

Metabolomics as a Tool for Rapid Urinary Tract Infection Diagnosis

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Summary:

Urinary tract infections caused by *Escherichia coli* are increasingly resistant to first-line antibiotics, yet current culture-based diagnostics require 2 to 4 days, driving empiric broad-spectrum therapy that accelerates resistance spread. Diagnostic gaps persist, with low-level and fastidious organisms frequently missed by standard methods, while rapid susceptibility testing remains unavailable in most settings. To address this challenge, we developed an untargeted and targeted metabolomics approach to identify urinary metabolite signatures specific to UTI-causing bacteria. Using 75 *E. coli* strains grown in artificial urine medium and analysed by complementary liquid chromatography–mass spectrometry methods, we identified differential metabolite production patterns including increased succinate and depleted mannitol in bacterial cultures. These metabolomic signatures offer potential biomarkers for rapid bacterial identification independent of culture. This approach could enable faster, more specific UTI diagnostics to reduce inappropriate antibiotic use and combat antimicrobial resistance.

Keywords: untargeted metabolomics, targeted metabolomics, UTI metabolites, UTI rapid diagnostics

Introduction

Urinary tract pathogens are increasingly resistant to first-line antibiotics, while empiric broad-spectrum therapy drives further resistance spread [1]. Meanwhile, diagnostic gaps persist, with low-level and fastidious organisms missed by standard methods, and rapid susceptibility testing technologies remain unavailable and not specific in most settings [2]. In our current efforts to address this issue, we aimed at developing an untargeted metabolomics approach that would screen for urinary metabolites in the presence of infection due to different urinary tract bacteria. At a second time we aimed at targeting specific amine and short chain fatty acids to quantify them, to check bacterial growth behavior specific to UTI bacteria. Finally, this data, completed with genomic sequence could draw a bigger picture into identifying robust metabolite biomarkers specific to different UTI infections.

Untargeted and targeted metabolomics for UTI

Two untargeted complementary methods (normal phase hydrophilic interaction liquid

chromatography HILIC and reverse-phase C18) were used to analyze 75 *E. coli* strains after ensuring robust growth in an artificial urine medium. After metabolite extraction, samples were analyzed in a high-resolution mass spectrometer (Orbitrap Exploris 240). The complementarity of these methods assured the identification of different metabolites, polar and apolar present in the 75 strains. Artificial urine served as a controllable medium and as a baseline for matrix effects to avoid false identification due to contamination.

Results

Growth of the 75 *E. coli* strains in the artificial urine was comparable with several outliers (data not shown). The medium of artificial urine presents a constant stress to the bacterial growth with abundant salts and a highly saturated solution which could potentially destabilize the bacterial cells. However, given that the growth was steady and comparable throughout strains we continued the metabolomics extraction of the extracellular metabolites for untargeted and targeted metabolomics (Fig.1).

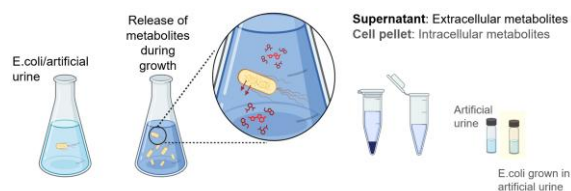


Fig. 1. Untargeted and targeted metabolomics of 75 *E. coli* strains grown in artificial urine for extracellular bacterial metabolites identification

Samples were extracted and polar metabolites were analyzed in HILIC conditions, where several total ion chromatograms were extracted and compared (Fig.2). In negative ionization HILIC conditions several different relative peak intensities were visible between blank (artificial urine) and grown *E. coli* in artificial urine (Fig.2). Succinate showed higher relative intensity in *E. coli* samples compared to artificial urine, potentially suggesting an overproduction due to bacterial growth. Conversely mannitol showed lower abundances in the *E. coli* samples suggesting that it was likely consumed by bacteria to ensure growth. Such differences in metabolite intensities need to be further confirmed with several standards to ensure a more accurate quantification. Nevertheless, this data suggests that following upon different changes of the bacterial metabolites one can possibly target those as specific to bacteria with the bigger aim of identifying bacterial infections based on metabolite production as a starting point.

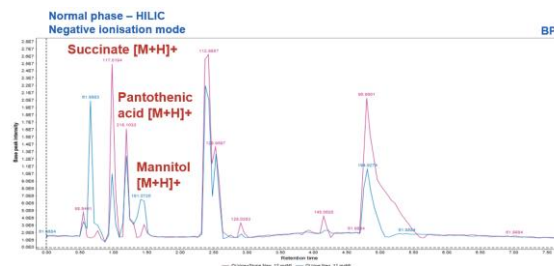


Fig. 2. Superimposed negative ionization chromatogram peaks of artificial urine (blank, blue) and *E. coli* grown in artificial urine (pink). BPI: baseline peak intensity

References

- [1] M. R. Timm, S. K. Russell, S. J. Hultgren, Urinary tract infections: pathogenesis, host susceptibility and emerging therapeutics, *Nature Reviews Microbiology* 23(2), 72–86 (2025); doi: 10.1038/s41579-024-01092-4
- [2] A. B. Bellankimath, S. Branders, I. Kegel et al., Metagenomic sequencing enables accurate pathogen and antimicrobial susceptibility profiling in complicated UTIs in approximately four hours, *Nature Communications* 17, 187 (2026); doi: 10.1038/s41467-025-66865-8