

# Precision Metagenomics for Broad Detection of Genitourinary Pathogens and Associated Antimicrobial Resistance Markers

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## Background

Urinary tract infections (UTIs) affect ~150 million people worldwide annually with estimated cost in the US of \$2.8 billion. More than 1 million patients suffer from recurrent or difficult-to-treat UTI annually in the US alone. Urine culture is designed only for the detection of the most common uropathogens and requires viable bacteria. In contrast, target-enriched next-generation sequencing (Precision Metagenomics, PM) can detect, quantify, and genotypically characterize hundreds of common and rare pathogens and thousands of antimicrobial resistance markers in a single test. We demonstrated analytical performance of a commercially available PM test (Urinary Pathogen ID/AMR Panel, UPIP, IDbyDNA) targeting 135 bacterial, 35 viral, 14 fungal and 7 protozoal pathogens plus >2,000 antimicrobial resistance (AMR) markers within 24 hours compared to automated urine culture.

## Methods

After conventional urine culture with or without antimicrobial susceptibility testing was performed, remnant urine samples were spiked with internal control (Bacteriophage T7, Microbiologics), DNA extracted (Quick-DNA™ Urine Kit, ZymoBionics), sequencing libraries prepared and target-enriched in triplex with UPIP and sequenced on the NextSeq platform (Illumina) to a depth of 1M reads/sample. Sequencing data was analyzed with the automated Explify Software Platform

## Results

Of 585 urine samples, 555 (95%) were positive for one or more potential genitourinary pathogens targeted by UPIP. The most common uropathogens detected by both PM and culture were *Escherichia coli*, Enterococcal species, *Klebsiella pneumoniae*, Staphylococcal species, *Pseudomonas aeruginosa* and *Proteus mirabilis*. Positive agreement for these most common uropathogens was 98% and UPIP detected additional positives in 101 samples (36%). Overall, 518 urines (88.5%) had at least one AMR marker detected by UPIP (Figure 1). AMR markers associated with resistance to commonly prescribed empiric antibiotics (amoxicillin and other beta-lactams, trimethoprim/sulfamethoxazole, fluoroquinolones) were prevalent (Table 1). The most frequently detected beta-lactamase gene families were ampC-type (22.7%), CfxA (14.0%), TEM (11.1%), SHV (9.4%) and OXA (6.0%).

## Conclusions

UPIP demonstrated high percent positive agreement with urine culture, detection of common uropathogens missed by culture, and profiling of AMR markers conferring resistance to widely used antibiotics. Precision Metagenomics provides a powerful addition to the diagnostic toolkit for recurrent or difficult-to-treat UTI.

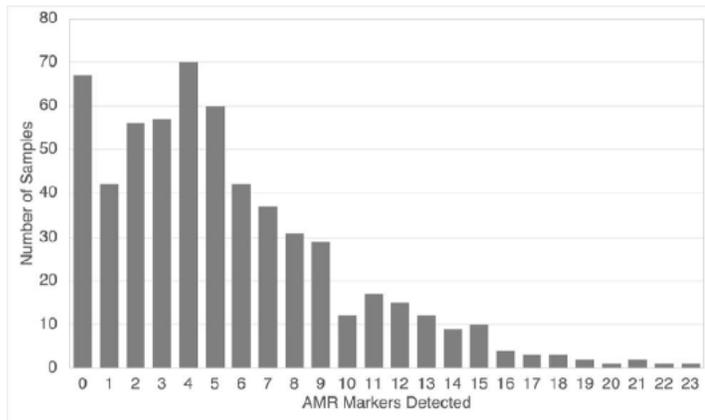
Keyword 1: Next-Generation Sequencing

Keyword 2: Urinary Tract Infection

Keyword 3: Antimicrobial Resistance

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Number of Samples by # AMR Markers Detected



Frequency of Most Common AMR Marker Families

AMR Gene Family	Count	Percent
Tetracycline-resistant ribosomal protection protein	317	54.2%
Erm 23S ribosomal RNA methyltransferase	225	38.5%
Major facilitator superfamily (MFS) antibiotic efflux pump	216	36.9%
Resistance-nodulation-cell division (RND) antibiotic efflux pump	205	35.0%
Trimethoprim resistant dihydrofolate reductase dfr	193	33.0%
ABC-F ATP-binding cassette ribosomal protection protein	159	27.2%
AmpC-type beta-lactamase	133	22.7%
General Bacterial Porin with reduced permeability to beta-lactams	130	22.2%
Sulfonamide resistant sul	119	20.3%
Antibiotic-resistant GlpT	114	19.5%

**Conflicts of Interest:**

Do you have any conflicts of interest to declare? - I have no potential conflict of interest to report.  
Other support - All authors were employees of IDbyDNA at the time of this work.