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Evaluation of an Antimicrobial Susceptibility Testing Algorithm for Gram-Positive Bacteria Directly from Positive Blood Culture Using Automated Microscopy Analysis of Susceptibility Patterns

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AMENDED ABSTRACT

Objectives: The performance of an antimicrobial susceptibility testing (AST) algorithm using automated microscopy (AM) analysis was evaluated. Beta-lactam MICs from susceptibility growth patterns (SGP) of *Staphylococcus aureus* (SA) and *Streptococcus pneumoniae* (SP) directly from positive blood culture (PBC) were compared to CLSI standard reference broth micro-dilution (BMD).

Methods: A total of 91 clinical isolates (44 SA with ceftaroline (CFT) and 47 SP with penicillin (PEN) and ceftriaxone (CRO)) 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. PBC aliquots were prepared by automated gel electrofiltration, then inoculated into separate flowcells of a multichannel cassette. Bacterial cells were immobilized on the surface of each channel, and challenged with a single concentration of antibiotic for 4.5 h. AM captured time-lapse images during antibiotic exposure. An algorithm converted bacterial growth or inhibition image data into an MIC. Results were compared to BMD.

Results: The AM bacterial SGP response profiles corresponded with MICs as shown in Fig. 4. The AM algorithm reported an MIC in < 4.5 h. The essential agreement of AM with BMD testing ranged from 95-98% with 1-4 minor errors for each combination tested.

Conclusion: The AM and BMD MIC results were concordant, demonstrating MIC determination was feasible using a single concentration of beta-lactam antibiotic with SA and SP. Further evaluation of the algorithm should include other classes of antibiotics.

INTRODUCTION

Antibiotic susceptibility testing (AST) results are critical to determine effective treatment for patients with bacterial infections. Conventional phenotypic antibiotic susceptibility testing methods such as broth micro-dilution (BMD) measure overnight bacterial growth in serial doubling dilutions of antibiotic concentrations in order to generate an MIC result. In this study, we tested the feasibility of an AST algorithm to produce an MIC result using bacterial growth in a single concentration of antibiotic measured by an innovative automated microscopy system. Microscopy MIC results were compared to BMD per CLSI standard guidelines.

METHODS

A set of SA and SP training isolates with known MICs was tested to select an optimal single concentration of each antibiotic (CFT, PEN or CRO) for MIC determination based on susceptibility growth patterns. A computer algorithm was established for each bug-drug combination to convert bacterial growth into an MIC value based on a mathematical regression model of those results.

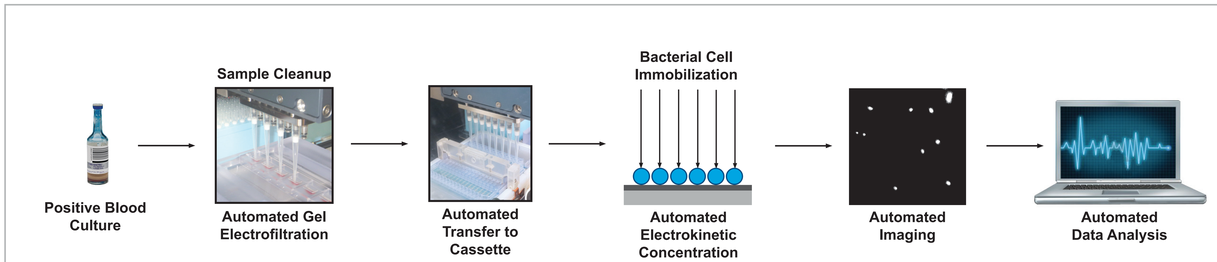

Figure 1: *Process flow including positve blood culture, sample cleanup by automated gel electrofiltration, automated transfer to the cassette for bacterial immobilization by electrokinetic concentration, automated imaging, and automated data analysis.*

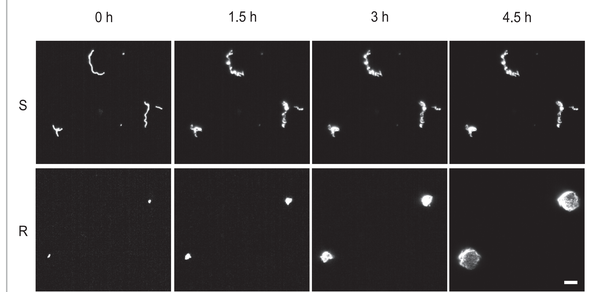

Figure 3: *Time-lapse images of susceptible (S) and resistant (R) S. pneumoniae isolates growing in penicillin at 0, 1.5, 3 and 4.5 h. Susceptible clones exhibit very slow elongated growth compared to resistant clones. Images zoomed in to view individual bacterial clones. Scale bar in the lower right image is 10 µm.*

Fig. 1 summarizes the procedures used to test a total of 91 clinical challenge isolates (44 SA with CFT and 47 SP with PEN and CRO). Simulated blood culture aliquots were diluted to produce an inoculum containing approximately 1 x 10^6 CFU/mL in lysis buffer. A pipetting robot performed sample cleanup 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.

Sample aliquots (20 µL) were pipetted into independent flowcells of a disposable 32-channel cassette. A low voltage was applied for 5 min to concentrate and immobilize the bacterial cells on the transparent lower surface of each flowcell channel. Single concentrations of antibiotic in cation-adjusted Mueller Hinton broth with 0.85% agar were introduced into each flowcell.

Automated microscopy used a custom engineering prototype (Accelerate Diagnostics Inc., Tucson, AZ USA) to take dark-field images of each flowcell every 10 min for 4.5 h. The microscope scanned 14 fields of view in each flowcell channel automatically (Fig. 2). Image analysis software analyzed the growth rates of each growing clone over the challenge period to produce bacterial cell population response profiles, and the computer algorithm converted those results into an MIC value. CLSI frozen BMD testing was performed in parallel. MIC results from the two methods were compared to determine essential agreement (EA).

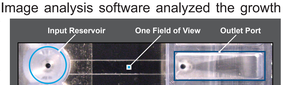

Figure 2: *Closeup of one flowcell in 32-channel flowcell cassette.*

RESULTS

Time-lapse images of susceptible and resistant SP isolates grown in the presence of PEN are shown in Figure 3. Data from time-lapse images were converted into bacterial cell response profiles, and an algorithm was established for each bug-drug combination to convert these data into an MIC. Examples of bacterial cell response profiles corresponding to different MIC values are shown in Figure 4. SA with CFT achieved EA of 98% with 4 minor errors observed (Table 1). SP with PEN had EA of 96% with 4 minor errors observed (Table 2). SP with CRO had EA of 95%. There was 1 minor error observed (Table 3). Automated microscopy produced MIC results from positive blood culture within 5 h compared to overnight pure colonies tested for BMD.

CONCLUSIONS

The results of this study demonstrate the feasibility of reporting an MIC result directly from positive blood culture in < 4.5 h using automated microscopy and single concentrations of antibiotics. Microscopy AST results were concordant with the standard reference methodology. Automated microscopy is a promising approach for providing earlier critical AST information directly from positive blood cultures to guide treatment for patients with bloodstream infections.

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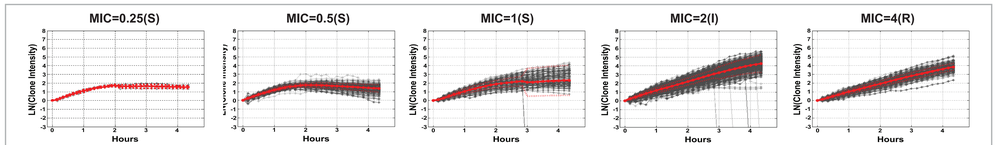

Figure 4: *Bacterial cell susceptibility pattern profiles for susceptible (S), intermediate (I), and resistant (R) S. aureus isolates growing in ceftaroline with MICs ranging from 0.25 µg/mL to 4 µg/mL. Each grey line is the growth curve for an individual bacterial clone. Solid red lines are the average and dotted red lines are two standard deviations from the average.*

Table 1: *SA with CFT test results. Essential Agreement=98% (n=44)†.*

Microscopy \ Broth Micro-Dilution	≤0.25	0.5	1	2	4	≥8
≤0.25	6	3	1	0	0	0
0.5	2	4	1	0	0	0
1	0	0	11	1	0	0
2	0	0	1	12	2	0
4	0	0	0	0	0	0
≥8	0	0	0	0	0	0

Table 2: *SP with PEN test results. Essential Agreement=96% (n=28)†.*

Microscopy \ Broth Micro-Dilution	≤0.5	1	2	4	8	≥16
≤0.5	6	0	0	0	0	0
1	0	5	1	0	0	0
2	0	0	2	0	0	0
4	0	0	2	7	0	0
8	0	0	0	1	2	0
≥16	0	0	0	1	0	1

Table 3: *SP with CRO test results. Essential Agreement=95% (n=19)†.*

Microscopy \ Broth Micro-Dilution	≤0.25	0.5	1	2	4	≥8
≤0.25	4	0	0	0	0	0
0.5	0	1	0	0	0	0
1	0	0	4	0	0	0
2	0	0	0	5	0	0
4	0	0	0	0	1	0
≥8	0	0	0	1	0	3

†2013 CLSI breakpoints in red