Background: Healthcare-associated bloodstream infections (HAI-BFIs) due to Non-Fermenting Gram-negative bacteria (NF-GNB), such as Pseudomonas aeruginosa (PA) and Acinetobacter baumannii (AB), which can develop drug resistance leading to infections with Multidrug Resistant Organisms (MDROs), is a growing concern. Septic patients infected with MDROs have been shown to have a higher risk of receiving inappropriate therapies and higher mortality due to the lack of availability of an automated system for rapid antibiotic susceptibility testing (AST) of PA and AB directly from positive blood culture.

Methods: 106 clinical isolates (80 PA and 26 AB) were pre-selected for MIC testing near the CLSI breakpoints. Simulated blood culture aliquots of 0.5% were performed in 384-well microtiter plates containing healthy donor blood. Isolates growing in the broth were washed, pelleted, and resuspended in lysis buffer to produce an inoculum containing approximately 1 x 10^6 CFU/mL. Briefly, 200 µL of inoculum were run on a 0.5% agarose gel for 20 min. Sample was electrokinetically extracted and immobilized on a poly-cationic coating on the lower surface of each flowcell. Immobilized bacterial suspensions were then challenged with pre-selected single concentration solutions of antibiotics prepared in CAMHB with 0.85% agar. Automated microscopy used a custom engineering 32-channel flowcell cassette. A 5-min low-voltage electric field immobilized bacteria on a disposable cassette. A 5-min low-voltage electric field immobilized bacteria on a disposable cassette. Automated imaging, and automated data analysis.

Results: Automated microscopy produced MIC results from positive blood culture within 5 h. A 1.5 h in vitro period culture was used for MIC of PA with CIP ranging from 0.5 to 32 µg/mL. Automated microscopy had a total turnaround time of 2538 min and a mean of 1.96 h compared to overnight pure colonies tested for BMD. Automated microscopy had a total turnaround time of 2538 min and a mean of 1.96 h compared to overnight pure colonies tested for BMD. Automated microscopy had a total turnaround time of 2538 min and a mean of 1.96 h compared to overnight pure colonies tested for BMD. PA with CIP achieved EA of 100% and CA of 96% with 2 minor errors observed (Table 1a). AB with CIP had EA of 100% and CA of 94% with 3 minor errors observed (Table 1b). PA with AMK had EA of 95% and CA of 93% with 3 minor errors observed (Table 2a). AB with AMK had EA of 98% and CA of 95% with 2 minor errors observed (Table 2b).

Conclusion: The results of this study demonstrate the feasibility of the automated microscopy technology to obtain AST results directly from positive blood culture within 5 h. Automated microscopy is a promising approach for providing MIC results directly from positive blood cultures sooner to guide treatment for patients with bloodstream infections due to NF-GNB, including potential MDROs. The growth rates of individual bacterial clones were analyzed by image analysis using automated microscopy and automated data analysis. The results of this study demonstrate the feasibility of the automated microscopy technology to obtain AST results directly from positive blood culture within 5 h. Automated microscopy is a promising approach for providing MIC results directly from positive blood cultures sooner to guide treatment for patients with bloodstream infections due to NF-GNB, including potential MDROs.