Prevalence of antimicrobial-resistant staphylococci in nares and affected sites of pet dogs with superficial pyoderma

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ABSTRACT. Currently, antimicrobial-resistant staphylococci, particularly methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), are frequently isolated from canine superficial pyoderma in Japan. However, little is known regarding the nasal prevalence of MRSP in pet dogs. Here, we determined the prevalence of antimicrobial-resistant staphylococci in nares and affected sites of pet dogs with superficial pyoderma. Of the 125 nares and 108 affected sites of pet dogs with superficial pyoderma, 107 (13 species) and 110 (eight species) staphylococci strains, respectively, were isolated. The isolation rate of *S. pseudintermedius* from pyoderma sites (82/110 strains, 74.5%) was significantly higher than that from nares (57/107 strains, 53.3%) (*P*<0.01). Notably, the prevalence of MRSP (18/57 strains, 31.6%) in nares was equivalent to that in pyoderma sites (28/82 strains, 34.1%). Furthermore, the phenotypes and genotypes of antimicrobial resistance in MRSP strains from nares were similar to those from pyoderma sites. Our findings revealed that the prevalence of antimicrobial-resistant staphylococci in the nares of pet dogs with superficial pyoderma is the same level as that in affected sites. Therefore, considerable attention should be paid to the antimicrobial resistance of commensal staphylococci in companion animals.

KEY WORDS: antimicrobial resistance, pet dog, *Staphylococcus aureus*, *Staphylococcus pseudintermedius*, *Staphylococcus schleiferi*

Staphylococci are widely disseminated as commensal bacteria in human and animal skins and mucosae. However, many species can serve as causative agents for infectious diseases. These bacteria are divided into coagulase-positive staphylococci (CoPS) and coagulase-negative staphylococci (CoNS). To date, at least 11 species (*Staphylococcus aureus*, *S. simiae*, *S. intermedius*, *S. delphini*, *S. lutrae*, *S. pseudintermedius*, *S. schleiferi*, *S. hyicus*, *S. agnetis*, *S. chromogenes*, and *S. felis*) have been identified as CoPS [2]. Generally, the virulence of CoPS is higher than that of CoNS, and *S. aureus*, *S. pseudintermedius*, and *S. schleiferi* are major pathogens for humans and dogs [4].

S. aureus, a typical CoPS, causes various infectious diseases in humans due to the production of various toxins [33]. In contrast, *S. pseudintermedius* is the major causative agent of superficial pyoderma in dogs [11]. Currently, the identification of methicillin-resistant *S. pseudintermedius* (MRSP) and methicillin-resistant *S. schleiferi* (MRSS) in canines with pyoderma is a problematic issue in the veterinary field, particularly in Japan [21]. Some MRSP strains show multidrug resistance due to the presence of aminoglycoside resistance gene (*aacA-aphD*), macrolide resistance gene (*ermB*), and tetracycline resistance genes (*tetM*, *tetK*) [25].

Commensal staphylococci in dogs may cause pyoderma on their skin [8]. Inadequate use of antimicrobial agents for pet dogs could lead to resistance in their commensal bacteria. We previously reported that antimicrobial-resistant bacteria were frequently found in commensal staphylococci in humans [32]. Exogenetic antimicrobial resistance determinants can transfer horizontally among staphylococci because they are located on mobile genetic elements [18]. Therefore, from a "One Health" perspective, we should pay attention to the commensal staphylococci of companion animals to prevent their transmission to humans. Here, we characterized staphylococci isolated from nares and affected sites in pet dogs with superficial pyoderma in Japan.

MATERIALS AND METHODS

Bacterial strains

We obtained informed consent from the owners of the pet dogs used in this study. Nare samples were collected from 125 pet dogs with pyoderma using sterilized swabs from July to September 2011 at a veterinary clinic in Tokyo, Japan. The pyoderma samples (affected area) were collected from 108 pet dogs, which were different dogs to those used for the nare samples, using sterilized swabs from July to September 2014 from three veterinary clinics in Tokyo (43 samples), Saitama (40 samples), and Chiba (25 samples), Japan (Supplementary Table 1). All veterinary clinics are primary care institutions. *S. pseudintermedius* LMG 22219, *S. schleiferi* JCM 7470, and *S. aureus* JCM 2874 were used as quality control strains for antimicrobial susceptibility testing. The MRSA N315 strain was used as a reference strain for a typical MRSA strain [16].

Growth conditions and bacterial identification

The samples, which were collected using Venturi Transystem® Culture Swab Transport System (Copan Diagnostics Inc., Murrieta, CA, USA), were cultured on mannitol salt agar (Oxoid, Hampshire, UK) under aerobic conditions at 35°C for 48 hr. All colonies with different colors and morphologies were selected and streak cultured on tryptone soy agar (Oxoid) under aerobic conditions at 35°C for 24 hr. Following, the isolates were tested using Gram staining, degradation of mannitol, and production of coagulase (PS LATEX; Eiken Chemical, Tokyo, Japan) [28]. CoPS species were determined using the multiplex PCR method developed by Sasaki *et al*. [26]. Strains that could not be identified using PCR were determined using 16S rRNA gene sequencing [9]. MRSP, MRSS, and MRSA were identified based on the presence of *mecA* [13].

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined using the agar doubling-dilution method, in accordance with the criteria proposed by the Clinical and Laboratory Standards Institute (CLSI) [5]. The following antimicrobial agents were used: ampicillin (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan), oxacillin (Sigma-Aldrich, St. Louis, MO, USA), cephalexin (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), imipenem (FUJIFILM Wako), fosfomycin (Sigma-Aldrich), enrofloxacin (Tokyo Chemical Industry), levofloxacin (FUJIFILM Wako), erythromycin (Sigma-Aldrich), lincomycin (Sigma-Aldrich), gentamicin (FUJIFILM Wako), minocycline (FUJIFILM Wako), chloramphenicol (FUJIFILM Wako), and vancomycin (FUJIFILM Wako). The breakpoints of these antimicrobial agents were determined using the interpretation criteria proposed by the CLSI [6].

PCR amplification

PCR for the detection of *mecA*, ET (*eta*, *etb*, and *etd*), SE (*sea, seb, sec, sed, see, seg, seh, sei,* and *sej*), TSST-1 (*tst*), hemolysin (*hla*, *hlb*, *hld*, *hlg*, and *hlg-2*), leukocidin (*lukS/F-PV*, *lukED*, and *lukM*), epidermal cell differentiation inhibitor (*edin*), ACME (*arcA* and *opp-3C*), macrolide resistance (*ermA*, *ermB*, and *ermC*), tetracycline resistance (*tetM* and *tetK*), lincomycin resistance (*lnuA*), and aminoglycoside resistance (*aacA-aphD*) genes was carried out as described previously [14, 17, 22, 27, 29, 30].

Multilocus sequence typing (MLST) for S. aureus

MLST for *S. aureus* was performed as described previously [7, 23].

Statistical analysis

Differences in the rates of gene possession and antimicrobial resistance were evaluated using the χ^2 or Fisher's exact test (n<10). *P* values of less than 0.05 were considered statistically significant.

RESULTS

Identification of species for staphylococci isolated from nares and pyoderma sites of dogs

Among the nare samples of 125 dogs, 92 (73.6%) were positive for staphylococci, from which we isolated 107 *Staphylococcus* strains. These staphylococci were classified into 13 species (Table 1). *S. pseudintermedius* (57/107 strains, 53.3%) was predominant, followed by *S. schleiferi* (26/107 strains, 24.3%) and *S. aureus* (5/107 strains, 4.7%). In contrast, 98 (90.7%) pyoderma samples from 108 dogs were positive for staphylococci, of which 110 *Staphylococcus* strains were isolated. These staphylococci were classified into eight species (Table

Table 1. Isolation rates of *Staphylococcus* species isolated from nares and pyoderma sites in pet dogs

Species	No. (%) of isolates		
	Nares (n=107)	Pyoderma (n=110)	Total (n=217)
S. aureus	5 (4.7)	1 (0.9)	6 (2.8)
S. capitis	2 (1.9)	0	2 (0.9)
S. caprae	2 (1.9)	0	2 (0.9)
S. chromogenes	1 (0.9)	0	1 (0.5)
S. cohnii ssp. *urealyticus*	2 (1.9)	0	2 (0.9)
S. epidermidis	0	3 (2.7)	3 (1.4)
S. haemolyticus	2 (1.9)	3 (2.7)	5 (2.3)
S. lugdunensis	3 (2.8)	0	3 (1.4)
S. pseudintermedius	57 (53.3)	82 (74.5)*	139 (64.1)
S. saprophyticus	1 (0.9)	1 (0.9)	2 (0.9)
S. schleiferi ssp. *coagulans*	26 (24.3)	18 (16.4)	44 (20.3)
S. sciuri	1 (0.9)	1 (0.9)	2 (0.9)
S. simulans	3 (2.8)	0	3 (1.4)
S. warneri	0	1 (0.9)	1 (0.5)
S. xylosus	1 (0.9)	0	1 (0.5)
Not determined[a]	1 (0.9)	0	1 (0.5)

Zero to two bacteria were detected in each subject. [a], Species of the isolates could not be determined. *$P<0.01$, vs. nares.

Table 2. Proportion of methicillin-resistant *Staphylococcus pseudintermedius*, methicillin-resistant *Staphylococcus shleiferi*, and methicillin-resistant *Staphylococcus aureus* isolated from nares and pyoderma sites in pet dogs

Species	Origin (n)	No. (%) of isolates	
		mecA-positive	*mecA*-negative
Staphylococcus pseudintermedius	Nares (57)	18 (31.6)	39 (68.4)
	Pyoderma (82)	28 (34.1)	54 (65.9)
S. shleiferi	Nares (26)	8 (30.8)	18 (69.2)
	Pyoderma (18)	5 (27.8)	13 (72.2)
S. aureus	Nares (5)	2 (40.0)	3 (60.0)
	Pyoderma (1)	0	1 (100.0)

1). *S. pseudintermedius* (82/110 strains, 74.5%) was predominant in the pyoderma samples, followed by *S. schleiferi* (18/110 strains, 16.4%). *S. aureus* was found in only one sample (0.9%) of pyoderma sites. The isolation rate of staphylococci in pyoderma samples was significantly higher than that of the nare samples ($P<0.001$). In particular, the isolation rate of *S. pseudintermedius* from pyoderma samples was significantly higher than that of the nare samples ($P<0.01$).

Detection of *mecA* was performed for *S. pseudintermedius*, *S. schleiferi*, and *S. aureus* strains, and methicillin-resistant strains were determined. As a result, 18 of 57 *S. pseudintermedius* strains (31.6%), eight of 26 *S. schleiferi* strains (30.8%), and two (40.0%) of five *S. aureus* strains from nares were identified as MRSP, MRSS, and MRSA, respectively (Table 2). On the other hand, MRSP and MRSS were found in 28 of 82 *S. pseudintermedius* strains (34.1%) and five of 18 *S. schleiferi* strains (27.8%) from pyoderma sites, respectively. The MRSA strain was not found in the pyoderma sites. No significant difference was found in the proportion of *mecA*-positive strains in *S. pseudintermedius* and *S. schleiferi* strains isolated from the nares and pyoderma sites of dogs ($P=0.75$ and 1.00, respectively).

Antimicrobial susceptibility of S. pseudintermedius and S. schleiferi strains isolated from nares and pyoderma sites

Antimicrobial susceptibilities of staphylococci isolated from nares and pyoderma sites were compared (Tables 3 and 4). No obvious difference was found in the antimicrobial resistance rates between strains originating from nares and pyoderma sites in either *S. pseudintermedius* or *S. schleiferi* strains. MRSP strains showed multidrug resistance against levofloxacin, erythromycin, gentamicin, and chloramphenicol (Table 3). Five of 39 strains (12.8%) of methicillin-susceptible *S. pseudintermedius* (MSSP) exhibited resistance against oxacillin despite being negative for *mecA*. Antimicrobial susceptibility against most agents of *S. schleiferi* strains was higher than that against *S. pseudintermedius* strains (Tables 3 and 4). Two MRSS strains (40.0%) from pyoderma sites were *mecA*-positive but susceptible to oxacillin. Similar to the above-mentioned MSSP strains, two methicillin-susceptible *S. schleiferi* (MSSS) strains (11.1%) exhibited resistance against oxacillin despite being negative for *mecA*.

Antimicrobial resistance genes in S. pseudintermedius and S. schleiferi strains isolated from nares and pyoderma sites

Antimicrobial resistance genes were detected (Tables 5 and 6). In addition to the results of antimicrobial susceptibility tests, no great difference was found in the detection rates of antimicrobial resistance genes between strains originating from nares and pyoderma sites in either *S. pseudintermedius* or *S. schleiferi* strains. The possession rate of *ermB* was consistent with the resistance rate of erythromycin, whereas the rates of *aacA-aphD* possession (e.g., 94.4% in MRSP strains from nares) and gentamicin resistance (e.g., 50.0% in MRSP strains from nares) differed widely (Tables 3 and 5). However, all *aacA-aphD*-positive strains showed decreased susceptibility to gentamicin (MICs >2 μg/ml). Likewise, the possession rate of *tetM* was not consistent with the rate of resistance to minocycline.

The possession rates of antimicrobial resistance genes in *S. schleiferi* were lower than those of *S. pseudintermedius* (Table 6). No great difference in the possession rates of antimicrobial resistance genes in *S. schleiferi* was found between the strains originating from nares and pyoderma sites.

Characterization of S. aureus isolated from pet dogs

In the present study, *S. aureus* strains were identified in five nare samples and one pyoderma sample (Table 1). Based on MLST analysis, we classified the strains from nares (NVM123, NVM146a, NVM151a, NVM178, and NVM183a) as ST30, 15, 5, 188, and 5, respectively (Table 7). The MV103 strain from pyoderma sites was classified as ST8. Among the strains from nares, NVM151a and NVM183a were MRSA. The antimicrobial resistance genes *ermA*, *lnuA*, *aacA-aphD*, and *tetM* were detected in three, one, two, and two strains, respectively. The presence of antimicrobial resistance genes was consistent with their susceptibilities (Supplementary Table 2). Notably, the ST5 MRSA strain showed high MIC values and multidrug resistance against β-lactams, macrolides, and lincomycin.

The possession patterns of virulence factors in *S. aureus* strains differed based on the clonal type. In particular, many virulence factors, such as *seb*, *sec*, *seg*, *sei*, *tst* (NVM183a), *lukED*, *hla*, *hld*, and *hlg-2*, were found in the ST5 MRSA strains.

Table 3. Comparison of the antimicrobial susceptibility of *Staphylococcus pseudintermedius* strains isolated from nares and pyoderma sites in pet dogs

Antimicrobial agent	Methicillin-resistant *S. pseudintermedius* (MRSP)				Methicillin-susceptible *S. pseudintermedius* (MSSP)			
	Nares (n=18)		Pyoderma (n=28)		Nares (n=39)		Pyoderma (n=54)	
	MIC_{50} / MIC_{90}	R (%)	MIC_{50} / MIC_{90}	R (%)	MIC_{50} / MIC_{90}	R (%)	MIC_{50} / MIC_{90}	R (%)
Ampicillin	0.25 / 4	-	0.5 / 4	-	≤0.06 / 0.25	-	≤0.06 / ≤0.06	-
Oxacillin	0.5 / ≥256	88.9	1 /≥256	82.1	0.13 / 0.5	12.8	≤0.06 / ≤0.06	1.9
Cephalexin	4 / 128	-	1 / 16	-	2 / 64	-	0.25 / 0.5	-
Imipenem	≤0.06 / ≤0.06	-	≤0.06 / ≤0.06	-	≤0.06 / ≤0.06	-	≤0.06 / ≤0.06	-
Fosfomycin	≤0.5 / ≥256	-	≤0.5 / 64	-	≤0.5 / 128	-	≤0.5 / ≤0.5	-
Enrofloxacin	16 / 32	-	16 / 32	-	0.5 / 16	-	≤0.06 / 16	-
Levofloxacin	8 / 8	94.4	8 / 16	89.3	0.25 / 8	35.9	≤0.06 / 8	24.1
Erythromycin	≥256 / ≥256	94.4	≥256 / ≥256	89.3	0.13 / ≥256	30.8	≤0.06 / ≥256	40.7
Lincomycin	≥256 / ≥256	-	≥256 / ≥256	-	0.5 / ≥256	-	0.5 / ≥256	-
Gentamicin	8 / 32	50.0	8 / 16	46.4	0.5 / 16	17.9	≤0.13 / 8	7.4
Minocycline	1 / 4	0.0	2 / 8	0.0	≤0.5 / 1	0.0	≤0.5 / 8	0.0
Chloramphenicol	4 / 64	38.9	64 / 64	60.7	2 / 32	10.3	4 / 64	14.8
Vancomycin	0.5 / 0.5	0.0	0.5 / 1	0.0	0.5 / 1	0.0	0.5 / 1	0.0

MIC_{50} / MIC_{90}, the minimum inhibitory concentrations (MICs) (µg/ml) that inhibit the growth of 50% / 90% of the strains. R, rate of resistant strains. The resistance breakpoints of the following antimicrobial agents were defined according to criteria from the CLSI [6]: oxacillin, ≥0.5 µg/ml; levofloxacin, ≥4 µg/ml; erythromycin, ≥8 µg/ml; gentamicin, ≥16 µg/ml; minocycline, ≥16 µg/ml; chloramphenicol, ≥32 µg/ml; vancomycin, ≥16 µg/ml. -, breakpoints were not defined.

Table 4. Comparison of the antimicrobial susceptibility of *Staphylococcus schleiferi* strains isolated from nares and pyoderma sites in pet dogs

Antimicrobial agent	Methicillin-resistant *S. schleiferi* (MRSS)				Methicillin-susceptible *S. schleiferi* (MSSS)			
	Nares (n=8)		Pyoderma (n=5)		Nares (n=18)		Pyoderma (n=13)	
	MIC_{50} / MIC_{90}	R (%)	MIC_{50} / MIC_{90}	R (%)	MIC_{50} / MIC_{90}	R (%)	MIC_{50} / MIC_{90}	R (%)
Ampicillin	0.13 / 0.25	-	1 / 8	-	≤0.06 / 0.5	-	≤0.06 / 0.25	-
Oxacillin	2 / 4	87.5	0.5 / 128	60.0	≤0.06 / 8	11.1	≤0.06 / ≤0.06	7.7
Cephalexin	8 / 16	-	4 / 64	-	2 / 2	-	0.25 / 0.5	-
Imipenem	≤0.06 / ≤0.06	-	≤0.06 / ≤0.06	-	≤0.06 / ≤0.06	-	≤0.06 / ≤0.06	-
Fosfomycin	1 / 8	-	≤0.5 /≥256	-	≤0.5 / 16	-	≤0.5 / ≤0.5	-
Enrofloxacin	0.5 / 2	-	0.13 / 1	-	0.5 / 16	-	0.25 / 8	-
Levofloxacin	0.25 / 2	0.0	0.25 / 1	0.0	0.25 / 8	38.9	0.13 / 8	23.1
Erythromycin	≤0.06 / 0.13	0.0	≤0.06 / ≤0.06	0.0	≤0.06 / 16	11.1	≤0.06 / ≤0.06	0.0
Lincomycin	0.13 / 0.25	-	0.25 / 32	-	0.25 /≥256	-	0.13 / 0.5	-
Gentamicin	0.5 / 8	0.0	≤0.13 / 32	20.0	0.5 / 1	5.6	≤0.13 / 0.5	0.0
Minocycline	≤0.5 / ≤0.5	0.0	≤0.5 / ≤0.5	0.0	≤0.5 / ≤0.5	0.0	≤0.5 / ≤0.5	0.0
Chloramphenicol	2 / 2	0.0	2 / 4	0.0	2 / 2	0.0	4 / 4	7.7
Vancomycin	0.5 / 1	0.0	1 / 1	0.0	0.5 / 1	0.0	0.5 / 1	0.0

MIC_{50} / MIC_{90}, the minimum inhibitory concentrations (MICs) (µg/ml) that inhibit the growth of 50% / 90% of the strains. R, rate of resistant strains. The resistance breakpoints of the following antimicrobial agents were defined according to criteria from the CLSI [6]: oxacillin, ≥0.5 µg/ml; levofloxacin, ≥4 µg/ml; erythromycin, ≥8 µg/ml; gentamicin, ≥16 µg/ml; minocycline, ≥16 µg/ml; chloramphenicol, ≥32 µg/ml; vancomycin, ≥16 µg/ml. -, breakpoints were not defined.

Table 5. Comparison of the possession rates of antimicrobial resistance genes in *Staphylococcus pseudintermedius* strains isolated from nares and pyoderma sites in pet dogs

Gene	No. (%) of strains			
	Methicillin-resistant *S. pseudintermedius* (MRSP)		Methicillin-susceptible *S. pseudintermedius* (MSSP)	
	Nares (n=18)	Pyoderma (n=28)	Nares (n=39)	Pyoderma (n=54)
aacA-aphD	17 (94.4)	25 (89.3)	12 (30.8)	17 (31.5)
tetM	12 (66.6)	17 (60.7)	13 (33.3)	24 (44.4)
tetK	4 (22.2)	2 (7.1)	3 (7.7)	1 (1.9)
ermB	16 (88.9)	25 (89.3)	11 (28.2)	23 (42.6)
lnuA	0	1 (3.6)	0	0

Table 6. Comparison of the possession rates of antimicrobial resistance genes in *Staphylococcus shleiferi* strains isolated from nares and pyoderma sites in pet dogs

Gene	No. (%) of strains			
	Methicillin-resistant *S. schleiferi* (MRSS)		Methicillin-susceptible *S. schleiferi* (MSSS)	
	Nares (n=8)	Pyoderma (n=5)	Nares (n=18)	Pyoderma (n=13)
aacA-aphD	2 (25.0)	1 (20.0)	0	0
tetM	0	0	1 (5.6)	0
lnuA	0	1 (20.0)	1 (5.6)	0

Table 7. Molecular epidemiological features of *Staphylococcus aureus* strains isolated from nares and pyoderma sites in pet dogs

Strain	Origin	Sequence type	Antimicrobial resistance gene					MSCRAMMs									Virulence factor										
			mecA	*ermA*	*lnuA*	*aacA-aphD*	*tetM*	*cna*	*fib*	*fnbA*	*fnbB*	*clfA*	*clfB*	*eno*	*ebps*	*bbp*	*seb*	*sec*	*seg*	*sei*	*tst*	*lukED*	*hla*	*hlb*	*hld*	*hlg*	*hlg-2*
NVM123	Nares	30	-	+	-	-	-	+	-	+	-	+	+	+	+	+	-	-	+	+	+	-	-	-	+	+	-
NVM146a	Nares	15	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+
NVM151a	Nares	5	+	+	-	-	+	-	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-	+	-	+
NVM178	Nares	188	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	+	-	+
NVM183a	Nares	5	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	+
MV103	Pyoderma	8	-	-	+	+	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-	+	-	+

MSCRAMMs, Microbial Surface Components Recognizing Adhesive Matrix Molecules.

DISCUSSION

This study revealed that the isolation rate of staphylococci from the pyoderma sites was significantly higher than that from the nares in pet dogs. Additionally, the proportion of *S. pseudintermedius* was higher in the pyoderma sites compared to in the nares. Maali *et al*. reported that the isolation rate of *S. pseudintermedius* was over 80% in the normal skin of dogs [19]. The detection rate of *S. pseudintermedius* in pet dogs in this study was lower than that presented in a previous study. *S. schleiferi* accounted for 20–30% of the staphylococci. The proportions of MRSP and MRSS in the strains isolated from nares were equivalent to those of pyoderma sites. The isolation rates (31.6–34.1%) of MRSP were significantly lower than those (66.5%) reported in a previous study in Japan (*P*<0.001) [15]. However, the proportion of MRSP in *S. pseudintermedius* isolated from dogs was 0–7% in other countries [31], indicating that the isolation rate of MRSP in Japanese dogs is higher than that in other countries.

Our data showed no difference in antimicrobial susceptibility between staphylococci isolated from nares and pyoderma sites. Importantly, MRSP strains existing not only in pyoderma sites but also in nares exhibited multidrug resistance. These results indicate that commensal staphylococci of pet dogs have acquired antimicrobial resistance. Several *mecA*-negative but oxacillin-resistant *S. pseudintermedius* and *S. schleiferi* strains were identified in this study. *mecB* and *mecC* are determinants of resistance (other than *mecA*) against oxacillin in staphylococci [1]. However, the *mecA*-negative oxacillin-resistant strains were negative for both *mecB* and *mecC* (data not shown). *S. aureus* strains with decreased susceptibility to oxacillin have been sporadically reported worldwide [12, 20]. These strains are *mecA*-negative, implying a different mechanism of resistance to that of MRSA. They are referred to as borderline oxacillin-resistant *S. aureus* (BORSA). Recently, we found that one of the mechanisms underlying decreased susceptibility to oxacillin involves a specific class A β-lactamase, BlaZ [24]. Therefore, the *mecA*-negative oxacillin-resistant strains identified in this study may possess novel resistance factors, in a manner similar to BORSA.

Aminoglycoside (*aacA-aphD*), macrolide (*ermB*), and tetracycline (*tetM*) resistance genes were frequently found in MRSP strains isolated from both nares and pyoderma sites. Furthermore, the lincomycin resistance gene (*lnuA*) was identified in MRSP and MRSS from pyoderma sites and MSSS from nares. To the best of our knowledge, this is the first report of the detection of *lnuA*-positive *S. schleiferi* strains. Additionally, *aacA-aphD*, *tetM*, and *lnuA* were detected in *S. aureus* strains. Further studies are necessary to determine whether *S. pseudintermedius* and *S. schleiferi* act as reservoirs of the antimicrobial resistance genes and exchange them with *S. aureus*.

ST5, 8, and 30 *S. aureus* strains, which are frequently found in human infectious diseases, were isolated from pet dogs. ST5 is a major type of hospital-acquired MRSA, and ST8 and 30 are major types of community-associated MRSA in Japan [10]. In particular, two strains of ST5 were identified as MRSA and carried multiple antimicrobial resistance genes and virulence factors. Boost *et al*. suggested that *S. aureus* strains may exchange between owners and pet dogs [3]. Further study is necessary to demonstrate whether *S. aureus* strains isolated from pet dogs act as causative agent of infectious diseases in humans or not.

In conclusion, we revealed that the prevalence of antimicrobial-resistant staphylococci in the nares of pet dogs with superficial pyoderma is the same as that in affected sites. Therefore, we attention should be paid to the antimicrobial resistance of commensal staphylococci in companion animals.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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