Background: Staphylococcus aureus (SA) is a major cause of healthcare-associated bloodstream infections. Although molecular techniques can detect SA resistance mechanisms, genetic detection may not consistently correlate with phenotypic expression. This study evaluated automated microscopy for the rapid detection of clinically important SA resistance phenotypes directly from positive blood cultures.

Methods: A total of 156 strains of previously isolated clinical SA isolates with known resistance mechanisms were tested. Automated microscopy (Accurate Imaging, Tucson, AZ USA) to take time-lapse dark-field images of each flowcell every 10 min for 4.5 h. The automated microscope scanned 14 fields of view in each flowcell (~20-24 h). To reduce debris, culture aliquots were processed by automated gel electrofiltration, where 200 µL of inoculum were run on a 0.5% agarose gel for 20 min. The gel was recovered from the gel well and diluted with L-DOPA buffer to lyse bacterial cells in the presence of antibiotics into a minimum inhibitory concentration (MIC) value or a positive/negative result. Microscopy results were compared to results from standard CLSI based broth microdilution (MD), disk diffusion (D), and E-test (E) susceptibility testing methods.

Results: The microscopy method was evaluated on 56 resistant SA isolates. The microscopy method exhibited sensitivity of 100% and specificity of 100% for all resistance mechanisms tested. The microscopy method showed sensitivity and specificity of 100% for VRSA, 95% for VSSA, 100% for MRSA (MIC=16) and MSSA (MIC=4) detection. The microscopy method showed sensitivity of 100% and specificity of 100% for MLSb detection.

Conclusion: Automated microscopy for rapid SA resistance detection is a promising alternative to molecular techniques, and results could potentially be used for earlier targeted treatment of SA bloodstream infections.

Staphylococcus aureus (SA) is a major cause of healthcare-associated bloodstream infections. Although molecular techniques can detect SA resistance mechanisms, genetic detection may not consistently correlate with phenotypic expression. Current methods for detecting resistance phenotypes are not capable of providing results immediately. The potential exists for automated microscopy to provide rapid antimicrobial susceptibility testing.

Methods: In this study, 156 clinical SA isolates were tested for known resistance mechanisms. These isolates were divided into two separate groups: group 1 and group 2. Group 1 strains were tested for VRSA, VSSA, MRSA (mecA/mecC), and D-tests (MLSb). Group 2 strains were tested for VRSA, VSSA, MRSA (mecA/mecC), and MSSA (MIC≤1 mg/L), vancomycin-resistant SA (VRSA=12, VSSA=43), methicillin-resistant SA (MRSA), and D-tests (MLSb).

Results: Each strain was tested in triplicate with a final inoculum of 5 x 10^5 to 5 x 10^6 CFU/mL. Sample collection was performed by a spinning robot using automated gel electrofiltration, where 200 µL of inoculum were run on a 0.5% agarose gel for 20 min. Sample was recovered from the gel well and diluted with L-DOPA buffer to lyse bacterial cells in the presence of antibiotics into a minimum inhibitory concentration (MIC) value or a positive/negative result. Microscopy results were compared to results from standard CLSI based broth microdilution (MD), disk diffusion (D), and E-test (E) susceptibility testing methods.

Conclusions: Automated microscopy for rapid SA resistance detection is a promising alternative to molecular techniques, and results could potentially be used for earlier targeted treatment of SA bloodstream infections.

**RESULTS**

**Table 1: Sensitivity and specificity of S. aureus resistance mechanisms.

<table>
<thead>
<tr>
<th>Resistance mechanism</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>VRSA</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>VSSA</td>
<td>95%</td>
<td>100%</td>
</tr>
<tr>
<td>MRSA</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>MSSA</td>
<td>100%</td>
<td>100%</td>
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</table>

**Figure 1:** Process flow including simulated blood culture preparation, sample collection by automated gel electrofiltration, automated transfer to the cassette for bacterial immobilization by electrokinetic concentration, automated imaging, and automated data analysis.

**Figure 2:** Clones of one Bacterial in 32-channel Brachial cassette.

**Figure 3:** A representative image of VRSA and MRSA resistance directly from patient blood cultures, highlighting the use of automated microscopy for rapid detection of clinically important SA resistance phenotypes.

**Figure 4:** Growth curves for individual representative clones of VRSA (MIC=16) and MSSA (MIC=4) for MLSb detection. Susceptibility pattern profiles for representative VRSA and MRSA isolates are shown. Disk diffusion results for VRSA and MRSA isolates are shown.

**Figure 5:** Sensitivity and specificity of S. aureus resistance mechanisms.