Theoretical Antimicrobial Selection Based on Precision Metagenomics Compared with Standard Urine Culture/Susceptibility: A Reliability and Inter-Rater Agreement Feasibility Analysis

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BACKGROUND

- Antimicrobial management of Urinary Tract Infections (UTI) is typically empiric or guided by culture and phenotypic antimicrobial susceptibility testing (AST).[1]
- Limitations of culture could be addressed by novel approaches, such as next generation sequencing (NGS), which provides concurrent quantitative detection of uropathogens and qualitative detection of antimicrobial resistance markers directly from urine samples. [2,3]
- Results of sequencing-based testing are complex.
- The purpose of this feasibility study was to interrogate reliability and inter-rater agreement between 4 infectious disease-trained physicians based on retrospective review of laboratory findings from a historical sample cohort.

METHODS

- De-identified remnant clinical urine samples with predicate culture + AST results (BD Phoenix) were previously analyzed with a Research Use Only (RUO) targeted NGS workflow (UPIP: Urinary Pathogen ID/AMR Panel with Explify analysis; Illumina) under a research protocol.[2]

- NGS results were not shared with the treating providers.
- Paired results from 25 samples were presented to 4 raters in randomized order and standard format (below).
- Raters independently assessed if and how the analytes detected by each method would have biased hypothetical result interpretation if found in the urine sample of a 40-year-old female with no allergies, no past medical history, and no recent medications.

Culture + AST

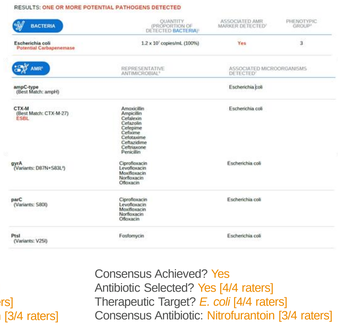
E. coli 50,000-100,000 CFU/mL

Antibiotic	E coli ESBL MIC	Interp
Amikacin	≤4	S
Ampicillin	>16	R
Aztreonam	4	R
Cefazolin	>32	R
Cefepime	2	S
Cefoxitin	≤4	S
Ceftazidime	4	R
Ceftriaxone	>32	R
Cefuroxime	>16	R
Ciprofloxacin	>2	R
Gentamicin	2	S
Imipenem	≤0.25	S
Levofloxacin	>4	R
Meropenem	≤0.125	S
Nitrofurantoin	≤16	S
Tetracycline	≤1	S
Tobramycin	2	S
Trimeth/Sulfa	≤0.5/9.5	S
Ertapenem	≤0.125	S
Piperacillin/Tazo	≤2/4	S

S = SUSCEPTIBLE I = INTERMEDIATE R = RESISTANT

Consensus Achieved? Yes
Antibiotic Selected? Yes [4/4 raters]
Therapeutic Target? *E. coli* [4/4 raters]
Consensus Antibiotic: Nitrofurantoin [3/4 raters]

UPIP RUO Report (abridged)



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METHODS, continued

- Alignment of organism and AMR targets identified by each method with selected antimicrobials were evaluated by an infectious disease-trained stewardship pharmacist.
- Consensus was defined as simple majority, i.e. agreement between ≥ 3 raters.
- The reliability of NGS to classify samples in a manner consistent with the reference method (culture + AST) was estimated by simple agreement. Inter-rater agreement was estimated using the irr package in R.

RESULTS

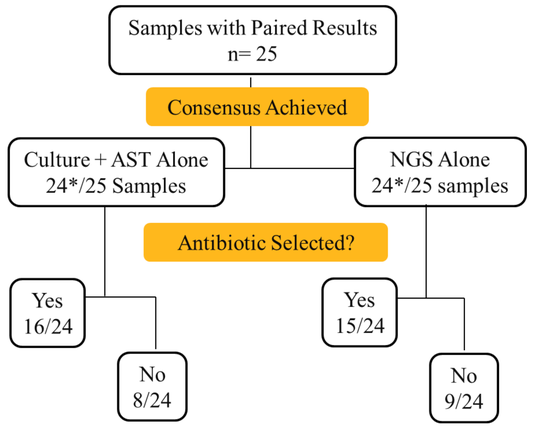

Figure 1: Consensus on Result Interpretation was Achieved for Most Samples, for Both NGS and Culture + AST Results.

* The NGS result and culture +AST result for which consensus was not achieved were from the same sample. Both methods identified MRSA at moderate abundance in this urine sample.

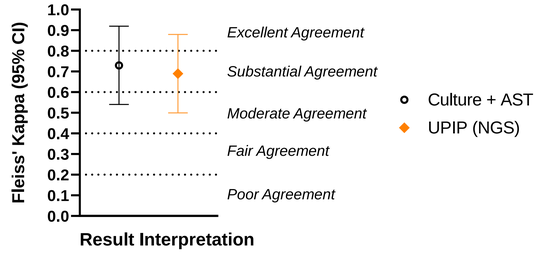

Figure 2: Inter-Rater Agreement is Comparable for Hypothetical Action Based on NGS vs Culture + AST Results.

RESULTS, continued

Table 1: Sample Observations Associated with Inter-Rater Disagreements

Observation	# Samples (% Cohort)
Agreement (NGS vs Culture) in the Direction of Consensus Action	
Antibiotic Selection Favored by Both Methods: uropathogens detected at moderate or high abundance	14/24 (58%)
No Antibiotic Selected for Either Method: culture-negative (n=6); low abundance mixed organisms (n=1)	7/24 (29%)
Disagreement (NGS vs Culture) in Direction of Consensus Action	
Antibiotic Selection Favored by NGS: UPIP detected bacterial uropathogens (culture only grew yeast)	1/24 (4%)
No Antibiotic Selected Based on NGS: UPIP detected multiple uropathogens; no single species predominant (n=1); UPIP detected no organisms but culture grew low abundance S. agalactiae (n=1)	2/24 (8%)

Table 2: Sample Observations Associated with Change in Hypothetical Antimicrobial Selection

Change in Selection based on NGS Results (Reference: Culture)	
Consensus Antimicrobial Identified by Both Methods	Notes
No Antibiotic Selected for Either Method	7/17
Same Antibiotic Selection Favored by Both Methods	5/17
Different Antibiotics Favored Based on NGS	
Trimethoprim-Sulfamethoxazole to Nitrofurantoin	1/17
Trimethoprim-Sulfamethoxazole to Ciprofloxacin	1/17
Trimethoprim-Sulfamethoxazole to Carbapenem	1/17
Ciprofloxacin to Nitrofurantoin	1/17
Antibiotic Selection Favored by NGS	
No antibiotic (culture) to Amoxicillin (NGS)	1/17
Consensus Not Achieved by ≥1 Method	
No Change in Primary Therapeutic Target(s)	5/7
Different Therapeutic Target Based on NGS	
UPIP detected no organisms	1/7
UPIP detected different coliform bacillus and additional uropathogen	1/7

DISCUSSION AND CONCLUSIONS

- Interpretation of urine culture results can be subjective and routine management of UTI is highly variable; 1 in 2 affected women may receive inappropriate antimicrobial therapy.[4]
- This study evaluated the inter-rater agreement and reliability of an RUO NGS-based assay with standardized bioinformatic analysis for the detection of uropathogens and AMR markers from urine, with standard urine culture as a comparator.
- In this pilot study, the level of agreement between raters for the interpretation of quantitative pathogen detection and qualitative pathogen characterization results was high and was comparable between an RUO NGS test and a standard culture-based test.
- Selection of a relevant antibiotic was no more variable based on raters' review of results of NGS vs standard methods.
- This pilot study had several limitations: small sample size, the participating providers do not all routinely see patients for UTI in their practice, and evaluation of intra-rater variability over time or "learning effects" of provider training was out of scope.
- Overall, these findings support the continued investigation of NGS-based testing as an adjunct method in settings where urine culture falls short. The establishment of evidence-based reporting and interpretation standards will be important for the future evaluation of NGS-based tests in clinical research studies to maintain consistency across multiple investigators and sites.

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