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 gene targets direct 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 preservative tubes at the Beth Israel Deaconess Medical Center Lab (Boston, MA, USA) (n=285) and the Intermountain Central Lab (Murray, UT, USA) (n=201). Samples were submitted to OpGen for testing with Acuitas and analysis by Acuitas Lighthouse software. Results were compared to bacterial identification and antibiotic susceptibility results generated 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 labs (150 EC, 82 KP, 50 EF, 18 PM, 16 PA) and 392 were detected by Acuitas (178 EC, 81 KP, 98 EF, 22 PM, 13 PA). For samples detected at >10^4 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, cefotaxime and ceftriaxone. 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.

Introduction

- The urinary tract is a common source of infection from bacteria resistant to multiple antibiotics such as Enterobacteriaceae with extended spectrum beta-lactamases.
- Urine culture and phenotypic susceptibility testing are the gold standard diagnostic tests for UTIs.
- A novel molecular test and analysis software (Acuitas® AMR Gene Panel , Acuitas Lighthouse®) are under development for detection of 5 common uropathogens (*E. coli* [EC], *Klebsiella pneumoniae* [KP], *Pseudomonas aeruginosa* [PA], *Proteus mirabilis* [PM] *Enterococcus faecalis* (EF) and 47 antibiotic resistance genes directly from urine and prediction of phenotypic non-susceptibility.
- We conducted a prospective, observational study to compare Acuitas® AMR Gene Panel and Acuitas Lighthouse® results with clinical culture identification and susceptibility results.

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 cultures: Urine specimens were collected into 4 mL urine preservative tubes with boric acid preservative (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 -80ºC 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)* is 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)* is 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

Total Isolates Submitted and Tested

Institution	Cultures	Phenotype data	Acuitas	# Predictions	# Assessments
Beth Israel	191	167	285	245	1457
Intermountain	216	129	201	184	1477
Total	407	296	486	429	2934

Performance Characteristics of Acuitas for Pathogen Identification

Organism	Accuracy	Sensitivity	Specificity	PPV	NPV
E. coli (n=150)	92%	91%	92%	81%	96%
K. Pneumoniae (n=82)	89%	91%	93%	83%	92%

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 urine pathogens (*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.

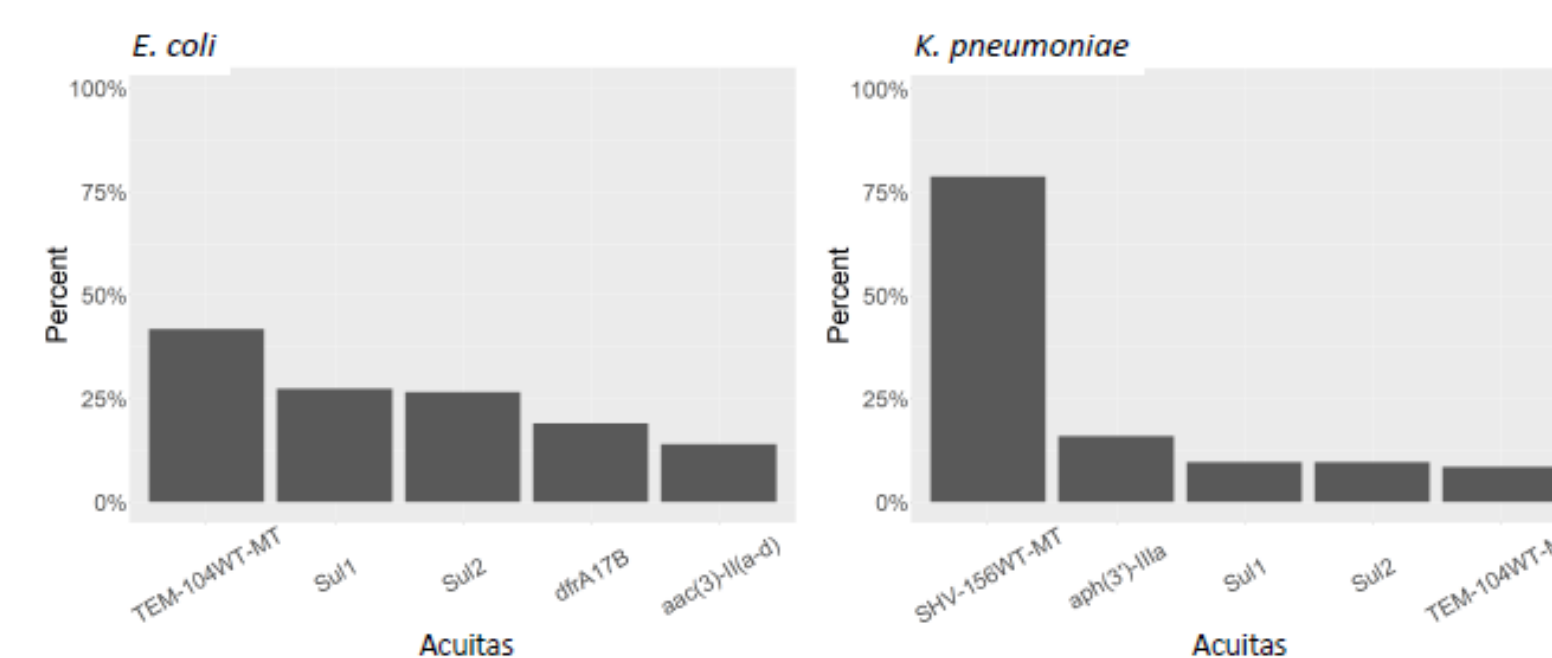**Figure.** Top 5 resistance genes by bacterial species.

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

Antibiotic	Accuracy	Sensitivity	Specificity	PPV	NPV
Aminoglycosides					
Gentamicin	96%	78%	99%	95%	96%
Tobramycin	97%	86%	99%	95%	98%
Beta-Lactams					
Aztreonam	94%	76%	98%	92%	95%
Ampicillin	94%	94%	95%	95%	93%
Cefazolin	91%	71%	96%	81%	93%
Cefotaxime	96%	85%	98%	92%	97%
Ceftazidime	92%	60%	98%	83%	93%
Ceftriaxone	94%	85%	96%	81%	97%
Cefepime	96%	85%	98%	92%	97%
Fluoroquinolones					
Ciprofloxacin	97%	92%	100%	100%	96%
Levofloxacin	99%	96%	100%	100%	98%
Folate Pathway Antagonist					
Trimethoprim/ Sulfamethoxazole	86%	68%	94%	85%	86%

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

Antibiotic	Accuracy	Sensitivity	Specificity	PPV	NPV
Aminoglycosides					
Gentamicin	94%	57%	97%	67%	96%
Tobramycin	95%	50%	99%	75%	96%
Beta-Lactams					
Aztreonam	94%	50%	99%	80%	95%
Cefazolin	95%	80%	97%	80%	97%
Cefotaxime	96%	67%	100%	100%	96%
Ceftazidime	96%	57%	100%	100%	96%
Ceftriaxone	94%	50%	100%	100%	93%
Cefepime	94%	50%	100%	100%	93%
Imipenem	97%	0%	100%	NA	97%
Fluoroquinolones					
Ciprofloxacin	87%	60%	89%	27%	97%
Levofloxacin	90%	50%	91%	13%	99%
Folate Pathway Antagonist					
Trimethoprim/ Sulfamethoxazole	89%	36%	100%	100%	88%

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