Verification of Real Time PCR for the Detection of Antibiotic-Resistance Markers and Semi-Quantification of Urinary Pathogen Traits from Urinary Samples


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INTRODUCTION
Urine tract infections (UTIs) are a major cause of hospital admissions and can be associated with mortality linked to sepsis. An increase in antimicrobial resistance patterns 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 9 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 markers for antibiotic resistance with UTI.

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 (FLU). 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 the qPCR on the q-PCR microplate was >10^3 CFU/mL, the performance of the Acuitas for organism ID yielded a positive agreement of 93% and negative agreement of 92%. 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 90% and 78%, respectively, and negative predictive value (NPV) was 94% and 95%, respectively. Performance of the phenotype predictions of antibiotic resistance E. coli and K. pneumoniae were calculated by averaging each by VP class. Cephalosporin had a TA of 93% with PPV of 96% (range: 78%-98%) and NPV of 97% (78%-97%). Fluoroquinolones and aminoglycosides had a TA of 92% and 97%, respectively. A total agreement of 90% with PPV of 90% (range: 76%-98%) and NPV of 94% (87%-99%).

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 to AST to targeted therapy for patients affected with UTI.

DISCUSSION
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.

LIMITATIONS
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 antimicrobial resistance genes directly from urine specimens. The Acuitas Lighthouse Software predicts phenotypic AST results. This multiplex project represents the first demonstration of an automated diagnostic platform for the Acuitas AMR Gene Panel using clinical samples. Further testing is warranted prior to clinical use.

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