Culture independent, molecular detection of uropathogens and antibiotic resistance genes direct from urine specimens

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ABSTRACT
Background: Infections from bacteria resistant to multiple antibiotics are a global problem with the urinary tract serving as a major site of infection. Acuitas® AMR Gene Panel (RUO)* (OpGen, Inc, Gaithersburg, MD, USA) is a molecular test developed to detect 5 common uropathogens (E. coli [EC], Klebsiella pneumoniae [KP], Pseudomonas aeruginosa [PA], Proteus mirabilis [PM], and Enterococcus faecalis [EF]) and 47 antibiotic resistance genes targets directly from urine samples. We conducted a prospective, observational study to determine the performance characteristics of the Acuitas test and Acuitas Lighthouse Software (RUO)*.

Materials/methods: Clinical urine samples, positive for target organisms, were collected in urine preservation tubes at the Beth Israel Deaconess Medical Center Lab (Boston, MA, USA) and the Intermountain Central Lab (Murray, UT, USA) (n=203). Samples were submitted to OpGen for testing with Acuitas and analysis by Acuitas Lighthouse software. Results were compared to bacterial identification and antibiotic susceptibility testing performed at the clinical labs using automated, phenotypic methods (Microscan Walkaway, Beckman Coulter, Brea, CA, USA). Evaluation of performance characteristics for predicting antibiotic resistance was limited to EC and KP due to smaller numbers of other organisms.

Results: A total of 316 target uropathogens were identified in urine samples by the clinical lab: 210 (EC, KP, 50 EF, 45 PM, 14 PM, and 92 KP were detected by Acuitas (178 EC, 81 KP, 98 EF, 22 PM, 13 PM). For samples detected ≥10^5 cfu/mL, the overall sensitivity/specificity/PPV/NPV (%) of Acuitas for detection of EC was 91/92/81/96, and KP was 81/93/83/92 respectively. Phenotype data was available for resistance prediction of 230 isolates (153 EC; 77 KP). The composite sensitivity/specificity/PPV/NPV for Acuitas Lighthouse resistance prediction for EC was 84/98/92/95 and 53/98/70/95 for KP for EC, performance was best with ampicillin, fluoroquinolones, cefalotin and cefotaxime. KP prediction was best with 3rd generation cephalosporins and cefepime. Conclusions: The Acuitas panel accurately detected common uropathogens for which Acuitas Lighthouse predicted susceptibilities to antibiotics commonly used to treat urinary tract infections (UTI) directly from urine samples. This test and software have the potential to serve as a rapid diagnostic test to guide early, empiric antibiotic use in patients presenting with complicated UTI.

Methods
Setting: Urine samples positive for target organisms were collected from two microbiology laboratories (Beth Israel Deaconess Medical Center, Boston, MA; Intermountain Healthcare Central Microbiology Laboratory, Murray, UT). Samples were submitted to OpGen (Gaithersburg, MD) for testing with Acuitas® AMR Gene Panel and analysis with Acuitas Lighthouse Software.

Urine culture: Urine specimens were collected into 4 mL urine preservative tubes with (bipotassium acidified BD Vacutainer Plus C&S). Cultures and susceptibility testing were performed at the collecting laboratories according to local standard operating procedures.

Specimens positive for target organisms were cryopreserved at −80°C and shipped to OpGen for molecular testing. Identification and susceptibility testing were performed on Microscan Walkaway System (Beckman Coulter). Results were submitted to OpGen.

Acuitas® AMR Gene Panel (RUO)*: A multiplexed, real-time PCR assay for the detection of 47 antibiotic resistance genes, spanning 9 antibiotic classes, and 5 pathogens (E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa and E. faecalis). It tests directly from urine or isolated colonies, and reports results in less than 3 hours.

Acuitas Lighthouse® Software (RUO)*: A cloud-based software capable of identifying and tracking antimicrobial resistant threats and is being developed to predict antimicrobial resistance from Acuitas AMR Gene Panel results to rapidly guide appropriate antibiotics.

Results

Table 1. Acuitas Lighthouse - Genotypic Predictions of Resistance in E. coli

<table>
<thead>
<tr>
<th>Total Isolates Submitted and Tested</th>
<th>Institution</th>
<th>Cultures</th>
<th>Phenotype data</th>
<th>Acuitas # Predictions</th>
<th># Assessments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beth Israel</td>
<td>191</td>
<td>167</td>
<td>285</td>
<td>245</td>
<td>1457</td>
</tr>
<tr>
<td>Intermountain</td>
<td>216</td>
<td>129</td>
<td>201</td>
<td>184</td>
<td>1477</td>
</tr>
<tr>
<td>Total</td>
<td>407</td>
<td>296</td>
<td>486</td>
<td>429</td>
<td>2934</td>
</tr>
</tbody>
</table>

Table 2. Acuitas Lighthouse - Genotypic Predictions of Resistance in K. pneumoniae

<table>
<thead>
<tr>
<th>Performance Characteristics of Acuitas for Pathogen Identification</th>
<th>Organism</th>
<th>Accuracy</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (n=1150)</td>
<td>92%</td>
<td>91%</td>
<td>92%</td>
<td>81%</td>
<td>96%</td>
<td></td>
</tr>
<tr>
<td>K. Pneumoniae (n=82)</td>
<td>89%</td>
<td>91%</td>
<td>93%</td>
<td>83%</td>
<td>92%</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions:
- Acuitas AMR Gene Panel and Acuitas Lighthouse accurately detected E. coli and K. pneumoniae in urine isolates cultured in clinical laboratories and also accurately predicted susceptibility patterns of commonly used antibiotics.
- More samples are needed to determine performance for other common uropathogens (Pseudomonas aeruginosa, Proteus mirabilis, Enterococcus faecalis).
- Molecular testing has the potential to rapidly detect resistant organisms directly from urine specimens to help guide antibiotic use and infection control practices.

*For Research Use Only. Not for use in diagnostic procedures.

References:

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Conflict of Interest Statement:
Bert K. Lopansri has received research support and speaker’s fees from OpGen; Stefan Riedel, MD, PhD is a member of the OpGen Clinical Advisory Board and a paid consultant to OpGen. This study was funded by OpGen.