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.

*S. 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 MSSA 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* (SCCmec) 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 − λ) and Pielou’s evenness index (*H*′). 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 SCCmec type II *tst*, *sec*, *seg*, and *sei* positive). Three strains with the New York clone genotype (USA100; *tst*-negative ST5 SCCmec type II) were also obtained. The CC8 MRSA strains showed significant genetic heterogeneity in MLST alleles, SCCmec types, and toxin profiles. No
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 clones, six STs which accounted for not less than 10% of clones in the population.

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 SCCmeC were excised retain resistance to macrolides at a high rate, probably via a Tn554 that is located on SCCmeC and contains a macrolide resistance-encoding ermA gene (2). Such SCCmeC-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 − λ = 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-

### Table 1: Diversity and evenness indexes of S. aureus isolates in various populations

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

ST(s) which accounted for not less than 10% of clones in the population.
estimated, 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

![Phylogenetic tree](http://jcm.asm.org/)
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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REFERENCES