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Rapid Detection of Clinically Important *Staphylococcus aureus* Resistance Phenotypes Directly from Positive Blood Cultures Using Automated Microscopy

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AMENDED ABSTRACT

Background: *Staphylococcus aureus* (SA) is one of the major causes 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: 156 strains of previously characterized clinical SA isolates with known resistance mechanisms: vancomycin-resistant SA (VRSA=12, VSSA=43), methicillin-resistant SA (MRSA=24, MSSA=22), macrolide-lincosamide-streptogramin-B resistance (MLSb pos=14, neg=25), and *mecC* MRSA (n=16) were tested. Aliquots of BD BACTEC Standard Aerobic media containing healthy donor blood were seeded with 10-100 bacterial cells and incubated ~20-24 h. To reduce debris, culture aliquots were processed by automated gel electrofiltration (20 min), then pipetted into independent flowcells of a disposable multichannel cassette for bacterial immobilization. Bacteria were challenged with single-concentration antibiotic solutions targeting the resistance phenotypes evaluated. Automated microscopy captured time-lapse images, and a software algorithm converted the growth or inhibition of individual bacterial cells in the presence of antibiotics into a minimum inhibitory concentration (MIC) or a phenotype positive/negative result. Microscopy results were compared to results from standard CLSI frozen broth micro-dilution (BMD), cefoxitin disk diffusion (*mecA/mecC*-mediated resistance, MRSA), and D-tests (MLSb).

Results: The microscopy method time to result was 5 h. Resistance mechanisms of previously characterized isolates were concordant with test results. Table 1 lists sensitivity and specificity of the test for each resistance mechanism. All 16 *mecC* isolates were detected as resistant by microscopy (100% agreement).

Conclusion: Results support the feasibility of automated microscopy for rapid SA resistance phenotyping directly from positive blood cultures within 5 h. The microscopy system offers a promising alternative to molecular techniques, and results could potentially be used to target treatment of SA bloodstream infections in a timely manner.

INTRODUCTION

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 phenotypic antimicrobial susceptibility testing (AST) typically require 2-4 days to achieve a result. Delays to appropriate therapy can increase the risk of mortality for sepsis patients with bloodstream infections. Automated microscopy has the potential to reduce the turnaround time to phenotypic AST results by directly analyzing live bacterial cells and excluding the overnight culturing step. This study evaluated the speed and accuracy of automated microscopy for the detection of clinically important SA resistance phenotypes directly from positive blood cultures.

METHODS

A total of 156 strains of clinical SA isolates with known resistance mechanisms were tested.

Figure 1 summarizes the process flow for testing. Simulated blood culture aliquots were diluted to produce an inoculum containing approximately 1 x 10^6 CFU/mL in lysis buffer. Sample cleanup was performed by a pipetting 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 a final inoculum of 5 x 10^5 to 5 x 10^6 CFU/mL.

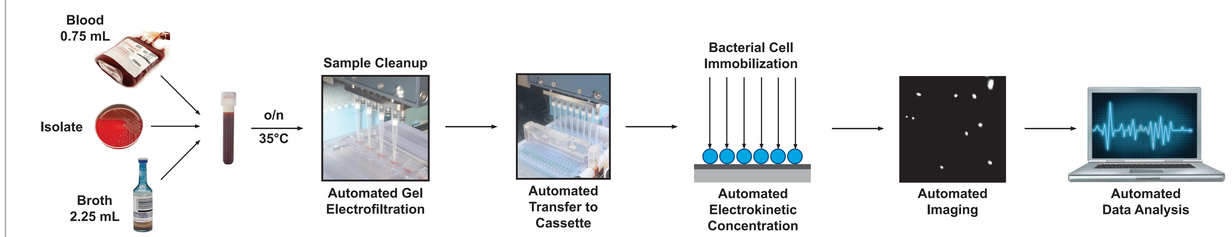

Figure 1: *Process flow including simulated blood culture preparation, sample cleanup by automated gel electrofiltration, automated transfer to the cassette for bacterial immobilization by electrokinetic concentration, automated imaging, and automated data analysis.*

Sample aliquots (20 µL) were pipetted into individual flowcell channels of a 32-channel disposable cassette. A 5-min low-voltage electric field immobilized bacteria on a poly-cationic coating on the lower surface of each flowcell. Immobilized bacterial cells were washed with cation-adjusted Mueller Hinton broth (CAMHB, BD), then challenged with pre-selected single concentration solutions of antibiotic prepared in CAMHB with 0.85% agar targeting the resistance mechanisms evaluated. Automated microscopy used a custom engineering prototype (Accelerate Diagnostics, Inc., 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 channel (Figure 2).

Image analysis software analyzed the growth rates of individual bacterial clones and a computer algorithm converted the data into an MIC value or a positive/negative result. Broth Micro-Dilution (BMD) per CLSI M07 and M100, cefoxitin disk diffusion (*mecA/mecC*-mediated resistance, MRSA), and D-tests (MLSb) were performed as references using overnight plated pure culture colonies, and results were compared to microscopy.

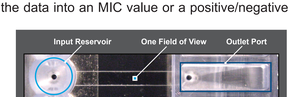

Figure 2: *Closeup of one flowcell in 32-channel flowcell cassette.*

RESULTS

Table 1: *Sensitivity and specificity by S. aureus resistance mechanism.*

Resistance Mechanism	Sensitivity	Specificity
VRSA	12/12 (100%)	41/43 (95%)
MRSA	24/24 (100%)	22/22 (100%)
MLSb	13/14 (93%)	24/25 (96%)

The microscopy method time to result was 5 h. Table 1 lists the sensitivity and specificity of the test for each resistance mechanism. Because genetic methods detect the presence of the *mecA* gene, the *mecC* mutant is miscategorized as MSSA by current commercial *mecA* detection methods, but all 16 *mecC* isolates were correctly detected as MRSA by the phenotypic microscopy method. Two VSSA strains were identified as VRSA by microscopy (false positives), and the MLSb test had one false negative and one false positive. Examples of time-lapse image data and susceptibility pattern profiles are shown in Figures 3 and 4, respectively.

CONCLUSION

Results support the feasibility of automated microscopy for rapid SA resistance phenotyping directly from positive blood cultures within 5 h. The microscopy system offers a promising alternative to molecular techniques, providing results that could potentially be used for earlier targeted treatment of SA bloodstream infections.

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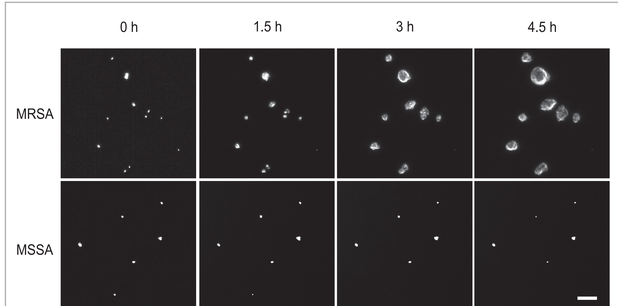

Figure 3: *Time-lapse images of MRSA and MSSA isolates growing in cefoxitin at 0, 1.5, 3 and 4.5 h. Resistant clones continue to grow while susceptible clones arrest or lyse. Images zoomed in to view individual bacterial clones. Scale bar in the lower right image is 20 µm.*

Figure 4: *Representative S. aureus bacterial cell susceptibility pattern profiles for each resistance mechanism. Each grey line represents a growth curve for an individual growing bacterial clone. Each solid red line is the average of the data, and the dotted red lines represent two standard deviations from the average. Bacterial growth in vancomycin was used for VRSA detection. Susceptibility pattern profiles for representative a) VRSA (MIC=16) and b) VSSA (MIC=0.5) isolates are shown. Bacterial growth in cefoxitin was used for MRSA detection. Susceptibility pattern profiles for representative c) MRSA (MIC=16) and MSSA (MIC=4) isolates are shown. Finally, a mixture of erythromycin and clindamycin in a single flowcell channel was used for MLSb detection. Susceptibility pattern profiles for representative e) MLSb positive and f) MLSb negative isolates are shown.*