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Predominance of methicillin-resistant *Staphylococcus aureus* SCC*mec* type II-CC5 and SCC*mec* type IV-CC1/CC8 among companion animal clinical isolates in Japan: Findings from phylogenetic comparison with human clinical isolates

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ABSTRACT

Objectives: To characterise the genotypic profiles of methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates from companion animals and to investigate their association with those from humans in Japan.
Methods: Non-duplicated MRSA clinical isolates recovered between July 2016 and January 2018 were analysed. The MRSA isolates were typed by polymerase chain reaction (PCR)-based open reading frame (ORF) typing (POT) scores, SCC*mec* types, multilocus sequence typing, and virulence gene profiles. Phylogenetic comparison of those isolates with previously described human isolates was performed.
Results: Among 56 MRSA isolates (33 cats, 20 dogs and three rabbits), 26 isolates with a POT1 score of 93, SCC*mec* type II mostly belonged to CC5, including ST5. Twenty-six isolates with a POT1 score of 106, SCC*mec* type IV showed diversity of STs: 15 isolates belonged to CC8, mainly including ST8, and 11 isolates belonged to CC1, including ST1 and newly identified STs 4768, 4775, and 4779. Two cat isolates were ST8-SCC*mec* type IV possessing *pvl*/ACME-*arcA*, presumed to be the hypervirulent community-associated MRSA (CA-MRSA) clone USA300. Notably, all three rabbit isolates belonged to ST4768. The POT1 score 106 CA-MRSA isolates from animals and humans were divided into two large clusters of CC1 and CC8, where host species-specific sub-clusters were not identified within each cluster. A large cluster of POT1 score 93 healthcare-associated MRSA (HA-MRSA) isolates from animals and humans consisted of sub-clusters formed exclusively by the vast majority of human isolates and those formed by animal and human isolates.
Conclusion: Companion animals could be potential reservoirs and vehicles for the transmission of CA-MRSA to humans, and could transmit companion animal-adaptive HA-MRSA lineages to humans as their second reservoirs.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is still of great concern in both healthcare and community settings because of the high frequency of multidrug resistance and production of many virulence factors. High adaptability to human host and clinical environments allows MRSA to persistently colonise and easily spread in both hospitals and communities, causing a variety of infections ranging from mild to severely life-threatening. Epidemiological trends in MRSA have been undergoing a change because of the intrusion of community-associated MRSA (CA-MRSA) lineages usually carrying staphylococcal cassette chromosome *mec* (SCC*mec*) types IV or V into hospital settings, which is displacing classical healthcare-associated MRSA (HA-MRSA) lineages carrying SCC*mec* types I, II or III. CA-MRSA is considered more virulent than HA-MRSA by frequently producing the Panton-Valentine leukocidin (PVL), harbouring the arginine catabolic

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mobile genetic element (ACME) in case of the USA300 clone, and showing higher activity of the accessory gene regulator controlling the expression of staphylococcal toxin genes [1]. Major HA-MRSA lineages worldwide include ST5 (clonal complex [CC] 5) including New York/Japan clone, ST239 (CC8), ST22 (CC22), and ST36 (CC30) [2]. In contrast, the main CA-MRSA clones are continent-specific, namely: ST8 (CC8) including USA300 and USA300-LV in the USA; ST80 (CC80) in Europe; ST30 (CC30) and ST59 (CC59) in Asia; and ST93 (CC93) and ST30 (CC30) in Australia [3]. The emergence of hybrid lineages such as ST239 or ST764 may be another situation for MRSA diversification [4,5].

Companion animals are occasionally colonised or infected with MRSA. MRSA infections are represented by skin and soft tissue infections, and commonly associated with wound infections, surgical site infections, otitis, and urinary tract infections. Clonal lineages of MRSA isolates recovered from companion animals are often the same as the human epidemic clones found in the same geographic region, such as ST22 in the United Kingdom, Germany, Portugal, and Australia; ST59 in China; and ST239 in Australia [6–11]. Such genetic similarity of MRSA isolates between companion animals and humans suggests their transmission between humans and companion animals, which could be reservoirs for human MRSA infections. Livestock-associated MRSA (LA-MRSA), evolving from human-adapted MRSA with several genetic changes has emerged globally in farm animals including pigs, cattle, horses, and poultry, and in humans [3,12–14]. Detection of LA-MRSA in companion animals and its transmission between humans and dogs has also been reported [15,16]. The prevalence of LA-MRSA lineages differs geographically, although CC398 is the most common globally; the most prevalent clonal complex in Europe is CC398, while it is CC9 in Asia [3].

This study reported the prevalence and molecular characterisation of MRSA clinical isolates of companion animal origin in Japan. Furthermore, the association of clonal lineages between animal isolates and human isolates was investigated by including previously described MRSA clinical isolates of human origin [17].

2. Materials and methods

2.1. Bacterial isolates

For bacterial isolation, urine samples were plated on TSAII with 5% sheep blood/Drigalski agar bi-plates (Nihon Becton Dickinson, Tokyo, Japan) and incubated for 24–48 h at 35 °C. Other samples were plated on TSAII with 5% sheep blood/ChocolateII agar bi-plates (Nihon Becton Dickinson) and incubated for 24–48 h in 5% CO2 at 35 °C. Presumptive *S. aureus* colonies were subjected to the MicroScan WalkAway plus system using Pos BP Combo 3.2 J panel for biochemical identification and antimicrobial susceptibility testing, according to the manufacturer's instructions (Beckman Coulter, Inc, Tokyo, Japan). The identification of *S. aureus* exhibiting resistance to oxacillin and/or cefoxitin was further confirmed by MALDI-TOF MS assay using $\geq$ 2.000 score cut-offs for species-level identification, as recommended by the manufacturer (Bruker Daltonics, Bremen, Germany) and by carriage of the *mecA* and *femA* genes. Those MRSA isolates were stored at −80 °C and subcultured on Müller-Hinton agar prior to DNA extraction.

2.2. Molecular typing

Genotyping of MRSA isolates was performed by the polymerase chain reaction (PCR)-based open reading frame (ORF) typing (POT) system using Cica Geneus Staph POT Kit (Kanto Chemical, Tokyo, Japan), as previously described [17]. Namely, 22 target genes, mainly including integrated prophages and SCC*mec* II and IV elements (as shown in Table S1), were amplified by multiplex-PCR using DNA extracted by the Cica Geneus DNA Extraction Reagent (Kanto Chemical), according to the manufacturer's instructions. The score for each of the POT1, 2 and 3 was calculated based on the presence or absence of the bands of PCR-amplified products in a binary manner. The POT1 was scored based on SCC*mec* elements and genomic islets, allowing estimation of clonal complexes (CCs) and identification of SCC*mec* types II and IV. The POT2 and POT3 were scored mainly based on prophage-derived ORFs, allowing discrimination of MRSA strains.

Additional analyses for detecting the SCC*mec* cassette recombinase genes *ccrA1* and *ccrC*, and *mec* gene complex class C for determining SCC*mec* types I and V, and the five small genomic islets for supporting the estimation of CCs were performed, as shown in Table S1, by multiplex PCR, as previously described [17].

Multilocus sequence typing (MLST) was performed on all MRSA isolates by using the primers specified on the MLST website (https://pubmlst.org/saureus/), and the STs were assigned through the same *S. aureus* MLST database [18].

2.3. Detecting virulence determinants

The presence of toxic shock syndrome toxin-1 (TSST-1) gene, *tst*, exfoliative toxin genes, *eta* and *etb*, and Panton-Valentine leukocidin gene, *pvl*, was investigated by using a multiplex PCR, as previously described [17]. A multiplex PCR was also carried out to detect staphylococcal enterotoxin genes, *sea*, *seb*, and sec, as shown in Table S1 [19]. The ACME-encoded *arcA* was detected by PCR using the primers ACME-arcAF (5'-GAGCCAGAAGTACGCGAG-3') and ACME-arcAR (5'-CACGTAACTTGCTAGAACGAG-3') [20].

2.4. Phylogenetic analyses

The genotypic relationships of 56 animal MRSA isolates were analysed based on the presence or absence of 37 genetic elements shown in Table S1, comprising 22 genes analysed in the POT system with an additional eight genes and seven virulence genes, except ACME-*arcA* gene, by constructing phylogenetic networks with SplitsTree version 4.14.4 using NeighborNet method [21]. For comparative analysis of the presence or absence of 37 genetic elements between companion animal and human MRSA isolates, previously reported results on epidemiologically-unlinked 353 human-derived MRSA isolates comprising 84 isolates in 1990, 91 isolates in 2004 and 178 isolates in 2016 were included [17].

2.5. Statistical analysis

The Pearson's χ^2 test was used to determine the prevalence differences in virulence genes between companion animal-derived MRSA with a POT1 score of 93 in this study and human-derived MRSA with a POT1 score of 93 in a previous study [17]. Statistical significance was defined as a *P*-value of < 0.05.

3. Results

3.1. Isolation and antimicrobial susceptibility of MRSA

A total of 163 non-duplicated *S. aureus* isolates were obtained from clinical samples of 163 companion animals at veterinary clinics in Japan from July 2016 to January 2018, as shown in Table 1. Among them, 56 MRSA isolates (34.4%) that were identified were subjected to analyses. Those MRSA isolates consisted of 33 MRSA of 94 *S. aureus* (35.1%) from cats, 20 MRSA of 49 *S. aureus* (40.8%) from dogs and three MRSA of nine *S. aureus* (33.3%) from rabbits. MRSA isolates were mainly obtained from pus (22, 39.3%), urine (10, 17.9%) and skin (six, 10.7%). The MRSA isolates displayed low susceptibility to erythromycin (8.9%), clarithromycin (8.9%),

Table 1
Isolation of *Staphylococcus aureus* and MRSA in companion animals.

Animal	Number of *S. aureus* isolates	Number of MRSA isolates (%)	Type of sample												
			Pus	Urine		Skin	Eye discharge	Ear discharge	Joint fluid	Buccal swab	Nasal discharge	Respiratory secretions	Puncture fluid	Tissue	Bone	Implant
				Spontaneous urine	Catheter urine/ urine obtained by bladder puncture											
Cat	94	33 (35.1)	12	2	4	3	2	3	1	2	2		1	1		
Dog	49	20 (40.8)	8		4	3			2			1			1	1
Rabbit	9	3 (33.3)	2				1									
Others	11[a]	0 (0.0)														
Total	163	56 (34.4)	22	2	8	6	3	3	3	2	2	1	1	1	1	1

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*.
[a] 11 *Staphylococcus aureus* isolates were from three chinchilla, three degu, two squirrels, one hedgehog, one guinea pig, and one flying phalanger.

clindamycin (12.5%), and levofloxacin (10.9%). In contrast, they exhibited 100% susceptibility to arbekacin, vancomycin, teicoplanin, and linezolid.

3.2. Clonality analysis of MRSA isolates

The distribution of POT1 scores among the 56 MRSA isolates is shown in Table 2. The POT1 scores of 106 and 93 found in 26 isolates each (46.4%) were predominant, and those of 64, 68, 85, and 100 were each observed in one isolate. All 26 isolates with a POT1 score of 93, which were characterised by harbouring SCC*mec* type II, mainly belonged to CC5, including ST5 (21 isolates; 11 dog and 10 cat isolates), followed by ST764 (two isolates). Newly identified ST4778 (CC9) was also found in an isolate from a dog. All 26 isolates with a POT1 score of 106 were characterised by harbouring SCC*mec* type IV, although the diversity of their STs was observed. Namely: 15 isolates belonged to CC8, including ST8 (eight isolates; five cat and three dog) and newly identified ST4777 (one isolate); and 11 isolates belonged to CC1, including ST1 (three isolates) and newly identified STs 4768 (three isolates), 4775 (one isolate) and 4779 (one isolate). Notably, all three rabbit isolates exhibiting mutually different POT scores (POT1-POT2-POT3) of 106-183-37, 106-191-37 and 106-129-5 were assigned ST4768. A new sequence type of ST4776 (CC5) was also identified in an isolate with a POT1 score of 68 harbouring SCC*mec* type V. LA-MRSA was not found among the 56 MRSA isolates analysed in this study. Newly identified STs (ST4768, ST4775, ST4776, ST4777, ST4778 and ST4779) in this study were deposited in the PubMLST database.

3.3. Virulence determinants among MRSA isolates

A high rate of *tst* + *sec* carriage (14 of 25 isolates, 56.0%) was found in CC5-SCC*mec* type II lineage (POT1 score 93) (Table 2). In this lineage, six isolates harbouring only *tst* (25.0 %) and one cat isolate harbouring *tst* + ACME-*arcA* were also noted. Among the CC1-SCC*mec* type IV lineage (POT1 score 106), a high prevalence of carriage of only *sea* (6 of 11 isolates, 54.5 %) was found, although there were no isolates harbouring other virulence determinants. Many isolates in the CC8-SCC*mec* type IV lineage (POT1 score 106) did not have virulence determinants; however, two cat isolates belonging to ST8 harboured *pvl* + ACME-*arcA* simultaneously. Comparison of virulence determinants between companion animal-derived MRSA with POT1 score 93 in this study and human-derived MRSA with POT1 score 93 in a previous study [17] revealed that *tst* occurred significantly more frequently among MRSA isolates of companion animal origin than in those of human origin in 2016 ($P = 0.030$) and 2004 ($P = 0.008$) (Table 3). The *tst* + *sec* combination was significantly more frequently detected in MRSA isolates than from humans in 2004 ($P = 0.049$) and more infrequently in those from humans in 2016 ($P = 0.004$) compared with isolates from companion animals. The *seb* gene was significantly more frequently detected in MRSA isolates from humans in 2016 ($P = 5.132 \times 10^{-8}$) and in 2004 ($P = 0.036$), whereas the gene was not detected in MRSA isolates from companion animals. Overall, frequencies of *tst*, *seb*, *sec*, and *tst*/*sec* were very similar between MRSA isolates from companion animals and humans in 1990, with no statistically significant differences observed. The POT1 score of 106 isolates from companion animals had significantly lower prevalence of *sea* gene (23%) than MRSA isolates from humans in 2016 (48%) (data not shown).

3.4. Phylogenetic network analysis

The phylogenetic network constructed for the 56 MRSA isolates of companion animal origin is shown in Fig. 1. Two large genetic clusters of POT1 scores 93 and 106, and three independent

Table 2
Molecular characteristics of 56 methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates from companion animals.

POT1 score	SCC*mec* type	No. of isolates	Clonal complex	Sequence type	Animal species (n)	No. of virulence gene-positive isolates									
						tst	*tst* + *sec*	*tst* + ACME-*arcA*	*sea*	*seb*	*sec*	*pvl* + ACME-*arcA*	*eta*	*etb*	ND
64	V	1	CC72	ST72	Dog (1)	0	1	0	0	0	0	0	0	0	0
68	V	1	CC5	ST4776[a]	Dog (1)	0	0	0	0	0	0	0	0	0	1
85	unidentified	1	CC5	ST5	Cat (1)	1	0	0	0	0	0	0	0	0	0
93	II	26	CC5	ST5	Dog (11)	3	7	0	0	0	0	0	0	0	1
					Cat (10)	2	6	1	0	0	0	0	0	0	1
				ST764	Cat (2)	0	0	0	0	0	0	0	0	0	2
				ST512	Cat (1)	0	1	0	0	0	0	0	0	0	0
				ST1863	Cat (1)	1	0	0	0	0	0	0	0	0	0
			CC9	ST4778[a]	Dog (1)	1	0	0	0	0	0	0	0	0	0
100	I	1	CC5	ST5	Cat (1)	0	0	0	0	1	0	0	0	0	0
106	IV	26	CC1	ST1	Cat (3)	0	0	0	2	0	0	0	0	0	1
				ST2725	Cat (2)	0	0	0	0	0	0	0	0	0	2
					Dog (1)	0	0	0	1	0	0	0	0	0	0
				ST4779[a]	Cat (1)	0	0	0	0	0	0	0	0	0	1
				ST4775[a]	Cat (1)	0	0	0	1	0	0	0	0	0	0
				ST4768[a]	Rabbit (3)	0	0	0	2	0	0	0	0	0	1
			CC8	ST8	Cat (5)	1	0	0	0	0	0	2	0	0	2
					Dog (3)	0	1	0	0	0	0	0	0	0	2
				ST4224	Cat (3)	2	0	0	0	0	0	0	0	0	1
				ST380	Dog (2)	0	0	0	0	0	0	0	0	0	2
					Cat (1)	0	0	0	0	0	0	0	0	0	1
				ST4777[a]	Cat (1)	0	0	0	0	0	0	0	0	0	1

Abbreviations: POT, polymerase chain reaction-based open reading frame typing; *tst*, toxic shock syndrome toxin-1 gene; *sea*, staphylococcal enterotoxin A gene; *seb*, staphylococcal enterotoxin B gene; *sec*, staphylococcal enterotoxin C gene; *pvl*, Panton-Valentine leukocidin gene; ACME-*arcA*, *arcA* gene within the arginine catabolic mobile element (ACME); *eta*, exfoliative toxin A gene; *etb*, exfoliative toxin B gene; ND, not detected.
[a] Sequence types newly assigned in this study.

Table 3
Comparison of virulence gene profile between companion animal-derived MRSA with POT1 score 93 in this study and human-derived MRSA with POT1 score 93 in a previous study.

Gene	Presence of virulence gene [n (%)]				*P*-value[b]		
	MRSA of companion animal origin (n = 26)	MRSA of human origin[a]			MRSA of companion animal origin vs. MRSA of human origin (in 2016)	MRSA of companion animal origin vs. MRSA of human origin (in 2004)	MRSA of companion animal origin vs. MRSA of human origin (in 1990)
		in 2016 (n = 72)	in 2004 (n = 73)	in 1990 (n = 35)			
tst	8 (31%)	8 (11%)	6 (8%)	16 (46%)	0.030[c]	0.008[c]	0.294
seb	0 (0%)	42 (58%)	13 (18%)	3 (9%)	5.132×10^{-8}[c]	0.036[c]	0.254
sec	0 (0%)	1 (1%)	0 (0%)	0 (0%)	1	1	1
tst + *sec*	14 (54%)	16 (22%)	55 (75%)	17 (49%)	0.004[c]	0.049[c]	0.797

Abbreviations: POT, polymerase chain reaction-based open reading frame typing; MRSA, methicillin-resistant *Staphylococcus aureus*.
[a] Previously published [17].
[b] *P*-value was calculated by Pearson's χ^2 test.
[c] Statistically significant difference ($P < 0.05$).

evolutionary lineages of POT1 scores 64, 68 and 100 were identified, with the formation of a reticular network indicative of recombination or horizontal gene transfer events. Those two large clusters of POT1 scores 93 and 106 were depicted with longer branch lengths between them, showing longer evolutionary distances. Among isolates with a POT1 score of 106, CC1 isolates including *sea* carriers, and CC8 isolates including *tst* or *pvl* + ACME-*arcA* carriers were clustered separately. Two CC8 cat isolates positive for *pvl* + ACME-*arcA* showed close genetic proximity. An isolate with a POT1 score of 85 belonging to ST5 was located within the cluster of POT1 score 93 isolates.

Phylogenetics of 56 companion animal isolates in comparison with 353 human isolates were assessed [17] (Fig. 2). In a phylogenetic network, the isolates with a POT1 score 106 of companion animal origin (26 isolates) and human origin (87 isolates) mainly formed two large clusters: one comprised CC1 companion animal-derived isolates and presumed CC1 human-derived isolates, and the other comprised CC8 companion animal-derived isolates and presumed CC8 human-derived isolates. Those isolates from companion animals, including cats, dogs and rabbits, and from humans did not form host-species-specific sub-clusters within each cluster.

The MRSA isolates with a POT1 score 93 from companion animals (26 isolates) and humans (180 isolates) formed a large cluster. The cluster was further divided into several sub-clusters (i.e. sub-clusters comprising cats and human isolates, sub-clusters comprising cats, dogs and human isolates, and sub-clusters exclusively comprising the vast majority of human isolates (Fig. 2). Thus, host-species-specific sub-clusters were not identified among cats or dogs isolates but human isolate-specific sub-clusters were noted.

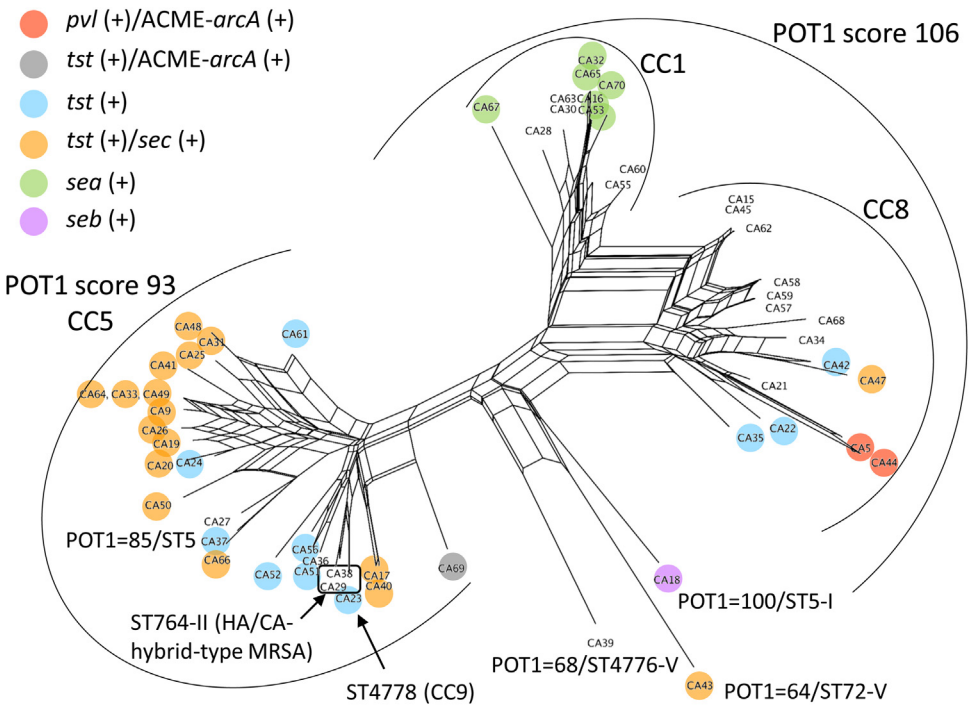

Fig. 1. Phylogenetic network between 56 MRSA isolates of companion animal origin. The diagram was created by SplitsTree based on 37 gene profiles comprising 22 genes from the POT system with an additional eight genes and seven toxin genes.
Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; POT, polymerase chain reaction-based open reading frame typing; *tst*, toxic shock syndrome toxin-1 gene; *sea*, staphylococcal enterotoxin A gene; *sec*, staphylococcal enterotoxin C gene; *pvl*, Panton-Valentine leukocidin gene; ACME-*arcA*, *arcA* gene within the arginine catabolic mobile element (ACME).

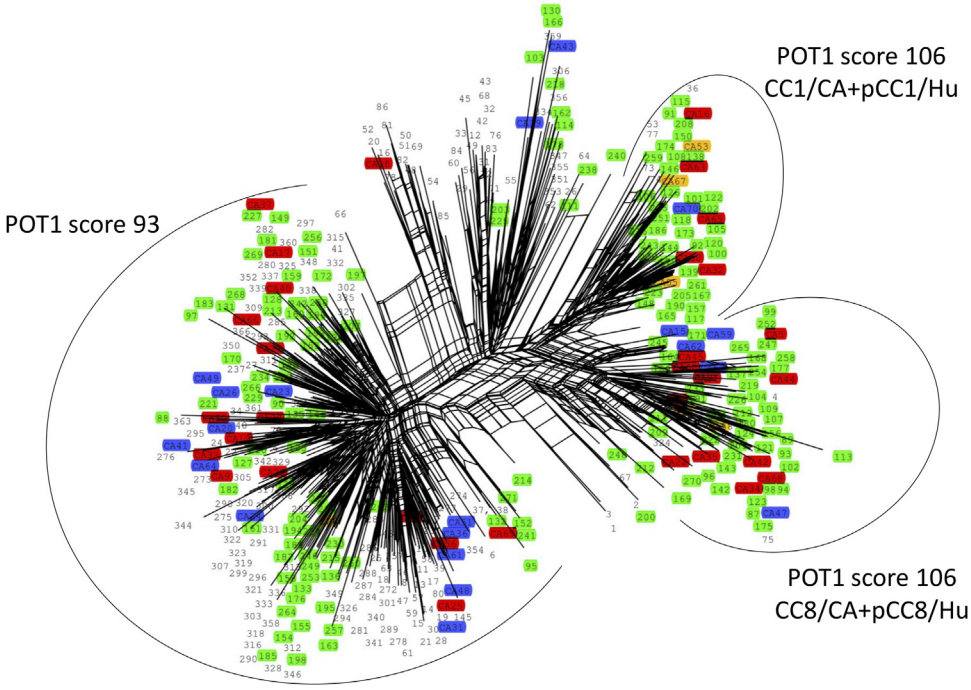

Fig. 2. Phylogenetic network of MRSA isolates comprising 56 isolates from companion animals and 353 isolates from humans generated by neighbour-net method using SplitsTree. Animal isolates from cat (red), dog (blue) and rabbit (orange) were separately indicated. For human isolates, 178 isolates in 2016 (green) and 175 isolates in 2004 and 1990 (no colour) were indicated [17].
Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; POT, polymerase chain reaction-based open reading frame typing; CC, clonal complex; pCC, presumed clonal complex; CA, companion animal; Hu, human.

4. Discussion

MRSA transmission between humans and companion animals has been increasingly recognised worldwide, where MRSA isolates recovered from companion animals and humans generally shared the same clonal lineages [3,22,23]. Thus, research to assess the public health importance of MRSA in companion animals has become imperative due to their close contact with humans and extensive exposure to antimicrobials. In a French study, of MRSA isolates from 34 cats and 28 dogs collected during 2010 and 2015, the most frequent clone in both animal species was CC8-SCC*mec* type IV, followed by CC398, whereas ST22 was extremely rare, despite its high prevalence in animals in other European countries [3,23,24]. In an active surveillance study in the US, USA100/ST5-SCC*mec* type II was most frequently isolated (32 of 37, 86.5%) followed by USA800/ST5-SCC*mec* type IV (5.4%) and USA500/ST8-SCC*mec* type IV (2.7%) from dogs [25]. There are limited studies in Japan, where three MRSA isolates from dogs, comprising two ST5-SCC*mec* type II and one ST30-SCC*mec* type IV, have been reported [26]. The present study aimed to investigate the molecular characteristics of MRSA from companion animals with a larger number of clinical isolates, to gain an in-depth understanding of the latest epidemiology of MRSA clones. Furthermore, comparative analysis of MRSA clonal lineages and virulence determinants was performed between those animal isolates and epidemiologically-unrelated human isolates that have been described in a previous study [17] to explore the link between animal MRSA lineages and human MRSA populations.

MRSA genotypes were mainly assessed by the POT system, which has been used for the genotypic analysis of previously described human MRSA isolates to conduct their comparative analysis [17]. Whole genome sequencing is without doubt a powerful tool for phylogenetic analysis of large numbers of isolates; however, it requires expensive upfront and operating costs and experience in computational bioinformatics for analysing massive quantities of data. The POT system – based on the detection of ORFs derived from lysogenic prophages, SCC*mec* types II and IV and genomic islets for CC estimation – has been evaluated as a simple and useful tool for analysing the genotypic characteristics of MRSA isolates. Although the POT system has been widely used in clinical settings in Japan, due to its high discriminatory power comparable to pulsed-field gel electrophoresis, the system has not been well recognised in other countries.

The current study identified new MLST sequence types – ST4768, ST4775, ST4776, ST4777, ST4778, and ST4779 – amongst MRSA isolates recovered from companion animals. Intriguingly, all three isolates from rabbits were assigned to ST4768 (CC1), suggesting that the ST4768 lineage is rabbit-specific. However, those isolates did not form independent sub-cluster on SplitsTree depiction. Internationally distributed *S. aureus* strains belonging to ST121 lineage, which is considered a virulent clone in humans, have been responsible for high-virulence diseases in farmed rabbits [27,28]. Two rabbit isolates were additionally obtained after the study period, one with a POT1 score of 106, ST1-SCC*mec* type IV possessing *sea* and the other with a POT1 score of 98, CC8 (ST8 like)- SCC*mec* type I (data not shown). Further studies are needed to identify a genetic epidemiological picture of companion rabbit MRSA populations.

Two cat isolates were identified as ST764 SCC*mec* type II, a variant of ST5 SCC*mec* type II. The MRSA ST764 lineage is a hybrid variant of ST5 HA-MRSA with CA-MRSA by acquiring virulence determinants, including the ACME-*arcA* and *seb* genes but lacking the *pvl*, *tst* and *sec* genes [5], and has been widely spread among human clinical isolates in Japan [29]. No virulence determinant analysed in this study was detected in the two ST764 cat isolates; however, the transmission of those isolates from humans to companion animals is most likely to occur because the ST764 lineage has so far not been reported in MRSA isolates from companion and food animals.

The ST5 SCC*mec* type II lineage known as New York/Japan clone, the previously dominant HA-MRSA clone in Japan, has been replaced by CA-MRSA clones with POT1 score 106 in the post-epidemic phase [17]. This ST5 SCC*mec* type II lineage has frequently been associated with *tst*- and *sec*-positive but *pvl*- and ACME-*arcA*-negative. Of the 21 isolates of ST5 SCC*mec* type II lineage in this study, 18 isolates were also found to be positive for *tst* and/or *sec*, and negative for *pvl* and ACME-*arcA*. However, one cat isolate of this lineage tested positive for *tst* and ACME-*arcA*, while negative for *sec* and *pvl*. Acquisition of ACME by ST5 SCC*mec* type II has already been described among human clinical isolates in Japan [30].

The ST8- SCC*mec* type IV characterised by possessing both *pvl* and ACME-*arcA*, known as the hypervirulent CA-MRSA clone USA300, is a highly successful lineage, which has been predominant in the USA and is spreading globally. The presumed USA300 clone has been identified with low frequency, four of 178 human clinical isolates in 2016 (2.2%) in Japan according to further analysis of a recent study [17]. Two cat isolates of 56 isolates (3.6%) were found to be presumed USA300 clone; therefore, a potential risk of transmission of those isolates to humans from companion animals should be considered.

The prevalence rates of isolates with POT1 score 93 HA-MRSA clone and 106 CA-MRSA clone were comparable between companion animals (46.4% each) and humans in 2016 (40.4% and 44.9%, respectively) [17]. A phylogenetic comparison of POT1 score 106 MRSA isolates of companion animal origin with those of human origin mainly comprising 2016 isolates showed that those isolates were divided into two large clusters of CC1 and CC8. Host-species-specific sub-clusters were not identified within each cluster, suggesting that those clonal lineages are likely adaptable to both humans and companion animals; therefore, companion animals can be potential reservoirs and vehicles in the transmission of those CA-MRSA lineages.

Comparative analysis of the frequency of virulence determinants among MRSA isolates with a POT1 score 93 from companion animals and humans in 2016 revealed that significantly higher rates of *seb* carriage were recorded among human isolates than animal isolates, although both isolates were collected during the same time period. In contrast, carriage rates of *tst* and *tst* + *sec* were significantly higher among animal isolates than human isolates. It is noticeable that the carriage rates of virulence determinants *tst*, *seb*, *sec*, and *tst* + *sec* among POT1 score 93 isolates of companion animal origin were rather closer to those of human origin in 1990, with no statistically significant difference. These findings led to the initial assumption that POT1 score 93 isolates from companion animals and humans in 1990 might be genetically homogeneous. However, phylogenetic analysis showed that those isolates did not form a distinct sub-cluster. On SplitsTree depiction, animal isolates formed sub-clusters together with human isolates. Thus, these HA-MRSA lineages could possibly transmit between humans and companion animals through adaptive genetic evolution. Further attention should be paid to companion animals as potential reservoirs for these HA-MRSA lineages. Notably, sub-clusters formed exclusively by almost all human isolates but containing no animal isolates were also depicted, implying that these are dominant CC5 HA-MRSA lineages circulating only among humans in Japan.

In conclusion, CC5-SCC*mec* type II HA-MRSA and CC1- or CC8-SCC*mec* type IV CA-MRSA were dominant in 56 MRSA isolates obtained from clinical samples of companion animals at veterinary clinics in Japan. This study is unique in that phylogenetic comparative analysis was conducted using companion animal clinical isolates and a large number of epidemiologically-unlinked human clinical isolates that were analysed in a previous study [17]. The findings support the role of companion animals as potential

reservoirs and vehicles for the circulation of CA-MRSA and HA-MRSA between companion animals and humans. In addition, sub-clusters formed exclusively by the vast majority of human isolates included in this study were identified within a CC5-SCC*mec* type II HA-MRSA cluster.

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Competing interests

None.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.08.016.

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