Development of a Culture-Free Diagnostic for Urosepsis Leveraging Whole Genome Sequencing and Machine Learning

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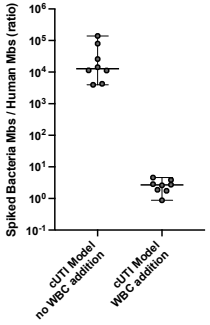
Introduction

Complicated urinary tract infections (cUTIs) can be life-threatening and occur most often in hospitalized patients. There are 2.8 million cases of cUTIs in the U.S. each year, with over 20% of cases progressing to urosepsis, causing nearly 150,000 deaths annually. Effective clinical management of cUTIs requires rapid identification (ID) of causative pathogens and reliable antibiotic susceptibility tests. Urine culture, the current gold-standard, exhausts vital turn-around time from specimen collection to actionable information for treatment. We developed **DZD-UroSeq** to address the need for a culture-free diagnostic, leveraging ultra-high enrichment (UHE) of pathogen DNA, whole-genome sequencing (WGS), and machine learning to deliver high resolution species ID with antimicrobial resistance and susceptibility profiling from patient urine.

DZD-UroSeq is a novel method of species agnostic bacterial enrichment and isolation direct from patient urine, followed by WGS on an Oxford Nanopore Technologies sequencing device for significantly reduced turn around time. WGS data is analyzed with Keynome® ID, our algorithm for species pathogen identification in mixed human-pathogen samples, and Keynome® *g*-AST (genomic antibiotic susceptibility testing), our machine learning algorithm for determining AST from genomic sequences to accurately predict resistance. After our first phase of DZD-UroSeq assay validation we demonstrate comprehensive capabilities for whole genome recovery, species identification, and AST determination direct from urine, with an end-to-end turnaround time under 6 hours.

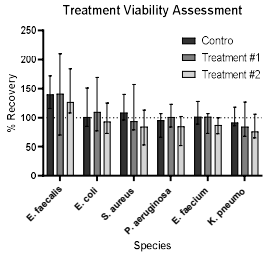
cUTI Model Development

A urine sample from a patient with a suspected cUTI is likely to be enriched in white blood cells (WBCs), a clinical sign of infection. To effectively model cUTI urine and determine whether WBCs compromise the efficiency of enrichment for bacterial DNA, we evaluated the recovery of bacterial DNA from mixed samples that contain both bacteria and a 5-fold excess of human WBCs (*i.e.*, 1x10^5 E. coli + 5x10^5 WBCs). We also note from the results post sample processing and whole genome sequencing that the presence of white blood cells significantly increases the amount of human DNA sequenced, suggesting that the bacterial enrichment strategy required further optimization for the DZD-UroSeq workflow.



UHE Optimization

The DZD-UroSeq workflow was further developed with the cUTI model described above. To ensure that our UHE strategy did not result in significant bacterial viability loss, we measured recovery of 6 key uropathogens with and without treatment strategies to facilitate human DNA removal. Results indicated that bacterial recovery in the form of viable cells are not considerably different between the treated samples and the control group.



Keynome® ID results

Bacterial Species	Donors tested	Tested Range (CFU/mL)	Keynome® ID Species Identification	Average Genome Coverage 1x
Escherichia coli	4	9.3x10^4 - 9.8x10^4	100%	98.26%
Enterococcus faecalis	4	1.0x10^5	100%	80.94%
Enterococcus faecium	4	1.1x10^5 - 1.2x10^5	100%	99.82%
Klebsiella pneumoniae	4	8.5x10^4 - 1.1x10^5	100%	93.10%
Pseudomonas aeruginosa	4	9.3x10^4	94%	45.05%
Proteus mirabilis	4	1.5x10^5	100%	99.04%
Staphylococcus aureus	4	9.5x10^4	100%	99.83%
*Total Accuracy**			99.1%	

*Keynome® ID accuracy across 112 samples (4 donors x 4 technical replicates per species) processed among 7 target pathogens.

DZD-UroSeq process was manually tested on 7 clinically relevant uropathogens. To demonstrate proof-of-concept, we selected 7 species that are representative of the pathogen diversity associated with cUTI and urosepsis: *E. coli*, *P. mirabilis, P. aeruginosa, K. pneumoniae, E. faecalis, E. faecium,* and *S. aureus*. These seven gram-negative and gram-positive pathogens of interest vary in morphology, membrane and surface structure and adhesion properties, and other genomic characteristics, such as genome GC content. We tested a total of 16 replicates of each of the 7 species (*n*=16 per species; 4 urine donors, 4 replicates each) through the DZD-UroSeq workflow, reaching a total accuracy of 99.1%

DZD-UroSeq Turnaround Time Tracking	
Assay steps	Time (minutes)
Bacteria enrichment	111
Bacteria lysis and amplification	63.5
DNA library prep	41
Sequencing	57
Computational Analysis (ID/g-AST)	69
Total	**341.5 (5.7 hours)**

Keynome® g-AST Results

Species	Drug	Accuracy
Enterococcus faecalis	ciprofloxacin	100%
Enterococcus faecalis	gentamicin (HL)	100%
Enterococcus faecalis	levofloxacin	100%
Enterococcus faecalis	streptomycin (HL)	100%
Enterococcus faecalis	tetracycline	100%
Enterococcus faecalis	vancomycin	100%
Escherichia coli	ampicillin	100%
Escherichia coli	aztreonam	100%
Escherichia coli	cefotaxime	100%
Escherichia coli	ceftriaxone	100%
Escherichia coli	ciprofloxacin	100%
Escherichia coli	gentamicin	100%
Escherichia coli	levofloxacin	100%
Escherichia coli	tobramycin	100%
Klebsiella pneumoniae	aztreonam	100%
Klebsiella pneumoniae	ceftazidime	100%
Klebsiella pneumoniae	ceftriaxone	100%
Klebsiella pneumoniae	gentamicin	100%
Klebsiella pneumoniae	imipenem	100%
Klebsiella pneumoniae	tobramycin	100%
Klebsiella pneumoniae	TMP/SMX	100%

DZD-UroSeq delivers accurate AST determination for three uropathogenic species. Using Keynome®*g*-AST we demonstrated high accuracy of antimicrobial susceptibility prediction models for 3 urosepsis species (21 bug/drug combinations). Our ongoing work is focused on further model development for a broad panel of bug/drug combinations that account for ≥90% of all cUTI infections.

DZD-UroSeq Turnaround Time

DZD-UroSeq turn-around time is less than six hours. Because the time between sample collection and data-driven diagnostic results is a key differentiator between the standard-of-care and DZD-UroSeq, we measured the amount of time each step in our workflow takes, demonstrating the potential of a sample-to-answer turn-around time in less than 6 hours, well within the first round (8–12 hours) of empiric therapy.

Conclusions

- We demonstrate the development of a test cUTI model system to develop and optimize our DZD-UroSeq diagnostic process.
- Our Keynome® ID pipeline allows accurate identification of 7 target uropathogens in urine samples.
- Keynome® *g*-AST pipeline resulted in accurate predictions of antibiotic resistance/susceptibility profiles for 21 bug/drug combinations.
- DZD-UroSeq turnaround time allows for timely decision making and antibiotic stewardship within the first round of empiric therapy.

Acknowledgments

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