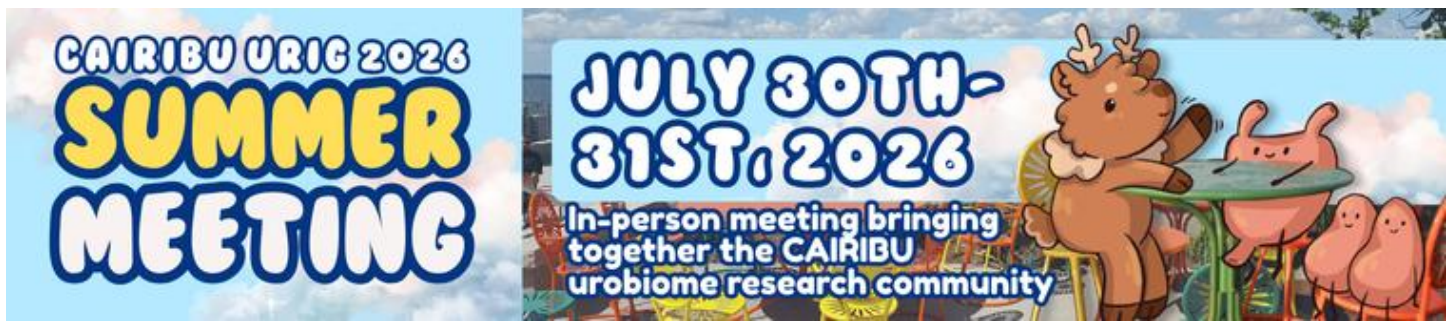




A LOGIC REGRESSION FRAMEWORK FOR FUTURE DISEASE PREDICTION WITH LONGITUDINAL UROBIOME DATA (poster #1)

Megan Marie Martinez¹, Niru Shanbhag¹, Gregory Matthews², Catherine Putonti¹

¹Bioinformatics Program, Loyola University Chicago; ²Department of Mathematics and Statistics, Loyola University Chicago

INTRODUCTION AND OBJECTIVE: Microbiome composition has been explored as possible indicators of health or disease, where machine-learning models are often employed for disease prediction. However, these prediction models can struggle with interpretability, either by only identifying associated taxa in isolation or obscuring the relationships between taxa. Using logic regression, a statistical method that creates logical combinations of binary predictors, bacterial relationships can be modeled in an interpretable manner. The objective of this study is to create a logic regression framework to analyze longitudinal urobiome data, elucidating taxon abundance thresholds that can be used to predict future infection or disease.

METHODS: A literature search via PubMed was performed identifying longitudinal urobiome studies including metagenomic relative abundance data for both asymptomatic and symptomatic participants. For this analysis, symptoms included urinary tract infections (UTIs) or anything associated with UTIs (recurrent UTIs, UTI-like symptoms, etc.). As missingness and uneven-sampling dates is a common challenge in longitudinal data, data imputation was performed using multivariate imputation by chained equations (MICE) via predictive means matching. Five imputed datasets were created and pooled for use in model training and testing. The top 10 most abundant taxa of each study will be included as predictors for the model. Taxa will be binarized as present/absent for use in logic regression based on threshold vector Φ , with optimal Φ values found using simulated annealing via rolling origin cross validation. Once an optimal Φ is found, a holdout set will be binarized and a final logic regression model will be fit. This process will be repeated for different tree and leaf combinations for predictors, and the combination with the lowest log loss will be selected.

RESULTS: Here we will present the model developed. It has thus far been tested on mock data and single-time point urinary microbiome data. With its application to urinary microbiome data, "usual suspects" of UTIs were identified. However, the temporal element should elucidate how far in advance these taxa can be used as an indicator of symptoms and what other taxa may contribute to future symptoms, in our case UTIs, even if they are not necessarily the causative uropathogen.

CONCLUSIONS: Using logic regression to forecast future symptoms and disease allows for a more detailed exploration of the dynamic microbial relationships present in the urobiome. Identifying the presence of particular taxa can provide the foundation for future molecular work to explore the mechanisms of interaction between these taxa.

HEALTH RELEVANCE: Determining bacterial taxa associated with symptoms and disease allows for earlier intervention or prevention, improving the quality of life of patients.

DNA FROM BACTERIAL BIOFILMS AND KIDNEY STONE FORMATION (poster #2)

Rena Yang^{a,b,c,d}, Rushil Pingalie^{f,g,h}, William C. Schmidt^{a,b,c,d}, Consuelo Huidor^f, Erik Luijtene^{g,h,i}, Gerard C. L. Wong^{a,b,c,d}, Kymora B. Scotland^f

^aDepartment of Bioengineering, University of California; ^bDepartment of Chemistry and Biochemistry, University of California; ^cDepartment of Microbiology, Immunology, & Molecular Genetics, University of California; ^dCalifornia NanoSystems Institute, University of California; ^eDepartment of Materials Science and Engineering, Northwestern University; ^fDepartment of Molecular, Cell, and Developmental Biology, University of California; ^gDepartment of Engineering Sciences and Applied Mathematics, Northwestern University; ^hDepartment of Physics and Astronomy, Northwestern University; ⁱDepartment of Chemistry, Northwestern University; ^jDepartment of Urology, University of California

INTRODUCTION AND OBJECTIVE: Calcium oxalate (CaOx) stones, which constitute more than 70% of all kidney stones, have traditionally been thought to be non-infectious. In our recent work, we have shown that kidney stones obtained from human patients, including clinically 'non-infectious' stones, possess an unexpected internal structure with intercalating bacterial extracellular DNA (eDNA) and polycrystalline mineral layers, even in patients without underlying urinary tract infections (UTIs).¹ In this work, we investigate the role that bacterial eDNA plays in kidney stone formation.

METHODS: Through a combination of fluorescence and electron microscopy, we identified voids in kidney stones with embedded bacteria. Fluorescent staining experiments identified bacterial biofilm components in stones, including areas preferentially enriched with eDNA. *In vitro* crystallization experiments and molecular dynamics simulations were conducted to examine CaOx crystal growth in the presence and absence of bacterial eDNA.

RESULTS: Our *in vitro* experiments and molecular simulations demonstrate that bacterial eDNA can act as a molecular template which promotes CaOx nucleation by spatially organizing Ca ions. Cleaving the eDNA into shorter fragments attenuates this effect.

CONCLUSIONS: Bacterial eDNA may enhance bacterial survival in the urinary environment while simultaneously promoting the formation of CaOx kidney stones. We show that stone formation can be related to the need for calcium (Ca) homeostasis in slow-growing bacteria. (Intracellular concentrations are at the micromolar range, while extracellular concentrations are in the mM range.) Pumping out the Ca via ATPases is not practical at low metabolic states. However, the high charge density of released eDNA serves as an anionic matrix that sequesters and organizes a large number of Ca ions via Manning condensation and drastically lowers the effective Ca concentration near the bacteria, creating a local environment more suited to bacterial survival.

HEALTH RELEVANCE: This conceptual framework for nephrolithiasis can in principle explain why kidney stones recur in some patients, and the triggering of UTIs or even sepsis after lithotripsy.

¹Schmidt WC, et al. Intercalated bacterial biofilms are intrinsic internal components of calcium-based kidney stones. *PNAS*. 2026 Feb 3;123(5):e2517066123. doi: 10.1073/pnas.2517066123. Epub 2026 Jan 26. PMID: 41587311; PMCID: PMC12867757.

URINARY MICROBIOME DIFFERENCES IN POSTMENOPAUSAL WOMEN WITH TYPE II DIABETES AND URINARY TRACT INFECTIONS (poster #3)

Ujjaini Basu¹, Michael L. Neugent¹, Sara B. Papp², Ceejay N. Saenz¹, Sara Begole², Jessica de Araujo Paula², Philippe E. Zimmern², Nicole J. De Nisco^{1,2}

¹Department of Biological Sciences, University of Texas at Dallas, Richardson;

²Department of Urology, University of Texas Southwestern Medical Center, Dallas

INTRODUCTION AND OBJECTIVE: Postmenopausal (PM) women with Type 2 Diabetes (T2DM) experience high rates of severe recurrent urinary tract infections (rUTIs), yet the biological drivers remain poorly understood. While a *Lactobacillus*-dominated female urinary microbiome (FUM) typically protects against infection, we hypothesize that the FUM of women with T2DM may be altered by diabetes-associated metabolic and immune dysregulation thus contributing to the UTI susceptibility in this population. This study aims to test this hypothesis by defining FUM alterations associated with T2DM both at baseline and during rUTI.

METHODS: Following IRB approval and informed consent, urine was collected from 100 PM women passing exclusion criteria for uncomplicated rUTI representing four groups: No UTI history/T2DM(-), rUTI(+)/T2DM(-), No UTI history/T2DM(+), UTI(+)/T2DM(+) (N=25 each). Genomic DNA was extracted using the Zymo DNA/RNA kit for shotgun metagenomic sequencing on an Illumina NextSeq2000. ReadQC was used for quality control and host-read removal. We used MetaPhlan 4.2 for read-based taxonomic assignment and ANCOM-BC for differential enrichment analysis.

RESULTS: The FUM of PM women with T2DM and no UTI history was significantly depleted of protective *Lactobacillus* species and enriched in BV-associated taxa like *Fannyhessea* compared to non-diabetic controls (Figure 1A). Fewer *Escherichia coli* UTIs were observed in T2DM women than non-diabetic women. Differential enrichment analysis revealed that fastidious FUM species not typically associated with UTI were enriched in women with T2DM experiencing active rUTI (Figure 1B).

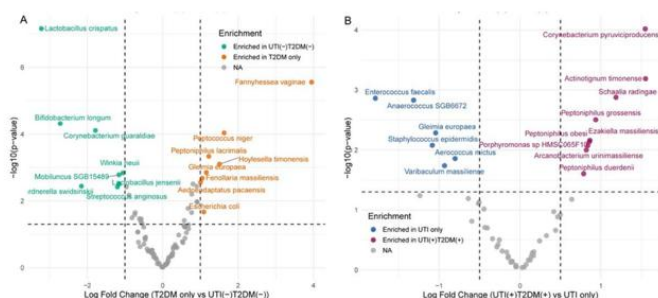


Figure 1: Volcano plot showing differentially enriched microbial taxa comparing T2DM-only patients to healthy controls (A) and UTI+/T2DM+ individuals to a UTI-only cohort (B). Points depict enrichment status and are labeled for significant species, with dashed lines representing the thresholds for log fold change and statistical significance.

CONCLUSIONS: The FUM of PM women with T2DM both with and without UTI differ significantly from their non-diabetic counterparts. T2DM urobiomes are depleted of beneficial *Lactobacillus* species, signifying transition to a dysbiotic state more susceptible to UTI. During infection, women with T2DM exhibited differences in uropathogen profiles and were characterized by an enrichment of fastidious species that may not be detected by standard urine culture (SUC).

HEALTH RELEVANCE: This research identifies differences in the T2DM FUM that may contribute to rUTI susceptibility and uncovers critical diagnostic gap: SUC may fail to detect the unique, fastidious uropathogens prevalent in diabetic women, leading to diagnostic blind spots and higher recurrence rates. These results advocate for personalized UTI management to improve clinical outcomes for PM women with T2DM.

ISOLATION AND CHARACTERIZATION OF ENTEROCOCCUS FAECALIS BACTERIOPHAGES FROM SEWAGE (poster #4)

Zoe R. Berge¹, Alan J. Wolfe¹, Catherine Putonti¹

¹Loyola University Chicago

INTRODUCTION AND OBJECTIVE: While *Enterococcus faecalis* is a commensal of the human gastrointestinal tract, it is an opportunistic pathogen of other anatomical sites. It is increasingly recognized as a uropathogen associated with complicated and recurrent urinary tract infections (rUTIs). Antibiotic resistance within *E. faecalis* is an important clinical issue in rUTIs, thus driving the need for alternative strategies like phage therapy. Because *E. faecalis* is frequently found in the gastrointestinal tracts of humans and many animals, wastewater provides an ideal reservoir of novel *E. faecalis* phages with potential therapeutic use. The objective of this study is to isolate and characterize viable wastewater phages capable of infecting urinary *E. faecalis* isolates.

METHODS: Wastewater samples collected from the Lake County Wastewater Treatment Plant (Libertyville, IL) were centrifuged to pellet cells and debris. The supernatant was then filtered through a 0.22 rUTIs µm filter to further remove remaining bacteria. Phage enrichment was performed by incubating the filtrate with multiple urinary *E. faecalis* isolates from our collection. Phages were isolated and purified using three rounds of the standard double-layer agar plaque assay.

RESULTS: Phages isolated and plated on our urinary *E. faecalis* strains represent a variety of plaque morphologies, likely indicating diversity of *E. faecalis* infecting phages from the samples. Further analysis is needed to determine host range specificity against the six distinct enterococcal strains. Genomic sequencing and transmission electron microscopy is in progress and will be instrumental in characterizing the *E. faecalis* infecting phages isolated.

CONCLUSIONS: Wastewater serves as a rich reservoir for viable *E. faecalis* phages. The isolated phages possess strong lytic capability against target, clinical strains. These phages thus have a potential for therapeutic use.

HEALTH RELEVANCE: Antibiotic resistance is a growing global concern, associated with treatment failure and recurrent infections, including rUTIs. Identifying bacteriophages that could be utilized in phage therapy expands our understanding of alternate treatments and broadens the available phages to be considered for therapy.

MULTI-OMIC INTEGRATION REVEALS MICROBIAL AND METABOLOMIC SIGNATURES OF LACTOBACILLUS-PREDOMINATED UROBIOMES (poster #5)

Nathan L. Boyer¹, Juliette Kudrick², Lindsey Burnett³, Nazema Y. Siddiqui⁴, Lisa Karstens^{1,2,5}

¹Division of Informatics, Clinical Epidemiology, & Translational Data Science, Oregon Health & Science University; ²Division of Oncological Science, Knight Cancer Institute;

³Department of Obstetrics, Gynecology and Reproductive Sciences, University of California San Diego; ⁴Department of Obstetrics & Gynecology, Duke University School of Medicine; ⁵Division of Urogynecology, Oregon Health & Science University

Introduction and Objective: Postmenopausal women experience increased rates of lower urinary tract disorders, potentially linked to dysbiosis of the bladder microbiome (urobiome). In premenopausal women, commensal *Lactobacillus* species typically predominate and are associated with urinary tract health. However, the molecular mechanisms underlying this protection, and species-specific contributions of *Lactobacilli*, remain unclear. We applied a multi-omic approach to identify molecular signatures associated with *Lactobacillus*-predominant (L-pred) versus *Lactobacillus*-minority (L-min) urobiomes.

Methods: Catheterized urine samples from 166 women (Duke IRB Pro00083917; OHSU IRB 10729) underwent DNA extraction, 16S rRNA V4 sequencing (MiSeq), species-level *Lactobacillus* identification via LoopSeq synthetic long-read sequencing, and untargeted metabolomics using ultra-high-performance liquid

chromatography–Orbitrap mass spectrometry. Urobiomes were classified as L-pred (>50% *Lactobacillus*) or L-min (<1%). Multi-omic features were identified using sparse generalized canonical correlation analysis (sgCCA; MintTea v1.1.1) and validated with DESeq2 (v1.48.2), including species-level comparisons of *L. crispatus*, *L. iners*, and *L. gasseri*.

Results: Among 138 participants with complete data, 27 were L-pred and 23 L-min. One multi-omic module, comprising 5 metabolites and 28 bacterial genera, was associated with L-pred. Two unannotated metabolites showed >8-fold increases in L-pred (padj < 0.05). In contrast, all significant taxonomic features—including *Klebsiella*, *Pseudomonas*, and *Streptococcus*—were decreased (1–4 log fold; padj < 0.05). Species-level analyses revealed distinct patterns: *L. gasseri*-predominant urobiomes were positively associated with *Bifidobacterium*, *Alloscardovia*, and *Brevundimonas*, while *L. iners*-predominant urobiomes were associated with the metabolite DMAPT (padj < 0.05).

Conclusions: Integrative multi-omic analysis identified system-level features linked to urobiome composition and potential protective mechanisms. *Lactobacillus*-predominant urobiomes exhibit distinct molecular signatures consistent with protection, with species-specific differences suggesting heterogeneous mechanisms.

STRAIN-LEVEL PHENOTYPIC AND TRANSCRIPTOMIC DIVERSITY IN CLINICALLY ISOLATED PROTEUS MIRABILIS (poster #6)

Megan A Cline¹, Jyothi Guruprasad¹, Ashley Zheng¹, Robert F Potter^{2,3}, Aayushi Uberoi¹

¹Division of Laboratory and Genomic Medicine, Department of Pathology and Immunology, Washington University in Saint Louis School of Medicine; ²Infectious Disease Diagnostics Laboratory, Children's Hospital of Pennsylvania; ³Department of Pathology & Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania

INTRODUCTION AND OBJECTIVE: *Proteus mirabilis* (*Pm*) is a leading cause of complicated urinary tract infections and is frequently isolated from acute and chronic skin wounds. Despite its prevalence in wound infections, little is known about how strain-level variation influences virulence and host responses. The objective of this study was to characterize phenotypic diversity among clinical wound-associated *Pm* isolates and determine how strain-specific differences influence epithelial responses.

METHODS: We analyzed 48 whole-genome-sequenced *Pm* isolates recovered from wounds and tissue abscesses across three medical centers. Strains were phenotyped for swarming, biofilm formation, and adherence to keratinocytes. Swarming was quantified by measuring colony expansion on blood agar plates over 6 hours. Biofilm formation was assessed via a crystal violet assay following 24-hour static growth. Adherence was determined by infecting HaCaT keratinocytes (MOI ~10) for 2 hours, quantifying adherent bacteria by CFU enumeration. Relationships among phenotypes were assessed using Spearman correlation analysis and principal component analysis (PCA) on scaled phenotypic data. Representative strains across the phenotypic landscape were selected to infect HaCaTs, followed by bulk RNA sequencing. Differential gene expression was identified using DESeq2.

RESULTS: *Pm* isolates exhibited large variation in swarming, biofilm formation, and epithelial adherence. Correlation analyses revealed weak relationships among individual phenotypes, indicating that these virulence-associated traits vary independently across strains. PCA identified a low-dimensional behavioral landscape, with PC1 and PC2 explaining 39.5% and 31.9% of phenotypic variance, respectively. Swarming and biofilm formation contributed most strongly to PC1, whereas epithelial adherence was the primary contributor to PC2, separating strains along ecological and host-interaction axes. RNA sequencing demonstrated strain-specific epithelial responses, including differential induction of inflammatory and wound-associated genes.

CONCLUSIONS: Clinical wound-associated *Pm* isolates display extensive phenotypic and transcriptomic heterogeneity despite close phylogeny. Organizing strains within a behavioral landscape revealed major axes of variation that distinguish ecological and host-interaction

phenotypes. These findings suggest that strain-level diversity is an important determinant of epithelial responses during wound infection.

HEALTH RELEVANCE: Understanding how strain-specific traits influence host responses may improve our ability to identify clinically relevant virulence mechanisms and develop more effective strategies for managing chronic wound infections.

STRAIN-LEVEL DISTINCTION OF UROBIOME MEMBERS USING GENOMIC DATA (poster #7)

Maria E. Steiling¹ and Catherine Putonti^{1,2}

¹Loyola University Chicago Bioinformatics Program; ²Loyola University Chicago Biology Department

INTRODUCTION AND OBJECTIVE: Average Nucleotide Identity (ANI) has become the gold standard for determining an isolate's genus and species. While there are several ANI tools available, nuanced differences distinguish the algorithms employed. Generally speaking, ANI computes the percent identity shared between two genomes. Large scale genomic analyses made recommendations for genus and species ANI value thresholds. However, an ANI threshold for distinguishing related from unrelated strains within a species has not yet been established. This distinction is critical for epidemiological investigations. In the context of the urobiome, establishing this distinction could provide important insights into the provenance of UTI-associated strains. This objective of this study is to first conduct a survey of available ANI tools and then, using these tools, examine the thresholds previously posited as they pertain to urobiome species. As a result of this work, we aim to be able to provide recommendations for thresholds for distinguishing between the same and related strains of bacteria.

METHODS: Publicly available sequencing reads of isolates of the genera *Lactobacillus*, *Klebsiella*, *Proteus*, and *Streptococcus* were retrieved from the SRA database and examined using the tool FastANI. ANI values were compared between unrelated and identical isolates. Genomes are assumed to be unrelated if submitted on different dates and/or from different institutions. Identical genomes were produced via subsampling raw sequencing reads and generating multiple assemblies of the same sequenced isolate.

RESULTS: ANI distributions generated for identical isolates are much more similar and greater than unrelated isolates, as expected. The difference between unrelated and related isolates can thus inform the threshold for a given species. Distributions, and the resulting threshold recommendations, are expected to be specific to the species being examined. We have selected bacteria that represent different doubling times and growth conditions.

CONCLUSIONS: This project will update the standards for classification and provide resources for the greater urobiome community.

HEALTH RELEVANCE: Bacterial classification is the cornerstone of treatment and study design. This study seeks to provide updated guidance on the accurate classification of bacterial strains.

COMPLEMENTARY IN VIVO AND IN VITRO APPROACHES TO INVESTIGATE UROBIOME METABOLISM OF ENVIRONMENTAL CHEMICALS AND SUBSEQUENT EFFECTS ON BLADDER HEALTH (poster #8)

Audra L. Crouch¹, Brandon McKenna^{1,3}, Andrew McGlynn^{1,3}, David Jegede², Madison Griffin^{4,5}, Sheryl S. Justice⁶, Craig Burd⁷, Ahmad El Hellani², Vanessa L. Hale¹

¹Veterinary Preventive Medicine, The Ohio State University; ²Environmental Health Sciences, College of Public Health, The Ohio State University; ³Department of Veterinary Medicine, The Ohio State University; ⁴College of Medicine, The Ohio State University; ⁵Student Urinary Tract Program in Education and Research, Nationwide Children's Hospital; ⁶College of Nursing, The Ohio State University; ⁷Department of Molecular Genetics, The Ohio State University

INTRODUCTION AND OBJECTIVE: Environmental chemical exposures play a role in many health conditions including bladder

diseases such as bladder cancer (BC). While some chemicals are known to have carcinogenic effects on host cells, the interaction between environmental chemicals and host-associated microbiomes, and how these interactions potentially impact the host has received little attention. Polycyclic aromatic hydrocarbons (PAHs) are byproducts of incomplete combustion and are prevalent in charred foods, automobile exhaust, and cigarette smoke, with diet and tobacco as primary exposure routes, and have been linked to BC. Benzo[a]pyrene (BaP), a prototypical PAH, can be metabolized into secondary metabolites that induce carcinogenic, mutagenic, and pro-inflammatory responses in host tissues. While human cells are known to metabolize BaP, it remains unclear whether bladder-associated microbes can metabolize BaP into reactive intermediates and contribute to carcinogenic or mutagenic effects in host tissues.

METHODS: To address these gaps, we developed an integrated *in vivo* and *in vitro* workflow. *In vivo*, mice (N =36) received daily BaP, peanut oil (vehicle), or water for four weeks, then we collected bladders. Urine and fecal samples were collected for metagenomics. In parallel, we conducted *in vitro* studies of *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans* co-cultured with HBLAK urothelial cells and BaP using a trans-well system allowed BaP diffusion without direct contact between microbes and urothelial cells. After co-culture experiments, HBLAK cells were assessed for cytotoxicity and proliferation.

RESULTS: BaP exposure increased HBLAK cytotoxicity in a dose-dependent manner, with greater effects in the presence of *P. aeruginosa* (5.5% vs. 17%). Although urothelial cytotoxicity increased in the presence of *P. aeruginosa* and BaP, cytotoxic levels are still considered low. *In vivo*, we found treatment-specific shifts in microbial communities, between vendors, host tissues, and taxa capable of PAH metabolism.

CONCLUSIONS: This framework enables controlled investigation of microbe–chemical–host interactions and demonstrates that microbial presence enhances BaP-associated cytotoxic and estrogenic effects. These findings support a role for microbiota in modulating PAH metabolism and host response.

HEALTH RELEVANCE: Microbial metabolism of environmental chemicals such as BaP may represent an underrecognized mechanism influencing bladder health and disease risk.

DISCOVERY OF *ESCHERICHIA COLI* PLASMID-DEPENDENT BACTERIOPHAGES IN THE FEMALE URINARY TRACT (poster #9)

Hillary E. Dapsauski¹, Catherine Putonti¹

¹Loyola University Chicago

INTRODUCTION AND OBJECTIVE: Plasmid-dependent phages (PDPs) represent a largely unexplored category of bacteriophages that rely on plasmid-encoded structures, such as plasmid-encoded pili within the host, to facilitate infection. One bacterial species that has been studied within the urobiome is *Escherichia coli* (*E. coli*), which is frequently associated with UTIs, antibiotic resistance, and carries a diverse set of plasmids. The overarching problem addressed by this study is bridging the knowledge gap regarding whether PDPs target urinary *E. coli* plasmids and what their diversity may be within the urobiome.

METHODS: Publicly available urinary *E. coli* genomes were analyzed to predict plasmid sequences using Deeplasid, a machine learning tool for plasmid identification in genome assemblies. Predicted plasmids were further characterized using agglomerative clustering to assess sequence similarity, incompatibility typing tools, plasmid isolation and sequencing to validate Deeplasid predictions, and plasmid PCR typing to confirm incompatibility classifications for a subset of these genomes within our collection. A modified Phage Discovery by Co-Culturing (Phage DisCo) protocol was then used to isolate phages from wastewater and healthy female urinary samples

capable of infecting plasmid-containing urinary *E. coli* strains. The samples were then sequenced and analyzed.

RESULTS: A total of 641 *E. coli* plasmid sequences were predicted from the 171 urinary genomes examined, revealing substantial abundance of plasmids within urinary *E. coli* strains. Furthermore, 231 unique plasmid sequences were identified, indicative of a diverse plasmidome representative of 10 different incompatibility types. Phages infectious of urinary *E. coli* strains were identified both from urine samples and wastewater samples using the Phage DisCo protocol. Genome sequencing of these phages provides insight into putative PDPs from both environments.

CONCLUSIONS: These findings provide evidence of extensive plasmid diversity among urinary *E. coli* isolates and establish a framework for investigating PDP-host interactions within the urobiome. Characterization of PDPs within the healthy female urobiome may improve understanding of microbial dynamics and support development of alternative approaches for targeting antibiotic-resistant bacteria.

HEALTH RELEVANCE: This work expands PDP research beyond environmental niches into human-associated microbial communities. Development of a robust toolkit for identifying and characterizing PDPs may support innovative therapeutic strategies for antibiotic resistant UTIs and improve understanding of phage-plasmid interactions relevant to human health.

CHARACTERIZING PUTATIVE GLYCOSYL HYDROLASES FROM TWO URINARY BACTERIA (poster #10)

Stella M. Davis¹, Monique Bittencourt Dias², Jean Philippe F. Gourdi^{1,2}

¹Biochemistry & Molecular Biology Program, Lewis & Clark College; ²Chemistry Department, Lewis & Clark College

INTRODUCTION AND OBJECTIVE: Since the recent confirmation that the bladder hosts a diverse array of microbes collectively known as the urinary microbiome (urobiome), researchers have sought to understand its homeostasis. Uromodulin (UMOD), a glycoprotein found at high concentrations in the bladder, plays a role in host defense by binding bacteria to form aggregates that can be expelled from hosts via micturition. Nevertheless, many bacteria from the urobiome possess glycosyl hydrolases (GHs) that can digest UMOD's glycans and utilize them as an energy source. The aim of this study is to clone, express, and functionally characterize putative GHs previously identified through bioinformatic analysis. Specifically, we aimed to kinetically characterize two putative GHs from the GH38 family (alpha-mannosidase activity), from *Bifidobacterium longum* (GH38 BL) and *Lactobacillus crispatus* (GH38 LC).

METHODS: GH38 constructs obtained through a collaboration with BioF-Great (University of Georgia, Athens) were expressed in T7 *Escherichia coli* cells and purified using standard His-tag purification methods. To characterize the GH38s' kinetic efficiency and alpha-mannosidase activity, a fluorescence dequenching assay with 4-Methylumbelliferyl α -D-mannopyranoside (4MUM), was used. Both GH38s were incubated with increasing concentrations of 4MUM in a 96-well black microplate. Release of 4-Methylumbelliferone (excitation 360nm, emission 450nm) was measured with Tecan Safire 2 microplate reader. Michaelis-Menten parameters for each GH38 construct were obtained using the Lineweaver-Burk model.

RESULTS: The activity assay showed increased fluorescence as 4MUM concentration increased. GH38 BL has a K_M value of 144.4 μ M and a V_{max} of 0.16 μ M min⁻¹. GH38 LC has a K_M value of 4484 μ M and a V_{max} of 0.11 μ M min⁻¹ (Figure 1).

CONCLUSIONS: GH38 BL had been previously established to have alpha-mannosidase activity, but we were able to further characterize it by determining its Michaelis-Menten parameters. The previously uncharacterized GH38 LC exhibited alpha-mannosidase activity but showed a higher K_M and lower V_{max} than GH38 BL, indicating lower efficiency. Overall, confirming two GH38s' alpha-mannosidase

activity and establishing kinetic parameters for both bacterial species indicates their possible role in the metabolism of the mannose-containing glycans from UMOD.

HEALTH RELEVANCE: Identifying and characterizing putative GHs from bacteria in our urobiome is crucial in elucidating the metabolic pathways that maintain the health and diversity of our urinary microbiota. Understanding bladder homeostasis allows for medical advancements to treat imbalance-related disorders such as urinary tract infections and overactive bladder.

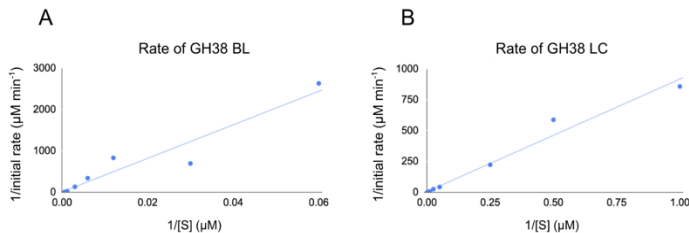


Figure 1. GH38 constructs from *B. longum* and *L. crispatus* exhibit alpha-mannosidase activity. Kinetic parameters of GH38 constructors were determined through a fluorescence dequenching assay that utilizes 4MUM, and the data was fit to the Lineweaver-Burk model. For both lines of best fit $R > .95$. The lines of best fit were used to calculate Michaelis-Menten values. GH38 BL has a K_m of 144.4 μM and a V_{max} of 0.16 $\mu\text{M min}^{-1}$, and GH38 LC has a K_m of 4484 μM and a V_{max} of 0.11 $\mu\text{M min}^{-1}$.

COMPARING SEQUENCING APPROACHES AND TEMPORAL DYNAMICS TO ELUCIDATE THE CLINICAL UTILITY OF THE PEDIATRIC UROBIOME (poster #11)

Keisha Harrison¹, Tatyana A. Sysoeva², Maryellen Kelly^{3,4}, Lisa Karstens^{5,6}

¹Division of Oncological Sciences, Knight Cancer Institute, Oregon Health Sciences University; ²Department of Biological Sciences, The University of Alabama in Huntsville; ³Healthcare of Women and Children, School of Nursing, Duke University; ⁴Department of Urology, Duke University; ⁵Division of Informatics, Clinical Epidemiology, & Translational Data Science, Oregon Health & Science University; ⁶Division of Urogynecology, Oregon Health & Science University.

INTRODUCTION: Urinary tract infections (UTIs) are the most common outpatient infection in the United States and represent one of the most serious bacterial infections encountered by pediatricians. Up to 50% of children that have experienced recurrent UTIs (rUTIs) have chronic health consequences. Despite this burden, the pediatric urobiome remains understudied, particularly in relation to its temporal stability, compositional differences between children with and without rUTIs and representative sampling practices.

METHODS: Two studies were conducted to investigate pediatric urobiome stability and methodological impacts on microbial detection (Duke IRB Pro00110530 and Pro00100031). In **Study 1**, six children provided voided urine samples on days 1–5, 14, 21, 28, and 60. The samples underwent 0.22 μm filtration to collect biomass and DNA was isolated and prepared for 16S rRNA V4 sequencing (MiSeq). In **Study 2**, 20 voided urine samples were collected from children - 10 with no UTI history and 10 with a history of rUTI. DNA was extracted following 0.22 μm filtration and sequenced using two methods: 16S rRNA V4 sequencing (MiSeq) and synthetic long-read 16S rRNA gene sequencing (LoopSeq).

RESULTS: Among the participants in Study 1, relative abundance profiles remained stable over time, with the predominant taxa within each individual persisting across multiple time points. Alpha diversity showed limited fluctuation, for example Shannon diversity varied less than 0.5 between the first and final collections, including in one participant who experienced an *E. coli* perturbation early in sampling. A linear mixed-effects model confirmed this quantitatively: subject identity accounted for 53–79% of variance across four alpha-diversity metrics (intraclass correlation: Shannon = 0.67; inverse Simpson = 0.53; observed ASVs = 0.73; Faith's phylogenetic diversity = 0.79), indicating a persistent per-individual urinary microbiome identity across the 60-day window. The predominant phyla were *Firmicutes* and *Bacteroidota*. In Study 2, *Actinobacteriota* were more abundant

in MiSeq data, while *Proteobacteria* appeared only in LoopSeq data. Across the methods, 86 genera were detected with both, 13 were unique to LoopSeq and 78 were unique to MiSeq; 34 of the MiSeq-unique genera represented potential environmental contaminants, reflecting the higher specificity and reduced contaminant amplification with LoopSeq.

CONCLUSIONS: The pediatric urobiome appears stable over two months within individuals, suggesting that cross sectional or limited sampling frequency is sufficient for urobiome studies. Sequencing methodology substantially influences microbial detection, with long-read approaches offering superior taxonomic resolution and lower amounts of environmental genera.

HEALTH RELEVANCE: These findings support the use of long-read 16S rRNA gene sequencing for distinguishing disease-state phenotypes or monitoring responses to therapeutic interventions.

PANGENOMIC AND PREDICTIVE METABOLOMIC CHARACTERIZATION OF CLINICAL UROGENITAL LACTOBACILLUS CRISPATUS ISOLATES TO IDENTIFY CANDIDATE LIVE BIOTHERAPEUTIC PRODUCT (LBP) STRAINS (poster #12)

S Ama Kwabia^{1,2}, Christina Bergemann², Silvia Grant-Beurmann², and Nazema Y Siddiqui²

¹Duke University, Department of Molecular Genetics and Microbiology; ²Duke University, Department of Obstetrics and Gynecology, Division of Urogynecology

INTRODUCTION AND OBJECTIVE: *Lactobacillus crispatus* has emerged as a promising Live Biotherapeutic Product (LBP) candidate for the treatment of recurrent urinary tract infection (rUTI). In postmenopausal individuals, who are disproportionately affected by rUTI, estrogen hormone therapy (EHT) is associated with genitourinary enrichment of *L. crispatus*. However, functional characterization of *L. crispatus* in the urinary niche, and in the context of EHT, remains limited. Using comparative genomics and *in silico* metabolomics, we characterized the pangenome of four *L. crispatus* clinical isolates. We aimed to identify baseline functional potential and strain-specific features relevant to LBP development.

METHODS: *Lactobacillus crispatus* strains 5009r-1B, 5029-1, 5016-2, and 5026-3 were isolated from urine collected by transurethral catheterization from UMICRO participants. Whole-genome sequencing and assembly were performed by Plasmidsaurus using long-read Oxford Nanopore Technology with automated *de novo* assembly and polishing. Genomes were annotated using BV-BRC, BASys2, and Prokka, and a pangenome was constructed using Roary. BV-BRC supported accessory genome pathway, plasmid, and phage analyses. Core and accessory metabolomes were predicted in RStudio from BASys2-derived predictions.

RESULTS: Analyses revealed a core genome comprising 36% of genes ($n = 1,463$), a core predicted metabolome comprising 91% of metabolites ($n = 367$), and comparable numbers of antibacterial resistance genes ($n < 20$) across all strains. Strain 5009r-1B was the most genomically divergent with no unique metabolites. The rUTI-derived strain 5026-3 was enriched for fatty acid biosynthesis and β -oxidation metabolites. Strains 5016-2 and 5029-1 were most similar by genome phylogeny but had the largest unique accessory metabolomes ($n = 12$). Strain 5016-2 showed enrichment in formaldehyde detoxification and oxidative stress response pathways, while strain 5029-1 showed enrichment in vitamin biosynthesis, c-di-GMP production, and nitrogen metabolism.

CONCLUSIONS: The presence of metabolic divergence despite genomic similarity suggests functional divergence and warrants further investigation. Strain 5016-2 may possess enhanced capacity to withstand chemical and oxidative stress, which could enhance resilience in states of disease or therapeutic exposure. Strain 5029-1 demonstrated the broadest metabolic potential, including a putative colonization determinant that may enhance its suitability as an LBP.

HEALTH RELEVANCE: As *L. crispatus*-based LBPs advance toward clinical use, understanding strain-specific functional potential within this niche and population will be crucial for optimizing therapeutic development and knowledge of rUTI pathophysiology.

THE STABILITY OF THE HEALTHY FEMALE LOWER URINARY TRACT VIROME (poster #13)

Lila Nelson¹, Catherine Putonti¹

¹Loyola University Chicago

INTRODUCTION AND OBJECTIVE: The healthy female lower urinary tract (LUT) harbors a community of both bacteria and viruses. The bacterial community, or microbiome, is relatively low biomass, but is highly dynamic and closely associated with urinary tract health. Previous studies using 16S rRNA sequencing and culture-based methods have demonstrated daily fluctuations in the urobiome. These methods have yet to be replicated utilizing shotgun metagenomic sequencing. While the viral community, or virome, of the LUT is less well characterized than the bacterial constituents, it contains bacteriophages, or viruses that infect bacteria, as well as eukaryotic viruses. To date there has not been a longitudinal study of the LUT virome. Through longitudinal sampling, this study examines the temporal dynamics of the healthy female LUT microbiome and virome.

METHODS: Daily urine samples were collected over a 14-day period from five healthy female participants and were separated into viral and bacterial fractions. These fractions were sequenced using shotgun metagenomic sequencing capturing all genetic material present in the samples. First, we explore the taxonomic and functional composition and stability of the bacterial fraction. We then characterize the viral fraction, assessing the stability and diversity of the LUT virome including both phage and eukaryotic virus populations.

RESULTS: Comparison of taxonomic stability and functional stability revealed a significantly greater functional stability than taxonomic stability of the bacterial populations. Analysis of the virome identified a large proportion of novel phage sequences. Some of these phage sequences are stable across days while many are transient showing varied stability within the LUT virome. Notably, both bacterial and viral communities were highly individualized.

CONCLUSIONS: This study expands current understanding of the functional stability within the LUT microbiome and provides the first longitudinal characterization of the LUT virome. Importantly, this study elucidates previously unexplored temporal dynamics within the healthy female LUT microbiome and virome.

HEALTH RELEVANCE: These findings establish a baseline for future studies of the urobiome in healthy females. More specifically, it has shown that there is not just one community composition that reflects health.

BUILDING A METAGENOME-ASSEMBLED GENOME CATALOG OF THE FEMALE URINARY MICROBIOME IN HEALTH AND DISEASE (poster #14)

Ceejay N. Saenz¹, Raheel S. Ahmed¹, Michael L. Neugent^{1,2}, Nicole J. DeNisco^{1,3}

¹The University of Texas at Dallas, Department of Biological Sciences; ²University of Rochester Medical Center, Department of Urology; ³The University of Texas Southwestern Medical Center, Department of Urology

INTRODUCTION AND OBJECTIVE: Recurrent urinary tract infection (rUTI) is a rising clinical concern in postmenopausal (PM) women. rUTI can persist for years and due to widespread antimicrobial resistance, front-line antibiotics are often ineffective. Estrogen hormone therapy is used for the management of rUTI in PM women because it may help promote colonization of protective lactobacilli in the female urinary microbiome (FUM). FUM modulation is a promising approach for rUTI management, but more research is needed to understand how the FUM functions in health and disease.

Here, we present a catalog of metagenome-assembled genomes (MAGs) representing the FUM of PM women in health and disease. This unique MAG catalog will enable further understanding of the functional roles of key FUM species.

METHODS: Shotgun metagenomic sequencing was performed on urine collected from 86 PM women falling into one of three cohorts defined by UTI history and infection status. Following quality control and host read removal, the MetaWRAP v1.3.2 pipeline was used for metagenome assembly and binning. CheckM2 was used to assess bin quality. Initial taxonomic identification was performed by Kraken2 and then refined with GTDB-tk. A custom parsing script was developed to decontaminate bins to increase bin purity.

RESULTS: The MetaWRAP pipeline produced 676 putative MAGs across the dataset. Using CheckM2, we found that 500 were classified as high-quality (>90% complete; <5% contamination), 60 were classified as medium-quality (>50% complete; <10% contamination), and 89 were classified as low-quality (<50% complete; <10% contamination). Out of 676 total bins, Kraken2 only resolved 324 bins to the species level while GTDB-tk resolved 501 bins to species level. *Finegoldia magna* (25 bins) was the most common species level FUM MAG recovered across the cohort.

CONCLUSIONS: Tool selection significantly impacted our results. Kraken2 failed to classify a large portion of MAGs, likely due to the underrepresentation of FUM species in its database, while GTDB-tk showed more comprehensive taxonomic resolution. The recovery of *F. magna* MAGs demonstrates its prevalence in the FUM and allows for functional genomic analysis that we anticipate will reveal mechanisms linked to its persistence and role in the FUM. This method will then be applied to other MAGs, allowing us to characterize FUM genomes across different health states.

HEALTH RELEVANCE: UTIs are among the most common infections globally, disproportionately affecting women, and treatment is complicated by antibiotic resistance. FUM MAGs will enable functional characterization of the FUM across health states, improving understanding of disease development and persistence, while supporting microbiome-focused UTI interventions.

UNDERSTANDING THE ROLE OF SEX AND AGE AS RISK FACTORS IN URINARY TRACT INFECTION SUSCEPTIBILITY USING A HUMAN BLADDER CHIP MODEL (poster #15)

Dasvit Shetty^{1,2}, Gretchen Carpenter¹, Zohreh Izadifar^{1,2,3}

¹Boston Children's Hospital; ²Harvard Medical School; ³Wyss Institute for Biologically Inspired Engineering

INTRODUCTION AND OBJECTIVE: Urinary tract infections (UTIs) disproportionately affect women and the elderly. Differences in anatomy, genetics, sex hormones, immune responses, and microbiome and changes with aging have been implicated to contribute to this disparity. However, mechanistic understanding of how sex and age affect the urinary tract mucosal physiology and UTI pathogenesis remain a major knowledge gap. Our objective is to use a human Bladder-on-Chip (Bladder Chip) in vitro model to investigate how sex and age, as biological variables, regulate physiology and responses of the bladder to infection.

METHODS: We culture donor-derived primary urothelial and stromal cells on either side of a porous membrane inside a dual-channel microfluidic chip (Emulate) to form a 3D urothelium-stroma interface under dynamic flow and stretch conditions. Using bladder tissue specimens from young (<45 y/o) and old (>60 y/o) male and female donors (n=3 per group), we isolate and expand primary urothelial and stromal cells to create individual Bladder Chips. The urothelium of engineered Bladder Chips will be infected with Uropathogenic *E. coli* (UPEC) (UTI89) at MOI 10 at day 7 of culture. Chip responses will be studied at 0.5, 2h, 24, 72h post infection. The molecular, cellular and functional properties of Bladder Chips will be studied, focusing on 3D cellular structure, phenotype (qPCR, immunostaining), barrier integrity, UPEC adhesion, invasion, intracellular bacterial community

formation, cell injury, and proinflammatory cytokine responses. We will compare responses in young versus old male versus female Bladder Chips with and without infection.

RESULTS: We established a protocol for reproducible isolation, expansion, and banking of primary human bladder urothelial and stromal cells with consistent purity similar to commercially available primary cells. We created a biobank of > 15 donors (male, female, 18-70 y/o). Our prototype of the human Bladder Chip recreates a 3D-differentiated urothelium, expressing key urothelial markers (uroplakin II, mucin I, etc.) and forming a tight-urine tolerant barrier, interfaced with a thick stromal tissue. We are now leveraging this platform to create sex and age specific Bladder Chips to investigate if and how age and sex contribute to disparity of UTI pathogenesis.

CONCLUSIONS: We anticipate that donor-derived Bladder Chips will provide a physiologically relevant, human-based platform to uncover novel mucosal mechanisms of bladder biology and help address key knowledge gaps underlying UTI disparities in women and older adults.

HEALTH RELEVANCE: By identifying why sex and aging increase susceptibility to UTIs, this project may uncover new therapeutic targets and support improved, patient-informed approaches to UTI prevention and care.

INVESTIGATING THE USE OF HUMAN READ REMOVAL TOOLS ON LONG READ MICROBIAL SEQUENCING DATA (poster #16)

Juliette Kudrick¹, Keisha Harrison¹, Lisa Karstens^{1,2}, Megan Carnes³, Silvia Grant-Beurmann⁴, Nazema Siddiqui⁴

¹Division of Oncological Sciences, Knight Cancer Institute, Oregon Health & Science University; ²Division of Informatics, Clinical Epidemiology, and Translational Data Science, Department of Medicine, Oregon Health & Science University; ³Genomics and Applied Public Health, RTI International; ⁴Duke University, Department of Obstetrics and Gynecology, Division of Urogynecology

INTRODUCTION AND OBJECTIVE: Long read 16S rRNA gene sequencing data has become increasingly common for studies of low microbial biomass environments. However, human read removal in long read 16S rRNA datasets remains largely unexplored. Our study addressed this by assessing the performance of two human read removal (HRR) tools on 5 sets of simulated long read data and 1 set of experimental PacBio long read data.

METHODS: To simulate data that accurately mimicked real long read sequences, we began by comparing PacBio 16S rRNA gene sequencing data from vaginal and urinary samples to the T2T CHM13v2.0 human reference genome using NCBI BLASTN. Sequences with percent identities ≥ 99.00 were extracted as examples of possible human contaminants. For microbial sequences, we extracted whole reference genomes of 9 common urogenital species from the NCBI RefSeq database. Using the above sequences as templates, we simulated 210,251 human reads and 295,347 microbial reads with PBSIM2. Sequences were randomly sampled with SeqKit to create 5 datasets (all $n = 200,000$), each with a unique microbe:human ratio. For each dataset, we ran Bowtie2 (a subtractive aligner) and BioBloom (a direct classifier), and then calculated accuracy, true positive rate (TPR), and negative predictive value (NPV). We also applied both HRR tools to a small dataset of low microbial biomass samples (4 from vaginal swabs, 4 from voided urine, and 7 from a mock microbial community dilution series).

RESULTS: Both tools performed well on the simulated data, with BioBloom's accuracy ranging from 99.989 – 100.00%, and Bowtie2's accuracy ranging from 99.995 – 100.00%. However, both tools saw slight declines in accuracy, TPR, and NPV as contamination increased. For all test sets, BioBloom was more likely to call human reads as bacterial, indicating it is less effective. Tools performed comparably on experimental data. Both methods flagged an average of 12% of urine and 2% of vaginal reads as human. For mock samples, BioBloom called an average of 0.003% of reads as human, while Bowtie2 called an average of 0.002%.

CONCLUSIONS: Results indicate that current HRR tools appear well equipped to handle long read 16S sequencing data. However, Bowtie2 does provide improved accuracy, TPR, and NPV over BioBloom. Notably, the pattern of increasing human contamination leading to worsening tool performance emphasizes the importance of contamination control at all stages of microbiome research, particularly when dealing low microbial biomass environments such as the urobiome.

HEALTH RELEVANCE: As the popularity of microbiome research grows, it is critical we ensure accurate and comprehensive HRR. This will protect participant privacy while also ensuring that the signatures mined from microbiome data are accurate and reproducible.

BIOFILM FORMATION, BUT NOT ANTIBIOTIC RESISTANCE, DEFINES KIDNEY STONE BACTERIA (poster #17)

Dhriti Tattari, Amber Wang, Mangesh V. Suryavanshi, and Aaron W. Miller

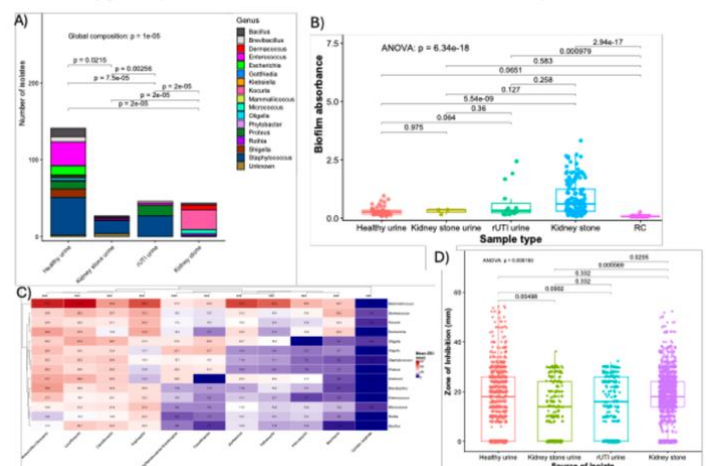
Department of Microbial Sciences in Health, Cleveland Clinic Research

INTRODUCTION AND OBJECTIVE: Emerging evidence indicates that biofilm formation and antibiotic resistance are common features of calcium-based metabolic stones, yet few studies have directly evaluated whether these associations are causal. The objective of the current study was to evaluate these features for 80 bacterial isolates derived from clinical kidney stone and urine specimens.

METHODS: Bacteria were isolated from the urine of those with no history of kidney stones or urinary tract infection, those with symptomatic stones, and those undergoing chronic treatment for recurrent urinary tract infections (rUTI) urine, along with calcium-based kidney stones. Bacteria were taxonomically identified and analyzed for biofilm formation and antibiotic resistance. Biofilm formation was quantified using a crystal violet microtiter plate assay. Antibiotic resistance was assessed by disk diffusion for eleven commonly used antibiotics.

RESULTS: The taxonomy of isolates differed among sample types (healthy urine, kidney stone urine, rUTI urine, and kidney stones, $P = 1 \times 10^{-5}$; Fig. 1a). Isolates from kidney stones exhibited significantly greater biofilm formation than healthy urine isolates ($P = 5.54 \times 10^{-9}$) and reagent controls ($P = 2.94 \times 10^{-17}$; Fig. 1b), independent of taxonomy. In contrast, antibiotic resistance was largely driven by taxonomy (Fig. 1c) rather than source of isolation (Fig. 1d). Additional isolates are being evaluated to reduce potential selection bias and increase robustness of results.

Figure 1. Characteristics of clinical urinary and kidney stone isolates. A) Taxonomy of isolates by isolation source; B) Quantitative biofilm formation among the 80 clinical isolates; C) Antibiotic resistance by genus. *** p-value < 0.001 by genus. D) Zone of inhibition for all isolates and all antibiotics combined, by isolation source.



CONCLUSIONS: Kidney stones harbor bacterial communities unique from the lower urinary tract that are primarily defined by biofilm formation rather than antibiotic resistance, independent of taxonomy. Our findings suggest that enhanced biofilm formation, rather than

increased antimicrobial resistance, is a defining characteristic of stone-associated bacteria that may act as a selective force for bacterial persistence at the site of stone formation.

HEALTH RELEVANCE: Biofilm formation may contribute to the formation and recurrence of calcium-based stones, which could provide a specific target for clinical intervention. Understanding microbial phenotypes associated with stone disease may inform future approaches for the prevention, diagnosis, and treatment of calcium-based stones.

ANAEROBIC URINARY BACTERIA USE UROMODULIN AS A GROWTH SUBSTRATE (poster #18)

Julia S. L. Tri^{1,2}, Elie Al Khoury¹, Jean-Phillippe F. Gourdine^{1,2}

¹Biochemistry & Molecular Biology Program, Lewis & Clark College; ²Chemistry Department, Lewis & Clark College

INTRODUCTION AND OBJECTIVE: Uromodulin (UMOD), the most abundant glycoprotein found in urine, has previously been shown to inhibit the colonization of bacteria associated with urinary tract infections (UTIs). Recently, isolated *Bifidobacterium longum*, an anaerobic, nonpathogenic bacterium present in the human urinary microbiome (urobiome), was found to degrade UMOD, suggesting that it may use UMOD as a nutrient source. This study assessed whether anaerobic bacteria present in urine pellets are capable of using UMOD as a growth substrate.

METHODS: Urine samples were obtained from two healthy donors, one male and one female, using midstream urine collection. Urinary bacterial pellets were grown independently under anaerobic conditions. The bacterial cultures consisted of minimal medium with varying concentrations of UMOD as the sole carbon source. After 10 days of incubation, bacterial growth was measured using optical density at 600 nm (OD₆₀₀). Following a significant analysis of variance (ANOVA) result ($p < 0.0005$), statistical significance between groups was evaluated with a Tukey's Honestly Significant Difference (HSD) test.

RESULTS: Bacterial growth showed a positive trend with increasing UMOD concentration compared to the control (Fig 1). UMOD concentrations as low as 25 µg/mL promoted bacterial growth. Although growth did not differ significantly between the 25 and 50 µg/mL conditions, a significant increase in growth was observed between the 50 and 125 µg/mL conditions. Bacterial growth plateaued at concentrations exceeding 125 µg/mL, possibly due to substrate saturation or limitation of other nutrients.

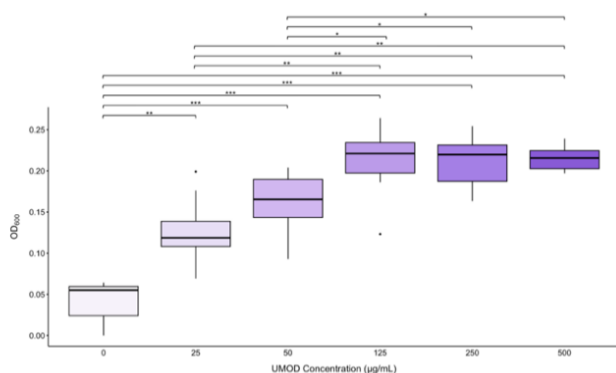


Fig 1. Boxplot representing urinary bacterial growth (OD₆₀₀) on varying concentrations of UMOD after 10 days of anaerobic incubation. Statistical significance was calculated using Tukey's HSD test (* $p < 0.05$, ** $p < 0.0005$, *** $p < 0.0000005$).

CONCLUSIONS: While previous research has established UMOD's antimicrobial role against pathogenic, UTI-associated bacteria, this study indicates that UMOD may also be used as a nutrient source for urinary bacteria under anaerobic conditions. This could suggest that another mechanism for UMOD in UTI prevention is through the proliferation of commensal bacteria, thereby increasing resource

competition and reducing the ability for pathogenic bacteria to colonize the bladder.

HEALTH RELEVANCE: These findings propose a novel role for UMOD as a nutrient source for native urinary bacteria to maintain urobiome homeostasis.

THE VAGINAL MICROBIOME IN WOMEN RECENTLY EXPERIENCING URINARY TRACT INFECTION AND BACTERIAL VAGINOSIS (poster #19)

Sonia N. Whang¹, Xinyue Wang^{1,2}, Krystal J. Thomas-White³, Genevieve Olmschenk³, John E. Garza⁴, Pita Navarro³, Nicole M. Gilbert^{1,5-7}

¹Department of Pediatrics, Washington University School of Medicine in St. Louis, MO; ²Institute for Informatics, Data Science and Biostatistics, Washington University School of Medicine in St. Louis; ³Evvy, New York; ⁴McDonnell Genome Institute, Washington University School of Medicine in St. Louis; ⁵Department of Obstetrics and Gynecology; ⁶Department of Molecular Microbiology; ⁷Center for Women's Infectious Disease Research, Washington University School of Medicine in St. Louis

INTRODUCTION AND OBJECTIVE: The vaginal microbiome (VMB) is linked not only to symptoms, but also to the risk of urogenital infections. Millions of women suffer from urogenital symptoms and infections, and current treatments often do not bring permanent relief. This academic-industry collaboration facilitated a large-scale evaluation of the VMB in 10,003 women across the US who reported experiencing two of the most common urogenital infections: bacterial vaginosis (BV) and urinary tract infection (UTI).

METHODS: We compared the VMB of women who reported UTI, BV, or both in the last 30 days to women reporting never having experienced either infection. We analyzed shotgun metagenomic profiles to compare vaginal community structure, classifying them into established Community State Types (CST) and VALENCIA categories. We compared alpha/beta diversities and utilized Linear Discriminant Analysis and MaAsLin2 to uncover which bacterial species were significantly associated with each group. We evaluated differences in the mean relative abundances of the key BV bacteria *Gardnerella* and a wide range of uropathogenic species.

RESULTS: Women reporting recent BV or UTI displayed distinct community structures, including higher prevalence of VALENCIA CST-IV subtypes, and significantly elevated alpha diversity compared with women who reported no prior diagnosis. Species-level *Gardnerella* profiling revealed that multiple *Gardnerella* species were enriched in BV but did not differ significantly between UTI and non-UTI groups. Uropathogens including *E. coli*, *E. faecalis*, and *S. saprophyticus* were at higher prevalence and relative abundance in women who experienced UTI, including among participants who reported recent antibiotic use.

CONCLUSIONS: With this sizeable VMB metagenomic dataset and associated health, life-course, symptom, and treatment information, we corroborate known associations at the population-level and deliver unique data and insights for future clinical and mechanistic research. These data suggest that the vagina can be a reservoir for uropathogens in women reporting to have UTI, even after antibiotic use. These findings demonstrate that microbial signatures associated with recent BV and UTI remain detectable at population scale, provide a high-resolution reference vaginal metagenomics, and offer new directions for preventions that consider the vaginal reservoir in recurrent UTI.

HEALTH RELEVANCE: Current clinical practice does not routinely consider the VMB when diagnosing and treating UTIs. Our observational study underscores the need to consider treatments directed toward eradicating the uropathogen and potential UTI co-conspirators from the vaginal reservoir in future personalized medicine approaches to prevent recurrent UTI.

CORRELATIVE IMAGING AND METAGENOMICS REVEAL BIOFILM-COMPATIBLE BACTERIAL NICHES IN HUMAN KIDNEY STONES (poster #20)

Mangesh V. Suryavanshi, Aaron W. Miller

Department of Microbial Sciences in Health, Cleveland Clinic Research

INTRODUCTION AND OBJECTIVE: Kidney stone disease is driven by urinary supersaturation, crystal nucleation, and mineral growth. Yet layered, porous stones may contain protected intrastone bacterial microenvironments. We tested whether stone microstructure and microbial functional gene signatures support bacterial participation in calcium oxalate lithogenesis.

METHODS: Surgically retrieved stones were analyzed by synchrotron radiation micro-computed tomography (SR- μ CT), Fourier-transform infrared spectroscopy, X-ray diffraction, field-emission scanning electron microscopy, energy-dispersive X-ray spectroscopy, and electron probe microanalysis. Comparative shotgun metagenomics of pure calcium oxalate stone formers and age- and sex-matched controls used four nonredundant gene databases to assess antimicrobial resistance, biofilm, toxin, and virulence functions; Wilcoxon rank-sum tests assessed group differences. Five representative isolates were tested during CaOx crystallization for biofilm formation and crystal entrapment.

RESULTS: Stones showed cores, radial concentric layers, porous channels, cracks, and mixed mineral interfaces not captured by bulk composition. SR- μ CT identified calcium phosphate nuclei within calcium oxalate monohydrate stones, while elemental mapping showed nonuniform calcium- and phosphorus-rich zones. Scanning electron microscopy revealed mineral-organic interfaces near porous regions and growth boundaries. Comparative metagenomics demonstrated distinct antimicrobial-resistance gene composition between groups. Calcium oxalate stone formers had higher antimicrobial-resistance genes/sample (Wilcoxon $p=0.016$), biofilm genes/sample ($p=0.032$), and virulence genes/sample ($p=0.016$); toxin genes were not significantly different ($p=0.095$). Most isolates showed multidrug resistance and strong surface adherence with 60% forming biofilms during CaOx crystallization and entrapped microcrystals.

CONCLUSIONS: These data support kidney stones as spatially organized biomineral systems in which bacteria may persist in mineral-organic microdomains. Biofilm-associated bacteria may provide local scaffolds for crystal adhesion and aggregation and become embedded during layered mineral accretion.

HEALTH RELEVANCE: Identifying biofilm-compatible microbial niches and antimicrobial-resistance gene enrichment may inform strategies to prevent recurrent calcium oxalate stone disease.

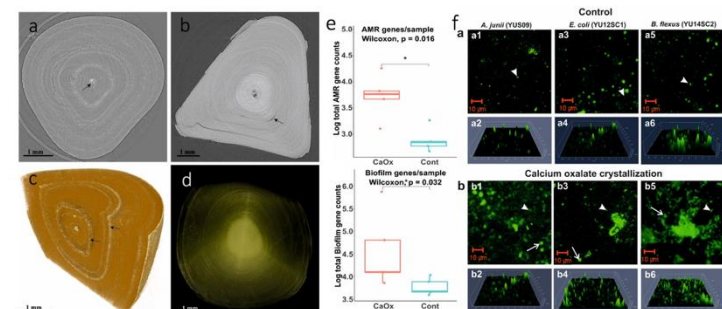


Figure: Calcium oxalate stone microstructure, urobiome gene signatures, and biofilm-associated crystal aggregation. (a, b) SR- μ CT slices showing radial concentric layers, calcium phosphate nuclei, and internal cracks. (c, d) Three-dimensional stone reconstructions. (e) Higher antimicrobial-resistance and biofilm-associated gene abundance in urine from calcium oxalate (CaOx) stone formers. (f) Biofilm formation during calcium oxalate crystallization by *Acinetobacter junii*, *Bacillus flexus*, and *Escherichia coli*. Arrows indicate bacteria; arrowheads indicate calcium oxalate crystal aggregates.

FINDING KLEBSIELLA PNEUMONIAE SUSCEPTIBLE PHAGES IN WASTEWATER (poster #21)

Sanjana Nekkanti, Catherine Putonti, Alan Wolfe

Loyola University of Chicago

INTRODUCTION AND OBJECTIVE: *Klebsiella pneumoniae* (*K. pneumoniae*), a common colonizer of the gastrointestinal tract and occasionally the upper respiratory tract, is a well-recognized opportunistic pathogen associated with urinary tract infections (UTIs) when found in the lower urinary tract. There is an increasing rate of antibiotic resistance in *K. pneumoniae*, so alternative strategies such as phage therapy have gained renewed interest in Western Medicine. Because phages are often strain specific, expanding catalogs of diverse phages targeting bacterial pathogens is essential for the broad implementation of phage therapy. Historically, wastewater has been a rich source of phages due to the high concentration and diversity of bacteria present. In this study, we have isolated phages from wastewater capable of lysing clinical *K. pneumoniae* isolates from the urinary tract.

METHODS: We were provided with wastewater samples from the Lake County Wastewater Treatment Plant in Libertyville, IL. Samples from the influent raw and aeration tanks were provided on 3 separate days. The wastewater samples were filtered to isolate phages present. The filtrate was next added to a mix of clinical *K. pneumoniae* isolates, incubated overnight to enrich phage particles, and then plated the next day to identify plaques. Individual phages have been purified through repeatedly picking individual plaques, culturing with host bacterium, and plating. Genomic sequencing and transmission electron microscopy is in progress and will be instrumental in characterizing the *K. pneumoniae* infecting phages isolated.

RESULTS: Plating phages isolated from our wastewater samples revealed a range of plaque morphologies, from small pinpoint plaques to larger diffuse plaques. The distinct plaques phenotypes likely reflect the presence of multiple species, and potentially multiple genera. Individual phages have subsequently been isolated and purified. These phages show varied efficacy in lysing and killing urinary *K. pneumoniae* isolates, further supporting the genetic and phenotypic diversity of the wastewater phages found. Whole-genome sequencing and transmission electron microscopy analyses are currently underway.

CONCLUSIONS: Individual phages that lyse clinical *K. pneumoniae* isolates in our lab have been identified from wastewater. These phages expand our catalog of resources for potential therapeutic use.

HEALTH RELEVANCE: Urinary tract infections have a significant impact on quality of life, particularly in females. Phages present a promising new treatment strategy in the face of the rise of antibiotic resistance.

CHARACTERIZING ANTIMICROBIAL RESISTANCE PATTERNS OF BACTERIURIA CAUSED BY ESCHERICHIA COLI WITH WHOLE GENOME SEQUENCING IN A PUBLIC HEALTHCARE INSTITUTION IN SAN FRANCISCO, CA (poster #22)

Eva Raphael¹, Esme Grenstam¹, Nicholas Hathaway¹, Dian Gu¹, Abraham Soto¹

University of California, San Francisco

INTRODUCTION AND OBJECTIVE: Urinary tract infections (UTIs) are the most common bacterial infections worldwide, with a majority caused by *Escherichia coli*. The prevalence of antimicrobial resistant uropathogenic *E. coli* is increasing. Extended spectrum beta-lactamase (ESBL) production is known to increase the risk of UTI complications. Additionally, many ST131 *E. coli* strains, one of the pandemic genotypes, carry ESBL genes, rendering them resistant to common antibiotics like cephalosporins and penicillins. Characterization of antimicrobial resistant *E. coli* causing bacteriuria

can advise treatment strategies, as well as provide insight into transmission mechanisms.

METHODS: We identified four pandemic genotypes (ST 69, 73, 95, and 131) by multiplex PCR in a collection of urine *E. coli* isolates (June 2022 to June 2025) from patients receiving care in a San Francisco public healthcare institution, including outpatient, inpatient, and skilled nursing facility. In this pilot study, we obtained whole genome sequence (WGS) data on 139 (44% inpatient) of 604 *E. coli* isolates collected from June to October 2022. We sent 81 ST131 genotypes, 28 non-ST131 ESBL-producing *E. coli*, and a comparative subset of 30 non-ST131 and non-ESBL isolates for WGS, which was analyzed with Bactopia. Known antibiotic genes were detected using the Comprehensive Antibiotic Resistance Database (CARD). We conducted phylogenetic analyses of ST131.

RESULTS: Of the 8 beta-lactamase families tested, only the presence of any of the plasmid-associated CTX-M genes was strongly associated with ESBL production ($p < 0.001$). ST131 predominated the ESBL producer population with 57% of ESBL-producing isolates being ST131. Fluoroquinolone resistance was associated with ST131 ($p < 0.001$) and ESBL isolates ($p = 0.001$), with ESBL-ST131 isolates being 94.6% fluoroquinolone resistant. We found multiple clusters of ST131, especially in isolates from the public skilled nursing facility.

CONCLUSIONS: We found that fluoroquinolone resistance was associated not only with ST131 but with ESBL producers in general. While ST131 is in part driving multidrug resistance prevalence, ESBL production, regardless of genotype, is associated with multidrug resistance, most likely due to presence of ESBL genes on mobile genetic elements. Phylogenetic clusters of ST131 from the skilled nursing facility confirms that such facilities act as reservoirs for antimicrobial resistant organisms.

HEALTH RELEVANCE: There are no surveillance programs for pandemic genotypes or ESBL production for *E. coli*, yet antimicrobial resistance burden in various clinical settings is clearly driven by those elements. Our findings support expanding surveillance programs for antimicrobial resistant organisms.



For more information about the CAIRIBU Interactions Core and the CAIRIBU Urobiome Research Interest Group, visit the CAIRIBU website at <https://cairibu.urology.wisc.edu> or contact the CAIRIBU Interactions Core by email at cairibu@urology.wisc.edu