Colonization with Multiple Staphylococcus aureus Strains among Patients in European Intensive Care Units

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Colonization with Multiple *Staphylococcus aureus* Strains among Patients in European Intensive Care Units

Patients and healthy individuals intermittently and inconsistently carry different methicillin-resistant *Staphylococcus aureus* (MRSA) subtypes. In the present study, we assessed the clonality of methicillin-susceptible *S. aureus* (MSSA) and MRSA strains in patients admitted to 1 of 6 intensive care units (ICUs), using *spa* typing and multilocus variable number of tandem repeats analysis (MLVA). The findings published here are additions to those of our previously published study, to which we refer for further information on patients, ICUs, and genetic characteristics of isolated *S. aureus* strains.

The study was undertaken in 6 European university and teaching hospitals during a period of 3 months per center. Nasal swab specimens were obtained from all patients within 48 h after admission and twice per week thereafter. Swabs were cultured for 24 h at 37°C in 4 mL of trypsin soy broth with a 5-mg/L concentration of aztreonam, plated on manitol salt agar and blood agar plates, and incubated for 48 h at 37°C.

All *S. aureus* strains were examined by a triplex polymerase chain reaction for 16S rRNA, *nuc*, and *mecA*. DNA was isolated using a Nucleospin tissue kit (Machinery-Nagel) according to manufacturer’s protocol. All *S. aureus* isolates were genotyped. MLVA was performed by polymerase chain reaction of 6 variable number tandem repeats, shown previously to be at least as discriminatory as pulsed-field gel electrophoresis for typing of *S. aureus*. Spa typing was performed using 5′-ACGAGTAGTGCCCTTTGCTT and 3′-GCTCAAGCACCAAAAGAGGA primers (Invitrogen) or 5′-TAAAGACGATCCCTCCGGTGAGC and 3′-CAGCAGTAGTGCCCTTTGCTTGT primers (Isogen Life Sciences). DNA sequencing was performed using the chain termination method (Baseclear BV). Bionumerics (Applied Maths) was used to analyze obtained sequences and assign *spa* types. Novel *spa* types were submitted online to the Ridom database (available at: http://www.SpaServer.ridom.de).

Isolates were considered to be unique *S. aureus* strains if MLVA detected a difference in more than 2 loci and/or if they had a different *spa* type. Isolates with an identical or closely related MLVA type (ie, if they differed by no more than 1 locus) and an identical *spa* type were considered to be identical. Isolates containing a double-locus difference in MLVA type and/or an identical or closely related *spa* type were considered to be closely related. In case a strain was not typeable by *spa* typing, only MLVA findings were assessed to determine relatedness.

Of 629 patients hospitalized in a participating ICU, 371 (52%) had more than 1 culture taken. A total of 224 (36%) had 1 or more *S. aureus*-positive cultures during their ICU stay, and 49 (8%) were carriers of MRSA (Table). In total, 952 swab specimens were collected from the 224 *S. aureus* carriers, of which 425 (45%) were positive for *S. aureus*.

In these isolates, 119 *spa* types were found. Ten isolates were not typeable by *spa* typing. All strains were typeable by MLVA typing. In total, 237 MLVA types were found.

Of the 224 patients with at least 1 *S. aureus*-positive culture, 87 had multiple swab specimens that were positive for *S. aureus*, and 137 patients had only 1 *S. aureus*-positive swab specimen. Thirty-five (6%) of 629 ICU patients carried either 1 strain or at least 2 genetically closely related isolates, and 35 (6%) carried 2 genetically distinct strains. For 2 patients with 2 genetically distinct strains, it is possible that cross-

### Table. Carriage of Multiple *Staphylococcus aureus* Strains Among Intensive Care Unit (ICU) Patients

<table>
<thead>
<tr>
<th>Center</th>
<th>Proportion (%) of patients carrying <em>S. aureus</em></th>
<th>No. of strains in patients with multiple <em>S. aureus</em>-positive cultures&lt;sup&gt;a&lt;/sup&gt;</th>
<th>1 strain</th>
<th>2 closely related strains</th>
<th>2 distinct strains</th>
<th>&gt;2 distinct strains</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57/136 (42)</td>
<td></td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>47/130 (36)</td>
<td></td>
<td>9</td>
<td>0</td>
<td>14</td>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>52/115 (45)</td>
<td></td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>51/151 (34)</td>
<td></td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>12/64 (19)</td>
<td></td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>5/33 (15)</td>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Overall</td>
<td>224/629 (36)</td>
<td></td>
<td>26/629 (4)</td>
<td>9/629 (1)</td>
<td>35/629 (6)</td>
<td>17/629 (3)</td>
<td>87/629 (14)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are number of patients with at least 1 *S. aureus*-positive culture / total number of patients (%).

<sup>b</sup> Data are number of patients with the specified number of strains, or number of patients with the specified number of strains / total number of patients (%).
transmission occurred, because a patient carrying an S. aureus strain that was genetically identical to 1 strain in each patient was admitted to the ICU during the 2-week period before the patients acquired S. aureus. The remaining 17 patients (3%) carried more than 2 strains during their ICU stay. Carriage in 5 of these 17 patients possibly involved cross-transmission. No patients carried both closely related isolates and different strains. For 7 patients (1%), MSSA isolates were detected on one day, and MRSA isolates (with different spa and MLVA types) were detected on another.

It has long been believed that patients with S. aureus carriage predominantly carry 1 distinct S. aureus strain. A number of contemporary studies have shown clonal diversity during S. aureus colonization. Vandenbergh et al studied persistent carriership among patients and concluded that only a fraction will carry a genetically identical strain for several years. Simultaneous carriage of clonally distinct MSSA and MRSA strains was shown in a subgroup of (previously) hospitalized persons. Lim et al reported that ICU patients carry clonally diverse MRSA strains during their ICU stay.

In total, 52 patients (8%) patients carried 2 or more distinct S. aureus strains during their ICU stay. This is comparable with the results reported by Maslow et al. Seven patients (1%) in our study carried both MRSA and MSSA during their ICU stay. The question is whether these different strains are endogenous or acquired isolates. Acquisition of an exogenous isolate (by patient-to-patient cross-transmission) was possibly the case in 7 of 52 patients from this study who carried multiple strains. In 5 of these cases, the patient was a carrier of 3 or more distinct S. aureus strains over time. In the remaining 45 cases, cross-transmission could not be shown. Because acquisition of S. aureus in the ICU is related to treatment with antibiotics, which are often receiving by a high proportion of patients in the ICU, and to poor health status,10 an exogenous source would be conceivable. However, our results do not strongly support this hypothesis. In the case of endogenous strains, the question is whether the strains are simultaneously and, thus, polyclonally colonizing the nasal tissue. Unfortunately, we did not analyze multiple colonies from S. aureus–positive swabs to ascertain polyclonal carriage.

In the present study, only 45% of swab specimens obtained from colonized patients were positive for S. aureus. This finding has 2 possible explanations. First, patients could lose nasal colonization and be recolonized during the same ICU stay. Second, the method used for determining nasal colonization status may lack analytical sensitivity. Lim et al reported a low concordance between cultures of 2 nasal swab specimens collected on the same day: in almost 60% of these cultures, one culture was negative for S. aureus and the other positive for S. aureus.

The clinical implications of our findings are apparent. Patients receiving treatment for S. aureus infection could become reinfected with another S. aureus strain, which the patient could be carrying simultaneously. In addition, the possible lack of sensitivity of culture of nasal swab specimens may result in an underestimation of MRSA colonization. This could lead to increased spread of MRSA and to an increased number of nosocomial infections.

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REFERENCES

