Population genetic structures of *Staphylococcus aureus* isolates from cats and dogs in Japan.

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ABSTRACT

We determined 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 a commensal, and can cause endogenous infections in felines. In contrast, S. aureus population does not likely include any clone that has tropism for domestic dogs. Even if S. aureus infections should occur in dogs, the pathologies are likely exogenous infections.
Staphylococcus aureus is a coagulase-positive staphylococci (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), 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 (ST) 9, 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 conspicuous 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 endemic MRSA clones in the countries (15). However, in canine and feline hosts, there has been no report on the population genetic structures of not MRSA but MSSA 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 (Table S1), each of which represent an independent individual. These bacteria were identified as *S. aureus* using a PCR method (11), and 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. The diversity and evenness of ST distribution in each host were calculated using the Simpson's diversity index (1-λ) and the 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 difference of sample size than the Shannon-Wiener's index (*H*'). These parameters have been generally used for 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 ver 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 (Table S1). Ten new STs, ST1250, ST1251, ST1252, ST1253, ST1332, ST1333, ST1408, ST1412, ST1441 and ST1837, were found and newly designated over the course of this study.

Among seventy-four *S. aureus* isolates of feline-origin, twenty MRSA and fifty-four MSSA strains were obtained. All feline MRSA strains belonged to one of two lineages, CC5 (n=15) or CC8 (n=5). 60% (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. CC8 MRSA showed significant genetic heterogeneity in MLST alleles, SCCmeC types and toxin profiles. No PVL-positive strain was isolated in this study. Among feline MSSA strains, ST133 (n=9) was the most frequent ST, followed by ST5 (n=6) and ST20 (n=5). Multiple strains of sequence types 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 were only 1.1% (5 of 459), more than half of them were relevant to hospitalization and/or drug resistance (Table S1). Of 19 canine S. aureus strains, six belonged to ST5. Three of these strains exhibited the Japanese HA-MRSA genotype: 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. Any correlation between clinical status and genotype were not found in canine strains.

Donnio et al. reported that MSSA strains from which SCCmeC was 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 Japanese HA-MRSA clone should be expected in veterinary hospital environments. ST5 MSSA strains also are 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 (0.858-0.931), and distinct from greater homogeneity seen for swine (0.692), bovine (0.336-0.769) 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 ST 97, 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 extremely high Pielou's evenness index ($J^* = 0.808$) compared to those of humans (0.515-0.681), cats (0.639), pigs (0.443), cows (0.198-0.444) 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 has tropism for domestic dogs in Japan.

Our results show that feline hosts allow diverse S. aureus clones to adapt as commensals. Interestingly, ST 133, 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 overestimated, because the predominant species of CoPS in dogs, *S. pseudintermedius* and *S. schleiferi*, could be misidentified as *S. aureus* by conventional identification systems that use biochemical characterization (11). Recently, Kawakami et al. have reported that no *S. aureus* strain was isolated from 190 cases of canine pyoderma using a molecular identification method (6, 11). Weese et al. 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 *S. aureus* population does not include any clone that has tropism for healthy domestic dogs. Even if *S. aureus* infections should occur in dogs, it is likely that such pathologies are exogenous infections by random or human-related clones associated with the endemic MRSA in the region. 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 an infectious source of MRSA zoonotic transmission.

In conclusion, multiple *S. aureus* clones naturally occur as a commensal 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


Figure legend.

Figure 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 ver. 5.05. The number of MSSA (black circle) and MRSA (white circle) strains are indicated.
ST1164
ST1164
ST361
ST745
Feline origin
in Japan
n=72

ST361
ST745
Canine origin
in Japan
n=19

ST1441
0.001

ST
ST144
4
1
0.001

ST14

ST1164

ST1164

ST361
ST745
Bovine origin
in Brazil
n=227

ST361
ST745
Human origin
in Switzerland
n=132

S
ST144

S
ST14

S
ST14
<table>
<thead>
<tr>
<th>Host</th>
<th>Country</th>
<th>Clinical status</th>
<th># of isolates</th>
<th># of STs (CCs)</th>
<th>Simpson's index (1 - \frac{1}{n^2})</th>
<th>Pielou's Index (J')</th>
<th>Predominant STs among MSSA isolates*</th>
<th>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>In 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>In 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>infections (children)</td>
<td>51</td>
<td>20 (12)</td>
<td>0.931</td>
<td>0.681</td>
<td></td>
<td>ST88, ST121, ST398</td>
<td>4</td>
</tr>
<tr>
<td>UK</td>
<td>Intravenous drug user lesions</td>
<td>28</td>
<td>12 (11)</td>
<td>0.910</td>
<td>0.680</td>
<td></td>
<td>ST59, ST5, ST12, ST30, ST45</td>
<td>7</td>
</tr>
<tr>
<td>Mali</td>
<td>nasal carriage (patients)</td>
<td>88</td>
<td>20 (15)</td>
<td>0.858</td>
<td>0.522</td>
<td></td>
<td>ST15, ST152</td>
<td>9</td>
</tr>
<tr>
<td>Gabon</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>
<td></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>USA</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>
<td></td>
</tr>
<tr>
<td>UK</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>
<td></td>
</tr>
<tr>
<td>Chile</td>
<td>bulk milk</td>
<td>20</td>
<td>5 (3)</td>
<td>0.568</td>
<td>0.260</td>
<td>ST97</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>bulk milk</td>
<td>227</td>
<td>11 (6)</td>
<td>0.496</td>
<td>0.207</td>
<td>ST126, ST97</td>
<td>8</td>
<td></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>

* STs which accounted for not less than 10% of the population