Utilization of Laser Light Scattering for Rapid Detection and Subsequent Antimicrobial Susceptibility Testing Directly from Urine Specimens

S. Collier, A. Tomaras, A. Skinner, F. Albariolo, and A. Harrington
Loyola University Medical Center, Maywood, IL, USA, 2BacterioScan, Inc., St. Louis, MO, USA

Abstract

Background

The high volumes and lengthy turnaround times required to process urine specimens present a workflow challenge and prompt the use of empiric therapy, including to patients whose specimens may be unremarked. Methods that return diagnostic information more rapidly would improve patient care and facilitate antibiotic stewardship. The BacterioScan 216x is a laser light scattering device that detects urinary tract infections (UTIs) more rapidly than current methods. Our lab evaluated the 216x’s ability to provide rapid antimicrobial susceptibility testing (AST) data using samples deemed positive during the UTI screening protocol.

Methods

Ninety-five urine specimens were analyzed using the 216x UTI detection protocol according to the manufacturer’s instructions. In vitro results from positive flagged specimens were spread onto pre-warmed blood and MacConkey agar plates and incubated at 37°C for 4 hours. MALDI-TOF MS (Bruker) identification was performed on the resulting agar surface film. Direct-from-positive specimen AST was also evaluated on 22 samples containing Gram negative pathogens using a panel of 4 antibiotics tested at clinical breakpoint concentrations. Curvett contents were diluted to ≤10^6 CFU/ml into cation-adjusted Mueller Hinton Broth prior to dispensing into antibiotic-containing cuvettes. AST runs in the 216x were conducted at 35°C for 16 hours, after which optical profiles were compared to a corresponding no drug control.

Results

Fifty-four specimens possessed ≥100,000 CFU/ml of a UTI pathogen, 51 of which were accurately detected by the 216x after 190 minutes. Despite contaminant presence in many of the specimens, MALDI-TOF MS analysis of positively-flagged cuvette material showed ≥75% agreement with SOD data, with the majority of failed samples harboring lower density infections. Gram positive pathogens, or possessing ≥1 UTI pathogen. The SOD AST method (Microscan) indicated non-susceptibility in 30.3, 36.3, 45.5, and/or of carbapenem (CPE), Levofoxacin (LVX), Ceftaxim (FEP), and Meropenem (MER), respectively. After 3-4 hours of analysis, the 216x demonstrated categorical agreement at 100% for CIP, FEP, and MER, and 98.4% for LVX.

Conclusions

Based on these data, the Bacterioscan 216x has the potential to offer accurate UTI detection and, when coupled with MALDI-TOF MS, robust pathogen ID AST for at least 75% of Gram negative infected urine specimens, within as little as 8 hours post-collection. Further validation is needed ongoing.

Background

One of the primary strategies being implemented in hospitals to combat the spread of antimicrobial resistance is antimicrobial stewardship, and guidelines and practices promoting the appropriate use of antibiotics have been developed by institutional stewardship committees. The effective implementation of these practices is inhibited by the lengthy time-to-result required of conventional diagnostic platforms. Antimicrobial susceptibility testing can take 24-48 hours, and results are often available as long as 3-5 days after initial specimen collection. While awaiting results, clinicians must use empiric antibiotic strategies, which facilitate the emergence of resistant pathogens and may negatively impact the morbidity and mortality of critically ill patients. New diagnostic platforms that can provide antimicrobial susceptibility testing (AST) results in a more timely manner have potential to improve patient care by optimizing antimicrobial therapy at an earlier time in the patient’s treatment course. Ideally, new assays could provide diagnostic information regarding bacterial identification and antimicrobial susceptibility directly from the specimen.

Methods

The 216x was designed and engineered to rapidly screen urine specimens to detect urinary tract infections (UTIs) and to eliminate uninfected samples from the culture-based workflow. This qualitative test application recently received 510(k) clearance from the FDA. While earlier stratification of negative urine specimens improves clinical laboratory workflow and antimicrobial avoidance, the additional benefits of more rapidly identifying infected specimens were also considered. Utilizing the 216x’s inherent capability to accurately identify specimens at ≥100,000 CFU/ml, positive/negative, the growing contents within each cuvette were hypothesized to serve as a reasonable substrate from which further pathogen information could be obtained. Specifically, the potential downstream applications of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for detecting organism identification, as well as rapid, phenotypic AST profiling were speculated to be achievable through the direct from 216x-positive AST samples. In this study, we evaluated this comprehensive process using clinical urine specimens and compared the performance of laser light-scattering methods to those obtained using conventional approaches.

Table 1. Comparative performance of the BacterioScan 216x and ChemVeloCTY automated urinary test systems (UA). For detection of bacterial density (stratified by bacterial density) in patient urine samples.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>% strains resistant/susceptible</th>
<th>Categorical Agreement</th>
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<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>36.8±3.2</td>
<td>100%</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>34.2±5.8</td>
<td>97.4%</td>
</tr>
<tr>
<td>Piperacillin-Tazobactam</td>
<td>7.9±2.1</td>
<td>89.5%</td>
</tr>
<tr>
<td>Ceftaxim</td>
<td>53.9±4.7</td>
<td>100%</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.1±0.0</td>
<td>100%</td>
</tr>
</tbody>
</table>

Conclusions

- Laser light-scattering can be a useful method for screening clinical urine specimens for the presence or absence of bacteria.
- The 216x UTI detection protocol demonstrates comparable sensitivity and substantially greater specificity when compared to automated urinalysis.
- Using an abbreviated incubation and surface film for bacterial growth, MALDI-TOF MS-based identification provided accurate and rapid identification of organisms for ~70% of the samples when compared to standard culture. Samples that did not provide accurate results using the rapid MS method either harbored multiple organisms or started as dense infection in the initial specimen. No instances of inaccurate pathogen identification were observed.
- Phenotypic AST profiling of five standard-of-care UTI antibiotics in the 216x resulted in categorical agreement values of 100% when tested directly from positively flagged UTI urine samples. These results were available after 2-4 hours of optical analysis, (depending on the growth rate of the organism).
- These results support the development of a single process that utilizes laser light-scattering to discriminate between infected and uninfected urine and provide pathogen characterization information in a fraction of the time conventional methods require.