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Methicillin-Resistant *Staphylococcus pseudintermedius* in a Veterinary Teaching Hospital▽

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We surveyed methicillin-resistant coagulase-positive staphylococcus (MRCPS) strains from 57 (26 inpatient and 31 outpatient) dogs and 20 veterinary staff in a veterinary teaching hospital. From the staff, three MRCPS strains were isolated, and two were methicillin-resistant *Staphylococcus aureus* (MRSA). In contrast, 18 MRCPS strains were detected in both inpatient (12 of 26 [46.2%]) and outpatient (6 of 31 [19.4%]) dogs. Among them, only one strain was MRSA. Using direct sequencing of *sodA* and *hsp60* genes, the 18 MRCPS strains other than MRSA from a staff and 17 dogs, were finally identified as *Staphylococcus pseudintermedius*, a novel species of *Staphylococcus* from a cat. All of the methicillin-resistant *S. pseudintermedius* (MRSP) strains were multidrug resistant to erythromycin, clindamycin, trimethoprim-sulfamethoxazole, and levofloxacin. Most of the MRSP strains showed high-level resistance to oxacillin (≥128 μg/ml, 15 of 18 [83.3%]), and 10 of 15 (66.7%) high-level oxacillin-resistant MRSP strains carried type III SCC*mec*. DNA fingerprinting of MRSP strains by pulsed-field gel electrophoresis yielded eight clusters: clone A with four subtypes, clone B with four subtypes, clone C with three subtypes, and five other different single clones. MRSP strains from the staff and some inpatient and outpatient dogs shared three major clones (clones A, B, and C), but the strains of the other five different clusters were distributed independently among inpatient or outpatient dogs. This genetic diversity suggested that the MRSP strains were not only acquired in this veterinary teaching hospital but also acquired in primary veterinary clinics in the community. To our knowledge, this is the first report of MRSP in dogs and humans in a veterinary institution.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important antimicrobial-resistant nosocomial pathogens of humans worldwide and has also emerged recently in patients without established risk factors in the community (8). MRSA has also been detected in veterinary medicine, especially in cows, poultry, horses, and pet dogs and cats (39). In human hospitals, healthcare-associated outbreaks of MRSA are well documented (8). Recently, suspected cases of human-to-animal or animal-to-human transmission of MRSA have been reported sporadically (29, 36, 41, 42, 48, 50). In methicillin resistance of coagulase-positive staphylococci (CPS) other than *S. aureus*, which are predominant in animals, there have been also a few reports of methicillin-resistant *S. intermedius* (MRSI) strains obtained from dogs (15, 20, 23, 49). However, MRSI has been uncommon as a nosocomial pathogen in a veterinary hospital.

Six species of CPS other than *S. aureus*—*S. intermedius*, *S. schleiferi*, *S. hyicus*, *S. lutrae*, *S. delphini*, and *S. pseudintermedius*—have previously been described (11). Since biochemical characteristics of *S. schleiferi* and *S. hyicus* are different from those of *S. intermedius*, *S. delphini*, and *S. pseudintermedius* (11), it is easy to identify *S. schleiferi* and *S. hyicus.* Commercial kits such as Rapid ID32 Staph (bioMérieux), VITEK2 ID-GPC, and ID-GP identification card (bioMérieux), or Phoenix ID panel PMIC/ID-13 (BD) are also available for identification of the latter two species (26). However, the phenotypic characteristics of *S. intermedius*, *S. delphini*, and *S. pseudintermedius* resemble one another, and commercial kits are not available for differentiation of these species (11). Furthermore, 16S rRNA genes of these species are >99% identical and could not be used to differentiate the species (1, 11, 43). The discriminating power of *sodA* or *hsp60* sequencing is superior to phenotypic characterization or 16S rRNA sequencing for species identification of staphylococci (24, 25, 38). Although *S. intermedius* has been reported to be prevalent among dogs, cats, horses, goats, and pigeons (7, 13), these *S. intermedius* strains with different biotypes might have included *S. delphini* and *S. pseudintermedius*.

In the present study, we screened CPS strains from dogs and staffs in a veterinary teaching hospital to determine the prevalence of methicillin-resistant CPS (MRCPS) and study their mode of nosocomial spread among dogs and humans. Furthermore, for definite species identification of CPS strains, we performed *sodA* and *hsp60* sequencing.

MATERIALS AND METHODS

Sampling from dogs and veterinary staffs, isolation, and phenotypic characterization of MRCPS. Samples from both the nostrils of 57 dogs (26 inpatients and 31 outpatients) and median septum of both nostrils of 20 veterinary staff members were collected using Seed Swab (Eiken Chemical Co., Ltd., Tokyo, Japan) from January to March 2006 at Nippon Veterinary and Life Science University Hospital. Informed consent of the owners and veterinary staffs were obtained prior to sampling. The Ethical Association of Nippon Veterinary and

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TABLE 1. Carriage of methicillin-resistant staphylococci in veterinary staff members and dogs

Group	No. tested	No. (%) of humans or dogs infected with:					
		S. aureus	MRSA	SIG	MRSIG	CPS	MRCPS[a]
Veterinary staff	20	5 (25.0)	2 (10.0)	1 (5.0)	1 (5.0)	6 (30.0)	3 (15.0)
Dogs							
Inpatient	26	2 (7.7)	1 (3.8)	13 (50.0)	11 (42.3)	15 (57.7)	12 (46.2)
Outpatient	31	3 (9.7)	0 (0)	12 (38.7)	6 (19.4)	15 (48.4)	6 (19.4)
Total	57	5 (8.8)	1 (1.75)	25 (43.9)	17 (29.8)	30 (52.6)	18 (31.6)

[a] MRCPS is equal to MRSA plus MRSIG.

Life Science University approved this study. All patient dogs in the Nippon Veterinary and Life Science University Hospital were transferred from primary care animal hospitals in the community. Swabs were immediately inoculated on mannitol-salt agar (Nissui Co., Ltd., Tokyo, Japan) directly and incubated at 37°C for 48 h for selective isolation of staphylococci. Catalase-positive, gram-positive cocci that were presumptively identified as staphylococci by colonial morphology were subcultured on Trypticase soy agar II with 5% sheep blood (TSAB; BD Japan, Co., Ltd., Tokyo, Japan). Tube coagulase tests with rabbit plasma (Denka Seiken Co., Ltd., Tokyo, Japan) were performed, and only CPS strains were selected for further investigation (12). Species identification of CPS was carried out by using the latex agglutination test for clumping factor and protein A (PS Test; Eiken Chemistry Co., Ltd, Tokyo, Japan), DNase test, and rapid ID32 Staph (bioMérieux, Marcy l'Etoile, France) (12, 26, 37). A strain of *S. intermedius* identified phenotypically was designated *S. intermedius* group (SIG) and was composed of *S. intermedius*, *S. delphini*, and *S. pseudintermedius* presumptively because it was very difficult to differentiate these three species by biochemical reactions (11). These strains were stored in 10% skim milk at −85°C until use and maintained on TSAB.

Antimicrobial susceptibility testing. Methicillin resistance was determined by measurement of MIC of oxacillin using the agar dilution method for all CPS strains (9). MIC tests for other antimicrobial agents were performed by broth microdilution method using the MIC-2000 system (Dry plate Eiken DP22; Eiken). The antibiotics tested included penicillin, ampicillin, oxacillin, cefazolin, cefotiam, cefaclor, flomoxef, imipenem, gentamicin, arbekacin, erythromycin, clindamycin, minocycline, levofloxacin, fosfomycin, vancomycin, teicoplanin, and trimethoprim-sulfamethoxazole. The MICs were interpreted as susceptible or resistant according to the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) recommendations (9). Breakpoints for human coagulase-negative staphylococci were applied to SIG strains according to recommendations of Bemis et al. (4). Oxacillin resistance was classified as low level (MIC of 0.5 to 64 μg/ml) or high level (MIC of ≥128 μg/ml).

DNA extraction for amplification. A single colony was suspended to a McFarland 1.0 standard in 100 μl of TE buffer (20 mM Tris, 2 mM EDTA [pH 7.5]) with 10 U of achromopeptidase (Wako Chemical, Co., Ltd., Osaka, Japan), and the suspension was incubated at 55°C for 10 min. After centrifugation at 18,500 × *g* for 5 min, the supernatants were used as crude DNA extracts for PCR (22).

Genetic identification of CPS. To confirm species identification by phenotypic characterization, we conducted molecular identification of staphylococci by detection of the *femB* gene encoding for an enzyme important in cross-linking peptidoglycan in *S. aureus* (19). Direct sequencing of *sodA* and *hsp60* genes was performed for the differentiation of SIG strains (24, 25, 38). Sequencing reactions were performed by using a Big Dye terminator (version 1.1) cycle sequencing kit (Applied Biosystems, Foster City, CA) with an ABI Prism 3100 genetic analyzer (Applied Biosystems). Multiple alignment was carried out by the CLUSTAL X program (46). The construction of the unrooted phylogenetic tree was performed by the neighbor-joining method (40).

Detection of *mecA* and SCC*mec* typing. *mecA* was determined for all of the CPS to confirm methicillin-resistance, and SCC*mec* typing was performed for all *mecA*-positive CPS strains by amplification of the regions within SCC*mec*, the *ccr* region (three classes of *ccr*), and the *mec* region (IS*1272*, *mecI* and *mecR1*, and *mecA*) by PCR as described previously (17, 18, 30).

Detection of enterotoxins, exfoliative toxins, TSST-1 and the Panton-Valentine leukocidin (PVL) genes. Localization of 11 exotoxin genes, encoding for staphylococcal enterotoxins SEA (*sea*), SEB (*seb*), SEC (*sec*), SED (*sed*), SEE (*see*), SEG (*seg*), SEH (*seh*), SEI (*sei*), SEJ (*sei*); exfoliative toxin A, B (ETA; *eta*, ETB; *etb*); TSST-1 (*tst*); Panton-Valentine leukocidin (*lukS* and *lukF*) were detected by PCR as reported previously (3, 28, 33).

PFGE analysis. Chromosomal DNAs of the CPS strains digested with SmaI, AscI, and XmaI (New England Biolabs, Beverly, MA) were analyzed by pulsed-field gel electrophoresis (PFGE) as described previously (37) with minor modifications. The PFGE conditions for SmaI-, XmaI-, and AscI-digested DNAs were as follows: a switch time of 3.0 to 9.0 s and a run time of 9 h and a switch time of 8.0 to 45.0 s and a run time of 12 h (for SmaI and XmaI); a switch time of 5.0 to 40.0 s and a run time of 22 h (for AscI); included angle, 120°; and voltage, 6 V/cm. The buffer temperature was maintained at 11.3°C. The PFGE patterns were interpreted according to the criteria of Tenover et al. (45). Isolates showing six or fewer fragment differences were considered to be subtypes of a pulse type (45).

RESULTS

Surveillance of CPS from dogs and veterinary staff members. Of 20 veterinary staff members, CPS strains were isolated from 6 (30.0%) and 3 of the 6 were MRCPS (Table 1). Of the three MRCPS strains, two were MRSA. Another MRCPS strain was identified as *S. intermedius* phenotypically and categorized as SIG. This strain was methicillin-resistant SIG (MRSIG).

In 57 dogs, CPS strains were isolated from 30 dogs (52.6%) were isolated, and 18 of the 30 strains were MRCPS. *S. aureus* strains were isolated from 5 dogs (8.8%), and SIG strains were isolated from 25 dogs (43.9%). A total of 20% (one of five) of *S. aureus* strains and 68% (17 of 25) of SIG strains showed methicillin resistance.

Species identification of MRSIG strains based on molecular phylogenetic analysis. All of the 18 MRCPS strains other than MRSA in the present study were phenotypically identified as MRSIG. The *sodA* sequence-based analysis revealed that all of the 18 strains had only 96 to 97% sequence similarities with *Staphylococcus delphini* CIP 103732^T and were quite different from *S. intermedius* CIP 8160^T, with 91 to 92% similarities. Furthermore, there was only 93 to 94% similarity of *hsp60* gene between these 18 strains with both *S. delphini* CIP 103732^T and *S. intermedius* ATCC 29663^T, respectively. These 18 strains belonged to the same cluster of *sodA* and *hsp60* gene sequence, and the sequence similarities were greater than 99% with *S. pseudintermedius* LMG 22219 ^{T} for both the *sodA* gene (Fig. 1) and the *hsp60* gene (Fig. 2). Thus, all 18 MRSIG strains were identified as *S. pseudintermedius* by phylogenetic analysis of the *sodA* and *hsp60* genes.

Antimicrobial susceptibility profiles. Among three MRSA strains isolated in the present study, two strains from a veterinary staff member and a dog were resistant to all β-lactams, minocycline, erythromycin, clindamycin, and levofloxacin but

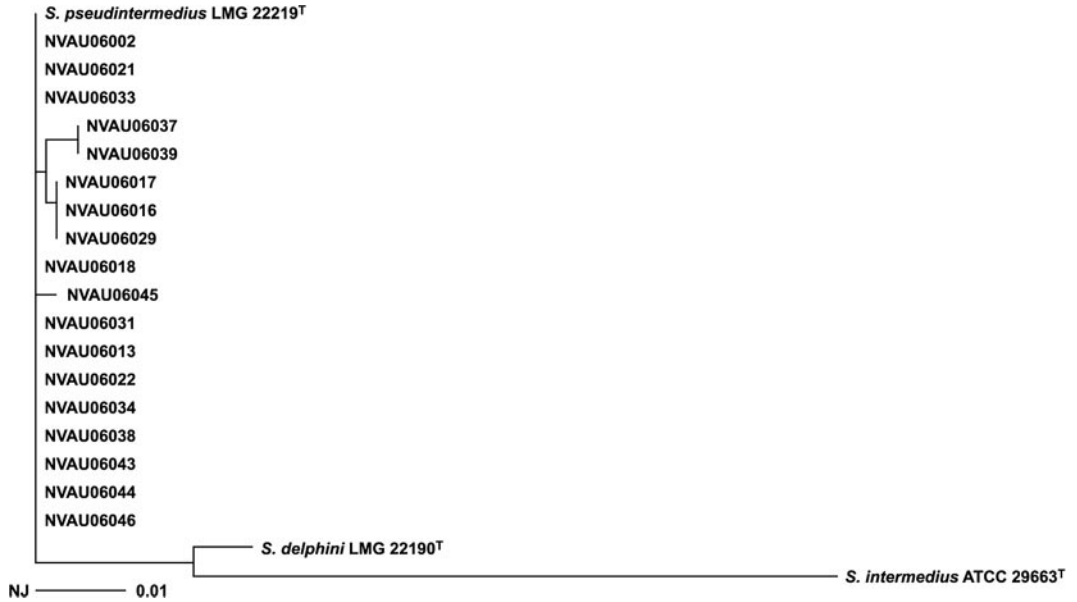

FIG. 1. Phylogenetic tree (unrooted) based on partial *sodA* gene sequences of the *S. intermedius* group isolated in the present study. The tree was constructed by the neighbor-joining method using CLUSTAL X.

susceptible to vancomycin, trimethoprim-sulfamethoxazole, teicoplanin, and gentamicin. In contrast, one strain from a staff member showed susceptibility to all antimicrobial agents other than β-lactams.

On the other hand, all of methicillin-resistant *S. pseudintermedius* (MRSP) strains were divided into two antibiogram types based on oxacillin susceptibility (Table 2.). The majority of the MRSP strains (15 of 18 [83.3%]) showed high-level resistance to oxacillin, and the rest of them (3 of 18 [16.7%]; 1 from an inpatient dog and 2 from outpatient dogs) were low-level resistant (2–4 μg/ml) despite being *mecA* positive. These three strains were more susceptible to cefazolin, cefotiam, cefaclor, and fosfomycin than high-level oxacillin-resistant strains were; nevertheless, they were resistant to minocycline. All of the MRSP strains were resistant to erythromycin, clindamycin, trimethoprim-sulfamethoxazole, gentamicin (except for one strain that was intermediate), and levofloxacin. Most of the dogs with MRSP nasal carriage (14 of 17; 82.4%) had received antimicrobial agents as cefmetazole, cefaclor, enrofloxacin, or fosfomycin, within the previous 6 months.

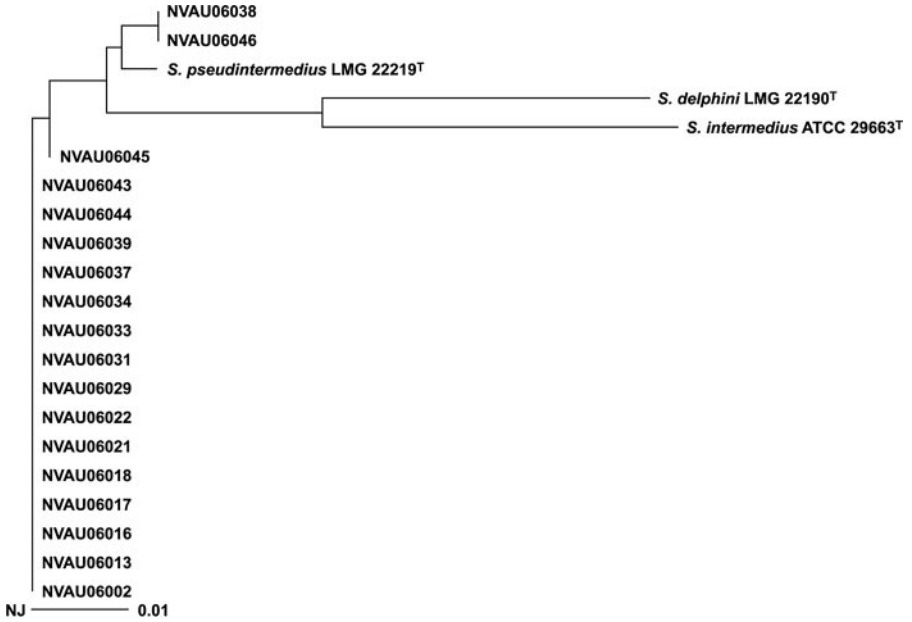

FIG. 2. Phylogenetic tree (unrooted) based on partial *hsp60* gene sequences of the *S. intermedius* group isolated in the present study. The tree was constructed by the neighbor-joining method using CLUSTAL X.

TABLE 2. MICs for antimicrobial agents against MRSP in a veterinary teaching hospital

Antimicrobial agent[a]	MIC (μg/ml) of antimicrobial agent against[b]:					
	H-MRSP (n = 15)			L-MRSP (n = 3)		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
OXA	>128	>128	>128	2–4	2	4
AMC	8–>16	>16	>16	8–>16	8	>16
CFZ	4–>32	4	8	≤0.5–2	≤0.5	2
CTM	≤0.5–>32	4	8	≤0.5–2	≤0.5	2
CEC	32–>32	>32	>32	2–32	2	32
FMX	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
IPM	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12	≤0.12
GEN	16–>16	16	>16	8–16	8	16
ABK	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
MIN	≤0.5	≤0.5	≤0.5	8–16	8	16
ERY	>8	>8	>8	>8	>8	>8
CLI	>4	>4	>4	>4	>4	>4
VAN	≤0.5–1	1	1	≤0.5–1	≤0.5	1
TEC	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
FOF	32–>32	32	>32	≤8	≤8	≤8
SXT	>76/4	>76/4	>76/4	>76/4	>76/4	>76/4
LVX	8–>8	8	>8	8–>8	8	>8

[a] OXA, oxacillin; AMC, ampicillin; CFZ, cefazolin; CTM, cefotiame; CEC, cefaclor; FMX, flomoxef; IPM, imipenem; GEN, gentamicin; ABK, arbekacin; MIN, minocycline; ERY, erythromycin; CLI, clindamycin; VAN, vancomycin; TEC, teicopranin; FOF, fosfomycin; SXT, sulfamethoxazole/trimethoprim (both values are given); LVX, levofloxacin.
[b] H, high-level resistance to OXA (MIC of ≥128 μg/ml); L, low-level resistance to OXA (MIC of ≤64 μg/ml). n, Number of strains.

Molecular characterization of MRCPS strains. Table 3 shows the genotypic characteristics of 21 MRCPS strains analyzed in the present study. Two MRSA strains with high-level resistance to oxacillin isolated from a staff member and a dog belonged to type II SCC*mec* and had *tst*, *sec*, *seg*, and *sei* genes. The PFGE patterns of these two strains (X1 and X2) were closely related to each other (Fig. 3). The remaining multidrug-susceptible MRSA strain belonged to type IV SCC*mec* and had no toxin genes. The PFGE pattern of this strain was different from those of the other two strains.

For MRSP strains, 10 of 18 strains (55.6%) carried a type III SCC*mec* element, one strain (5.6%) had a type V SCC*mec* element, and 7 strains (38.9%) were nontypeable. PFGE analysis of AscI-digested DNA was less discriminative than that of SmaI-digested DNA because all of the MRSP strains were considered to be subtypes within the same cluster (Fig. 4). The

TABLE 3. Molecular characterization of MRCPS

Strain	Species	Group[a]	Resistance to OXA[b]	SCC*mec* type	Toxin type	PFGE pattern	
						SmaI	AscI
NVAU06003	MRSA	Human	H	II	*tst*, *sec*, *seg*, *sei*	X1	ND[d]
NVAU06030	MRSA	Dog-i	H	II	*tst*, *sec*, *seg*, *sei*	X2	ND
NVAU06032	MRSA	Human	H	IV	None	Y	ND
NVAU06013	MRSP	Human	H	III	None	A4	a1
NVAU06021	MRSP	Dog-o	H	III	None	B2	a4
NVAU06029	MRSP	Dog-o	H	III	None	C2	a1
NVAU06002	MRSP	Dog-o	H	III	None	D	a1
NVAU06018	MRSP	Dog-o	H	III	None	D	a1
NVAU06037	MRSP	Dog-o	H	NT[c]	None	E	a3
NVAU06046	MRSP	Dog-o	L	NT	None	H	a6
NVAU06016	MRSP	Dog-i	H	III	None	A2	a1
NVAU06017	MRSP	Dog-i	H	III	None	A3	a1
NVAU06022	MRSP	Dog-i	H	III	None	B3	a3
NVAU06031	MRSP	Dog-i	H	III	None	C1	a1
NVAU06039	MRSP	Dog-i	H	III	None	C3	a1
NVAU06045	MRSP	Dog-i	L	V	None	F	a5
NVAU06043	MRSP	Dog-i	H	NT	None	A1	a1
NVAU06044	MRSP	Dog-i	H	NT	None	A1	a1
NVAU06034	MRSP	Dog-I	H	NT	None	B1	a1
NVAU06033	MRSP	Dog-i	H	NT	None	B4	a2
NVAU06038	MRSP	Dog-i	L	NT	None	G	a6

[a] Dog-o and Dog-i refer to outpatient and inpatient dogs, respectively.
[b] H, high-level resistance to OXA (MIC of ≥128 μg/ml); L, low-level resistance to OXA (MIC of 0.5 to 64 μg/ml).
[c] NT, nontypeable.
[d] ND, not done.

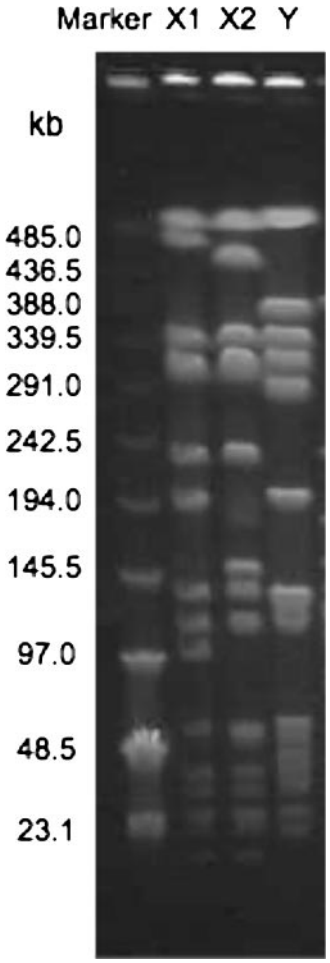

FIG. 3. PFGE patterns of SmaI-digested MRSA strains in the veterinary hospital. Low-molecular-weight marker DNA λ is shown on the left side. The three lanes on the right display results obtained with strains. Each lane shows a different PFGE type.

PFGE patterns of SmaI-digested DNA from the MRSP strains were differentiated into eight clones (A to H). Clones A to C were divided into several subtypes as A1 to A4, B1 to B4, and C1 to C3, respectively (Fig. 4). Only two pairs of strains shared patterns identical to each other (A1 and D). The genomic DNAs from strain NVAU06046 and NVAU06038 could not be digested by SmaI. The neoschizomer XmaI, which cuts the same recognition sequence of SmaI but at a different position, also could not digest the two DNAs (data not shown). Although the number of bands in their PFGE profiles was too small to match the criteria (45), each PFGE type was designated type G and type H tentatively. Clones with PFGE types A to E showed high-level resistance to oxacillin. PFGE types A and B were predominant in inpatient dogs (7 of 11 [63.6%]). One MRSP strain, NVAU06013, isolated from a veterinary staff member, also belonged to clone A with type III SCC*mec*. Both PFGE types B and C were detected in inpatient and outpatient dogs. In contrast, PFGE types D to H were only isolated from outpatient dogs, and PFGE types F and G were only found in inpatient dogs.

PFGE type H strain with nontypeable SCC*mec*, PFGE type F strain with type V SCC*mec*, and PFGE type G strain with nontypeable SCC*mec* showed low-level resistance to oxacillin. No toxin genes were detected in the MRSP strains.

DISCUSSION

To our knowledge, this is the first report on the prevalence of MRSP in dogs and humans in a veterinary institution. In dogs, the majority of CPS strains from nasal, oral, or ear commensal flora or pyoderma specimens have been reported to be *S. intermedius* (7, 39). In the present study, there was a low rate of *S. aureus* carriage in dogs (only 5 of 57 [8.8%]) compared to that observed in humans; only one MRSA strain was detected from a dog with MRSA infection. In dogs, *S. intermedius* is considered to be more predominant than *S. aureus* (7, 39), but we could not find any MRSI in the present study. Surprisingly, all of the MRSIG strains were identified as *S. pseudintermedius* using *sodA* or *hsp60* sequencing. Meyer and Schleifer described how different biotypes of *S. intermedius* strains showed only 50 to 65% DNA homology using DNA-DNA reassociation tests, indicating that they belonged to different species (31). Other authors also reported that *S. intermedius* isolates from different sources could be genotypically or phenotypically differentiated (6, 13, 14). In the phylogenetic point of view, these results suggest that *S. pseudintermedius* is not a novel emerging species among dogs but might have been recognized as one of the different biotypes of *S. intermedius*.

SIG strains did not seem to be found frequently in humans (only one MRSP was isolated in the present study). This human MRSP strain showed susceptibility patterns and genotypes (PFGE pattern and type III SCC*mec*) similar to those of dog-derived MRSP strains. This suggests dog-to-human transmission. Similar reports have been published previously (16, 21, 44).

The prevalence of MRSIG isolates from healthy or sick dogs has still been uncommon in previous reports (15, 23, 47, 49); however, the rate of methicillin resistance among SIG isolates observed in the present study was higher (17 of 25 [68%]) than those determined in previous reports. The use of cephalosporins, carbapenems, and fluoroquinolones is a well-known risk factor for acquisition of MRSA in humans (51). Since broad-spectrum antimicrobials were used for 14 of 17 dogs with MRSP in the present study, antimicrobial selective pressure might have contributed to this prevalence of methicillin resistance.

There was no type I, II, or IV SCC*mec* in MRSP strains. In Japan, the majority of human healthcare-associated MRSA strains carry type II SCC*mec*, and type III SCC*mec* is detected in major HA-MRSA strains from South America, East Europe, and Asian countries other than Japan and South Korea (10, 35). No type III SCC*mec* has been reported in Japanese MRSA strains (10). From the evolutionary point of view, the difference of SCC*mec* element between MRSA and MRSP suggests that the acquisition and spread of *mecA* gene in *S. aureus* and *S. pseudintermedius* occurred via independent transmission routes.

A minority of MRSP strains (3 of 18) with low-level oxacillin-resistance showed different PFGE patterns from those of any high-level oxacillin-resistant strains. Kania et al. reported that 22 (40%) of 55 methicillin-susceptible *S. intermedius*

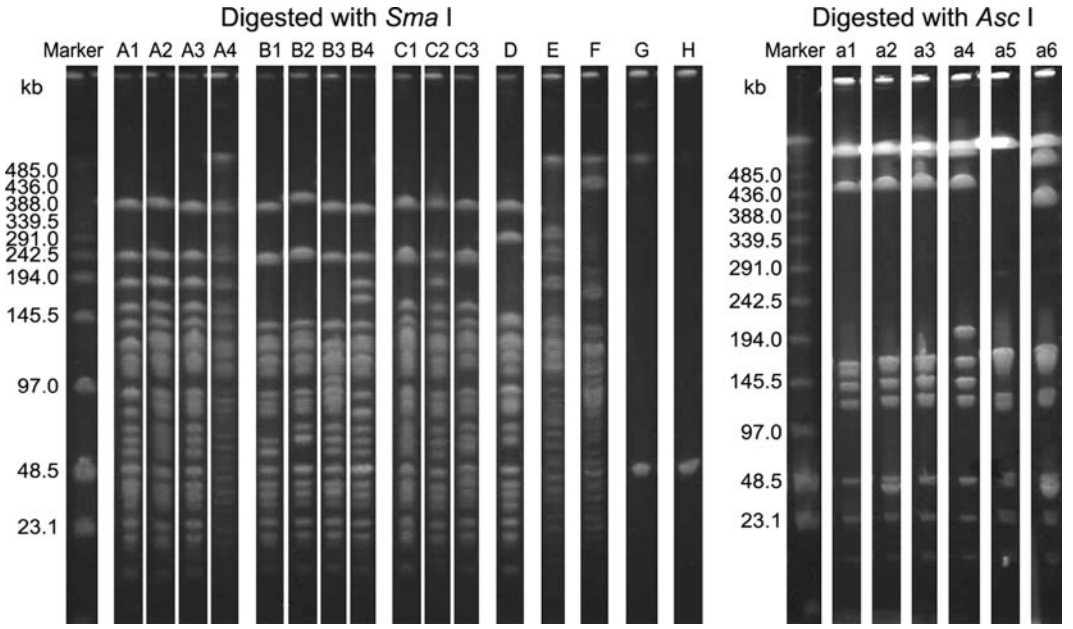

FIG. 4. PFGE patterns of MRSP strains digested with SmaI and AscI in the veterinary hospital. Low-molecular-weight marker DNA λ is shown on the left. The lanes on the right side display results obtained with strains. Each lane shows a different PFGE type.

strains determined by the disk diffusion method had the *mecA* gene (20). Furthermore, Bemis et al. found that the breakpoint of oxacillin for *S. aureus* was too low for the detection of oxacillin resistance in *S. intermedius* and *S. schleiferi* strains (4). These results suggest that low-level oxacillin resistance in *S. intermedius* and *S. schleiferi* strains might be underestimated or unrecognized in the veterinary laboratories. Heterogeneous resistance is well known in many species of *mecA*-positive staphylococci, but the mechanism of the different resistance level has not been elucidated yet. Future studies are needed to clarify this mechanism.

In our study, all staphylococcal enterotoxin genes, toxic shock syndrome toxin 1, Pantone-Valentine leukocidin, and exfoliative toxin genes were not detected in the MRSP strains. Becker et al., using the same PCR system that was used in the present study, reported that 33 of 281 (11.3%) *S. intermedius* strains had SEC genes (2). The reason for this discrepancy is uncertain, but it may be due to the difference in species; ours were identified as *S. pseudintermedius*, a species distinct from *S. intermedius.* Further testing of the enterotoxigenic potential among *S. intermedius*, *S. delphini*, and *S. pseudintermedius* should be carried out.

The genomic DNAs from strains NVAU06046 and NVAU06038 could not be digested by SmaI and XmaI; however, AscI-digested DNAs of both strains yielded banding patterns denoted as A6. This result might be due to an unknown restriction and/or methylation system in the recognition sequence of the SmaI and XmaI site. Although Bens et al. reported similar findings (5), the DNA modification enzyme in our strains was different from the ones sited in that report due to the protection of both SmaI and XmaI digestion.

The PFGE diversities of MRSP strains suggest that they were not only acquired in this veterinary teaching hospital but also acquired in primary veterinary clinics in the community. Although other comparable data of MRSP strains are not available, Middleton reported that MRSA isolates in seven veterinary teaching hospitals showed large genetic diversities (32).

MRSA strains in the veterinary environment have been described in several reports (27, 29, 36, 41, 42, 48, 49). In the present study, three MRSA strains isolated from two staff members and a dog were also isolated. They had profiles similar to the MRSA strains that have been recently predominant in healthcare-associated or community-acquired MRSA in Japan (22, 34, 37). Loeffler et al. also reported that most MRSA isolates from the veterinary hospital setting were related to human epidemic MRSA clone EMRSA-15 in the United Kingdom (29). Molecular epidemiology suggests that MRSA isolates from dogs are derived from humans.

In conclusion, the prevalence of not MRSI but MRSP carriage in dogs in this veterinary teaching hospital was unexpected since *S. pseudintermedius* is a new species established in 2005 (11). Further investigations to reclassify canine CPS based on genetic identification such as *sodA* or *hsp60* sequencing should be carried out.

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