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Population Genetic Structures of *Staphylococcus aureus* Isolates from Cats and Dogs in Japan

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We determined the population genetic structures of feline and canine *Staphylococcus aureus* strains in Japan by multilocus sequence typing (MLST). Ecological analyses suggested that multiple feline-related *S. aureus* clones, including ST133, naturally occur as commensals and can cause endogenous infections in felines. In contrast, *S. aureus* populations do not likely include any clone that exhibits tropism in domestic dogs. Even if *S. aureus* infections occur in dogs, the pathologies are likely exogenous infections.

Staphylococcus aureus is a coagulase-positive staphylococcus (CoPS) and is present in normal skin and nasal flora but opportunistically causes a wide range of infections in humans and animals. According to multilocus sequence typing (MLST) data, there are four major clonal complexes (CCs), CC97, CC126, CC133, and CC151, among bovine *S. aureus* isolates worldwide (5, 8, 13). Pig-associated strains exhibited sequence type 9 (ST9), ST398, and ST433 (1). These specific clones are not always common in natural populations of human *S. aureus* (4, 7, 9, 10, 12), suggesting that *S. aureus* clones have evolved host specifically.

Methicillin-resistant *S. aureus* (MRSA), which is one of the most conspicuously nosocomial pathogens in humans, is also now increasingly common in veterinary medicine. ST398 and ST9 MRSA clones have been a matter of zoonotic concern in many countries; these clones were generated from within swine-related methicillin-susceptible *S. aureus* (MSSA) clones in pig hosts (1, 15). Thus, to trace the original infectious source of MRSA zoonotic transmission, we need to understand the population structures of *S. aureus* clones in various animal species. There have been many reports involving domestic dogs and cats in outbreaks of human MRSA infections in countries where the clones are endemic (15). However, in canine and feline hosts, there has been no report on the population genetic structures of MSSA (not MRSA) strains, which reflect the natural habitation of *S. aureus* clones in the host species.

Here, we characterize feline and canine *S. aureus* strains by molecular methods and compare the strains from various host animal species. To obtain feline and canine *S. aureus* strains, we conducted the detection of *S. aureus* strains for 402 carriage specimens (dogs, $n = 232$; cats, $n = 170$) and 580 cases diagnosed as staphylococcal infection (dogs, $n = 459$; cats, $n = 121$) in eastern Japan from 2002 to 2010. We used 93 *S. aureus* strains isolated from 74 cats and 19 dogs (see Table S1 in the supplemental material), with each representing an independent individual. The bacteria were identified as *S. aureus* using a PCR method (11) and were characterized using MLST (3). Toxin typing, detection of *mecA*, and staphylococcal cassette chromosome *mec* (SCC*mec*) typing were also performed. All strains were tested for resistance to macrolides, aminoglycosides, and fluoroquinolones by the disk diffusion method based on CLSI guidelines (1a). The diversity and evenness of ST distribution in each host were calculated using Simpson's diversity index ($1 - \lambda$) and Pielou's evenness index (J'). Both values range from 0 (no diversity or evenness) to 1 (extreme diversity or evenness) and are more insusceptible to the difference of sample size than Shannon-Wiener's index (H'). These parameters have generally been used for the comparison of biodiversity between geographically separated environments. The values for feline and canine strains were compared with those previously reported for strains from humans, pigs, cows, and goats (1, 4, 5, 7–10, 12, 13). To visualize differences of diversity among host species, phylogenetic trees based on concatenate sequences of the seven genes used in MLST were constructed by MEGA version 5.05 (14).

Twenty-four unique STs and two nontypeable strains were identified among the 74 feline *S. aureus* strains: 14 unique STs were identified among the 19 canine strains (see Table S1 in the supplemental material), and 10 new STs, ST1250, ST1251, ST1252, ST1253, ST1332, ST1333, ST1408, ST1412, ST1441, and ST1837, were found and described over the course of this study.

Among the 74 *S. aureus* isolates of feline origin, 20 MRSA and 54 MSSA strains were obtained. All feline MRSA strains belonged to one of two lineages, CC5 ($n = 15$) or CC8 ($n = 5$). Sixty percent (9 of 15) of the CC5 MRSA strains exhibited the Japanese hospital-associated MRSA (HA-MRSA) genotype (ST5 SCC*mec* type II *tst*, *sec*, *seg*, and *sei* positive). Three strains with the New York clone genotype (USA100; *tst*-negative ST5 SCC*mec* type II) were also obtained. The CC8 MRSA strains showed significant genetic heterogeneity in MLST alleles, SCC*mec* types, and toxin profiles. No

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TABLE 1 Diversity and evenness indexes of *S. aureus* isolates in various populations

Host	Country	Clinical status (human population)	No. of isolates	No. of STs (CC)	Simpson's index	Pielou's index	Predominant ST(s) among MSSA isolates[a]	Source or reference
Dog	Japan	Carriage and infections	19	14 (9)	0.912	0.808	ST5	This study
Cat	Japan	Carriage and infections	74	26 (15)	0.908	0.639	ST133	This study
Human	Switzerland	Nasal carriage (adults)	132	37 (21)	0.918	0.603	ST45, ST30	10
	China	Nasal carriage (children)	147	25 (17)	0.875	0.515	ST121, ST59	4
	China	Infections (children)	51	20 (12)	0.931	0.681	ST88, ST121, ST398	4
	United Kingdom	Intravenous drug users lesion	28	12 (11)	0.910	0.680	ST59, ST5, ST12, ST30, ST45	7
	Mali	Nasal carriage (emergency patients)	88	20 (15)	0.858	0.522	ST15, ST152	9
	Gabon	Nasal carriage	34	10	0.891	0.605	ST30, ST15, ST72, ST80, ST88	12
Pig	France	Infections	14	4 (4)	0.692	0.443	ST398, ST9, ST433	1
Cow	Norway	Bulk milk	101	22 (5)	0.769	0.444	ST132, ST133	5
	United States	Bulk milk	116	16 (10)	0.633	0.334	ST124, ST126	13
	United Kingdom	Bulk milk	11	2 (2)	0.336	0.198	ST151, ST9	13
	Chile	Bulk milk	20	5 (3)	0.368	0.260	ST97	13
	Brazil	Bulk milk	227	11 (6)	0.496	0.207	ST126, ST97	8
Goat	Norway	Bulk milk	38	5 (3)	0.521	0.265	ST133, ST130	5

[a] ST(s) which accounted for not less than 10% of clones in the population.

Panton-Valentine leukocidin (PVL)-positive strain was isolated in this study. Among the feline MSSA strains, ST133 ($n = 9$) was the most frequent ST, followed by ST5 ($n = 6$) and ST20 ($n = 5$). Multiple strains of ST188 ($n = 4$), ST508 ($n = 4$), ST25 ($n = 3$), ST1251 ($n = 3$), ST8 ($n = 2$), ST12 ($n = 2$), and ST97 ($n = 2$) were also identified. CC5 and CC8 *S. aureus* clones were not found among carriage isolates. Many of the CC5 and CC8 isolates were derived from infected wounds in inpatients or urinary tract infections and exhibited multidrug resistance. Aside from the CC5 and CC8 strains, we did not find any correlation between clinical status and genotype.

Most occurrences of *S. aureus* in dogs were cases of carriage in hospital patients. Among all cases diagnosed as staphylococcal infection in dogs, those from which *S. aureus* were isolated accounted for only 1.1% (5 of 459), and more than half of them were relevant to hospitalization and/or drug resistance (see Table S1 in the supplemental material). Of the 19 canine *S. aureus* strains, six belonged to ST5. Three of these strains exhibited the Japanese HA-MRSA genotype and three other ST5 strains were MSSA, but two had the same genotype as Japanese HA-MRSA, and one exhibited the same genotype as USA100. All of the remaining canine strains had distinct STs from one another. No correlation was found between clinical status and genotype in canine strains.

Donnio et al. reported that MSSA strains from which SCC*mec* was excised retain resistance to macrolides at a high rate, probably via a Tn*554* that is located on SCC*mec* and contains a macrolide resistance-encoding *ermA* gene (2). Such SCC*mec*-excised strains also frequently exhibited resistance to aminoglycosides and/or fluoroquinolones, resulting in the emergence and epidemic diffusion of multidrug-resistant MSSA (MR-MSSA) in hospital environments (2). In the current study, 77.8% (7 of 9) of ST5 MSSA strains exhibited erythromycin resistance and were also resistant to levofloxacin and/or gentamicin. Therefore, epidemic diffusion of ST5 MR-MSSA strains derived from the Japanese HA-MRSA clone should be expected in veterinary hospital environments. ST5 MSSA strains are also linked with antimicrobial use, suggesting that ST5 *S. aureus* clones are not naturally distributed in dogs and cats.

Populations of canine and feline *S. aureus* strains showed high diversity index values ($1 - \lambda = 0.912$ and 0.908, respectively). These high diversity index values are comparable to those of human strains (0.858 to 0.931) and distinct from greater homogeneity seen for swine (0.692), bovine (0.336 to 0.769), and caprine strains (0.521) (Table 1). As shown in Fig. 1, *S. aureus* strains of bovine origin in Brazil (8) showed relatively uneven and aggregated distribution of specific STs, ST126 and ST97, which have a strong tropism for bovine hosts. Strains from humans in Switzerland (10) and those of feline origin in the present study varied less from ST to ST than those of bovine origin. Our canine *S. aureus* strains showed an extremely high Pielou's evenness index ($J' = 0.808$) compared to those of humans (0.515 to 0.681), cats (0.639), pigs (0.443), cows (0.198 to 0.444), and goats (0.265) and did not reveal concentrated distribution of any STs other than ST5. High values of both diversity and evenness indexes in the dog strains indicate that the distribution of *S. aureus* clones in canine hosts formed a random pattern, suggesting that no *S. aureus* clone exhibits tropism in domestic dogs in Japan.

Our results show that feline hosts allow diverse *S. aureus* clones to adapt as commensals. Interestingly, ST133, which was the most frequent ST in cats in Japan, had been recognized as a host-specific clone in ruminant animals (5). The existence of substantial geographic structure has been reported in bacterial isolates from human and bovine hosts (5, 8, 13). Further studies in other geographic areas will be required to evaluate the adaptation of *S. aureus* clones in feline hosts.

The occurrence of *S. aureus* in dogs has probably been overes-

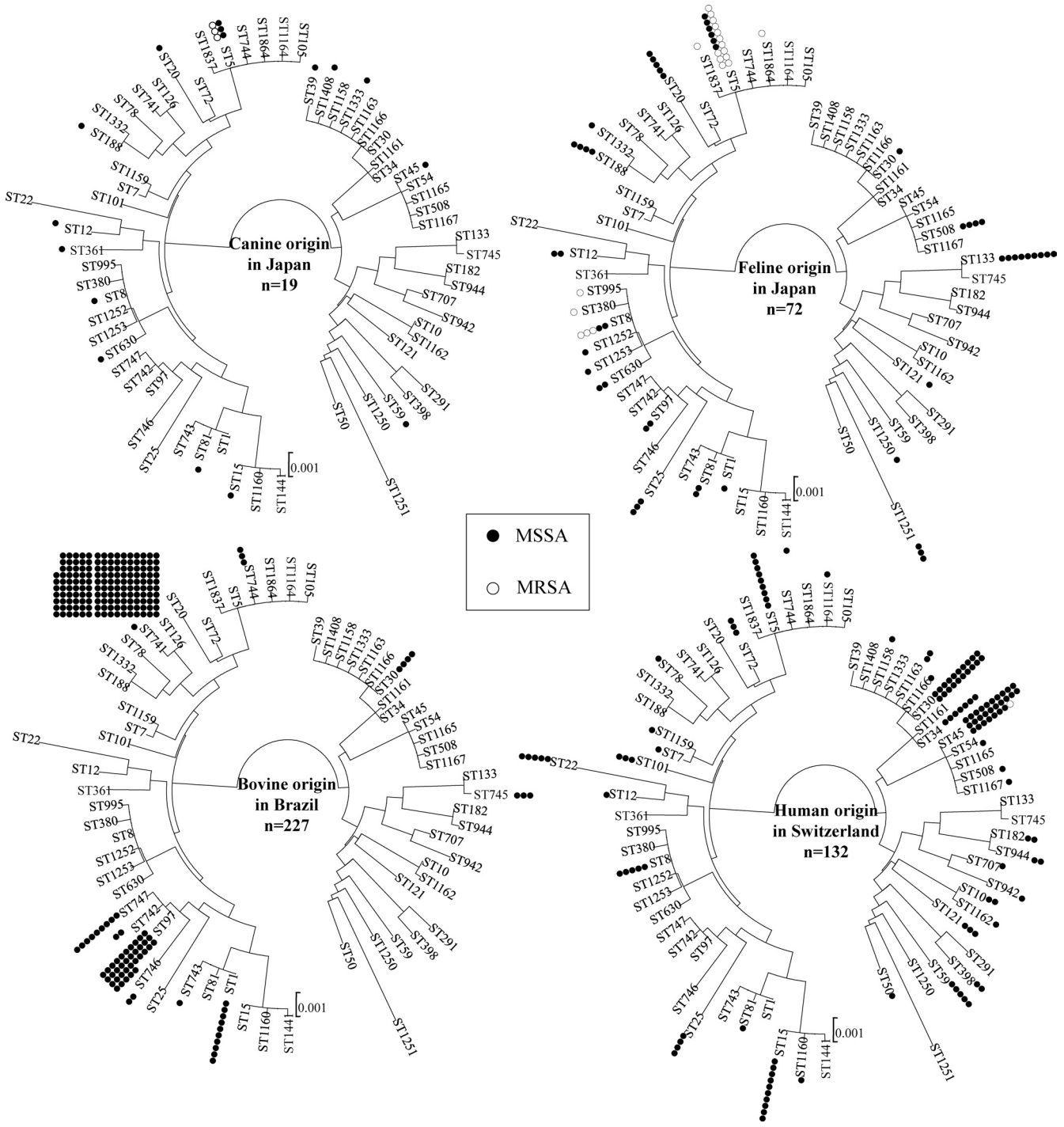

FIG 1 Phylogenetic tree based on concatenated *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL* sequences and distribution of strains from cats, dogs, humans (10), and cows (8) in population genetic structures of *S. aureus*. These trees were constructed by the neighbor-joining method using MEGA version 5.05. The numbers of MSSA and MRSA strains are indicated.

timated, because the predominant species of CoPS in dogs, *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi*, could be misidentified as *S. aureus* by conventional identification systems that use biochemical characterization (11). Recently, Kawakami et al. reported that no *S. aureus* strain was isolated from 190 cases of canine pyoderma by a molecular identification method (6, 11). Weese and van Duijkeren also speculated that *S. aureus* is not naturally a predominant commensal in dogs, based on evidence that MRSA colonization was transient in canine hosts (15). These reports support the hypothesis that the *S. aureus* population does not include any clone that has tropism for healthy domestic dogs. Even if *S. aureus* infections occur in dogs, it is likely that such

pathologies are exogenous infections caused by random or human-related clones associated with the regions where MRSA is endemic. Thus, in contrast to the case in pigs, dog-related MRSA clones will likely not be generated in canine hosts, given the lack of *S. aureus* clones adapted to domestic dogs. In the context of public health, dogs likely have low potential as a source of transmission of infectious, zoonotic MRSA.

In conclusion, multiple *S. aureus* clones naturally occur as commensals in cats and can also cause endogenous infections in felines. In contrast, domestic dogs likely acquire *S. aureus* strains from exogenous sources. These data are expected to contribute to public health and research findings on the molecular mechanisms underlying host specificity.

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