Chicago KUH FORWARD Meeting
hosted by Loyola University Chicago

March 1, 2021
<table>
<thead>
<tr>
<th>Session</th>
<th>Speaker</th>
<th>Duration</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welcome and logistics</td>
<td>Tamara Isakova</td>
<td>5 min</td>
<td>11:00 – 11:05</td>
</tr>
<tr>
<td>Introduction of objectives</td>
<td>Bennett Goldberg</td>
<td>5 min</td>
<td>11:05 – 11:10</td>
</tr>
<tr>
<td>Brief overview of bladder physiology and pathophysiology</td>
<td>Lauren Folgosa Cooley</td>
<td>10 min</td>
<td>11:10 – 11:20</td>
</tr>
<tr>
<td>The Human Urobiome: The Female Bladder and Urethra Harbor Distinct Microbial Communities</td>
<td>Alan Wolfe</td>
<td>10 min</td>
<td>11:20 – 11:30</td>
</tr>
<tr>
<td>Rethinking UTI</td>
<td>Baylie Hochstedler</td>
<td>10 min</td>
<td>11:30 – 11:40</td>
</tr>
<tr>
<td>Investigating the bactericidal supernatant of Lactobacillus crispatus</td>
<td>Omar Abdul-Rahim</td>
<td>10 min</td>
<td>11:40 – 11:50</td>
</tr>
<tr>
<td>Q&amp;A</td>
<td></td>
<td>10 min</td>
<td>11:50 – 12:00</td>
</tr>
</tbody>
</table>
Meeting Logistics

• As you log in, please feel free to introduce yourself in the chat and/or modify your name in Zoom through use of rename feature
• Please mute your microphone during presentations
• Please submit questions and comments through the Zoom chat feature
• You can ask questions at any time; we will do our best to answer questions at the end
• During the Q&A at the end, we will prioritize questions from trainees and early stage investigators
• We will follow up with an evaluation, but if you have ideas immediately following the session please email tamara.isakova@northwestern.edu
Objectives of Chicago KUH FORWARD research talks

- Familiarize members with research that is ongoing across the network
- Motivate members to identify areas of scientific overlap
- Prompt introspection re commonalities across perspectives, techniques and outcomes
- Make connections across institutions, departments and career stages

Operationalizing these objectives: Note connections across several dimensions:
- Research knowledge – what types of knowledge and understandings are connected
- Research techniques – what types of approaches and methods are connected
- Interstitial voids – what research question fill the void between disciplines and approaches
Chicago KUH Forward:

Bladder Physiology

Lauren Folgosa Cooley, MD PhD
PGY-4 Urology Resident
March 1, 2021
Bladder Physiology

• Location:

Female

Male
Bladder Physiology

- **Bladder lumen**
- **Urethra**
- **Ureter**
- **Glycosaminoglycan (GAG) layer**
- **Urothelium**
- **Lamina Propria Muscularis**
- **Superficial (Umbrella) cells**
- **Intermediate cells**
- **Basal cells**
- **Basement membrane**
- **Serosa**

**Umbrella Cells**: tight junctions

Bladder Physiology

- **Purpose**: Store and expel urine
  - Low pressure system
  - Expel urine with voluntary control
Innervation to the Bladder

- Bladder and external sphincter (outlet) receives innervation from 3 sources:
  1. Sympathetic nervous system ("fight or flight")
  2. Parasympathetic nervous system ("rest and digest")
  3. Somatic nervous system (voluntary control)

Fowler et al. Nat Rev Neurosci, 2008
Physiology of Micturition

Filling Phase

- Compliant Bladder
- Sympathetic efferent (T11-L2)
  - Detrusor relaxation
  - Bladder neck closure
- S2 - S4 (somatic fibers)
  - Voluntary contraction of external sphincter

Voiding Phase

- Parasympathetic (S2 – S4)
- Detrusor contraction Center (Barrington’s Nucleus)
  - (−) sympathetic
  - (+) parasympathetic
- S2 - S4 (somatic fibers)
  - Voluntary relaxation of external sphincter
Urologic Conditions Affected by the Urinary Microbiome

Kidney
• Nephrolithiasis (kidney stones)

Prostate
• Prostatitis
• Benign Prostatic Hyperplasia (BPH)
• Prostate Cancer

Bladder
• Urinary tract infection
• Bladder Cancer
• Overactive Bladder
• Voiding dysfunction / incontinence
• Interstitial Cystitis / Bladder Pain Syndrome

Male infertility

Wound healing
Interstitial Cystitis / Bladder Pain Syndrome

• **Symptoms**
  – Urinary frequency and urgency
  – Bladder and pelvic pain (especially with distension, relief with voiding)
  – Associated symptoms – anxiety, IBS, fibromyalgia

• **Mechanism**
  – Loss of protective glycosaminoglycan layer
  – Defect in urothelium rearrangement with distension
  – Immune – increased mast cell activation
  – Urinary and gut microbiome - sensitization pelvic nerves, some bacteria may have analgesic vs anti-analgesic properties

Whiteside et al. Nat Rev Urol 2015
The Human Urobiome:
The Female Bladder and Urethra Harbor Distinct Microbial Communities

KUH
March 1, 2021
The Human Urobiome

Why you should care

• The urothelium is a neural organ
• Now add bacteria
  o Have surface proteins that can bind epithelial cell receptors
  o Secrete small molecules that function as ligands for receptors
    ➢ Acetylcholine = muscarinic receptors
    ➢ ATP = purinergic receptors
    ➢ Glutamate/glutamine = glutamate receptors
Roadmap

• Old dogma vs New reality
• Debunking the old dogma
• Bladder and urethral microbiota are distinct
  • Why it matters
**The old dogma**
The bladder is sterile
Bacteria are not involved in most urinary disorders
Urinary tract infection when a uropathogen invades sterile bladder
*E. coli*-centric view

**The new reality**
Bladders have resident microbiota
Urinary disorders likely result from dysbioses
It’s way more than *E. coli*
Why we hypothesized the bladder was NOT sterile

- The human microbiome
  - 1 bacterial cell for every human cell
  - Walking talking incubators
  - Cannot live without them
  - Feed & protect us

- Other organs considered sterile were NOT Lungs

- Most bacteria don’t grow under standard urine culture conditions
Some of our Findings

- DNA evidence of bacteria in standard urine culture-negative samples obtained from the bladder
  - (Wolfe et al., 2012 PMC3318548)

- Evidence that bacteria are alive – development of enhanced urine culture method superior to standard
  - (Hilt et al., 2014 PMID: 24371246)

- Microbiota are distinct between Urgency Urinary Incontinence (UUI) & non-UUI
  - (Pearce et al., 2014 PMC4161260)

- Microbiota are distinct between Urinary Tract Infection (UTI) & non-UTI
  - (Price et al., 2016 PMC4844725)

- Microbiota impact OAB medication efficacy
  - (Thomas-White et al., 2015 PMC5119460)

- Microbiota are associated with post-operative UTI risk
  - (Thomas-White et al., 2018 PMC6527134)

- Microbiota are associated with urolithiasis
  - (Dornbier et al., 2020 PMID: 31240349)
Some of our Findings

• DNA evidence of bacteria in standard urine culture-negative samples obtained from the bladder
  o (Wolfe et al., 2012 PMC3318548)

• Evidence that bacteria are alive – development of enhanced urine culture method superior to standard
  o (Hilt et al., 2014 PMID: 24371246)

• Microbiota are distinct between Urgency Urinary Incontinence (UUI) & non-UUI
  o (Pearce et al., 2014 PMC4161260)

• Microbiota are distinct between Urinary Tract Infection (UTI) & non-UTI
  o (Price et al., 2016 PMC4844725)

• Microbiota impact OAB medication efficacy
  o (Thomas-White et al., 2015 PMC5119460)

• Microbiota are associated with post-operative UTI risk
  o (Thomas-White et al., 2018 PMC6527134)

• Microbiota are associated with urolithiasis
  o (Dornbier et al., 2020 PMID: 31240349)
# Standard Urine Culture (SUC) vs Expanded Quantitative Urine Culture (EQUC)

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Volume</th>
<th>Media</th>
<th>Atmospheric Conditions</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Urine Culture (SUC)</td>
<td>1 uL urine</td>
<td>Blood Agar, MacConkey Agar</td>
<td>Aerobic</td>
<td>24 hrs 35°</td>
</tr>
<tr>
<td>Expanded Quantitative Urine Culture (EQUC)</td>
<td>100 uL urine</td>
<td>Blood Agar, Chocolate Agar, CNA Agar, Anaerobic Blood Agar</td>
<td>Aerobic CO₂ Anaerobic</td>
<td>48 hrs 35°</td>
</tr>
</tbody>
</table>
This urine is not sterile

SUC
Blood agar
1 ul
24 hours

EQUC
Blood agar
100 ul
48 hours
Participants (N=50) answered demographic questionnaires prior to sample collection. All sample types were cultured via EQUC, with bacterial isolates identified by MALDI-TOF MS. The microbial communities of the female bladder and urethra were analyzed by diversity measures and CFU/mL comparison.

### Participant Profile (N=49)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median age (range)</strong></td>
<td>55 (21-85)</td>
</tr>
<tr>
<td><strong>Ethnicity (%):</strong></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>36 (74%)</td>
</tr>
<tr>
<td>Black</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (2%)</td>
</tr>
<tr>
<td><strong>Menopausal status (%)</strong>:</td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>Post</td>
<td>37 (76%)</td>
</tr>
<tr>
<td><strong>Sexually active ( %)</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26 (53%)</td>
</tr>
<tr>
<td>No</td>
<td>23 (47%)</td>
</tr>
</tbody>
</table>
The female bladder microbiota differ from urethral, peri-urethral and midstream voided urine

Bray-Curtiss Dissimilarity Index
similarity/dissimilarity of two samples
1 = completely different
0 = identical

B = bladder (transurethral catheterized urine)
U = urethra
P = peri-urethra
V = midstream voided urine

Voided urine is more often similar to peri-urethral skin than the lower urinary tract
some microbiota reside preferentially within the lower urinary tract

**Escherichia**, **Corynebacterium**, **Streptococcus**

while other microbiota are found at similar levels throughout

**Lactobacillus**, **Gardnerella**, **Aerococcus**, **Actinomyces**
Summary

• Lower urinary tract is not sterile
  • Bladder and urethral microbiota are distinct
  • Some bacteria are specialists; others are generalists

• Why does this matter?
Bacteria interact with the urothelium
Loyola Urinary Education and Research Collaboration (LUEREC)
https://ssom.luc.edu/luerec/
https://twitter.com/Urobiome

**Clinical:**
- Urogynecology
  - Linda Brubaker, MD
  - Elizabeth Mueller, MD, MSME
  - Thythy Pham, MD
  - Marian Acevedo Alvarez, MD
  - Colleen Fitzgerald, MD
  - Hayley Barnes, MD
  - Lauren Westbay, MD
  - Matt Grevelinger, MD
  - Mary Tulke, RN
- Urology
  - Larissa Bresler, MD
  - Ahmer Farooq, DO
  - Bob Flanigan, MD
  - Ryan Dornbier, MD
  - Chirag Doshi, MD
  - Petar Bajic, MD
  - Michelle Van Kuiken, MD
- MFM
  - Jean Goodman, MD
  - Jules Sung, MD
  - Emily Houlthaus, MD
- ID
  - Fritzie Albarillo, MD

**Basic Science:**
- Wolfe Lab
  - Alan Wolfe, PhD
  - Thomas Halverson, BS
  - Mark Khemmani, BS
  - Baylie Hochstedler, MS
  - Cesar Montelongo, MS
  - Omar Abdul-Rahim, MS
  - Brian Choi, MS
  - Jingjie Du, MS
  - Megan Pearce, PhD MPH
  - Krystal Thomas-White, PhD
  - Danielle Johansen, MS
  - Giuseppe Pistone, MS
  - Evann Hilt, PhD
  - Travis Price, PhD
  - Vibha Ahuja, PhD
  - Nancy Sloan, MS
  - Michael Bochert, MS
  - Carine Mores, MS

- Others
  - Phong Le, PhD
  - Katherine Radek, PhD

**Genomics/Bioinformatics:**
- Qunfeng Dong, PhD
- Catherine Putonti, PhD
- Xiang Gao, PhD
- Roberto Limeira, MS
- Cara Joyce, PhD
- Jason Shapiro, PhD
- Taylor Miller-Ensminger
- Genevieve Johnson
- Adriana Ene
- Xiaowu Gai, PhD
- Stephanie Kliethermes, PhD
- Amy Rosenfeld, PhD
- Gina Kuffel, BSc
- Andrea Garretto, MS
- Eddi Lin, MS
- Michael Zilliox, PhD

**Clinical Microbiology:**
- Amanda Harrington, PhD, D(ABMM)
- Paul Schreckenberger, PhD, D(ABMM)
- Kathleen McKinley, BSc

**Non-Loyola Collaborators:**
- Andy Schwaderer, MD - IUPUI
- Douglas Storm, MD – Iowa
- Hillary Copp, MD – UCSF
- Marjon de Vos, PhD – U Groningen
- Tim Bugni, PhD – UW-Madison
- Katie Forster – CHOP
- Suzanne Groah – Medstar
- Steve Walker – Wake Forest
  - Dave Nelson, PhD - IUPUI
  - UITN
  - PFDN
  - SWHR

**FUNDING**
- NIH
- KCC
- VBTech
- Loyola

**Clinical Microbiology:**
- Amanda Harrington, PhD, D(ABMM)
- Paul Schreckenberger, PhD, D(ABMM)
- Kathleen McKinley, BSc

**Non-Loyola Collaborators:**
- Andy Schwaderer, MD - IUPUI
- Douglas Storm, MD – Iowa
- Hillary Copp, MD – UCSF
- Marjon de Vos, PhD – U Groningen
- Tim Bugni, PhD – UW-Madison
- Katie Forster – CHOP
- Suzanne Groah – Medstar
- Steve Walker – Wake Forest
  - Dave Nelson, PhD - IUPUI
  - UITN
  - PFDN
  - SWHR
Urinary microbiome of recurrent UTI: new evidence against old dogma

Baylie Hochstedler
Wolfe Lab

LOYOLA UNIVERSITY CHICAGO
Old dogma: UTI is an *E. coli* problem
Recurrent UTI (RUTI) Study

- Adult women with clinical history of RUTI were recruited for this study (N=43)
- Participants provided paired catheterized (n=43) and voided urine (n=42) samples
- Each urine sample was cultured via standard urine culture (SUC) and expanded quantitative urine culture (EQUC)
- Bacterial isolates were identified via MALDI-TOF MS and bacterial composition was compared between urine collection and culture methods

<table>
<thead>
<tr>
<th>Comparison:</th>
<th>Culture Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SUC</td>
</tr>
<tr>
<td>Media</td>
<td>BAP MacConkey Agar</td>
</tr>
<tr>
<td>Amount of Urine: Catheterized Voided</td>
<td>1uL 1uL</td>
</tr>
<tr>
<td>Atmospheric Conditions</td>
<td>Aerobic at 37C</td>
</tr>
<tr>
<td>Length of Incubation</td>
<td>24 hours</td>
</tr>
</tbody>
</table>
RUTI Study

Part 1: Microbiome Analysis  Part 2: Symptom Analysis
Part 1: Microbiome Analysis

- What is the urinary microbiome of women with RUTI?
- How do urine collection and culture methods alter microbiome results?
Frequency of uropathogen detection by collection and culture method
EQUC detects more likely uropathogens than SUC
EQUC detects more likely uropathogens than SUC
EQUC detects more likely uropathogens than SUC
EQUC detects more likely uropathogens than SUC

*E. faecalis* is the most frequently detected likely uropathogen
Part 1: Microbiome Analysis

- SUC fails to reproducibly detect multiple uropathogens in this RUTI cohort
  - This includes most frequently detected uropathogen *E. faecalis*
  - Some uropathogens were only detected by EQUC
- EQUC of catheterized urine yields the highest total detection rate of uropathogens
  - EQUC detected higher rates of polymicrobial infections

Part 2: Symptom Analysis

- Do microbiome profiles correlate with certain urinary symptoms?
RUTI Symptoms Analysis

- Principal component analysis of patient UTISA surveys and clinical profiles
  - Clinical factors: Vaginal estrogen use, sexual activity status, overactive bladder
RUTI Symptoms Analysis

- Principal component analysis of patient UTISA surveys and clinical profiles
  - Clinical factors: Vaginal estrogen use, sexual activity status, overactive bladder
  - Five distinct symptom profiles (A-E)

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Symptom and Clinical Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (3)</td>
<td>Odor, cloudiness, and vaginal estrogen use</td>
</tr>
<tr>
<td>B (11)</td>
<td>Frequency, back pain, incomplete emptying, and vaginal estrogen use</td>
</tr>
<tr>
<td>C (9)</td>
<td>Pain/burning, odor, cloudiness, and urgency</td>
</tr>
<tr>
<td>D (7)</td>
<td>Frequency, urgency, pain/burning, and vaginal estrogen use</td>
</tr>
<tr>
<td>E (10)</td>
<td>Frequency, urgency, pain/burning, odor, OAB, and sexual activity</td>
</tr>
<tr>
<td>Other (3)</td>
<td>NA</td>
</tr>
</tbody>
</table>
RUTI Symptoms Analysis

- Principal component analysis of patient UTISA surveys and clinical profiles
  - Clinical factors: Vaginal estrogen use, sexual activity status, overactive bladder
  - Five distinct symptom profiles (A-E)
- Culture results were compared across symptom profiles groups
  - EQUC of catheterized urine

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Symptom and Clinical Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (3)</td>
<td>Odor, cloudiness, and vaginal estrogen use</td>
</tr>
<tr>
<td>B (11)</td>
<td>Frequency, back pain, incomplete emptying, and vaginal estrogen use</td>
</tr>
<tr>
<td>C (9)</td>
<td>Pain/burning, odor, cloudiness, and urgency</td>
</tr>
<tr>
<td>D (7)</td>
<td>Frequency, urgency, pain/burning, and vaginal estrogen use</td>
</tr>
<tr>
<td>E (10)</td>
<td>Frequency, urgency, pain/burning, odor, OAB, and sexual activity</td>
</tr>
<tr>
<td>Other (3)</td>
<td>NA</td>
</tr>
</tbody>
</table>
Distinct symptom profiles are associated with specific urinary microbes in women with RUTI.
Part 1: Microbiome Analysis

- SUC fails to reproducibly detect multiple uropathogens in this RUTI cohort
  - This includes most frequently detected uropathogen *E. faecalis*
  - Some uropathogens were only detected by EQUC
- EQUC of catheterized urine yields the highest total detection rate of uropathogens
  - EQUC detected higher rates of polymicrobial infections

Part 2: Symptom Analysis

- Distinct clinical profiles (symptoms and clinical factors) correlated with presence/absence of specific microbes
  - Single urinary symptoms/demographic factors did not correlate with presence of UTI-associated microbes
  - Groups did not differ by other demographic data
- The presence of *E. coli* was seen throughout each symptom profile and was not associated with certain urinary symptoms
Part 1: Microbiome Analysis

- SUC fails to reproducibly detect multiple uropathogens in this RUTI cohort
  - This includes most frequently detected uropathogen *E. faecalis*
  - Some uropathogens were only detected by EQUC
- EQUC of catheterized urine yields the highest total detection rate of uropathogens
  - EQUC detected higher rates of polymicrobial infections

Part 2: Symptom Analysis

- Distinct clinical profiles (symptoms and clinical factors) correlated with presence/absence of specific microbes
  - Single urinary symptoms/demographic factors did not correlate with presence of UTI-associated microbes
  - Groups did not differ by other demographic data
- The presence of *E. coli* was seen throughout each symptom profile and was not associated with certain urinary symptoms

Growing evidence that *E. coli* is not associated with specific UTI symptoms; instead, overall composition of urinary microbiome results in unique symptom profiles