Uropathogen Detection by Precision Metagenomics in Culture-Positive, Culture-Negative, and Volunteer Urine

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Background
Urinary tract infections (UTIs) are among the most common bacterial infections. Urine culture is often ordered, but its utility in guiding therapy is complicated by turnaround time and poor sensitivity for less common uropathogens, anaerobic and slow-growing microorganisms under standard conditions, with missed detections in up to 50% of symptomatic women. Quantitative molecular detection of uropathogens can be achieved directly from urine through next-generation sequencing (NGS), but interpretation requires clinical correlation data and improved understanding of the urobiome. We demonstrated differential microbial detection and load in remnant clinical samples and urine from asymptomatic volunteers of both genders using a commercially available precision metagenomics (PM) test targeting 135 bacterial, 35 viral, 14 fungal and 7 protozoal pathogens plus >2,000 antimicrobial resistance (AMR) markers.

Methods
Urine culture was performed using routine methods. Urine samples leftover after urine culture ("culture remnant samples", n=196) and from asymptomatic volunteers ("volunteer samples", n=63) were spiked with internal control (Bacteriophage T7, Microbiologics), DNA extracted (Quick-DNA Urine Kit, ZymoBiomics), sequencing libraries prepared and target-enriched with the Urinary Pathogen ID/AMR Panel (UPIP, IDbyDNA), and sequenced on the NextSeq platform (Illumina) to a target depth of 3M reads/library. Sequencing data were analyzed with the automated Explify Software Platform.

Results
The median microbial load of common uropathogens (copies/mL) was significantly higher in culture-positive remnant samples than in volunteer samples ($p=0.0012$, Mann-Whitney test) and in culture-positive vs. culture-negative remnant samples ($p<0.0001$, Mann-Whitney test) (Figure 1). Enterococci and Enterobacterales dominated detections in clinical samples; in contrast, Gram-positive flora predominated in the asymptomatic cohort. Potentially uropathogenic Gram-negative bacteria were detected in only 4 (6.3%) volunteer samples (Figure 2). AMR marker detection was infrequent in the asymptomatic cohort (12/63) and associated with concurrent detection of >1 bacterial microorganism in most (9/12) samples.

Conclusions
UPIP provides a powerful tool for comprehensive detection, quantification, and AMR profiling of genitourinary pathogens. These data support the feasibility of empirically deriving informative quantitative thresholds. Future prospective studies will focus on validating thresholds and evaluating the positive and negative predictive values of PM.

Keyword 1: Next-Generation Sequencing
Keyword 2: Urinary Tract Infection
Keyword 3: Asymptomatic Bacteriuria

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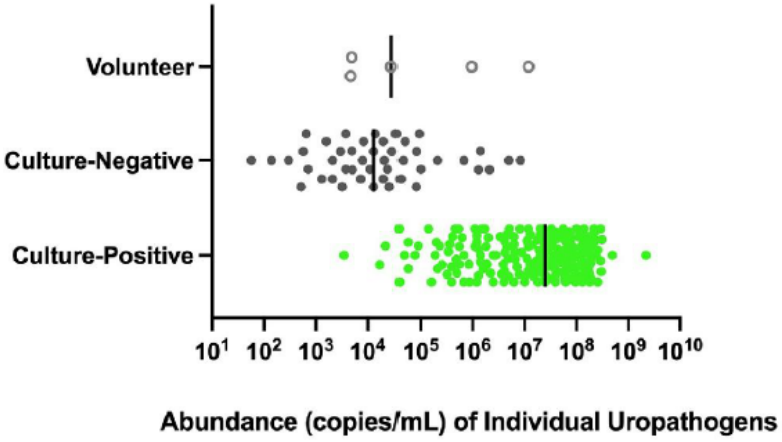
Median and Distribution of Uropathogen Load



Summary of Most Frequently Detected Bacteria

	# Unique Detections	% Samples
Asymptomatic (n=63)		
Staphylococcus epidermidis	6	10%
Corynebacterium spp	6	10%
Propionimicrobium lymphophilum	5	8%
Enterococcus faecalis	2	3%
Escherichia coli	2	3%
Enterobacter cloacae complex	1	2%
No microorganism detected	42	67%
Clinical, Culture-Positive (n=100)		
Escherichia coli	47	47%
Enterococcus spp.	45	45%
Enterobacter spp.	36	36%
Corynebacterium spp	35	35%
Klebsiella pneumoniae	10	10%
Staphylococcus aureus	3	3%
Staphylococcus saprophyticus	3	3%
Pseudomonas aeruginosa	3	3%
Proteus spp.	3	3%
No microorganism detected	1	1%
Clinical, Culture-Negative (n=96 samples)		
Prevotella spp	31	32%
Enterococcus spp.	30	31%
Aerococcus spp	18	19%
Corynebacterium spp	17	18%
Escherichia coli	11	11%
Veillonella spp	2	2%
No microorganism detected	23	24%

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.

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