Small Colony Variant of Methicillin-Resistant *Staphylococcus pseudintermedius* ST71 Presenting as a Sticky Phenotype

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We first observed the phenomenon of small colony variants (SCVs) in a *Staphylococcus pseudintermedius* sequence type 71 (ST71) strain, isolated from a non-pet owner. Although we found that small-sized colonies share main features with *Staphylococcus aureus* SCVs, they nevertheless show a novel, particular, and sticky phenotype, whose expression was extremely stable, even after subcultivation.

Improvements in the identification and classification of bacteria have recently shed light on coagulase-positive staphylococci (CoPS) other than *Staphylococcus aureus*. Among these organisms, *Staphylococcus pseudintermedius*, although primarily affecting dogs and cats, has also occasionally been recognized as a human pathogen. Most worrying is the emergence of human infections by methicillin-resistant *S. pseudintermedius* (MRSP) strains (1, 2). However, the interpretative criteria for methicillin resistance in this species are available only in the Clinical and Laboratory Standards Institute (CLSI) guidelines for bacterial pathogens from animals, which do not recommend cefoxitin as a surrogate antibiotic in the prediction of mecA-mediated methicillin resistance. Instead, oxacillin breakpoints have been defined (3). Since cefoxitin is an indicator for methicillin resistance. Instead, oxacillin breakpoints have been defined.

Nasal swabs were performed as a pre-bone marrow transplantation screening in a leukemic female patient with no rhinosinusitis. CoPS colonies (the coagulase test was positive at 4 h of incubation) with double-zone hemolysis on Trypticase soy agar containing 5% sheep blood (Liofilchem, Italy) were massively grown in pure culture as the putative colonizers of both nasal cavities (isolates from the left and right nostrils were named S84 and S86, respectively), and showed mannitol fermentation on mannitol salt agar (Liofilchem) after 24 h of incubation. The Vitek 2 (bioMérieux, France) identified them as *Staphylococcus interme-
dius*, and a specific multiplex PCR based on the analysis of the *mec* gene (10), as well as the restriction fragment length polymorphism analysis (11) placed them within the *S. pseudintermedius* species. It is noteworthy that, although *S. pseudintermedius* nasal colonization in humans is mainly associated with pet owners and veterinarians (12), the patient, even though living in a country environment, did not have any companion or farm animals.

Multilocus sequence typing (MLST) (13) revealed that strains S84 and S86 belong to sequence type 71 (ST71), the most common MRSP clone spreading in Europe in dogs and cats, which has also been associated with infections in humans (2, 14).

A broth microdilution method performed with the Sensititre plates NLEUST1 and EUST (TREK Diagnostic Systems, United Kingdom) revealed that the isolates shared the same multidrug resistance profile (Table 1), and *mec* gene detection, carried out by PCR as described previously (15), allowed us to label both as MRSP.

An agar disc test with cefoxitin provided a 31-mm IZD for both strains; after 48 h of incubation, however, microcolonies were observed within the S84 inhibition zone. This novel isolate...
TABLE 1 MICs of methicillin-resistant S. pseudintermedius ST71 strains S84, S86, and the SCV strain S85 as determined by broth microdilution method

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Measured conc ranges (µg/ml)</th>
<th>Resistance breakpoints (µg/ml)</th>
<th>MICs (µg/ml) and R and S profiles for strain:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S84</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>0.12–2</td>
<td>≥0.25</td>
<td>R, &gt;2</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>0.5–16</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>0.25–8</td>
<td>≥0.5</td>
<td>R, &gt;8</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.25–8</td>
<td>≥4</td>
<td>R, &gt;8</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.25–8</td>
<td>≥8</td>
<td>R, &gt;8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1–16</td>
<td>≥16</td>
<td>R, &gt;16</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.5–16</td>
<td>≥16</td>
<td>R, &gt;16</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.12–4</td>
<td>≥4</td>
<td>R, &gt;4</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>2–32</td>
<td>≥16</td>
<td>R, &gt;32</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>64–512</td>
<td>≥512</td>
<td>R, &gt;512</td>
</tr>
<tr>
<td>Mupirocin</td>
<td>0.5–256</td>
<td>≥256f</td>
<td>S, ≤0.5</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>4–64</td>
<td>≥32</td>
<td>S, 8</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>0.5–4</td>
<td>NA</td>
<td>≤0.5</td>
</tr>
<tr>
<td>Rifampin</td>
<td>0.016–0.5</td>
<td>≥4</td>
<td>S, ≤0.016</td>
</tr>
<tr>
<td>Linezolid</td>
<td>1–8</td>
<td>≥8</td>
<td>S, ≤1</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1–16</td>
<td>≥32</td>
<td>S, ≤1</td>
</tr>
</tbody>
</table>

* MICs were interpreted according to CLSI document M100-S23 (18); as an exception, the oxacillin breakpoints defined in the CLSI supplement VET01-S2 (3) for bacteria from animals were used.
* R, resistance; S, susceptibility.
* NA, no breakpoints available for cefoxitin screen and fusidic acid.
* f Phenotypically expressed resistance displayed by the SCV only, not by the parent strain.
* Breakpoint relates to nasal decolonization of S. aureus (18).

(named S85) showed poor and slow growth, with much smaller colonies (<0.5 mm) than the mother culture (1 and 2 mm) (Fig. 1) after subcultivation on sheep blood for 24 h at 37°C. S85 colonies were manniitol negative at 24 h, coagulase negative at 4 h (still, after 24 h of incubation, the isolate only weakly coagulated rabbit plasma), rubbery, adherent, confluent, and sticky (similar to colonies formed by Rothia mucilaginosa). S85 was identified as a mecA-positive S. pseudintermedius ST71 using the above-mentioned molecular methods and surprisingly displayed high-level resistance to cefoxitin, with an MIC of >16 µg/ml (Table 1) and a 12-mm IZD.

The three isolates had a unique molecular fingerprint if analyzed with pulsed-field gel electrophoresis (PFGE), semiautomated repetitive element palindromic PCR (rep-PCR) (DiversiLab; bioMérieux), and random amplified polymorphic DNA (RAPD), using previously described primers (NP2 being the most discriminant) (16) (Fig. 2), confirming that S85 is an SCV derived from the parental strain S84 after prolonged cefoxitin exposure.

After 48 h of incubation, SCV colonies reached the size of the parent strain, and they were less sticky and began to ferment mannitol. However, when recultivated from the 48-h-incubated plates, they started growing as an SCV again.

This first observation of an SCV in S. pseudintermedius is intriguing, although to date, it is impossible to know whether these variants play a part in the persistence of human and animal...
colonization and infections. Nevertheless, it is known that SCVs in Staphylococcus aureus have an increased ability to survive within nonprofessional phagocytes that then behave as reservoirs for recurrent and chronic diseases and reduce exposure to certain antibiotics (6, 7, 17).

Similarly to S. aureus SCVs, S85 showed decreased colony size and reduced coagulase activity. Unlike SCVs formed by S. aureus, however, those produced by S. pseudintermedius had an unchanged hemolysin pattern: both S84 and S85 formed internal completely hemolytic and external incompletely hemolytic zones (Fig. 1) whose diameters were, however, proportional to the colony size after 24 h of incubation. The SCV isolate S85 did not revert to the large-colony size, and the small phenotype was maintained after subcultivations and upon defrosting from −80°C storage. It is well known, conversely, that SCVs in S. aureus usually revert after discontinuation of the hostile conditions (6, 7, 17).

Unlike most S. pseudintermedius isolates, S85 was resistant to both cefoxitin and oxacillin and showed delayed mannitol fermentation and coagulase activity (Table 1); again, the most impressive feature was that the SCV colonies looked like R. mucilaginosa, being confluent, attached each other, filamentous, and strongly adherent to the agar.

This report suggests that similarly to those formed by S. aureus, S. pseudintermedius SCVs may represent a mechanism for survival under hostile conditions. Although both S. aureus and S. pseudintermedius small-sized variants display slower growth and decreased antibiotic susceptibility, lack of reversibility along with the sticky, confluent phenotype are the main differences between the SCVs of these two CoPS species.

There is still poor understanding of S. pseudintermedius colonization and infections in humans, as well as of the clinical impact of bacterial SCVs; nonetheless, a small-sized phenotype appeared to be an additional previously undescribed feature in this organism that may enable it to survive host defenses and antibiotic treatment. The strong reduction in the growth rate makes it difficult to detect SCVs within a mixed population in routine clinical practice. Hence, an underestimation of antibiotic resistance and subsequent drug clinical failure may unfortunately occur (7). Further studies will be necessary to better understand the role of SCVs in S. pseudintermedius host adaptation and pathogenicity in both humans and animals.

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