Predicting Antibiotic Resistance in Gram-Negative Bacilli by Rapid Detection of Resistance Genes

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BACKGROUND: Phenotypic antibiotic susceptibility testing (AST) is the standard guide for antibiotic therapy. However, because AST takes days, it leads to empiric therapy with broad-spectrum antibiotics. Molecular tests rapidly detect resistance genes but not for routine prediction of phenotypic antibiotic resistance.

METHODS: We evaluated a total of 7200 clinical isolates of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and *Pseudomonas aeruginosa* from Intermountain Medical Center, Murray, UT and the Merck Study for Monitoring Antimicrobial Resistance Trends (SMART) by phenotypic AST and a high-throughput PCR assay for 126 antibiotic resistance genes. Statistical algorithms were developed to predict phenotypic resistance across 17 antibiotics using 47 resistance genes. We subsequently developed a Research Use Only semi-quantitative PCR test that detects *E. coli, K. pneumoniae, P. aeruginosa, P. mirabilis* and *E. faecalis* and the 47 resistance genes in urine specimens, the Acuitas® AMR Gene Panel u5.47. We also developed the Acuitas Lighthouse® Knowledgebase to interpret results from the AMR Gene Panel for prediction of phenotypic antibiotic resistance.

RESULTS: Agreement between measured phenotypic AST and genotypic prediction of phenotypic susceptibility using 47 resistance genes and the Acuitas Lighthouse prediction algorithms was 79 to 97% for *E. coli* across the 17 antibiotics. Corresponding results were *K. pneumoniae* (78 - 90%), *P. mirabilis* (76 - 99%) and *P. aeruginosa* (46 - 84%). The semi-quantitative PCR test detects 5 bacterial pathogens in urine specimens and detects 47 antimicrobial resistance genes. The Acuitas Lighthouse Knowledgebase predicted phenotypic antibiotic resistance for 17 antibiotics based on 47 resistance genes.

CONCLUSIONS: PCR detection of pathogen levels and resistance genes using the AMR Gene Panel could provide useful information about patient infections. Analysis of the AMR Gene Panel results using the Acuitas Lighthouse Knowledgebase can potentially aid in the prediction of phenotypic resistance to antibiotics days ahead of AST. Early indication of molecular antibiotic resistance may improve antibiotic stewardship and patient outcomes.

Agreement (%) between phenotypic antibiotic susceptibility and genotypic prediction of susceptibility using 47 resistance genes

	E. coli (n=2643)	*K. pneumoniae* (n = 2027)	*P. aeruginosa* (n = 1550)	*P. mirabilis* (n = 1022)
Gentamicin	92	88	71	91
Tobramycin	91	90	79	85
Ciprofloxacin	94	78	84	NA
Levofloxacin	94	85	83	NA
Trimeth.Sulfa	89	88	NA	85
Ertapenem	93	83	NA	99
Imipenem	96	87	56	NA
Meropenem	97	87	62	99
Cefazolin	85	89	NA	76
Cefepime	89	89	54	90
Cefotaxime	89	87	NA	81
Ceftazidime	79	83	60	88
Ceftriaxone	89	89	NA	78
Ampicillin	94	NA	NA	86
Piperacillin	94	86	64	89
Aztreonam	84	87	46	89

Species Identification

ID Assay	Isolates Correctly Identified	Expected Cross-Activity
E. coli	88 of 88 (100%)	some *Shigella* spp.
E. faecalis	65 of 65 (100%)	
K. pneumoniae/variicola	87 of 88 (99%)	
P. aeruginosa	91 of 91 (100%)	
P. mirabilis/vulgaris	84 of 85 (99%)	

Test Protocol
2.5 hours

Automated extraction of bacterial DNA from urine

Real-time multiplex PCR followed by prediction of antibiotic resistance in Lighthouse

- *E. coli*
- *E. faecalis*
- *K. pneumoniae*
- *P. mirabilis*
- *P. aeruginosa*