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RECEIVED 07 October 2024 ACCEPTED 15 November 2024 PUBLISHED 18 December 2024

#### CITATION

Chan CCY, Groves RA, Rydzak T and Lewis IA (2024) Metabolomics survey of uropathogenic bacteria in human urine. *Front. Microbiol.* 15:1507561. doi: 10.3389/fmicb.2024.1507561

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# Metabolomics survey of uropathogenic bacteria in human urine

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**Introduction:** Urinary tract infections (UTIs) are one of the most prevalent infections in North America and are caused by a diverse range of bacterial species. Although uropathogenesis has been studied extensively in the context of macromolecular interactions, the degree to which metabolism may contribute to infection is unclear. Currently, most of what is known about the metabolic capacity of uropathogens has been derived from genomics, genetic knockout studies or transcriptomic analyses. However, there are currently very little empirical data on the metabolic activity of uropathogens when grown in urine.

**Methods:** To address this gap, we conducted a systematic survey of the metabolic activities of eight of the most common uropathogenic bacterial species that collectively represent 99% of uncomplicated UTIs.

**Results:** Liquid chromatography-mass spectrometry (LC–MS) analyses of human urine cultures revealed that uropathogens have four distinct metabolic clades. We generalized these clades as serine consumers (*Escherichia coli, Klebsiella pneumoniae*, and *Proteus mirabilis*), glutamine consumers (*Pseudomonas aeruginosa*), amino acid abstainers (*Enterococcus faecalis* and *Streptococcus agalactiae*), and amino acid minimalists (*Staphylococcus aureus* and *Staphylococcus saprophyticus*). These metabolic classifications can be further subdivided on a species-to-species level.

**Discussion:** This survey provides a framework to understanding the metabolic activity of the diverse range of uropathogens and how these species use divergent metabolic strategies to occupy the same niche.

#### KEYWORDS

urinary tract infection, uropathogens, bacterial metabolism, metabolomics, liquid chromatography-mass spectrometry

# **1** Introduction

Urinary tract infections (UTIs) are one of the most common infections, affecting around 400 million people worldwide (Zeng et al., 2022). UTIs can be caused by a diverse range of microorganisms, but in an outpatient setting, eight bacterial species are responsible for 99% of infections (Flores-Mireles et al., 2015; Wagenlehner et al., 2020). This relatively small number of species is somewhat surprising given the significant diversity of species identified in human feces, the presumed source of uropathogens (Tannock, 1999; Segata et al., 2012). This suggests that selective forces are at play, and one contributing factor may be nutritional selection.

Most of what we know about UTIs comes from *Escherichia coli*, which is frequently used as the model pathogen in studies since it is responsible for around 75% of uncomplicated UTIs (Flores-Mireles et al., 2015; Wagenlehner et al., 2020). Information about uropathogenic *E. coli* metabolism comes from genomic mapping, genetic knockouts, or transcriptional studies (Chan and Lewis, 2022). The consensus in the literature is that

uropathogenic *E. coli* must take up a wide range of urinary amino acids to fuel central carbon metabolism (Alteri et al., 2009; Chan and Lewis, 2022). In particular, serine catabolism is essential for *E. coli* growth in urinary environments (Hull and Hull, 1997; Roesch et al., 2003; Anfora and Welch, 2006; Anfora et al., 2007). Uropathogenic *E. coli* has also been shown to salvage various nucleotides, presumably to support gene replication (Russo et al., 1996; Vejborg et al., 2012; Shaffer et al., 2017; Andersen-Civil et al., 2018; Ma et al., 2018). In addition, *E. coli* also relays on urinary ethanolamine as a nitrogen source (Sintsova et al., 2018; Dadswell et al., 2019).

Although the metabolic requirements for uropathogenic E. coli are now emerging, this is only one of the many uropathogenic species, and thus, represents an incomplete picture of the metabolic selective strategies that could contribute to infection. Other uropathogenic species have yet to be metabolically characterized, despite collectively accounting for the remaining 25% of infections (Flores-Mireles et al., 2015; Wagenlehner et al., 2020). These species include Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis, Streptococcus agalactiae (group B streptococcus), Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida spp. (Flores-Mireles et al., 2015; Wagenlehner et al., 2020). Of these rarer uropathogens, infections caused by P. mirabilis have been attracting an increasing degree of attention because of its ability to produce crystalline biofilms which leads to complicated catheter-associated UTIs (Norsworthy and Pearson, 2017; Gmiter and Kaca, 2022). Similar to E. coli, P. mirabilis was also found to preferentially catabolize serine in human urine (Brauer et al., 2019, 2022). However, the metabolic needs of all uropathogens outside of E. coli are poorly understood. Moreover, there have not been any in-depth investigations of the metabolic distinctions between different uropathogenic species.

Recent advances of high-resolution liquid chromatography-mass spectrometry (LC–MS) have radically expanded the complement of metabolic activities that can be tracked in routine studies. Moreover, our group has recently developed a specialized metabolic boundary flux analysis strategy used to characterize the metabolic phenotypes of microbes based on the rates of nutrient uptake and waste product excretion (Lewis, 2024). These metabolic profiles provide a convenient mechanism for making interspecific comparisons of metabolic activities and are useful for identifying metabolic phenotypes that distinguish closely related species (Rydzak et al., 2022). Herein, we harnessed this boundary flux analysis strategy to conduct a systematic metabolomics survey of the eight most common uropathogenic species to provide a systematic review of the nutritional strategies used by these species when grown in human urine.

## 2 Materials and methods

# 2.1 Human urine collection and bacterial strains

Human urine was collected and pooled from five healthy adult donors (three females, two males) via a one-time collection, following institutional ethics board approval (REB19-0442). Immediately after collection, the pooled urine stock was filter sterilized, aliquoted, and stored at  $-20^{\circ}$ C. In preparation for experiments, aliquots of the

pooled urine were thawed at room temperature and centrifuged  $(4,200 \times g, 15 \text{ min})$ . This pooled stock was used for all experiments presented in this study.

Bacterial species most frequently responsible for uncomplicated UTIs—each contributing to at least 1% of infections in outpatient setting—were selected for our metabolomics survey. A total of eight species were evaluated including four Gram-negative species (*E. coli, K. pneumoniae, P. aeruginosa,* and *P. mirabilis*) and four Gram-positive species (*E. faecalis, S. agalactiae, S. aureus,* and *S. saprophyticus*). For each species, six diverse strains were selected from different isolation origins, including laboratory controls and clinical isolates from UTIs and other infections (Supplementary Table S1).

# 2.2 *In vitro* bacterial growth in pooled human urine

A total of 48 bacterial strains were grown in vitro in pooled, sterile-filtered human urine. All bacterial strains were first cultured overnight in Mueller Hinton (MH) medium (BD Difco, Mississauga, ON, Canada) at 37°C with shaking (180 rpm). Overnight cultures were centrifuged (4,200  $\times$  g, 15 min) and washed twice with phosphate buffered saline (PBS) to remove contaminating metabolites. Strains were then inoculated at an  $OD_{600}$  (optical density at 600 nm) of 0.1 in pooled human urine (Mutiskan<sup>TM</sup> GO Microplate Spectrophotometer, Thermo Scientific), producing 200 µL bacterial cultures on a 96-well plate. Thus, all urine cultures were normalized to approximately  $1 \times 10^5$  colony-forming units per milliliter (CFU/mL) at the start of the experiment. The urine cultures were incubated at 37°C with 5%  $\mathrm{CO}_2$  and 21%  $\mathrm{O}_2$  (Heracell^TM VIOS 250i Tri-Gas Incubator, Thermo Scientific) for 4 h and the OD<sub>600</sub> of the cultures were monitored at 30-min intervals (Mutiskan<sup>™</sup> GO Microplate Spectrophotometer, Thermo Scientific). Using the same growth protocol, an eight-hour growth course with the same strains was conducted in a separate experiment (Supplementary Figure S1).

Extracellular samples of the cultures were taken at the start and end of the growth course, and then diluted in HPLC (highperformance liquid chromatography)-grade methanol at a 1:1 ratio and frozen at  $-80^{\circ}$ C. Additionally, at the end of the growth course, the cultures were plated on MH agar plates (except for *S. agalactiae* cultures that were plated on Columbia blood agar instead) at  $10^{5}$  and  $10^{6}$  dilutions. The agar plates were incubated overnight at  $37^{\circ}$ C, and then the resultant colonies were counted.

# 2.3 Liquid chromatography-mass spectrometry analysis

LC–MS analysis was conducted at the Calgary Metabolomics Research Facility. In preparation for metabolomics analysis, extracellular samples collected from urine cultures were thawed at room temperature, centrifuged (4,200 × g, 10 min), and diluted 10-fold with 50% HPLC-grade methanol before undergoing LC–MS analysis. Our LC–MS methods has been described in detail elsewhere (Groves et al., 2022; Rydzak et al., 2022). Briefly, metabolites in the samples were resolved with a Syncronis<sup>TM</sup> Hydrophilic Interaction Liquid Chromatography (HILIC) Column (2.1 mm × 100 mm × 1.7  $\mu$ m, Thermo Scientific) on a Vanquish<sup>TM</sup> Ultra-High-Performance Liquid Chromatography (UHPLC) platform (Thermo Scientific) using a 15-min two-solvent gradient method (20 mM ammonium formate at pH 3.0 in HPLC-grade water and HPLC-grade acetonitrile with 0.1% formic acid). Mass spectrometry data were acquired in full scan in both positive and negative modes on a Q Exactive<sup>™</sup> HF Hybrid Quadrupole-Orbitrap<sup>™</sup> Mass Spectrometer (Thermo Scientific). All acquired LC–MS data were analyzed with El-MAVEN v0.12.0 software (Agrawal et al., 2019).

Metabolites were assigned using an in-house library of chemical standards on the basis of exact mass and chromatographic retention time. Each assignment was verified using a commercial standard from Sigma-Aldrich. Chemical standards were also used to prepare a concentration calibration reference mixture for absolute quantification, and metabolite concentrations were computed following established methods (Ponce et al., 2024). Metabolic activity exhibited by each species was identified based on the consumption or production of metabolites. These were defined as threshold differences relative to uninoculated urine controls. Significantly consumed or secreted metabolites were identified using Welch's two-sample *t*-test followed by Bonferroni correction ( $\alpha = 0.00625$ ).

# 2.4 Characterization of a cohort of clinical polymicrobial urine samples

To establish the frequency of which certain species co-segregated in polymicrobial UTIs, we acquired the species information of a cohort of patient urine samples sent to Alberta Precision Laboratories over the course of a week. The demographics of UTIs from the Calgary health region has been previously described (Laupland et al., 2007; Gregson et al., 2021). A total of 1,462 urine samples were identified as growth-positive (>10<sup>7</sup> CFU/L), which is defined as a UTI based on clinical guidelines (Alberta Precision Laboratories, 2021). Within this cohort, only 81 samples contained two causative species, and these causative organisms were identified to the species level. To determine if the observed species distribution was expected by chance, the top three pairs of species underwent individual chi-square goodnessof-fit tests.

# **3** Results

#### 3.1 Metabolic composition of human urine

The chemical composition of human urine can vary considerably between individuals and over time (Rasmussen et al., 2011; Bouatra et al., 2013; Virgiliou et al., 2021). Thus, to minimize variability, urine samples were pooled into one stock and this stock was used for all experiments. As expected, metabolomics analysis of our pooled urine stock revealed that it contained high concentrations of amino acids and relatively low concentrations of carbohydrates and nucleic acids (Supplementary Table S2). The concentrations of individual metabolites in our pooled urine stock were within the normal ranges reported in the literature, with a few exceptions including isoleucine, leucine, cystine, 4-hydroxyproline, uracil, and succinate, which were approximately 10 µM more or less abundant than published normal ranges (Bouatra et al., 2013).

# 3.2 Metabolomics analysis and identification of metabolic clades

For each species, six distinct isolates were seeded at  $1 \times 10^5$  CFU/ mL in pooled human urine and grown for 4 h, reaching  $3.0 \times 10^6$  to  $3.6 \times 10^8$  CFU/mL (Supplementary Figure S2). The metabolic profiles observed in the cultures over time were captured by LC-MS using our previously established strategy (Rydzak et al., 2022; Lewis, 2024) (Figure 1). The metabolite abundances in the cultures were compared to those in the urine controls to determine changes in metabolite concentration and identify metabolites that were consumed or produced (Supplementary Figure S3; Supplementary Table S3). As expected, many of the metabolic phenotypes that we observed were consistent with those reported in the literature. For example, E. coli is well-known for utilizing glucose as its preferred carbon source and secreting succinate (Holms, 1996; Thakker et al., 2012), a phenotype which we also observed when it was grown in urine (Supplementary Table S3). Similarly, tyramine production is wellcharacterized in E. faecalis (Connil et al., 2002; Perez et al., 2015), and we also observe its production in E. faecalis urine cultures (Supplementary Table S3).

Our metabolomics approach enabled us to capture a more comprehensive transect of metabolites across our panel of microorganisms. As reported previously (Rydzak et al., 2022), bacterial strains of the same species showed consistent metabolic phenotypes (Figure 1). However, each species exhibited a unique metabolic profile of consumed versus secreted metabolites (Supplementary Figure S3). Multivariate analyses of the metabolic profiles observed in these uropathogens showed that although individual species-related clusters occurred, broader phenotypes were also observed across groups of species (Figure 2). As expected, the most significant metabolic differences were between Gram-negative species (E. coli, K. pneumoniae, and P. mirabilis) and Gram-positive species (E. faecalis, S. agalactiae, S. aureus, and S. saprophyticus) with P. aeruginosa being a notable exception that occupies a unique metabolic space distinct from all other uropathogens (Figure 2A). Based on similarities and differences in metabolic profiles, uropathogens were generalized into four metabolic clades: (1) serine consumers, (2) glutamine consumers, (3) amino acid abstainers, and (4) amino acid minimalists (Figure 2B). Each of these labels are proxies to describe a complex suite of metabolic phenotypes that generally clusters these uropathogenic species.

The cluster of "serine consumers" included E. coli, K. pneumoniae, and P. mirabilis. All of these Gram-negative Enterobacterales species consumed between 90 and 98% of urinary serine (Figure 3A). Within the serine consumers, P. mirabilis metabolically deviated from E. coli and K. pneumoniae, which aligned with the separation observed in the genetic phylogenetic tree (Figure 2). A few distinct metabolic phenotypes drove this separation, including thymine production and arginine consumption (Supplementary Figure S4). P. aeruginosa is an outlier with regards to the Gram-negative group as it failed to consume serine (Figure 3A). P. aeruginosa was also the only species not to consume glucose, instead consuming higher concentrations glutamine (95%) and hypoxanthine (97%) (Figure 3A). Due to its distinct profile, P. aeruginosa was categorized into a metabolic clade of its own as a "glutamine consumer," despite displaying a few shared phenotypes with P. mirabilis, such as high arginine and succinate consumption (Supplementary Figure S4).



The clusters of "amino acid abstainers" and "amino acid minimalists" were exclusively made up of Gram-positive organisms that consumed very few amino acids compared to Gram-negative species (Figure 3B). Of these, the amino acid abstainers (*E. faecalis* and *S. agalactiae*) only consumed between 1 and 2 amino acids out of the 19 amino acids detected, while other species across our cohort consumed between 8 and 14 (Figure 3B). Whereas, the amino acid minimalists (*S. saprophyticus* and *S. aureus*) consumed a broader range of amino acids than amino acid abstainers but at a lower quantity than the Gram-negative bacteria (Figure 3B).

Most of the bacterial species evaluated consumed 319.50– 664.10  $\mu$ M of the total amino acids present (Supplementary Table S3). The exceptions were the amino acid abstainers (*E. faecalis* and *S. agalactiae*), which only consumed 22.04  $\mu$ M and 6.24  $\mu$ M of amino acids, respectively (Supplementary Table S3). Nearly all observed amino acids (16/19 amino acids) were consumed by at least one bacterial species (Supplementary Table S3). Urinary arginine, asparagine, glutamine, isoleucine, methionine, serine, threonine, tryptophan, tyrosine, and valine were consumed by over half of all species assessed ( $\geq$ 4/8 species) (Supplementary Table S3). Neither glycine nor histidine were consumed by any species, despite being the top two most abundant amino acids in urine (Supplementary Table S3). In contrast, the next two most abundant amino acids, serine and glutamine, were consumed by six out of eight species (Supplementary Table S3). As noted previously, *K. pneumoniae*, *P. mirabilis*, and *E. coli* consumed higher quantities of serine (90–98%), while *P. aeruginosa* consumed higher quantities of glutamine (95%) relative to other species (Supplementary Table S3).

As expected, urinary glucose was consumed by seven out of the eight species, each consuming between 84 and 96% (Supplementary Table S3). *P. aeruginosa* was the only exception, which did not significantly consume glucose (Figure 3A). Another prevalent carbon source in urine was succinate, and between 86 and 99% was consumed by *P. aeruginosa* and *P. mirabilis* (Supplementary Figure S4; Supplementary Table S3). Interestingly, succinate was also produced by *E. coli* (1003.44  $\mu$ M), *S. saprophyticus* (47.74  $\mu$ M), and *S. aureus* (184.60  $\mu$ M) (Supplementary Figure S4; Supplementary Table S3).

Nearly all available urinary nucleosides (9/11 nucleosides) were taken up by most species ( $\geq$ 5/8 species), including adenosine, cytidine, deoxyadenosine, deoxyguanosine, guanosine, inosine,



FIGURE 2

Clustering of the metabolic profiles of uropathogens. (A) Clustering of uropathogens according to their genomic and metabolic phylogenies. Genomic phylogenies were derived from phyloT v2 (https://phylot.biobyte.de/) and were compared to the metabolic classifications derived from hierarchical clustering. (B) Clusters of metabolic phenotypes from principal component analysis (PCA). Score plot distributions of eight species were shown with the metabolic clades illustrated by circles. PCA-biplot distribution of important metabolites that are contributing to the segregation of the scores. Red metabolites denote phenotypes that were identified as significant after Bonferroni correction in univariate analyses.

thymidine, and uridine (Supplementary Table S3). In contrast, nucleobases were rarely consumed (2/6 nucleobases), except for hypoxanthine and xanthine (Supplementary Table S3). A few species secreted nucleic acids, and the most pronounced example of this was the unique thymine production phenotype observed in *P. mirabilis* (203.62  $\mu$ M) (Supplementary Figure S4; Supplementary Table S3). This metabolic phenotype appeared to be specific to *P. mirabilis* urine culture, as it was not observed when it was grown in other growth media such as MH broth (Supplementary Figure S5).

# 3.3 Prevalence of species in polymicrobial UTIs

A significant fraction of UTIs are polymicrobial (Gaston et al., 2021). Given the distinct metabolic clades observed in this study,



Prominent metabolic features that defined each metabolic clade. Metabolic phenotypes distinctive to the metabolic clades are displayed. **(A)** The concentrations of serine, glucose, glutamine, and hypoxanthine differentiated Gram-negative species into SC and GC. **(B)** The total amino acid concentration consumed and the number of different amino acids consumed differentiated Gram-positive species into AAA and AAM. Statistical significance is denoted by an asterisk (\*, two-sample t-test with Bonferroni correction  $\alpha = 0.00625$ ). Error bars represent one standard deviation. SC, serine consumers; GC, glutamine consumers; AAA, amino acids abstainers; AAM, amino acids minimalists.

we speculated that polymicrobial infections may be more prevalent in species with complimentary metabolic niches. To test this hypothesis, we collected the species breakdown of all polymicrobial infections observed in the Calgary health zone over a one-week period, and in this cohort, 5.5% were identified as polymicrobial (81/1,462). The top three most frequent pairings in this cohort were *E. coli* and *E. faecalis* (32.1%), *E. coli* and *K. pneumoniae* (8.6%), and *E. coli* and *Streptococcus* 



*viridans* (8.6%) (Supplementary Table S4). To better understand if the co-segregation of these species followed the expected distribution based on their individual prevalence, we conducted chi-square goodness-of-fit tests on the top three pairs (Supplementary Table S4). The *E. coli* and *E. faecalis* pair (p = 0.0026) and the *E. coli* and *S. viridans* pair (p = 0.014) occurred significantly more frequently than expected, while the prevalence of the *E. coli* and *K. pneumoniae* pair (p = 0.48) followed the distribution expected by chance (Supplementary Table S4).

### 4 Discussion

The primary goal of this study was to provide empirical evidence for the metabolic preferences of common uropathogens when grown in human urine. To the best of our knowledge, this is the first study that provides the foundational metabolic data needed to understand the diverse nutritional strategies used by these uropathogens. Our metabolomics data showed that the eight most common uropathogens followed four nutritional strategies which we characterized as serine consumers, glutamine consumers, amino acid abstainers, and amino acid minimalists (Figure 2B). These categorical designations are not exhaustive lists of the metabolic differences between the clades but serves as convenient descriptors of the most distinctive metabolic features in each group (Figure 3). The nutritional strategies generally unique to each metabolic clade are illustrated in Figure 4.

As reported in similar studies (Rydzak et al., 2022), our metabolomics analysis showed that the metabolic features within

strains of the same species were minimal relative to interspecific differences (Figure 1). The metabolic distinctions between species tended to co-segregate according to phylogeny (Figure 2A). The most dramatic metabolic distinction observed in this dataset were those that separated Gram-negative (*E. coli, K. pneumoniae, P. mirabilis,* and *P. aeruginosa*) and Gram-positive species (*E. faecalis, S. agalactiae, S. aureus,* and *S. saprophyticus*) (Figure 2B). Gram-negative species are evidently well-adapted to using a wide range of urinary amino acids, while the Gram-positive species appear to use little to no amino acid catabolism to support their growth (Figure 3B). Few studies have performed metabolomics analysis on clinical urine samples, but one study quantified amines and amino acids in clinical urine specimens and reported lower concentrations of serine and asparagine in UTI patients with *E. coli* compared to healthy individuals (Puebla-Barragan et al., 2020), which aligned with our findings.

Divergence in nutritional strategies may provide a selective advantage for uropathogens, especially when competing against fast-growing organisms such as *E. coli*. This may be relevant in the context of polymicrobial infections, wherein two or more species co-exist in the urinary tract (Azevedo et al., 2017; De Vos et al., 2017; Gaston et al., 2021). Our analysis of the species breakdown of polymicrobial infections in the Calgary health zone appears to support this hypothesis. We observed significant co-segregation of *E. coli* and *E. faecalis* (p = 0.0026, chi-square goodness-of-fit test) (Supplementary Table S4) and this pairing was the most prevalent in other documented cohorts as well (Cardone et al., 2018; Folaranmi et al., 2022; Nye et al., 2024). This significant co-segregation may occur because *E. coli* and *E. faecalis* occupy compliment metabolic niches with *E. coli* as a serine consumer and

*E. faecalis* as an amino acid abstainer. *E. coli* depends heavily on amino acid catabolism, consuming 14 out of 19 amino acids totaling to 643.43  $\mu$ M, whereas *E. faecalis* does not, consuming only two amino acids totaling to 22.04  $\mu$ M (Figure 3B; Supplementary Table S3). This metabolic distinction may allow *E. faecalis* to establish infections in the urinary tract without competing in the same metabolic niche as *E. coli*. There is evidence to support this hypothesis. Previous studies co-cultured *E. coli* and *E. faecalis* in artificial urine medium and found that co-culturing either had a neutral or positive effect on bacterial viability (Galván et al., 2016; Nye et al., 2024).

One interesting application of our dataset is as a tool to help refine artificial urine. Artificial urine is used routinely to create a more physiological environment for *in vitro* studies of urinary tract diseases (Grases et al., 1996; Jacobs et al., 2001; Jones et al., 2007; Ma et al., 2018; Juarez et al., 2020). However, we note that the metabolic compositions in current artificial urine formulations are neither defined nor reflective of the metabolite abundances observed in human urine (Brooks and Keevil, 1997; Chutipongtanate and Thongboonkerd, 2010; Ipe et al., 2016; Sarigul et al., 2019). This is problematic because many of the metabolites we observed as preferred nutrients (outlined in Supplementary Table S2) are absent or not chemically defined in established formulas. Therefore, supplementing artificial urine with these key metabolites can potentially help bring these formulas closer to physiological relevance.

In summary, we conducted a systematic metabolomics survey of the eight most common uropathogens and identified four distinct metabolic clades that differentiate uropathogens. We observed significant differences in nutritional styles that may help explain the co-segregation of species in polymicrobial infections and pave the way for creating a more precisely defined artificial urine media. Overall, this dataset provides a foundation for future metabolomics analyses of uropathogens and clearly demonstrated that the nutritional strategies of uropathogens should be considered in future UTI studies.

#### Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

#### **Ethics statement**

The studies involving humans were approved by Conjoint Health Research Ethics Board (REB19-0442). The studies were conducted in accordance with the local legislation and institutional requirements. Informed consent was waived for participants in this study. Research test results are delinked from patient demographic information.

# Author contributions

CC: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. RG: Data curation, Methodology, Writing – review & editing. TR: Methodology, Writing – review & editing. IL: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

# Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was funded by the Natural Sciences and Engineering Research Council (NSERC; DG 04547). Data were acquired at the Calgary Metabolomics Research Facility (CMRF) at the Alberta Centre for Advanced Diagnostics (ACAD), which is supported by PrairiesCan (000022734). CCYC is funded by a Canada Graduate Scholarship from the Canadian Institute of Health Research. IAL is supported by a UCalgary Research Excellence Chair. This work was made possible in part by a research collaboration agreement with Thermo Fisher Scientific.

# Acknowledgments

Special thanks to Dr. Daniel B. Gregson for providing us with data on the prevalence of different species in polymicrobial UTIs. We also thank Dr. Stephanie Bishop for providing the chemical standard mixes for quantitative LC–MS analysis, and Keir Pittman for critical review and editorial assistance in preparing this manuscript.

# **Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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### Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2024.1507561/ full#supplementary-material

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