Analysis of Morphologically Similar *Staphylococcus aureus* Colonies to Assess Phenotypic and Genotypic Correlation

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**Abstract**

**Background:** When performing surveillance cultures from non-airline sites for *Staphylococcus aureus* (S. aureus), a single bacterial isolate is selected as representative of the cultured strain. Standard practice assumes that morphology and other colonies are the same and that these are phenotypically and genotypically identical. The objective of this study was to determine how frequently colonies with the same morphology are phenotypically and genotypically diverse.

**Methods:** As part of a larger study examining parent to child transmission of *S. aureus*, swabs were collected and cultured from multiple body sites. Cultures were inoculated into tryptic soy broth with 6% NaCl (BD Diagnostics, Sparks, MD), incubated overnight and subcultured onto 5% sheep blood agar (BD, Sparks, MD), and chromogenic media 204A (BioFire, Hercules, CA). For positive cultures, a set of five separate isolates for each unique colony morphology was characterized using pulsed-field gel electrophoresis (PFGE). Additionally, the antimicrobial susceptibility (AST) profile and identification of each isolate was determined using the Phoenix PMIC ID-105 panel (BD).

**Results:** Fourteen neonate and adult participants had positive *S. aureus* cultures that yielded 100 isolates (2 isolates tested for each of the 41 obtained morphologies). Of the 205 isolates, 204 (99.5%) had the same PFGE pattern as other isolates recovered from the same group of patients. One isolate had a one band difference from the others in its group. AST testing showed that 19 of the 204 isolates tested had the same antibiotic profile and the antimicrobial susceptibility testing of each isolate was determined using the Phoenix PMIC ID-105 panel (BD).

**Conclusions:** A majority of the isolates tested had the same antibiotic profile. Further study is needed to determine if there are additional factors that may contribute to the variability of the isolates tested.

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**Materials and Methods**

- 4 samples were collected from each neonate: Nasal, Umbilicus, Groin, Pen-anal
- 4 samples were collected from each parent: Nares, Throat, Groin, Pen-anal
- 10 μL of each sample were aliquoted into Tryptic Soy Broth and incubated for 16-24 h
- 10 μL of each sample were aliquoted onto a SAselSt™ chromogenic media plate and incubated at 37°C for 16-24 h
- 100 μL of each sample were aliquoted onto Trypic Soy Broth and then plated onto SAselSt™ chromogenic media and sheep blood agar and incubated at 37°C for 16-24 h
- 10 μL of each sample were aliquoted onto sheep blood agar and then incubated at 37°C (SAselSt™, 16-24 h; SBA, 24-48 h)
- 5 separate colonies/ morphotype body side were subcultured to SBA then frozen

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**FIGURE 1: Dendrogram of Isolates From Five Different Subjects**

**FIGURE 2: Isolates on SAselSt™ and Sheep Blood Agar Plates**

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**TABLE 1: Selected AST Data**

<table>
<thead>
<tr>
<th>Antibiotics Tested</th>
<th>Categorical Agreement (%)</th>
<th>Essential Agreement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>36/36* (100)</td>
<td>36/36* (95)</td>
</tr>
<tr>
<td>Cefadroxil</td>
<td>41/41 (100)</td>
<td>41/41 (100)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>37/41 (90)</td>
<td>36/40* (90)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>41/41 (100)</td>
<td>40/41 (98)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>41/41 (100)</td>
<td>40/41 (100)</td>
</tr>
</tbody>
</table>

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**Results (cont.)**

- A combination of fourteen neonates and adult participants had positive *S. aureus* cultures that yielded 100 isolates (2 isolates from each participant for each morphology).
- 99.5% (204 of 205) of the isolates had identical PFGE patterns as the other isolates from the same subject.
- One isolate in one group had a one band difference from the other 4 isolates. These are considered epidemiologically significant.
- AST testing indicated that 95% (19 of 20) of the isolates tested had the same antimicrobial profile.
- The 5% difference noted in the proportion of morphotypes with exact agreement is (0.3, 1.1) using an exact Binomial Confidence Interval.

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**Conclusions**

- All morphologically similar colonies from a single *S. aureus* culture can be considered genetically identical, as determined by PFGE.
- For the purposes of this study, 2 isolates from each of the 41 observed morphotypes yielded categorical agreement of S-R except for Erythromycin.
- AST profiles are not discriminating enough to assess genetic differences or relatedness. PFGE was shown to be the more reproducible method.
- Standard practice of using one isolate from a culture as a representative sample is inappropriate for epidemiological studies of patients with *S. aureus* colonization.