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Detection of methicillin-resistant *Staphylococcus pseudintermedius* ST169 and novel ST354 SCC*mec* II–III isolates related to the worldwide ST71 clone

K. ISHIHARA[1,2], A. KOIZUMI[2], M. SAITO[2], Y. MURAMATSU[2] AND Y. TAMURA[2]*

[1] *Department of Veterinary Medicine, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan*
[2] *School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan*

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SUMMARY

The recent appearance of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is a concern for both veterinary and human healthcare. MRSP clonal lineages with sequence type (ST) 71-*spa* t02-staphylococcal cassette chromosome *mec* (SCC*mec*) II–III and ST68-*spa* t06-SCC*mec* V have spread throughout Europe and North America, respectively. The current study compared the molecular characteristics of 43 MRSP isolates from dogs in Japan with those of MRSP from previous reports using multilocus sequence typing based on seven housekeeping genes, SCC*mec* typing, and detection of antimicrobial resistance genes. Three related clonal lineages, ST71, ST169, and the newly registered ST354, were observed in SCC*mec* II–III isolates from Japan, despite MRSP SCC*mec* II–III isolates being thought to belong to a single clonal lineage. The majority of SCC*mec* II–III isolates belonging to ST169 (9/11) and ST354 (3/3), but not ST71 (0/11), harboured *tetM*. Four STs were observed for the SCC*mec* V isolates; however, neither ST68 nor related STs were found in the Japanese MRSP isolates. In conclusion, MRSP SCC*mec* II–III isolates from Japan belonged to ST71 and related STs (ST169 and ST354). A variety of MRSP SCC*mec* V clones, including some novel clones, were identified.

Key words: Japan, methicillin resistance, MLST, *Staphylococcus pseudintermedius*.

INTRODUCTION

Staphylococcus pseudintermedius is part of the normal microbiota of dogs and cats, but can cause pyoderma and other opportunistic infections [1]. However, it rarely causes zoonotic infections in humans [2]. Methicillin-resistant *S. pseudintermedius* (MRSP) strains have recently been reported [3], and are increasingly being isolated from dogs [4]. MRSP isolates are not only resistant to β-lactam antibiotics, but show little or no susceptibility to various other antimicrobials, including aminoglycosides, macrolides, tetracycline, and fluoroquinolones [5–7], limiting the treatment options for MRSP infections. Molecular analysis of MRSP isolates using multilocus sequence typing (MLST) based on four housekeeping genes in addition to the 16S rRNA gene (MLST-4) [3], *spa* typing [8], and staphylococcal cassette chromosome *mec* (SCC*mec*) typing [7, 9] revealed that sequence type (ST)71-*spa* t02-SCC*mec* II–III and ST68-*spa* t06-SCC*mec* V are the major MRSP clones in Europe and North America, respectively [7]. SCC*mec* II–III and SCC*mec* V MRSP isolates have also been obtained from dogs and veterinarians in Japan [10, 11]. MLST-4 analysis of MRSP isolates

* Author for correspondence: Professor Y. Tamura, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan.
(Email: tamuray@rakuno.ac.jp)

from dogs and cats with dermatitis demonstrated that the ST71 lineage of SCC*mec* II–III MRSP is widespread in Japan [10]. MLST-4, which was developed for discrimination of *S. intermedius* groups, was applied in these previous studies on MRSP [7, 10, 12]. A new *S. pseudintermedius*-specific MLST method based on seven housekeeping genes (MLST-7) has been established to increase discrimination between isolates [13].

To compare the characteristics of MRSP isolates from Japan with those from Europe, North America, and other countries, the current study examined canine MRSP isolates from Japan using MLST-7, SCC*mec* typing, pulsed-field gel electrophoresis (PFGE), *spa* typing, antimicrobial susceptibility testing, and detection of antimicrobial resistance genes.

METHODS

Bacterial isolation and identification

Methicillin-resistant staphylococci were isolated from buccal mucosal samples from 292 dogs (225 dog patients brought to veterinary clinics for veterinary care or health maintenance. Twenty-two dogs and nine dogs out of 225 dog patients had a major complaint of dermatosis and external otitis, respectively; these dog patients included those admitted for vaccination and prevention of filariasis; 35 blood donor dogs; 13 dogs owned by veterinary staff; and 19 healthy dogs brought to veterinary clinics for purposes other than veterinary care) using CHROMagar MRSA (Kanto Kagaku Co., Japan). The samples were collected as part of a previous study [14] from 69 private veterinary clinics in the Ishikari region around Sapporo, Hokkaido Prefecture, Japan, during April and June 2008. As part of the previous study, *mecA*-positive isolates were confirmed by polymerase chain reaction (PCR) [14]. All isolates containing *mecA*, other than methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, which were examined previously [14], were tested using the ID32 STAPH system (Sysmex bioMérieux Co., Japan) according to the manufacturer's instructions. DNA was extracted from cultures using InstaGene Matrix (Bio-Rad, USA). Isolates classified as *S. intermedius* by ID32 STAPH were also analysed by PCR–restriction fragment length polymorphism (RFLP) of their *pta* genes, which can discriminate *S. pseudintermedius* from *S. intermedius* and *S. aureus*, as described previously [15]. Confirmed *S. pseudintermedius* isolates were then examined using the following tests.

Analysis of antibiotic resistance

Minimum inhibitory concentration (MIC) analysis was performed as described in the Clinical and Laboratory Standards Institute guidelines [16] using the broth micro-dilution method on Eiken Frozen Plates (Eiken Chemistry Co., Japan). The following antimicrobials were tested: oxacillin, cefazolin, cefotiam, imipenem, streptomycin, kanamycin, gentamicin, arbekacin, erythromycin, tetracycline, minocycline, chloramphenicol, ciprofloxacin, vancomycin, teicoplanin, quinupristin-dalfopristin, and linezolid.

The following antimicrobial resistance genes were screened by PCR using Go *Taq* Green Master Mix (Promega, Japan), as described previously for the detection of MRSP isolates [7]: *mecA* [11], *blaZ* [17], *aac(6′)-Ie-aph(2′)-Ia* [18], *aph(3′)-III* [18], *ant(6′)-Ia* [19], *sat4* [19], *ermB* [20], *dfrG* [21], *lnuA* [22, 23], *tetK* [24], *tetM* [25], and cat_{pC221} [26]. The primer sequences are listed in Table 1. PCR products were purified using a High Pure PCR Cleanup Micro kit (Roche Diagnostics GmbH, Germany), and products corresponding to the resistance gene fragments were confirmed by sequencing by FASMAC Co. (Japan). The PCR primers were also used to sequence these resistance genes. For sequencing of *tetM*, a 1862-bp fragment was amplified [27] and sequenced using the same primers and internal primers (Table 1).

Molecular characterization

MLST-7 analysis was conducted for all isolates as described previously [13]. The STs were determined using the *S. pseudintermedius* MLST database (http://pubmlst.org/spseudintermedius/).

SCC*mec* typing was performed by PCR amplification of the *mec* (classes A, B, C) and *ccr* (types 1, 2, 3, 5) gene regions [9]. In addition, the structure of SCC*mec* was determined using Oliveira's strategy [28]. To discriminate SCC*mec* II–III from SCC*mec* III, the cadmium resistance gene in the J2 region and the structure of the J1 region were examined by PCR as described previously [7, 29].

PFGE analysis of *Sma*I-digested DNA was performed as previously described [11, 30]. PFGE was performed using a CHEF-DR III system (Bio-Rad), as described previously [30].

spa genes were amplified using previously described primers and conditions [7, 8, 12]. DNA sequences of the *spa* genes were determined as described above. A *S. pseudintermedius spa* database, developed

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Table 1. *Primers used in this study*

Target gene	Primer sequence (5′–3′)	Amplicon size (bp)	Ref.
mecA	TGT CCG TAA CCT GAA TCA GC TGC TAT CCA CCC TCA AAC AG	519	[11]
blaZ	GAT AAG AGA TTT GCC TAT GC GCA TAT GTT ATT GCT TGA CC	533	[17]
aac(6′)-Ie-aph(2′) -Ia	CAG AGC CTT GGG AAG ATG AAG CCT CGT GTA ATT CAT GTT CTG GC	348	[18]
aph(3′)-III	GGC TAA AAT GAG AAT ATC ACC GG CTT TAA AAA ATC ATA CAG CTC GCG	523	[18]
ant(6′)-Ia	AAT TGT GAC CCT TGA GGG GGC ATA TGT GCT ATC CAG	814	[19]
sat4	CGA TAA ACC CAG CGA ACC ATA ACA TAG TAT CGA CGG	449	[19]
ermB	GAA AAG GTA CTC AAC CAA ATA AGT AAC GGT ACT TAA ATT GTT TAC	639	[20]
dfrG	TGC TGC GAT GGA TAA GAA TGG GCA AAT ACC TCA TTC C	405	[21]
lnuA	GGT GGC TGG GGG GTA GAT GTA TTA ACT GG GCT TCT TTT GAA ATA CAT GGT ATT TTT CGA TC	323	[22, 23]
tetK	TAG GGG GAA TAA TAG CAC ATT AAT CCG CCC ATA ACA AAT A	613	[24]
tetM	GTG GAC AAA GGT ACA ACG AG CGG TAA AGT TCG TCA CAC AC	406	[25]
	AGT TTT AGC TCA TGT TGA TG* TCC GAC TAT TTA GAC GAC GG*	1862	[27]
	TTG CGG AAA TGT CTT CAA AA† ATC CTT TCT GGG CTT CCA TT† GCG TAT CCC TTC CAT AAC TGC†	– – –	This study
cat_{pC221}	ATT TAT GCA ATT ATG GAA GTT G TGA AGC ATG GTA ACC ATC AC	435	[26]

* Used to amplify *tetM* for sequencing.
† Used as an internal primer for sequencing of *tetM*.

by Dr A. Moodley of the University of Copenhagen (personal communication), was used to determine *spa* types [8].

RESULTS

Identification

Forty-three isolates, each from a different dog (43/292, 14·7%), were classified as *S. intermedius* by ID32 STAPH. All of these isolates were further confirmed as *S. pseudintermedius* by PCR–RFLP, and all were methicillin resistant. These MRSP isolates were obtained from 23 dog patients (23/225, 10·2%; including three dogs with a major complaint of dermatosis), 15 blood donor dogs (15/35, 42·9%), and five dogs owned by veterinary staff (5/13, 38·5%). None of the 19 healthy dogs carried MRSP. The MRSP-positive samples came from 20 different veterinary clinics (20/69, 29·0%).

Molecular characteristics

ST275 ($n = 4$), ST276 ($n = 9$), ST323 ($n = 2$), ST325 ($n = 1$), ST324 ($n = 1$) and ST354 ($n = 3$) were detected in this study and assigned as novel STs in the *S. pseudintermedius* MLST database. ST71 ($n = 11$), ST169 ($n = 11$) and ST121 ($n = 1$) were also detected (Table 2). The SCC*mec* types of these 43 isolates are given in Table 2. The predominant SCC*mec* type was II–III ($n = 25$), followed by V ($n = 13$). SCC*mec* II–III isolates were classified as ST71, ST169, and ST354. The clonal relationships in MRSP SCC*mec* II–III STs of isolates from this study and others were predicted by BURST analysis using the *S. pseudintermedius* MLST database (http://pubmlst.org/spseudintermedius/), as shown in Figure 1.

All 25 SCC*mec* II–III isolates harboured open reading frames (ORFs) identical to those of SCC*mec* III-MRSA in the J1 region and *dcs* in the J3 region, but the cadmium resistance gene was absent from

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Table 2. *Summary of MRSP genotypes*

ST by MLST-7	Allele no.	SCC*mec* type	No. of isolates							
			spa type						Sub-total	No. of VCs†
			t02	t06	t58	t60	t62	n.d.*		
71	3–9–1–2–1–2–1	II–III	10	1					11	8
169	2–9–1–2–1–1–1	II–III				1		10	11	7
354	2–9–1–2–13–1–1	II–III						3	3	1
275	3–27–1–1–3–10–2	III						3	3	1‡
325	3–2–2–1–14–2–1	IV						1	1	1
121	2–9–3–1–1–2–1	V		1					1	1
276	1–8–2–4–5–1–2	V						9	9	3
323	7–10–1–1–13–1–2	V			1		1		2	1
324	4–10–2–1–1–5–2	V	1						1	1
275	3–27–1–1–3–10–2	Untypable§						1	1	1†
Total			11	2	1	1	1	27	43	20‖

MRSP, Methicillin-resistant *Staphylococcus pseudintermedius*; ST, sequence type; MLST-7, multilocus sequence typing based on seven housekeeping genes; VCs, veterinary clinics; n.d., not determined.
* *spa* could not be amplified using any of the primer pairs, therefore *spa* type was not determined.
† No. of VCs where MRSP isolates were obtained from dogs.
‡ MRSP-ST275 isolates obtained from one VC.
§ Although class A *mec* complex was determined, *ccr* was not amplified.
‖ Two different STs were obtained for isolates from four VCs.

the J2 region. The SCC*mec* structure confirmed by PCR [7, 9, 28, 29] of these 25 SCC*mec* II–III MRSP isolates matched the SCC*mec* II–III DNA sequence available from GenBank (accession no. AM904732). Three SCC*mec* III isolates harboured both *dcs* and a cadmium resistance gene, but not the ORF identical to that of SCC*mec* III-MRSA in the J1 region. All 13 SCC*mec* V isolates harboured Tn554.

spa types could only be determined for 16 (37·2%) of 43 isolates (Table 2) because the remaining isolates did not yield a product from *spa* PCR analysis using the various primer pairs. The predominant *spa* type was t02 (11 isolates), and new *spa* types t58, t60, and t62 were also identified (Table 2).

PFGE divided MRSP isolates into eight clusters (clusters A–H), with similarities within each cluster of ⩾60% (data not shown). Two SCC*mec* V isolates did not belong to any cluster. Molecular characteristics of MRSP isolates are given in Table 3. Clusters A ($n = 6$) and E ($n = 4$) contained only ST71-SCC*mec* II–III isolates. Cluster D contained only five ST169-SCC*mec* II–III isolates. Cluster C contained only SCC*mec* II–III isolates; however, it included ST71 ($n = 1$), ST169 ($n = 6$), and ST354 ($n = 3$) isolates. Cluster C included two sub-clusters; the first sub-cluster contained ST71-SCC*mec* II–III and ST169-SCC*mec* II–III isolates, while the second sub-cluster contained ST354-SCC*mec* II–III isolates. The similarity between PFGE band patterns of the four clusters containing SCC*mec* II–III isolates was

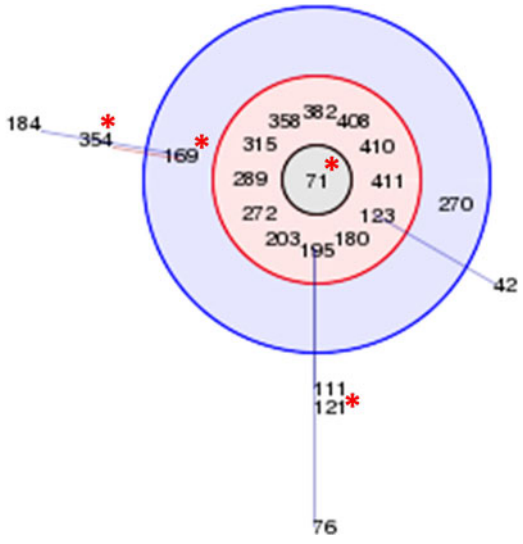

Fig. 1. Clonal relatedness of *Staphylococcus pseudintermedius* sequence types (STs) as predicted by BURST analysis. STs with ⩾4 loci matching those of ST169 (2–9–1–2–1–1–1) were selected for this analysis. The group including STs of methicillin-resistant *Staphylococcus pseudintermedius* isolates obtained in this study (* ST71, ST121, ST169, ST354) is shown.

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Table 3. *Molecular characteristics and antimicrobial resistance of MRSP isolates from dogs in Japan*

ST*	*spa* type	SCC*mec* type	PFGE†	Antimicrobial resistance genes detected by PCR											CIP	No. of isolates	No. of VCs‡
71	t02	II–III	A	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*				R§	3	2
71	t02	II–III	A	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*			cat_{pC221}	R	1	1
71	t02	II–III	A	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*	*tetK*		cat_{pC221}	R	2	2
71	t02	II–III	C	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*				R	1	1
71	t02	II–III	E	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*				R	1	1
71	t02	II–III	E	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*			cat_{pC221}	R	1	1
71	t02	II–III	E	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*	*tetK*			R	1	1
71	t06	II–III	E	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*	*tetK*		cat_{pC221}	R	1	1
169	n.d.	II–III	C	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*	*tetK*			R	2	1
169	n.d.	II–III	C	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*	*tetK*	*tetM*		R	3	2
169	t60	II–III	C	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*	*tetK*	*tetM*	cat_{pC221}	R	1	1
169	n.d.	II–III	D	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*	*tetK*	*tetM*		R	2	1
169	n.d.	II–III	D	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*	*tetK*	*tetM*	cat_{pC221}	R	3	3
354	n.d.	II–III	C	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*	*tetK*	*tetM*	cat_{pC221}	R	3	1
275	n.d.	III	B	*mecA*	*blaZ*		*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*			*tetM*		R	3	1
275	n.d.	UT	B	*mecA*	*blaZ*		*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*			*tetM*		R	1	1
325	n.d.	IV	G	*mecA*	*blaZ*								*tetM*		S	1	1
276	n.d.	V	F	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*		*tetM*		S	8	3
276	n.d.	V	–‖	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*		*tetM*		S	1	1

Table 3 (*cont.*)

ST*	*spa* type	SCC*mec* type	PFGE†	Antimicrobial resistance genes detected by PCR								CIP	No. of isolates	No. of VCs‡
121	t06	V	G	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*	*tetM*	*cat*$_{pC221}$	R	1	1
323	t58	V	H	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*					*tetM*		R	1	1
323	t62	V	H	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*	*tetM*	R	1	1
324	t02	V	‖	*mecA*	*blaZ*	*aac(6′)-Ie-aph (2′)-Ia*	*aph (3′)-III*	*ant (6′)-Ia*	*sat4*	*ermB*	*dfrG*	*lnuA*	*tetM*	R	1	1

MRSP, Methicillin-resistant *Staphylococcus pseudintermedius*; ST, sequence type; PFGE, pulsed-field gel electrophoresis; CIP, ciprofloxacin; VCs, veterinary clinics; UT, untypable; n.d., not determined.

* ST determined by multilocus sequence typing based on seven housekeeping genes.

† Clusters assigned by PFGE.

‡ No. of VCs where MRSP isolates were obtained from dogs.

§ R, resistant; S, susceptible.

‖ These isolates were not included in any clusters.

⩾50%. SCC*mec* V isolates were divided between clusters F ($n = 8$, ST276), G ($n = 1$, ST121), H ($n = 2$, ST323), and others ($n = 2$, ST276 and ST324).

Antimicrobial resistance

Antimicrobial resistance gene profiles and susceptibility to ciprofloxacin results are given in Table 3. All 25 SCC*mec* II–III isolates contained *mecA, blaZ, aac (6′)-Ie-aph(2′)-Ia, aph(3′)-III, ant(6′)-Ia, sat4, ermB*, and *dfrG*, and were resistant to ciprofloxacin (MIC range 4–32 μg/ml). None of the ST71-SCC*mec* II–III isolates harboured *tetM*, while nine out of 11 ST169-SCC*mec* II–III isolates and all ST354-SCC*mec* II–III isolates ($n = 3$) contained *tetM* (Table 3). Nine (69·2%) of the 13 SCC*mec* V isolates were susceptible to ciprofloxacin (⩽0·125 μg/ml).

The *tetM* sequences (1671 bp) from 30 MRSP isolates with SCC*mec* II–III, III, IV or V were determined, and phylogenetic analysis of these sequences revealed three homology groups (Fig. 2). The *tetM* sequences were identical in isolates belonging to the same ST. Moreover, the *tetM* sequence of ST354 isolates was identical to that of ST169 isolates.

All isolates examined in the current study were susceptible to arbekacin (MIC range 0·25–2 μg/ml), minocycline (0·25–4 μg/ml), vancomycin (0·25–1 μg/ml), teicoplanin (⩽0·125–2 μg/ml), quinupristin-dalfopristin (0·25 μg/ml), and linezolid (0·25–1 μg/ml). The antimicrobial resistance patterns determined by phenotypic analysis mainly agreed with patterns of detected antimicrobial resistance genes. Only two SCC*mec* V isolates without *cat*$_{pC221}$ were resistant to chloramphenicol (16–32 μg/ml). Although almost all isolates were resistant to ⩾2 antimicrobials in addition to β-lactam antibiotics, the MRSP SCC*mec* IV isolate was only resistant to β-lactam antibiotics and tetracycline.

DISCUSSION

ST71-*spa* t02-SCC*mec* II–III and ST68-*spa* t06-SCC*mec* V have been identified as the genotypes of major MRSP clonal lineages in Europe and North America, respectively [7]. A previous study classified MRSP isolates by MLST based on four housekeeping genes and the 16S rRNA gene (MLST-4) [3]. In the current study, MRSP isolates were discriminated using a recently developed MLST method based on seven housekeeping genes (MLST-7) [13]. The ST68 and ST71 designations

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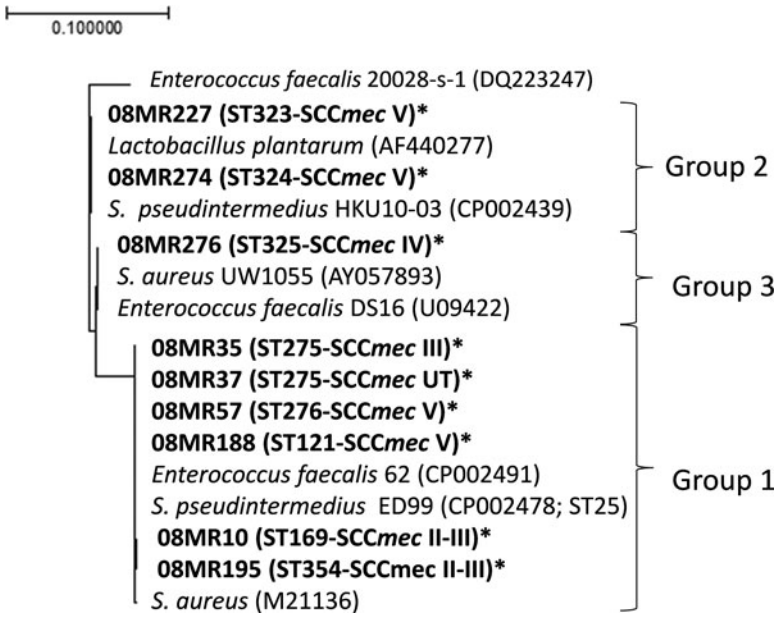

Fig. 2. Phylogenetic tree based on DNA sequences of *tetM* genes. *tetM* DNA sequences (1671 bp) from 30 methicillin-resistant *Staphylococcus pseudintermedius* isolates obtained from dogs in Japan were determined in this study. The sequence of nine representative isolates (* bold font, sequence type (ST) determined by multilocus sequence typing based on seven genes, along with the SCC*mec* type is shown in parentheses) were used for this phylogenetic tree. The remaining sequences were obtained from the GenBank database [bacterial species, strain code, and accession number (in parentheses) are shown]. The tree was constructed using the neighbour-joining method of GENETYX-tree (Genetyx Corp., Japan)

were maintained to provide continuity between the MLST-4 and MLST-7 techniques [13].

Because all MRSP SCC*mec* II–III isolates obtained from European countries [7, 12], North China [31], and Japan [10] have previously been typed as ST71 by MLST-4, all MRSP SCC*mec* II–III isolates were thought to belong to one clonal lineage that had spread worldwide. Thereafter, all MRSP SCC*mec* II–III isolates from Europe [13] and Brazil [32] were also typed as ST71 by MLST-7, supporting the theory of a single clonal lineage. However, in this study we confirmed that in Japan, SCC*mec* II–III elements are present in three related clonal lineages: ST71, ST169, and ST354. Recently, two MRSP ST169-SCC*mec* II–III isolates have also been reported in Thailand [5].

The current analysis showed that the allele sequences of two loci (*ack* and *sar*) differ between ST71 and ST169 isolates. These molecular characteristics suggest that ST71 and ST169 clones have been derived from a common ancestral clone, or one clone might be derived from the other through an unknown third clone. The common ancestor or third clone would be arranged in the red zone surrounding ST71 in Figure 1. In either case, neither clone was directly derived from the other. However, ST354 is probably a variant clone of ST169, the predominant ST in Japan, because only one locus (*purA*) differed between ST169 and ST354.

The majority of ST169 and ST354 isolates harboured *tetM*, while none of the ST71 isolates contained this gene. Although *tetM* genes from the MRSP isolates were divided into three groups in the current study, *tetM* sequences in ST169 ($n = 9$) and ST354 ($n = 3$) isolates were identical to each other. Therefore, it is suspected that the ancestor clone of ST169 already contained *tetM*, or that ST169 isolates acquired *tetM* prior to dissemination. The antimicrobial resistance gene patterns, other than for *tetM*, were similar in ST71, ST169, and ST354 isolates in Japan and ST71 isolates in Europe [7].

The present study detected three novel STs (ST276, ST323, ST324), in MRSP SCC*mec* V isolates from dogs in Japan, with ST276 being the major type in SCC*mec* V isolates in this study. Other SCC*mec* V isolates (ST121, ST323, ST324) were unlikely to be related to the ST276 clone, as allele sequences from these isolates differed from those of ST276 at $\geqslant 5$ loci. In 2007, 4507 (61·9%) of 7281 dogs that passed quarantine and gained entry into Japan came from

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North America (USA, including Hawaii and Canada) (Animal Quarantine Service, http://www.maff.go.jp/aqs/tokei/toukei.html). However, the present study showed that while various MRSP SCC*mec* V clones were present in dogs in Japan, neither ST68 nor related STs that are more common in North America were found in the tested isolates. A further 1654 (22·7%) dogs were from Asia (Taiwan, 611; Korea, 413; China, 193; Thailand, 160; Singapore, 89; and 'other', 188), and 578 (7·9%) dogs were from Europe (UK, 160; Germany, 104; France, 93; Italy, 44, Sweden, 34; and 'other', 143). These numbers suggest that the genotypes of MRSP isolates in Japan do not reflect the origins of their canine hosts.

Like the current study, two previous molecular analyses of Japanese MRSP isolates from clinical samples of companion animals revealed that SCC*mec* II–III and V were predominant types in clinical MRSP isolates in Japan [10, 33]. Moreover, all MRSP SCC*mec* II–III isolates were classified as ST71 by MLST-4 [10]. Therefore, MRSP isolates obtained from buccal samples in the current study are likely to be closely related to these previous clinical MRSP isolates.

In our original study, we evaluated animal patients as a potential source of MRSA contamination of veterinary staff. Therefore, buccal swabs from dog patients were collected to reveal the prevalence of MRSA in dogs which were cared for in veterinary clinics [14]. However, MRSA carriage was rare (3/292) in the dogs [14]. On the other hand, many *mecA*-positive non-MRSA staphylococcal isolates were obtained from these animals. Nearly two-thirds of the *mecA*-positive isolates were identified as *S. peudintermedius*, and these isolates were examined in the current study.

The prevalence of MRSP in blood donor dogs (42·9%, 15/35; $P<0·01$) and dogs owned by veterinary staff (38·5%, 5/13; $P<0·05$) was significantly higher than for dog patients (10·2%, 23/225). Moreover, common STs (ST275, ST276, ST354) of MRSP isolates were detected from $\geqslant 2$ blood donor dogs or dogs owned by veterinary staff of the same veterinary clinics. These results suggest that MRSP is transmitted between dogs in veterinary clinics, and that preventative measures should be implemented.

spa typing of *S. pseudintermedius* divided MRSP ST71-SCC*mec* II–III isolates obtained in Europe into multiple types [7, 8, 12]. The major *spa* type of ST71 isolates in this study was t02 (10/11), which is also the major type in Europe [7]. *spa* genes were not amplified from 27 MRSP isolates by the various primers used in this study. Feng *et al.* also reported that *spa* types could only be confirmed for 15 (21·7%) out of 69 MRSP isolates obtained in South China [6].

In conclusion, MRSP SCC*mec* II–III isolates from Japan were divided into three related STs: ST71, ST169, and the newly registered ST354. Of SCC*mec* V isolates, various novel STs (ST276, ST323, ST324) and ST121 were observed; however, ST68, which is a major genotype in North America, was not found.

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DECLARATION OF INTEREST

None.

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