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Verification of Real Time PCR for the Detection of Antibiotic-Resistance Markers and Semi-Quantitation of Urinary Tract Pathogens from Urine Samples

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ABSTRACT

INTRODUCTION
Urinary tract infections (UTI) are a major cause of hospital admissions and can be associated with mortality linked to urosepsis. An increase in antimicrobial resistant pathogens led to a need for rapid diagnostic panels to identify uropathogens and antibiotic resistance. In fewer than 3 hours, the Acuitas® AMR Gene Panel (ROU)† assay (OpGen, Gaithersburg, MD) can detect, and semi-quantify, the 5 most common causes of UTI directly from urine: *E. coli, K. pneumoniae, P. aeruginosa, P. mirabilis,* and *E. faecalis*. The panel also identifies 47 genetic makers for antibiotic resistance.

METHODS
Three clinical sites completed a method verification of the Acuitas panel compared to culture identification (ID), quantitation, and phenotypic antibiotic susceptibility testing (AST). Urine samples were collected by clean catch or catheterization. DNA extracted from urine was combined with the Acuitas master mix and the PCR was performed on a QuantStudio 5 thermocycler. Data analysis was performed by the Acuitas Lighthouse® Software (RUO)†. A total of 531 remnant samples were obtained, 305 samples had a positive and quantifiable ID by culture, and 255 had reportable AST results.

RESULTS
When quantitation of the 5 on-panel microbes was >10^4 CFU/ml, the performance of the Acuitas for organism ID yielded a positive agreement of 94% and negative agreement of 97%. For the 2 most common species, *E. coli* and *K. pneumoniae,* the total agreement (TA) for phenotype predictions of antibiotic resistance was 93% and 92%, respectively; positive predictive value (PPV) was 92% and 78%, respectively and negative predictive value (NPV) was 94% and 95%, respectively. Performance of the phenotype predictions of antibiotic resistance for *E. coli* and *K. pneumoniae* were calculated by averaging each antibiotic class. Cephalosporins had a TA of 93% with PPV of 90% (range: 76%-98%) and NPV of 94% (87%-99%). Fluoroquinolones and aminoglycosides had a TA of 92% and 97%, with a PPV of 87% and 91% and NPV of 94% and 98%, respectively. The most prevalent resistance genes identified were extended-spectrum β-lactamases (ESBLs), class TEM for *E. coli,* and SHV for *K. pneumoniae*.

CONCLUSIONS
The Acuitas AMR Gene Panel and Acuitas Lighthouse are designed to provide a rapid and accurate ID, semi-quantitation, and antibiotic resistance prediction, directly from urine specimens for the 5 most common UTI pathogens. Further assessment of the clinical impact of the panel is warranted and could lead to reduction in time from ID/AST to targeted therapy for patients afflicted with UTI.

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

EXPERIMENTAL DESIGN

Method comparison to assess diagnostic accuracy
- Acuitas panel vs. Vitek MS (MALDI-TOF mass spectrometry)
  - Identification of Bacteria
- Lighthouse Antibiotic Software vs. Site Reference Method
  - Phenotypic Antimicrobial Susceptibility Testing (AST)

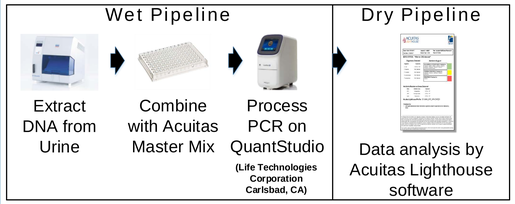
Setting for method verification
- Geisinger Medical Center (Danville, PA)
- Intermountain Medical Center (Murray, UT)
- Beth Israel Deaconess Medical Center (Boston, MA)

Sample size by category:
- 531 = Total samples collected
- 305 = Positive identification by Vitek MS
- 255 = Reportable AST results

MATERIALS AND METHODS

Figure 1

Population: patients suspected of UTI
Samples: 3.6mL of remnant urine (Jun-Aug 2018)



Acuitas Result Categories Used for Analysis
(Numerical values are listed as genome copies/mL urine)
Negative; < 10^4; 10^4 to 10^5; > 10^5

Figure 2: Antibiotics Tested for Resistance by Class

Aminoglycosides	Fluoroquinolones	Cephalosporins	Miscellaneous
Gentamicin	Ciprofloxacin	Cefazolin	Ampicillin
Tobramycin	Levofloxacin	Cefepime	Aztreonam
		Cefotaxime	Trimethoprim/ Sulfamethoxazole
		Ceftazidime	
		Ceftriaxone	

Figure 3: Antimicrobial Resistance Genes in the Acuitas AMR Gene Panel

†multiple genes/mutations detected	Aminoglycoside modifying enzyme	Modified fluoroquinolone binding site	Ribosomal methyltransferase	Carbapenemase	AmpC beta-lactamase	Phosphatidy-ethanolamine transferase	Cephalosporinase	Penicillinase	Dihydrofolate reductase binding	Dihydropteroate synthase inhibition	Modified glycopeptide binding site
AAC†, ANT†, APH†	×										
Gyrase mutations†		×									
armA, RMT†			×								
IMP, KPC, NDM, OXA-48, OXA-9, SPM, VIM				×							
CMY-2, CMY-41, DHA					×						
MCR-1, MCR-2						×					
CTX-M-1, CTX-M-2 , CTX-M-9, GES, PER, SHV, TEM, VEB							×				
OXA-1, SHV, TEM								×			
DFR†									×		
SUL†										×	
vanA											×

RESULTS

Table 1: Performance of Acuitas vs. Vitek MALDI-TOF MS for ID

Species	Vitek+/Acuitas+	Vitek-/Acuitas+	Vitek+/Acuitas-	Vitek-/Acuitas-	PA‡	NA‡	PPV	NPV	Total Agreement
E. coli	191	12	10	318	95%	96%	94%	97%	96%
K. pneumoniae	69	9	7	446	91%	98%	89%	99%	97%
P. mirabilis	12	7	0	512	100%	99%	63%	100%	99%
P. aeruginosa	5	4	1	521	83%	99%	56%	100%	99%
E. faecalis	28	35	0	468	100%	93%	44%	100%	93%
Total	305	67	18	2265	94%	97%	82%	99%	97%

‡Positive Agreement (PA), Negative Agreement (NA)

Table 2 & 3: Performance of Phenotypic Predictions of Most Prevalent Species

Table 2: E. coli		Phenotype	
		R/I	S
Acuitas Lighthouse Prediction	R	540	46
	S	88	1287

- TA: 93%
- PA: 86%
- NA: 97%
- PPV: 92%
- NPV: 94%

Table 3: K. pneumoniae		Phenotype	
		R/I	S
Acuitas Lighthouse Prediction	R	76	21
	S	33	567

- TA: 92%
- PA: 70%
- NA: 96%
- PPV: 78%
- NPV: 95%

Table 4: Performance of Phenotypic Predictions of Antibiotic Resistance of Most Prevalent Microbial Species by Antibiotic Class

Antibiotic	Total Agreement	PA	NA	PPV	NPV	TP§	FP§	FN§	TN§
Gentamicin	98% (n=258)	86%	100%	100%	98%	25	0	4	229
Tobramycin	96% (n=257)	88%	97%	83%	98%	29	6	4	218
Average	**97%**	**87%**	**99%**	**91%**	**98%**				
Range	**(96%-98%)**	**(86%-88%)**	**(97%-100%)**	**(83%-100%)**	**98%**				
Ciprofloxacin	92% (n=257)	86%	95%	86%	95%	59	10	10	178
Levofloxacin	91% (n=188)	83%	95%	88%	92%	49	7	10	122
Average	**92%**	**85%**	**95%**	**87%**	**94%**				
Range	**(91%-92%)**	**(83%-86%)**	**95%**	**(86%-88%)**	**(92%-95%)**				
Cefazolin	88% (n=242)	74%	96%	90%	87%	63	7	22	150
Cefepime	98% (n=258)	95%	99%	95%	99%	55	3	3	197
Cefotaxime	96% (n=166)	90%	99%	98%	95%	54	1	6	105
Ceftazidime	89% (n=258)	69%	94%	76%	92%	38	12	17	191
Ceftriaxone	95% (n=258)	92%	96%	90%	97%	60	7	5	186
Average	**93%**	**84%**	**97%**	**90%**	**94%**				
Range	**(88%-98%)**	**(69%-95%)**	**(94%-99%)**	**(76%-98%)**	**(87%-99%)**				
Ampicillin	97% (n=92)	100%	0%	97%	NA	89	3	0	0
Aztreonam	95% (n=166)	94%	95%	89%	97%	49	6	3	108
Trimeth.Sulfa	84% (n=258)	55%	97%	90%	82%	46	5	37	170
Average	**92%**	**83%**	**64%**	**92%**	**90%**				
Range	**(84%-97%)**	**(55%-100%)**	**(0%-97%)**	**(89%-97%)**	**(82%-97%)**				

§ True Positive (TP), False Positive (FP), False Negative (FN), True Negative (TN)

Table 5 & 6: Most Prevalent AMR Genes in Acuitas AMR Gene Panel

Table 5: E. coli

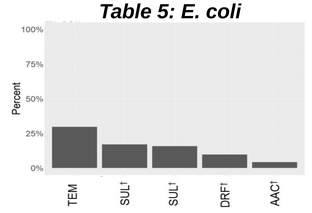
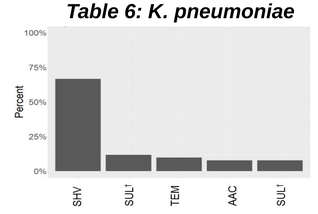


Table 6: K. pneumoniae



† multiple genes/mutations detected

DISCUSSION

The total time from collection of sample to result of the Acuitas panel is considerably shorter than current culture methods. Rapid and accurate ID and AST results could reduce the time to targeted therapy for certain pathogens. Targeted therapy could lead to decreases in length of stay and mortality. Further assessment of the panel is required to calculate clinical impact(s).

LIMITATIONS

While the total agreement for ID is promising, the accuracy of phenotypic AST predictions will need to improve to avoid therapeutic errors or results will need to be confirmed by traditional AST. Some species are represented by a very small sample size.

CONCLUSIONS

The Acuitas AMR Gene Panel is a rapid diagnostic tool with a total test time of approximately 3 hours. The Gene Panel detects 5 common uropathogens and 47 antibiotic resistance genes directly from urine specimens. The Acuitas Lighthouse Software predicts phenotypic AST results. This multisite project represents the first assessment of accuracy for the Acuitas AMR Gene Panel using clinical samples. Further testing is warranted prior to clinical use.

Diagnostic Accuracy Summary
- PA for ID: 94%
- NA for ID: 97%
- TA Phenotypic AST *E.coli* : 93%
- TA Phenotypic AST *K. pneumoniae:* 94%
- See Table 1 for additional organisms

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