176

Rapid Phenotypic Antibiotic Susceptibility Testing Using Forward-Angle Laser Light Scattering

Andrew P. Norgan[1] and Andrew P. Tomaras[2]

[1]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA; [2]BacterioScan, Inc., St. Louis, MO, USA

Abstract

Background:
Rapid antimicrobial susceptibility testing (AST) enables clinicians to prescribe targeted therapies more quickly, thereby improving patient care and promoting antibiotic stewardship. Forward-angle laser light scattering (FLLS) is a sensitive technique for detection of microbial growth at lower limits of detection than most conventional methods. The objective of this study was to evaluate the effectiveness of FLLS to provide rapid phenotypic susceptibility information for clinical Gram-negative urine isolates.
Methods:
A BacterioScan™ 216Dx™ FLLS instrument was used to assess bacterial growth. Disposable multicuvettes were pre-filled, up to 30 days before use, with breakpoint concentrations of ciprofloxacin (CIP), trimethoprim-sulfamethoxazole (SXT), nitrofurantoin (NIT), and ceftriaxone (CRO). Standardized inocula of urine isolates were prepared in Mueller-Hinton broth, and dispensed into the multicuvettes. The inoculated multicuvettes were analyzed in the 216Dx for 20 hours at 37°C. Growth in the presence of antibiotics, as measured by FLLS, was compared to a no-antibiotic control to facilitate susceptible/intermediate/resistant interpretations by a blinded observer. Susceptibility profiles generated by FLLS were then compared to a standard agar dilution method.
Results:
A total of 20 pathogens were evaluated in this study, including strains of *E. coli*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, and *Citrobacter* species. Overall, the experimental and reference data were in agreement, with 6 minor errors and 4 major errors committed. Detection times ranged from 3-5 hours for both susceptible and resistant strains (Figure 1A and 1B). This time frame was also suitable for pathogens known to require longer incubation times, such as *P. aeruginosa* (Figure 1C).
Conclusion:
FLLS can provide phenotypic AST information with a faster time-to-result than most current clinical AST methods (e.g., disc diffusion, broth microdilution, agar dilution). Further evaluation of additional clinical isolates and isolate-antibiotic combinations will be required for validation of this technique, and the development of a computational algorithm for susceptible/intermediate/resistant interpretation of FLLS growth curves.

Introduction

The process of definitively determining pathogen resistance/susceptibility can take 24-48 hours beyond isolation of the organism, which is time that critically-ill patients do not have. Thus the need for new diagnostic platforms that can provide antimicrobial susceptibility testing (AST) results with a more expedient time-to-result is greater than ever. Narrow angle forward laser scattering is a sensitive optical method for measuring particulates suspended in liquid, and can be used to estimate the density of microbes in a liquid sample at levels well below thresholds detectable with conventional methods. The BacterioScan 216Dx™ is a Laser Microbial Growth Monitor that delivers precise and repeatable quantification of microorganisms in liquid samples, with the ability to provide automated measurements during long-term incubation. The instrument measures the density of bacteria suspended in a liquid sample by measuring changes in both light scattering properties and optical density, enabling a lower limit of detection of ~$1x10^4$ CFU/ml, which is about twice the background level for purified water. The instrument uses a disposable multicuvette that holds up to 4 samples of 3.0 ml each, and can hold 4 multicuvettes at a time for a total of up to 16 samples. The system is random access, and cuvettes can be added or removed at any time.

In this study, we examined the potential use of laser light scattering as a platform for rapid AST of various Gram-positive and Gram-negative pathogens. Our preliminary panel consisted of 3 standard-of-care antibiotics used to treat urinary tract infections (UTIs), and was formatted to provide a categorical resistance/susceptibility output based on current CLSI breakpoint concentrations for each drug. The results presented here demonstrate the ability of the 216Dx to return accurate phenotypic resistance/susceptibility interpretations with a considerable improvement in time-to-result over traditional methods.

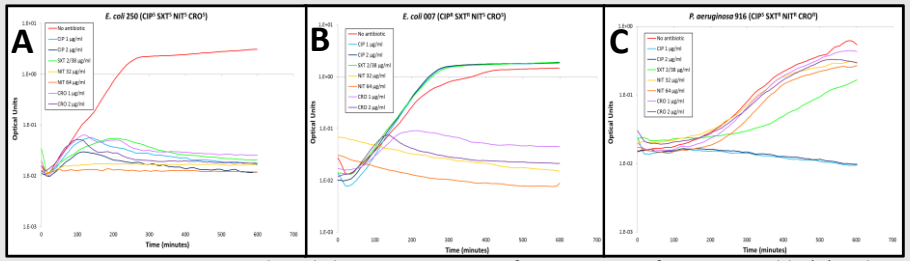

Figure 1. BacterioScan 216Dx laser light-scattering output for AST testing of pan-susceptible (A) and resistant (B) UTI clinical isolates of *E. coli*, as well as a fluoroquinolone-susceptible isolate of *P. aeruginosa* (C).

Table 1. Summary table for BacterioScan 216Dx laser light-scattering AST results.

Organism		Strains Tested	Total Tests	Correct	Minor Errors	Major Errors	Very Major Errors
Gram-negatives							
	E. coli	52	208	203	2	3	0
	Klebsiella spp.	17	61	52	6	2	1
	Pseudomonas spp.	9	25	24	0	1	0
	Enterobacter spp.	4	16	14	2	0	0
	Citrobacter spp.	4	16	15	1	0	0
Gram-positives							
	Enterococcus spp.	10	21	18	1	2	0
	Staphylococcus spp.	6	17	16	1	0	0
	Streptococcus spp.	2	2	2	0	0	0
Totals		104	366	344	13	8	1
% of Total Tests				94.0	3.6	2.2	0.3

Methods

- For all AST studies, stock solutions of each antibiotic were prepared according to the manufacturer's instructions, and the appropriate volume of each solution was dispensed to individual slots of 216Dx multicuvettes (Figure 2C).
- Bacterial isolates were grown overnight on blood agar plates prior to preparing suspensions equivalent to 0.5 McFarland turbidity standards. Suspensions were diluted 1:400 into cation-adjusted Mueller Hinton Broth (CAMHB; Difco) prior to dispensing 2.5ml into antibiotic-containing slots of multicuvettes.
- For direct-from-urine AST studies, a culture-negative urine specimen was spiked with 10^5 CFU/ml bacteria and processed in the BacterioScan 216Dx for 3 hours. Cuvette contents at the conclusion of this processing were diluted 1:10 in CAMHB and dispensed to antibiotic-containing multicuvettes.
- For direct-from-positive blood culture bottle AST, bacteria were spiked into BACTEC (Becton Dickinson) blood culture bottles at a starting density of ~10^2 CFU/ml and incubated according to the manufacturer's instructions. Upon successful indication of culture positivity, bottle contents were withdrawn, diluted 1:1000 in CAMHB, and dispensed to antibiotic-containing multicuvettes.

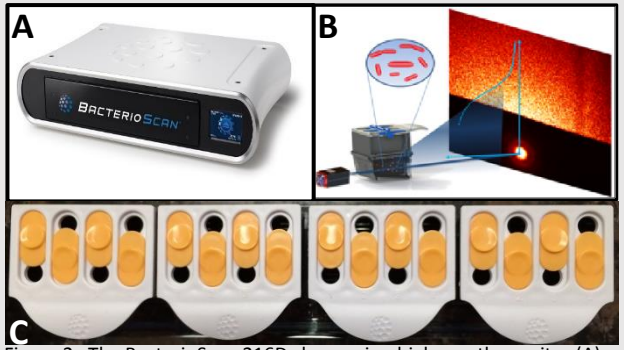

Figure 2. The BacterioScan 216Dx laser microbial growth monitor (A) uses forward-angle laser light-scattering (B) to detect particles in liquid samples. Each antibiotic at the indicated concentrations were dispensed to individual slots of the 216Dx disposable cartridges (C).

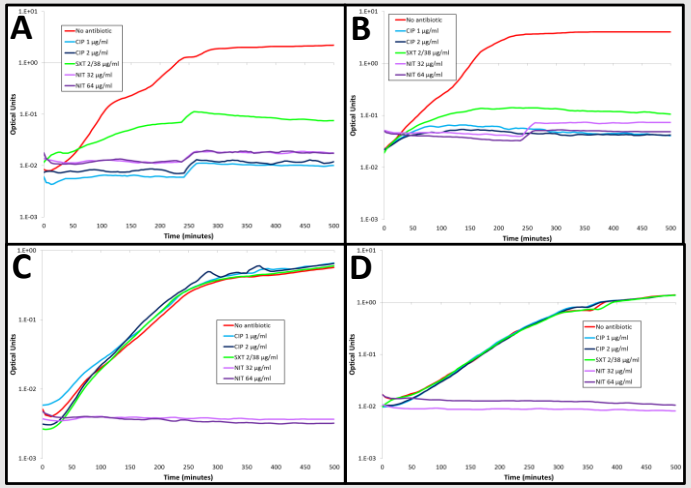

Figure 3. Direct-from-positive urine specimen AST testing of urine specimens initially detected as positives by the 216Dx. Comparisons between from-isolate (A,C) and direct-from-specimen (B,D) of pan-susceptible *E. coli* M0224 (A,B) and multidrug-resistant *K. pneumoniae* M0194 (C,D) were performed in the 216Dx.

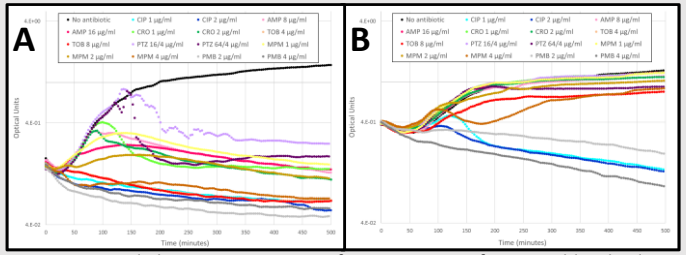

Figure 4. Laser light-scattering output for AST testing of positive blood culture bottles spiked with either a pan-susceptible (A) and KPC-expressing clinical isolate (B) of *E. coli* using 7 different antibiotics representing different chemical classes.

Conclusions

- Laser light-scattering provides phenotypic AST results with a high degree of accuracy and a more rapid time-to-result than conventional methods.
- AST results were comparable when cultured isolates and direct-from-positive urine specimen inocula were analyzed using 3 standard-of-care UTI antibiotics.
- Rapid differentiation between resistance and susceptibility to a variety of chemically-diverse antibiotics was observed when positive blood culture bottle contents were assessed.

Acknowledgements

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