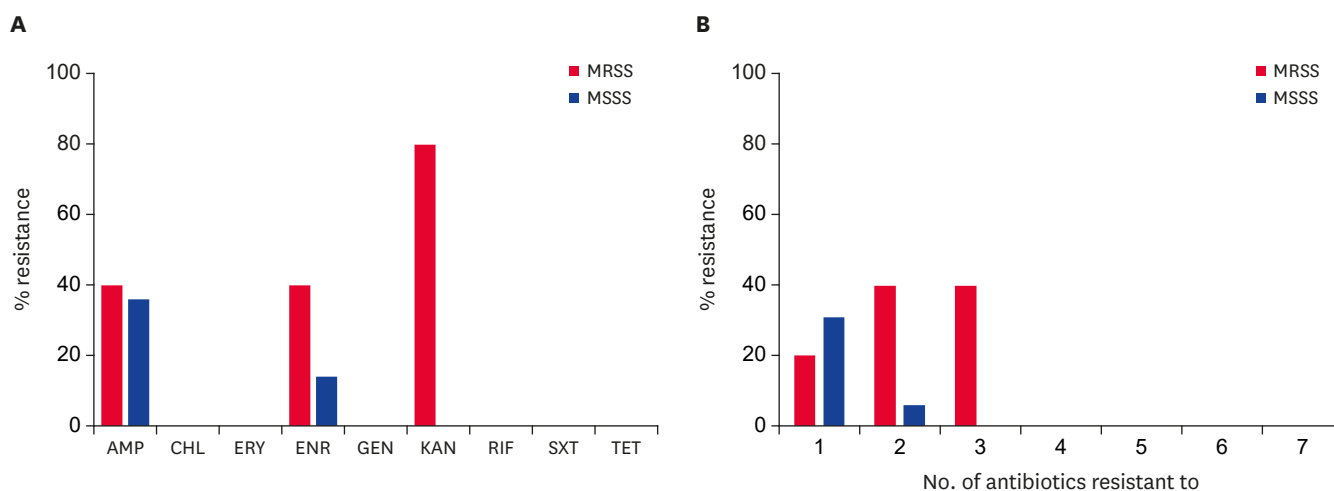
A

B

Fig. 1. Antimicrobial susceptibility of *S. schleiferi* isolates from dogs with otitis externa (A) and frequency of multidrug resistance among the *S. schleiferi* isolates (B). The percentages indicate the rates of resistant isolates among the *S. schleiferi* isolates. Susceptibility assays were performed using the disc diffusion methods according to the 2017 Clinical and Laboratory Standards Institute guidelines [14].
AMP, ampicillin; CHL, chloramphenicol; ERY, erythromycin; ENR, enrofloxacin; GEN, gentamicin; KAN, kanamycin; RIF, rifampicin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; MRSS, methicillin-resistant *S. schleiferi*; MSSS, methicillin-susceptible *S. schleiferi*.

status, all 21 of the *S. shcleiferi* isolates were susceptible to CHL, ERY, GEN, RIF, SXT, and TET. The two *S. schleiferi* isolates from healthy dogs were susceptible to all nine antibiotics tested.

As shown in **Fig. 1B** and **Table 2**, two MRSS isolates (SS4 and SS6) exhibited MDR, displaying resistance to ≥ 3 different classes of antibiotics.

Table 2. Antimicrobial resistance profiles, SCC*mec* types, and SE genes of *Staphylococcus schleiferi* isolates from dogs*

Samples No.	Clinical symptoms	SCC*mec*	*mecA*	Antimicrobial resistance profiles	OXA MICs[†]	SEs
SS1		-	-	ENR	0.125	*seg, sei, selk, sell, selm, selq*
SS2		V	+	KAN-OXA	**4**	*seg, sei, sell, selm, selq*
SS3		V	+	KAN-OXA	**4**	*seg, sei, sell, selm, selq*
SS4		V	+	AMP-ENR-KAN-OXA	**8**	*seg, sei, sell, selm, selq*
SS5		-	+	-	0.125	*seg, sei, sell, selm, selq*
SS6		VII	+	AMP-ENR-KAN-OXA	**2**	*seg, sei, sell, selq*
SS7		-	-	-	0.125	-
SS8		-	+	AMP	0.25	*seg, sei, selm*
SS9		-	+	AMP-ENR	0.25	*seg, sei, selm*
SS10	Otitis externa	V	+	OXA	**0.5**	*seg, sei, selm*
SS11		-	+	-	0.125	*selm*
SS12		-	-	-	0.125	*selm*
SS13		-	-	-	0.25	-
SS14		-	+	-	0.19	-
SS15		-	-	-	0.19	*seg, sei, selm*
SS16		-	-	-	0.19	*selo*
SS17		-	-	AMP	0.25	*selk, selm*
SS18		-	-	AMP	0.25	*selm*
SS19		-	-	AMP	0.25	*selm*
SS20	Healthy	-	+	-	0.125	*sell, selm, selq, selu*
SS21		-	+	-	0.125	*selm, seln, selu*

SCC*mec*, staphylococcal cassette chromosome *mec*; ENR, enrofloxacin; KAN, kanamycin; OXA, oxacillin; AMP, ampicillin; SE, staphylococcal enterotoxin; MIC, minimal inhibitory concentration.
*Methicillin-resistant *Staphylococcus schleiferi* isolates (OXA MICs ≥ 0.5) are represented by shading; [†]MICs to OXA were determined by broth microdilution method as described in Materials and Methods (the values in bold are greater than or equal to 0.5).
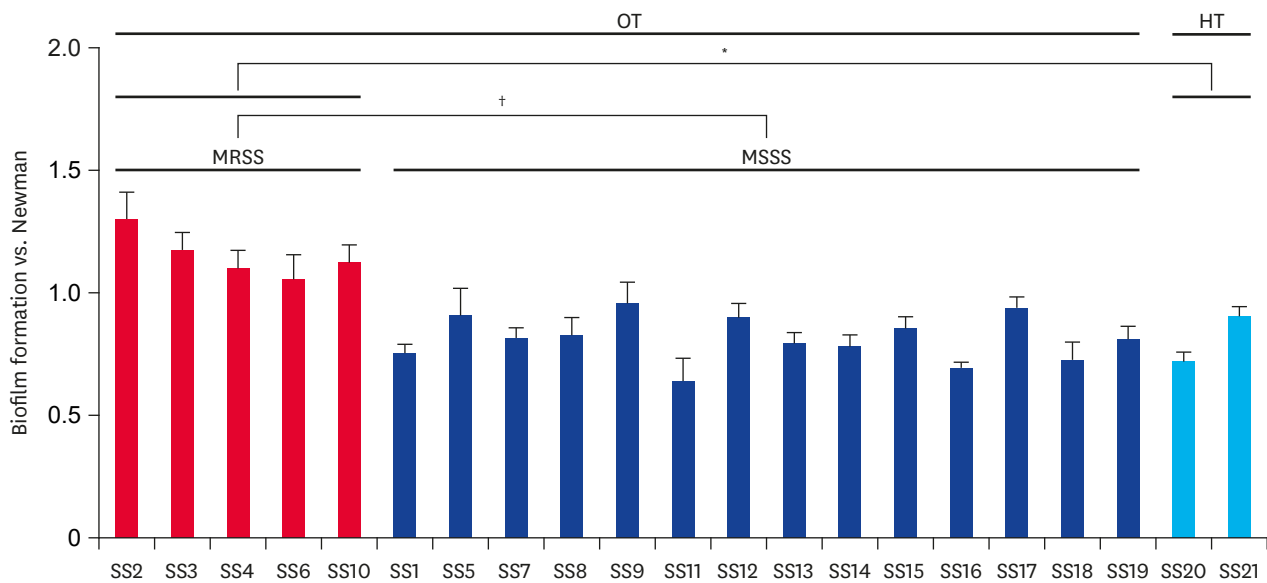
Fig. 2. Biofilm formation of *S. schleiferi* isolates. The data was normalized to the wild-type *S. aureus* Newman strain. Data represent the means (± standard deviation) from three independent experiments.
MRSS, methicillin-resistant *S. schleiferi*; MSSS, methicillin-susceptible *S. schleiferi*; OT, dogs with otitis externa; HT, healthy dogs.
*$p < 0.05$, †$p < 0.01$.

Biofilm formation

As noted in **Fig. 2**, the MRSS isolates exhibited significantly enhanced biofilm formation compared with the MSSS isolates ($p < 0.01$). Interestingly, although only two *S. schleiferi* strains isolated from healthy dogs were included in the biofilm assays, the MRSS isolates also showed significantly increased biofilm formation versus the two MSSS isolates ($p < 0.05$).

Prevalence and distribution of SEs

Of the 21 *S. schleiferi* isolates tested for SEs, 18 isolates (86%) carried at least one SE gene (**Table 2**). All the 5 MRSS isolates carried more than three SE genes (5 SE genes in SS2, SS3, and SS4 strains; 4 SE genes in SS6; and 3 SE genes in SS10). Most of the *S. schleiferi* isolates carried multiple SE genes regardless of MR or level of MDR. The two *S. schleiferi* isolates from healthy dogs, SS20 and SS21, also had 4 and 3 SE genes, respectively. Among the 19 SE genes screened, *selm* gene was most frequently carried by canine-associated *S. schleiferi* isolates (76%) followed by *seg* and *sei* genes (48% each). None of the *S. schleiferi* isolates carried *sea, seb, sec, sed, see, tsst-1, seh, selj, selp*, and *selr*.

In vitro susceptibilities to K9CATH and LL-37

There were no significant differences in susceptibilities to K9CATH and LL-37 between MRSS and MSSS strains isolated from canine otitis externa (**Fig. 3**). However, the MRSS isolates from dogs with otitis externa showed significantly reduced susceptibility to killing by K9CATH as compared to the two MSSS isolates from healthy dogs.

DISCUSSION

S. schleiferi has frequently been implicated as an opportunistic pathogen of canine skin and ear infections [9,16,29]. Although at a lower prevalence than in dogs, *S. shcleiferi* has also been associated with skin infections in cats and small birds [2,30]. *S. pseudintermedius* has
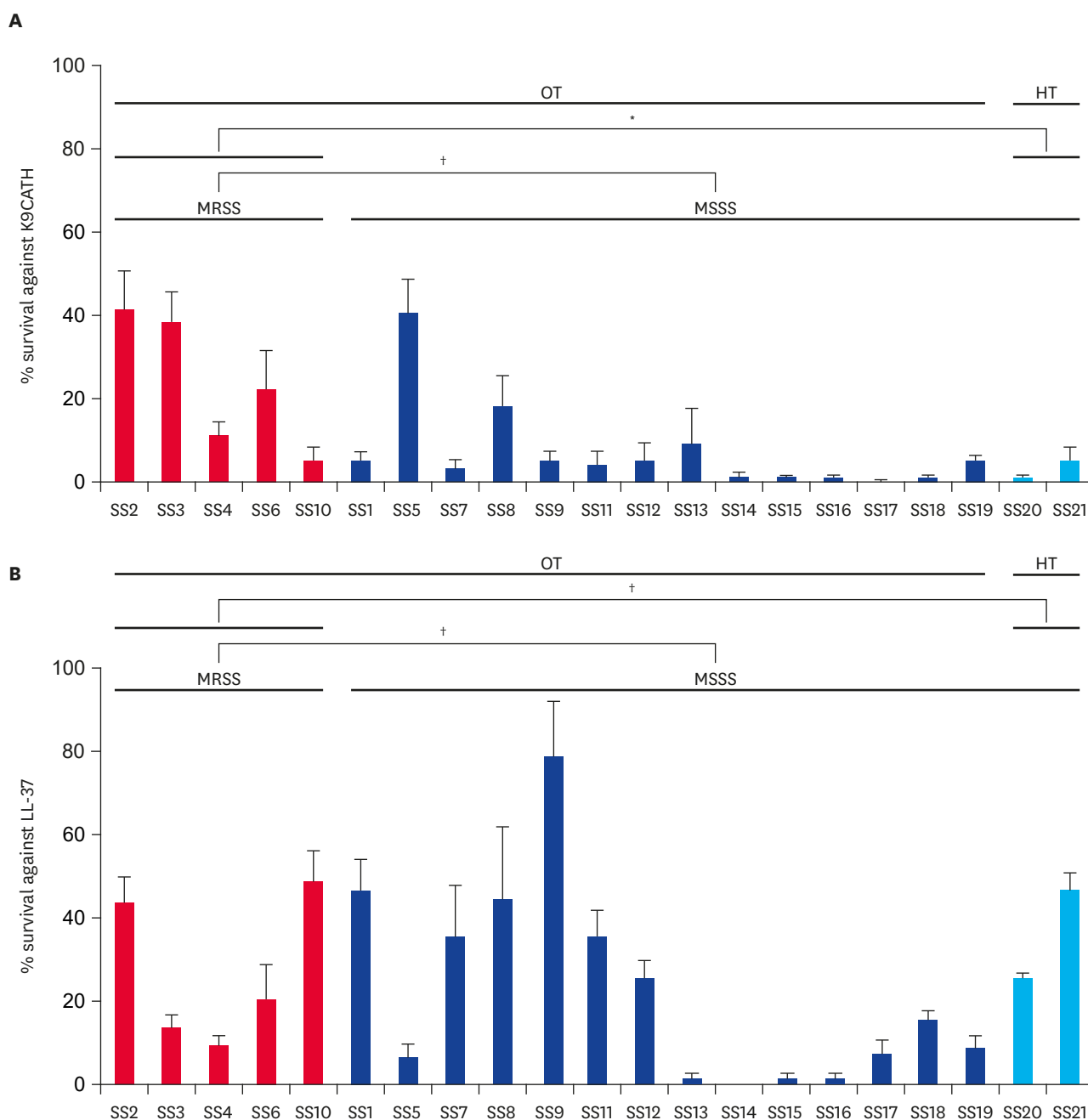
Fig. 3. *In vitro* susceptibility profiles of *S. schleiferi* strains to K9CATH (A) and LL-37 (B). *In vitro* cell survival assays were performed with K9CATH (3 μg/mL) and LL-37 (1 μg/mL) as described previously [26,27]. The *in vitro* survival assays were performed at least three times in triplicate. Data represent means (± standard deviation). MRSS, methicillin-resistant *S. schleiferi*; MSSS, methicillin-susceptible *S. schleiferi*; OT, dogs with otitis externa; HT, healthy dogs. *p < 0.01; †not significant.

been most frequently associated with canine otitis and/or other skin infections in small companion animals [8]. However, recent studies have demonstrated that CoNS [3,31], particularly *S. schleiferi*, are becoming an emerging zoonotic pathogen causing a growing concern in both human and veterinary medicine [1,5,30,31]. While several previous studies have investigated the prevalence and molecular characteristics of *S. pseudintermedius* isolates originating from dogs in Korea [8,10], the carriage of *S. schleiferi* among dogs, their antibiotic resistance profiles, and virulence factors associated with skin infections have not been studied in detail.

In the current investigation, in agreement with previous studies [8,10,32], *S. pseudintermedius* was the most frequent staphylococcal isolates from dogs with otitis externa and clinically healthy dogs (50% and 31%, respectively; **Table 1**). The current study, in support of those by May et al. [9] and Penna et al. [33], also demonstrated the importance of *S. schleiferi* in canine otitis externa, with 39% of the dogs culture-positive for *S. schleiferi*. However, unlike the study by Penna et al. [29], which reported a culture-positivity for *S. schleiferi* of ~32% among healthy dogs in Brazil, only two *S. schleiferi* isolates were recovered from two of the 42 healthy dogs tested in our investigation. Except for the two major staphylococci, *S. pseudintermedius* and *S. schleiferi*, only three other isolates of staphylococci (*Staphylococcus capitis*, *Staphylococcus epidermidis*, and *S. aureus*) were associated with canine otitis externa (**Table 1**).

Recent publications in Korea have reported MR rates for *S. pseudintermedius* isolates from clinically healthy dogs or those with skin infections ranging from 34% to 68% [8,10]. In line with these studies, our *S. pseudintermedius* isolates exhibited a 59% of MR rate overall (62% and 53% for the otitis externa and healthy groups, respectively; data not shown). Although the rate was lower than that of *S. pseudintermedius*, *S. schleiferi* isolates recovered from dogs with otitis externa displayed an MRSS prevalence of ~26%. Since the *mecA* gene lies within the SCC*mec* element, SCC*mec* typing was also conducted on all methicillin-resistant *S. pseudintermedius* (MRSP) and MRSS isolates. Consistent with previous findings in Korea [8,10], the majority of the MRSP isolates (22/24 isolates, 92%) harbored SCC*mec* V (data not shown). Importantly, 4/5 MRSS isolates (80%) also had SCC*mec* V present, suggesting that MR may have been disseminated within and between CoPS and CoNS in Korea.

In addition to the MR, MRSS tended to have increased levels of MDR compared to MSSS isolates (**Fig. 1A and B**). This association between an increased MDR phenotype with *mecA*-positive *S. schleiferi* isolates has not been reported before in Korea. Although prior antibiotic use is a major predisposing factors for the selection of antibiotic resistant *S. schleiferi*, the full history of antibiotic use earlier than 30 days before the ear swab, particularly in dogs with otitis externa, could not be included in the current investigation.

It has been reported that the ability of staphylococci to bind to extracellular adhesion molecules and host skin cells is an important virulence factor for inflammatory skin infections and otitis externa [34,35]. Interestingly, static biofilm assays revealed that the extent of biofilm formation in MRSS isolates were significantly enhanced compared with the MSSS isolates from dogs with otitis externa or healthy dogs (**Fig. 2**). Since the *S. schleiferi* biofilm formation during host infection can restrict the access of many clinically important antimicrobial drugs, it is conceivable that MRSS isolates with increased levels of MDR can survive even during antimicrobial treatment, especially against β-lactam antibiotics, and cause persistent infections.

Cathelicidins, typically amphipathic peptides (< 50 amino acids) with a net positive charge, are found in neutrophils, monocytes, and epithelial cells of the skin, gastrointestinal and respiratory epithelial cells. The persistence and progression of skin and soft tissue *S. schleiferi* infections would require the organism to resist the bactericidal action of cathelicidins. In contrast to the biofilm formation, there were no significant differences in *in vitro* susceptibilities to two cathelicidins of human and canine origins (LL-37 and K9CATH, respectively) between MRSS and MSSS strains isolated from dogs with otitis externa (**Fig. 3A and B**). However, the 5 MRSS strains isolated from dogs with otitis externa exhibited significantly higher resistance to K9CATH than the two MSSS strains from healthy dogs. Although this difference was observed

in a small number of strains, it is possible that the enhanced ability to form biofilm in MRSS isolates might also reduce the exposure of *S. schleiferi* cell community within the biofilm mass to host defense CAMPs. Current studies are ongoing in our laboratory to address these hypothesis.

SEs and toxic shock syndrome toxin-1 were originally identified and characterized in *S. aureus* [18]. Five different types of classical SE genes (*sea, seb, sec, sed,* and *see*) had been identified and 19 new types of SEs (*seg* through *sely*) have been reported in *S. aureus* [18]. Although SE genes have frequently been studied in coagulase-positive *S. aureus*, recent studies demonstrated that classical and newer enterotoxin genes were also carried by CoNS [19,36,37]. In addition, it has been reported that staphylococcal skin colonization and superficial infection were highly correlated with the presence of some of the SEs, possibly through the immunomodulatory activity of the enterotoxins [38]. In the present study, most of the canine-associated *S. schleiferi* isolates carried SE genes, especially the newer SEs. Of note, 16 of the 21 *S. schleiferi* strains (76.2%) were positive for *selm* gene. The enterotoxin gene clusters (*egc* locus) have been known to harbor several SE genes (*seg, sei, selm, seln,* and *selo*) together in staphylococci [37]. In line with this report, 10/16 *selm*-positive *S. schleiferi* strains (62.5%) were also positive for *seg, sei, seln, or selo* (**Table 2**). Although the enterotoxin genes were found in *S. schleiferi* strains regardless of disease status or antimicrobial resistance phenotypes, it might be still possible that the immunomodulatory/superantigenic activities of the SEs contribute virulence of the MRSS isolates in combination with enhanced biofilm formation and MDR phenotype.

We recognize that there are some limitations in the current investigation. Due to the limited sample size, we examined a relatively small number of staphylococcal isolates and phenotypes. Moreover, genetic factors associated with enhanced biofilm formation in MRSS isolates were not defined in the present studies. Nonetheless, in conclusion, our results provide important information on the prevalence of *S. schleiferi* among CoPS and CoNS in healthy dogs and dogs with otitis externa. The widespread presence of SCC*mec* type V among MRSS and MRSP also suggests that the SCC*mec* type V could have disseminated between CoPS and CoNS, particularly between *S. pseudintermedius* and *S. schleiferi*. In addition, increased level of MDR phenotype as well as higher level of biofilm formation was observed in MRSS compared to the MSSS isolates. Finally, this is the first study to report the prevalence of *S. schleiferi* among dogs in Korea, particularly MRSS which harbor SCC*mec* type V.

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