A USA300 variant and other human-related methicillin-resistant Staphylococcus aureus strains infecting cats and dogs in France

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Objectives: To characterize methicillin-resistant Staphylococcus aureus (MRSA) clinical strains from cats and dogs in France, and to compare the clones identified with the distribution of French human MRSA.

Methods: Susceptibilities to antimicrobials were assessed by disc diffusion. Resistance and virulence genes were screened using a microarray-based assay. Isolates were additionally characterized by Smal macrorestriction analysis and spa typing.

Results: From 2006 to 2010, the proportion of MRSA infections in pets in France was low (1.8%), but most isolates (87.0%, 20/23) belonged to human clones. The most common clones were the Lyon clone (69.6%, 16/23), the livestock-associated CC398 (13.0%, 3/23) and the Geraldine clone (8.7%, 2/23). Interestingly, we report the first USA300 clone infecting a European dog, which was probably imported by a US patient.

Conclusions: Over a 5 year period, the proportion of MRSA infections in pets appears low (<2%) in France, but the distribution of the clones mostly mirrors the epidemiology of human invasive clones. These data highlight the role of pets as both victims and reservoirs of endemic, epidemic and/or invasive MRSA.

Keywords: animal infection, MRSA, antibiotic resistance epidemiology, microarrays

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) infections in humans are associated with hospital settings (hospital-acquired (HA-MRSA)), but also increasingly with non-clinical settings (community-associated (CA-MRSA) or livestock-associated (LA-MRSA)).1,2 In this respect, the ST398 clone has been repeatedly reported in food-producing animals, such as pigs, but also in humans that were in close contact with ST398-carrying animals. In pets, staphylococcal infections are largely due to Staphylococcus pseudintermedius. Only a few studies have focused on well-characterized MRSA and the data suggested either a human-to-animal transmission of clones corresponding to locally prevalent human MRSA or a direct transmission by an infected person.3–5 Here, we intended to estimate the proportion of MRSA infections in pets over a 5 year period in France and to extensively characterize the clones involved.

Materials and methods

Bacterial strains

From 2006 to 2010, antibiogram results of 1250 coagulase-positive staphylococci isolated from cats and dogs were collected through the Resapath network, which ensures the surveillance of antimicrobial resistance in diseased animals in France (www.resapath.anses.fr). All cefoxitin-intermediate or cefoxitin-resistant isolates were sent to the Anses laboratory in Lyon, where they were cultured on blood agar and confirmed as S. aureus by standard procedures and species-specific PCR. Twenty-three infectious isolates were identified as MRSA and further processed. MRSA were collected from cats (n=7) suffering from skin infections (n=2), otitis (n=2), urinary infections (n=2) and nasal inflammation (n=1), whereas dogs (n=16) suffered from skin infections (n=8), joint infections (n=2), otitis (n=1), vaginal infection (n=1) or nasal inflammation (n=1) (Table S1, available as Supplementary data at JAC Online). Three MRSA isolated from dogs were of unknown origin. Whenever available, epidemiological data were retrospectively gathered.
Susceptibility testing

Antimicrobial susceptibility was tested by the disc diffusion method according to the guidelines of the Anti-infectious Committee of the French Society for Microbiology (www.sfsm-microbiologie.fr). The following antimicrobials were tested: penicillin, cefoxitin, fusidic acid, enrofloxacin, lincomycin, erythromycin, spiramycin, pristinamycin, chloramphenicol, fleroxacin, tetracycline, vancomycin, teicoplanin, kanamycin, gentamicin and tobramycin. Bacteria were classified as susceptible, intermediate or resistant according to the approved clinical breakpoints. S. aureus ATCC 25923 was used as the quality control.

Molecular typing

The presence of the mecA gene was systematically confirmed and SCCmec typing was performed by PCR. All mecA-positive strains were characterized by a microarray-based assay (S. aureus genotyping; Identibac, Alere), allowing detection of virulence and resistance genes, and assignment to clones or clonal complexes (CCs). PFGE was performed on SmaI-digested DNA and band patterns were analysed using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium). The repeat region of the spa gene was amplified according to described methods and spa types were assigned with an online spa database (http://www.spaserver.ridom.de/). A CC398-specific PCR was performed on isolates assigned to ST398 on the basis of the DNA array.

Results and discussion

A total of 23 MRSA (1.8%) were recovered from unrelated cats (n=7) and dogs (n=16) originating from 13 French districts and from various infection sites (Table S1). Sixteen MRSA (69.6%; Table 1) were assigned to the Lyon clone (n=10) or related variants (n=6) on the basis of conserved features (agr allele 1, SCCmec type IV and spa type t008 or related (t024, t068, t622 or t1171)) and close PFGE profiles (>80% homology) (Figure 1). Strains displayed five different susceptibility patterns and six different enterotoxin profiles, a diversity already described within this clone, which is the most prevalent human HA-MRSA circulating in France.7 A direct link with hospital settings could be established for one dog (#21219), whose owner was a young healthcare worker.

Additionally, two unrelated isolates (8.7%) belonged to the Geraldine clone, a mixed CA-MRSA/HA-MRSA clone that has recently emerged in France, where it represents >7% of all invasive MRSA strains.7,8 The two isolates shared the typical features of this clone (agr 2, spa type t002, SCCmec type I-truncated and tst+) and close but non-identical (80% homology) PFGE patterns. Both presented a susceptibility profile classically reported for this clone (with resistances to kanamycin, tobramycin and fusidic acid)8 and one isolate presented an additional resistance to chloramphenicol conferred by the cat gene.

In addition to these typically French human clones, three isolates (13.0%) from the Manche (n=1, spa type t899) and Gard districts (n=2, spa types t899 and t458) were assigned to CC398 on the basis of DNA microarrays and CC398-specific PCR.6 CC398-associated infections have rarely been reported in pets so far9 and always with a clear link with pigs or livestock farms. Here, none of the dogs lived on a farm, although they originated from small cities surrounded by countryside. Moreover, two isolates harboured the immune evasion cluster type B (sak, cph and scn, but not sea, genes), which is considered a human virulence factor and has recently been described in French calves,10 whereas CC398 MRSA have never been reported yet in humans in France.

Lastly, two isolates were assigned to typically human clones that have never been or are rarely reported in France. The first one, isolated from a dog suffering from pyoderma, exhibited agr allele 1, SCCmec type IV, spa type t032, a large set of enterotoxin genes and resistances to methicillin and fluoroquinolones only (Table 1). It belonged to the Barnim clone.

Table 1. Resistance and virulence profiles of the 23 MRSA isolates

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>Phenotypic resistance</th>
<th>Resistance genes</th>
<th>Toxinsa</th>
<th>Assigned clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>PEN, FOX, ERY, SPI, LIN, KAN, TOB, ENR</td>
<td>mecA, blaZ, erm(A), aadD, qacA, fosB</td>
<td>sea</td>
<td>Lyon clone</td>
</tr>
<tr>
<td>3</td>
<td>PEN, FOX, KAN, TOB, ENR</td>
<td>mecA, blaZ, aadD, fosB</td>
<td>se-d, j</td>
<td>Lyon clone</td>
</tr>
<tr>
<td>2</td>
<td>PEN, FOX, SPI, LIN, KAN, TOB, ENR</td>
<td>mecA, erm(A), aadD, fosB</td>
<td>sea</td>
<td>Lyon clone</td>
</tr>
<tr>
<td>1</td>
<td>PEN, FOX, ERY, SPI, LIN, KAN, TOB, ENR</td>
<td>mecA, blaZ, erm(A), aadD, tet(K), fosB</td>
<td>se-a, d, j</td>
<td>Lyon clone</td>
</tr>
<tr>
<td>1</td>
<td>PEN, FOX, ENR</td>
<td>mecA, blaZ, fosB</td>
<td>se-d, j</td>
<td>Lyon clone</td>
</tr>
<tr>
<td>1</td>
<td>PEN, FOX, SPI, LIN, TET, KAN, TOB, ENR</td>
<td>mecA, blaZ, erm(A), aadD, fosB</td>
<td>se-a, d</td>
<td>Lyon clone</td>
</tr>
<tr>
<td>1</td>
<td>PEN, FOX, ERY, SPI, LIN, KAN, TOB, ENR</td>
<td>mecA, blaZ, erm(A), aadD, qacA, fosB</td>
<td>none</td>
<td>Lyon clone</td>
</tr>
<tr>
<td>1</td>
<td>PEN, FOX, KAN, TOB, CHL, ENR</td>
<td>mecA, blaZ, aadD, cat, fosB</td>
<td>se-a, d, j, r</td>
<td>Lyon clone</td>
</tr>
<tr>
<td>1</td>
<td>PEN, FOX, KAN, TOB, CHL, FUS</td>
<td>mecA, blaZ, aadD, fosB</td>
<td>tst, se-c, d, g, l, i, j, m, n, o, u</td>
<td>Geraldine clone</td>
</tr>
<tr>
<td>1</td>
<td>PEN, FOEx, CHL</td>
<td>mecA, blaZ, fosB</td>
<td>se-c, g, i, l, m, n, o, u</td>
<td>Barnim clone</td>
</tr>
<tr>
<td>2</td>
<td>PEN, FOX, TET</td>
<td>mecA, blaZ, vga(A), dfrA, tet(M)</td>
<td>none</td>
<td>CC398</td>
</tr>
<tr>
<td>1</td>
<td>PEN, FOX</td>
<td>mecA, blaZ, vga(A), dfrA</td>
<td>none</td>
<td>CC398</td>
</tr>
</tbody>
</table>

PEN, penicillin; FOX, cefoxitin; ERY, erythromycin; SPI, spiramycin; LIN, lincomycin; KAN, kanamycin; TOB, tobramycin; ENR, enrofloxacin; TET, tetracycline; CHL, chloramphenicol; FUS, fusidic acid.

aEnterotoxins (se-), toxic shock syndrome toxin (tst) and Panton–Valentine toxins (lukF-PV and lukS-PV).
which is a common clone in England and Germany, where it has been responsible for nosocomial infections in human and pets.\textsuperscript{3,4} In this case, the owners of the dog regularly hunted in the Schwarzwald region of Germany. This suggests that the isolate may have been imported after contact with an autochthonous huntsman or, even more likely, with another huntsman’s dog carrier.

The second isolate was Panton–Valentine leucocidin-positive and found in a dog that developed an infection after limb surgery, which was successfully treated with clindamycin. This ACME-negative isolate (where ACME stands for arginine catabolic mobile element) was considered a USA300 variant based on molecular characteristics (\textit{agr}\textsuperscript{3}, \textit{spa} type t008, SCC\textit{mec} type IV and the presence of \textit{lukF-PV}/\textit{lukS-PV}, sek and seq genes) and a PFGE pattern closely related (>80% homology) to the USA300-114 reference strain. Moreover, epidemiological data argue in favour of this hypothesis. Indeed, the owners never travelled outside France, but, at the time of surgery, the veterinarian in charge of the dog was hosting a family member who was recovering from recent peritonitis after a long-term hospitalization in the USA, where he was living. Considering that this clone is uncommon in France, but widely distributed in the USA, respectively, where the corresponding clones are endemic. Altogether, pets represent a reservoir of invasive human MRSA that should not be neglected, especially in cases of recurrent infections in humans, for which animals could be a source of contamination and/or recontamination. This specifically raises the question of the decontamination of colonized or infected pets in such a context. In line with other results suggesting the transmission of human MRSA to cattle,\textsuperscript{10} these data further highlight the contribution of human clones to the spread of resistance in animals.\textsuperscript{11} Finally, the increasing prevalence in pets of methicillin-resistant \textit{S. pseudintermedius},\textsuperscript{12} a species closely related to \textit{S. aureus}, might also be a source of genetic diversification of ‘human’ MRSA in terms of resistance genes and virulence factors, whose risk for human health should be documented.

Over a 5 year period, the proportion of severe MRSA infections in pets appears low (<2%) in France, but the distribution of the clones tightly mirrors the epidemiology of human HA-MRSA/CA-MRSA. Moreover, 65.0% (13/20) of the isolates suspected to be of human origin presented a truncated \textit{hlb} gene (Table S1), which is typical of human isolates. Nevertheless, clones that are unusual in France, such as the Barnim clone, were also identified. In particular, this study reports the first description of a clinical infection with a USA300 variant in a dog in Europe. In both cases, the strains were probably imported from Germany and the USA, respectively, where the corresponding clones are endemic. Altogether, pets represent a reservoir of invasive human MRSA that should not be neglected, especially in cases of recurrent infections in humans, for which animals could be a source of contamination and/or recontamination. This specifically raises the question of the decontamination of colonized or infected pets in such a context. In line with other results suggesting the transmission of human MRSA to cattle,\textsuperscript{10} these data further highlight the contribution of human clones to the spread of resistance in animals.\textsuperscript{11} Finally, the increasing prevalence in pets of methicillin-resistant \textit{S. pseudintermedius},\textsuperscript{12} a species closely related to \textit{S. aureus}, might also be a source of genetic diversification of ‘human’ MRSA in terms of resistance genes and virulence factors, whose risk for human health should be documented.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{SmaI PFGE patterns and cluster analysis for the 20 pet-derived human MRSA clones. Strain 26152 corresponds to the USA300-114 reference strain.}
\end{figure}

\begin{table}
\centering
\begin{tabular}{|l|l|l|}
\hline
Strain number & \textit{spa} type & Clone \\
\hline
24436 & t008 & Lyon clone \\
24817 & t008 & Lyon clone \\
24506 & t008 & Lyon clone \\
22037 & t008 & Lyon clone \\
24508 & t008 & Lyon clone \\
24614 & t622 & Lyon clone \\
24503 & t024 & Lyon clone \\
22047 & t008 & Lyon clone \\
24441 & t024 & Lyon clone \\
24440 & t008 & Lyon clone \\
15910 & t068 & Lyon clone \\
20479 & t1171 & Lyon clone \\
23783 & t008 & Lyon clone \\
24437 & t008 & Lyon clone \\
21219 & t1171 & Lyon clone \\
24502 & t008 & Lyon clone \\
24504 & t008 & USA300 variant \\
26152 & — & USA300 \\
20462 & t002 & Geraldine clone \\
23284 & t002 & Geraldine clone \\
24501 & t032 & Barnim clone \\
\hline
\end{tabular}
\end{table}
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Transparency declarations
None to declare.

Supplementary data
Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References