Genotypic and Phenotypic Characterization of *Staphylococcus aureus* Isolates from Wild Boars

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Eight *Staphylococcus aureus* isolates collected from 117 wild boars were characterized and compared to livestock isolates. They belonged to sequence types ST133, ST425, and the new type ST1643. The *spa* types were t1181, t6782, and the new types t6384, t6385, and t6386. Antimicrobial susceptibility testing and microarray-based genotyping confirmed the absence of important virulence/resistance genes.

*Staphylococcus aureus* is an abundant bacterium occurring as commensal flora of humans and various animal species (1). Beyond asymptomatic carriage, *S. aureus* is associated with a variety of diseases (2). Due to their ability to cause clinical conditions including life-threatening infections, staphylococci, especially methicillin-resistant *S. aureus* (MRSA), are deemed to comprise one of the most important nosocomial pathogens in humans and are considered a major public health concern (3, 4). More recently, their importance in veterinary medicine has also been described (5). Besides companion animals and horses (6), MRSA was found to colonize or infect important livestock species, including cows, pigs, and poultry (1, 7, 8). During recent years, a particular focus was laid on MRSA strains from domestic pigs, which belong predominantly to the clonal complex 398 (CC398) (9). These strains are highly prevalent among domestic pig herds and other livestock species in Europe and North America (10), and recent evidence implies their potential to cause infections in humans (7, 11, 12). Although some animal clones, like CC398, can colonize or infect multiple host species, modern typing techniques and genetic analyses of *S. aureus* populations have demonstrated the existence of several host-specific clonal lineages and imply an adaptive evolutionary host restriction (2, 7).

Nevertheless, studies determining the prevalence of *S. aureus* in wild game and game meat are rare (13, 14). Given the recent rise of CC398 MRSA in domestic pigs, it would be interesting to determine whether wild boars harbor methicillin-susceptible CC398 precursor strains or if they are already affected in the CC398 MRSA epidemic, too.

Nasal swabs were collected from 117 wild boars hunted in eight different regions across Germany during the years 2008 and 2009. Swabs were plated on Columbia blood agar (Oxoid, Wesel, Germany), Columbia blood agar with colistin and nalidixic acid (Heidph Dr. Müller, Eppelheim, Germany), and selective MRSA CHROMagar (Becton, Dickinson, Heidelberg, Germany). Colonies were identified using the SlideStaph Plus and API ID32 Staph systems (both from bioMérieux, Marcy-l’Étoile, France). In addition, an *S. aureus*-specific PCR assay targeting the *eap* gene was carried out as described previously (15). Among the 117 nasal swabs, 8 (6.8%) were positive for *S. aureus* originating in four federal states in Germany in 2008 and 2009 (Table 1). In a previous investigation, neither *S. aureus* nor MRSA strains were detected in

### TABLE 1 Characteristics and geographic origins of *S. aureus* isolates from wild boars

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Origin</th>
<th>Datea</th>
<th>PFGE type</th>
<th>MLST</th>
<th>spa type</th>
<th><em>agr</em> group</th>
<th>Resistance geneb</th>
<th>Capsule type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lower Saxony</td>
<td>10/16/2008</td>
<td>A</td>
<td>ST425</td>
<td>t6386</td>
<td>II</td>
<td>fosB</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Saarland</td>
<td>10/24/2008</td>
<td>B1</td>
<td>ST133</td>
<td>t1181</td>
<td>I</td>
<td>fosB</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Hesse</td>
<td>10/25/2008</td>
<td>B2</td>
<td>ST133</td>
<td>t6384</td>
<td>I</td>
<td>fosB</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>Saarland</td>
<td>11/08/2008</td>
<td>C</td>
<td>ST1643</td>
<td>t6385</td>
<td>II</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Saarland</td>
<td>11/08/2008</td>
<td>C</td>
<td>ST1643</td>
<td>t6385</td>
<td>II</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Lower Saxony</td>
<td>12/11/2008</td>
<td>A</td>
<td>ST425</td>
<td>t6386</td>
<td>II</td>
<td>fosB</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>Rhineland-Palatinate</td>
<td>12/14/2009</td>
<td>D</td>
<td>ST245</td>
<td>t6782</td>
<td>II</td>
<td>fosB</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>Rhineland-Palatinate</td>
<td>12/14/2009</td>
<td>B3</td>
<td>ST133</td>
<td>t1181</td>
<td>I</td>
<td>fosB</td>
<td>8</td>
</tr>
</tbody>
</table>

a Month/day/year.

b No correlation between the presence of the *fosB* gene and elevated MICs of fosfomycin could be detected.

c —, not detected.
nasal swabs from 120 wild boars (16). The small number of S. aureus strains isolated in the present study corroborates the rarity of S. aureus as a nasal colonizer of wild boars.

Macrorestriction analysis according to the Harmony protocol was performed to investigate the clonalities of the isolates (17). Four different macrorestriction patterns were detected, designated types A to D. Each type was represented by 1 to 3 isolates exhibiting indistinguishable or very similar fragment patterns (Table 1). Antimicrobial susceptibility testing by broth microdilution and Etest (bioMérieux) following Clinical and Laboratory Standards Institute (CLSI) recommendations (18, 19) revealed that the isolates were susceptible (or exhibited low MICs) to all 17 antibiotics/antibiotic combinations tested, including oxacillin. Thus, they differed from CC398 livestock isolates, which often are resistant to beta-lactams and tetracycline and which, in some cases, show additional resistance to macrolides, lincosamides and aminoglycosides (7, 9).

Microarray analysis was done using the StaphType kit (Alere Technologies, Jena, Germany) according to the manufacturer’s instructions (2). ST425 isolates were also tested using an experimental array that additionally harbored probes for mecC (20) and a SCCmec-XI-associated blaZ allele. Analyses confirmed the presence of S. aureus species markers. None of the isolates harbored virulence genes encoding staphylococcal enterotoxins, exfoliative toxins (eta, etb, and etd), the toxic shock syndrome toxin (tsst), epidermal cell differentiation inhibitors (edin-A, edin-B, and edin-C), genes encoding immune evasion components (sak, chp, and scn), or the Panton-Valentine leukocidin (lukF-PV and lukS-PV). Except for fosB (a putative fosfomycin/bleomycin resistance gene) (21), which was present in six isolates (Table 1), no antibiotic
resistance genes were detected. However, the fosB-carrying isolates exhibited MICs of ≤4 mg/liter fosfomycin, indicating a susceptible phenotype. PCR experiments with the primers fosB-fw (5′-CTTTACTGACCTGTAGGT-3′) and fosB-rv (5′-TAAATCT GTTCTCAAGTTGTC-3′) (61 bp) and subsequent sequencing of the amplicons confirmed the presence of fosB. Nevertheless, the mechanism responsible for the functional inactivity of fosB remains to be clarified.

To further characterize the isolates, multilocus sequence typing (MLST) and spa typing were performed (22, 23). New types were assigned by the spa and MLST database curators, respectively. Three MLSTs, ST133, ST425, and the novel type ST1643, were detected (Table 1). The relatedness to other MLSTs from animals and humans is shown in Fig. 1. Two of the ST133 isolates belonged to spa type t1181, whereas the remaining ST133 isolate belonged to the novel type t6384. ST133 appears to be an ungulate-animal-specific genotype largely without association with humans (24). Among ST425 isolates, the spa types 8782 and novel type 6386 were detected (Table 1). ST425 is a well-known animal-associated lineage, and MRSA isolates of this sequence type originating with bovine milk samples and humans were recently found to carry a mecA homologue, the mecC gene (25, 26). The mecC gene was absent from all three boar isolates from this study. In contrast, ST1643 was so far detected only in wild boars. A single S. aureus ST1643 strain, associated with a skin infection of wild boar, was isolated about 40 years ago in Germany (http://saureus.mlst.net/sql/burstspadvanced.asp; identification no. 3286). The allelic profile for this isolate was previously assigned as ST856 and was amended due to a change in the trim length of the gene gmk, which is used for MLST analysis (http://saureus.beta.mlst.net/trim.html). Both ST1643 isolates detected during this study carried the novel spa type t6385.

None of the wild boars carried MRSA CC398, which is widely distributed among industrially raised pigs and which can be spread from pig farms into the environment (27, 28). Nevertheless, the low concentrations of MRSA detected in the vicinity of pig farms and the absence of antibiotic selective pressure are two factors that might reduce the probability of transmission. A similar observation was made by Cuny et al. (2012), who could not detect strains that might reduce the probability of transmission. A similar occurrence is also seen in pigs reared in an alternative system. Appl. Environ. Microbiol. 2007. High prevalence of methicillin resistant Staphylococcus aureus in pigs. Vet. Microbiol. 122:366–372.

In conclusion, S. aureus seems to be a rare nasal colonizer of wild boars, and isolates differ distinctly in their genotypes and resistance phenotypes from common livestock isolates. Apparently, given the small sample size, CC398 MRSA appears not to be abundant yet among wild boars in Germany, but further studies are required to confirm this observation and to observe possible future developments.

ACKNOWLEDGMENTS

We thank Regina Tegeler for helpful discussions and Inna Pahl and Vera Nöding for excellent technical assistance. S.M. and R.E. are employees of Alere Technologies. This had no influence on the study design, analysis, or interpretation of the data.

REFERENCES


