Emerging trends and challenges in microbiome research

Seong Jin Jeong
Seoul National University Bundang Hospital
1. Microbiome in the urinary tract
2. Trends in clinical microbiome research
3. Clinical studies & Results
4. Microbiota & LUTS
5. Limitations & future directions
What are microbiomes?

- A community of microorganisms including fungi, bacteria, viruses
- 500-100 bacterial species in humans
- Microbiota (microorganisms) vs. microbiome (collection of genomes)

**ROLES**
- Maintains homeostasis in humans
- Protective function against pathogens
- Formation of physical barrier
- Contribute to immune system
- Pathogenesis, implicated in obesity, IBD, UC, CD, NAFLD, DM, Alzheimer’s...

https://www.genome.gov/genetics-glossary/Microbiome
Novel culture methods → new discovery

**CULTURE-BASED METHODS**

**Standard Urine Culture**
- Microbial growth in culture media and bacterial identification
- Blood agar
- MacConkey agar
- 24 hours, 35°C, Aerobic conditions

**Enhanced Quantitative Urine Culture**
- Microbial growth in culture media and bacterial identification
- Blood agar
- Chocolate agar
- CNA blood agar
- 48 hours, 35°C, 5% CO₂

**SEQUENCING-BASED METHODS (NGS techniques)**

**Amplicon Sequencing**
- 16S rRNA gene
- a) DNA extraction and PCR amplification
- b) Sequencing and data analysis

**Shotgun sequencing**
- a) DNA extraction and fragmentation
- b) Sequencing and data analysis

**Designed for fast detection of few uropathogens (e.g., E.coli)**

→ **cannot detect slow-growing, nutrient-specific, anaerobic, or small-colonizing bacteria**
A Paradigm Shift

“Sterile Urine” and the Presence of Bacteria

Alan J. Wolfe a,*, Linda Brubaker b

a Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, Maywood, IL, USA; b Departments of Obstetrics and Gynecology and Urology, Stritch School of Medicine, Loyola University Chicago, Maywood, IL, USA

- Urinary tract considered sterile – microbiota found in asymptomatic urine (90% false negative)
- Limitations of culture-dependent methods of bacterial detection
- Vast majority of bacteria are not or cannot be cultured by standard lab techniques
- Introduction of 16S amplicon rRNA sequencing & EQUC (expanded quantitative urine culture) allows isolation & identification of previously missed organisms (x100 more urine used, various media & atmospheric conditions)
Explosive growth in literature

*Microbiome literature search in pubmed

![Chart showing the growth in Microbiome literature from 2003 to 2023. The number of publications more than doubled each year from 2015 to 2022. The total number of publications from 2003 to 2022 is 27,159.]
Urinary microbiome?

*urinary microbiome literature search in pubmed
Market projection in microbiome research

Global microbiome market:
- $269,000,000 in 2023
- $1,370,000,000 by 2029
Human microbiome in Asia

MarketsandMarkets, Human Microbiome Market, 2020

*2015-2022까지 8년동안 총 3,198 국책 과제 추진, 5055억 정부투자연구비 투입

MarketsandMarkets, Human Microbiome Market, 2020
연구개발특구진흥재단
Small but growing role of urinary microbiomes

A review of 10 years of human microbiome research activities at the US National Institutes of Health, Fiscal Years 2007-2016

NIH Human Microbiome Portfolio Analysis Team

A  Projects on the Role of the Microbiome in Specific Diseases, FY12-16.

B  Annual Trends in Projects on the Role of the Microbiome in Specific Diseases, FY12-16.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample collection method</th>
<th>Sample size (n)</th>
<th>Sample technique</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kogan et al., 2015</td>
<td>Midstream urine</td>
<td>24 ♀</td>
<td>Set of culture media and biochemical tests for bacterial identification</td>
<td><strong>FEMALE URINARY MICROBIOTA</strong>&lt;br&gt;Lactobacillus, Coagulase-negative Staphylococci, <em>Peptococcus</em>, <em>Corynebacterium</em>, <em>Propionibacterium</em>, <em>Eubacterium</em>, <em>Peptostreptococcus</em>, <em>Candida</em>, <em>Bacteroides</em>, <em>Bacillus</em>, <em>Veillonella</em>, <em>Enterobacteriaceae</em>, <em>Staphylococcus aureus</em>, <em>Enterococcus</em>, <em>Micrococcus</em>, <em>Prevotella</em>, <em>Actinomyces</em>, <em>Streptococcus</em></td>
</tr>
</tbody>
</table>

- Most genera shared
- Common: *Prevotella*, *Escherichia*, *Enterococcus*, *Streptococcus* or *Citrobacter*
- *Pseudomonas* only in men
- *Corynebacterium* and *Streptococcus* more abundant in men
- *Lactobacillus* more abundant in women
Microbiome in UTI

- Most researched
- UTI has been commonly associated with *Escherichia coli* (80% of the cases) but other commensal members of the gut microbiota, such as *Enterococcus*, and *Staphylococcus*, are also involved
- A correlation between an increase in the intestinal abundance of these genera and a higher prevalence of UTI
- Increase in uropathogens + imbalance of microbiota as a cause of UTI
Microbiome in cancer – TCC

Urinary microbiota – a potential biomarker and therapeutic target for bladder cancer

Hai Bi†, Ya Tian‡, Chuan Song‡, Jiari Li‡, Tingting Liu‡, Zhen Chen‡, Chen Chen‡, Yi Huang* and Yuanyuan Zhang**

- Urothelial cancer
- Chronic UTI as a risk factor
- 29 BCa vs. 26 control

**Actinomyces europaeus** enriched in BCa → a potential biomarker

Bacterial composition & alpha diversity significantly different

Perez-Carrasco (2021) Front Cell Infect Microbiol
Microbiome in cancer – PCa

Profilining the Urinary Microbiome in Men with Positive versus Negative Biopsies for Prostate Cancer

Eva Shrestha, James R. White*, Shu-Han Yu, Ibrahim Kulac, Onur Ertunc, Angelo M. De Marzo, Srinivasan Yegnasubramanian, Leslie A. Mangold, Alan W. Partin, and Karen S. Sfanos†

- Prostate cancer
- Chronic inflammation & prostatitis as a risk factor for cancer & progression

Enriched Actinobaculum schaalii, Anaerococcus lactolyticus, Varibaculum cambriense, Propionimicrobium lymphophilum and Ureaplasma species

- Presence of pro-inflammatory or pathogenic bacteria favors the development of prostate cancer

Shrestha (2018) J Urol
Urinary Incontinence – UUI

The Female Urinary Microbiome: a Comparison of Women with and without Urgency Urinary Incontinence

Meghan M. Pearce, Evann E. Hilt, Amy B. Rosenfeld, Michael J. Zilliox, Krystal Thomas-White, Cynthia Fok, Stephanie Kliethermes, Paul C. Schreckenberger, Linda Brubaker, Xiaowu Gai, Alan J. Wolfe

- UUI patients showed a **lower abundance** of *Lactobacillus* and a **higher abundance** of *Gardnerella*, along with other genera such as *Actinobaculum, Actinomyces, Aerococcus, Arthrobacter, Corynebacterium, Oligella, Staphylococcus* and *Streptococcus*

- 60 females with UUI vs. 58 control
- 16S rRNA + EQUC

Pearce (2014) mBio
Patients with MUI showed a decreased relative abundance of *Lactobacillus* and an increase in *Gardenerella* and *Prevotella*, similar to UUI patients.

- **Potential effect of UUI-associated components**

No difference by SUI sx.

- 123 females with MUI vs. 84 control
- 16S rRNA
### Table 3. Microbial strains identified in urine samples collected from individuals with overactive bladder symptoms

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Study</th>
<th>Year</th>
<th>Microbial strains</th>
<th>Decrease/Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>Burnett et al. [51]</td>
<td>2021</td>
<td>Few related studies in UTI, <em>Lactobacillus</em> or <em>Klebsiella</em> increased culture group has ‘frequency’ symptoms</td>
<td></td>
</tr>
<tr>
<td>Nocturia</td>
<td>Holland et al. [52]</td>
<td>2020</td>
<td>Urinary OUT related with <em>Lachnospiraceae Blautia</em> (protective correlation [negative correlation with symptom])</td>
<td></td>
</tr>
<tr>
<td>Urgency</td>
<td>Okamoto et al. [34]</td>
<td>2021</td>
<td><em>Bifidobacterium</em> group was low, and the <em>Faecalit</em> bacterium group was abundant in gut microbiome (not urobiome)</td>
<td></td>
</tr>
<tr>
<td>Urges incontinence</td>
<td>Pearce et al. [29]</td>
<td>2014</td>
<td>Increased <em>Gardnerella</em> and decreased <em>Lactobacillus</em> in UUI</td>
<td>Increased <em>Gardnerella</em> and decreased <em>Lactobacillus</em> in UUI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Lactobacillus</em> subgroup, <em>Lactobacillus crispatus</em> low and <em>Lactobacillus gasseri</em> high in UUI</td>
<td><em>Lactobacillus</em> subgroup, <em>Lactobacillus crispatus</em> low and <em>Lactobacillus gasseri</em> high in UUI</td>
</tr>
<tr>
<td></td>
<td>Thomas-White et al. [60]</td>
<td>2020</td>
<td>After vaginal estrogen therapy, decreased diversity and increased <em>Lactobacillus</em></td>
<td>After vaginal estrogen therapy, decreased diversity and increased <em>Lactobacillus</em></td>
</tr>
<tr>
<td></td>
<td>Abbasion et al. [61]</td>
<td>2019</td>
<td><em>Lactobacillus</em> inhibit ATP and calcium ion release from urothelium and inhibit UUI decreased <em>Lactobacillus</em>, cannot inhibit bladder contraction, and UUI happen</td>
<td><em>Lactobacillus</em> inhibit ATP and calcium ion release from urothelium and inhibit UUI decreased <em>Lactobacillus</em>, cannot inhibit bladder contraction, and UUI happen</td>
</tr>
<tr>
<td></td>
<td>Karstens et al. [56]</td>
<td>2016</td>
<td>14 Bacterial species identification in healthy and UUI decreased diversity has effects to symptom severity</td>
<td>14 Bacterial species identification in healthy and UUI decreased diversity has effects to symptom severity</td>
</tr>
</tbody>
</table>

UTI, urinary tract infection; UUI, urge urinary incontinence; ATP, adenosine triphosphate.

**Differences based on sex & study design → Inconclusive**

*Lactobacillus* species mainly identified in women

Bae S & Chung H. (2022) Int Neurourol J
Microbiome in neuropathic bladder

Distinguishing features of the urinary bacterial microbiome in patients with neurogenic lower urinary tract dysfunction

Giulia Lane¹,², Alyssa Gracey¹, Christine Bassis², Stephen E. Greiman², Paholo Barboglio Romo¹, J. Quentin Clemens¹, Priyanka Gupta¹, Diana O'Dell¹, John T. Stoffel¹, Anne P. Cameron¹

Enterobacteriaceae and Escherichia were the most abundant genera in NB. Pseudomonas was higher among patients using indwelling catheters relative to CIC. Aerococcus was at a higher relative abundance among males compared to females.

Neuropathic bladder
= urinary dysfunction derived from alterations in the CNS, which impair correct urine storage and emptying of the urinary bladder
Microbiome in IC/BPS

Integrated microbiome and metabolome analysis reveals novel urinary microenvironmental signatures in interstitial cystitis/bladder pain syndrome patients

- **Interstitial cystitis/Bladder pain syndrome** = pain and discomfort in the bladder and lower urinary tract, with absence of other pathology
- 30 IC/BPS & 30 control
- 16s rRNA + shotgun metabolomics

- **Lactobacillus** and **Escherichia-Shigella** was significantly higher in the urine of female IC/BPS patients and healthy controls compared to males, while **Bacteroides** and **Acinetobacter** were lower than in males. **Lactobacillus** genus may be protective against IC/BPS, whereas **Sphingomonas** may be a pathogenic factor.
- **Theophylline** (an anti-inflammatory metabolite) may downregulate the inflammatory response of IC/BPS.
Microbiome in CP/CPPS

- A wide range of samples – EPS, semen, urine… NOT well controlled nor replicated
- Generally increased overall bacterial diversity
- Increase in anaerobes, such as Clostridia, Bacteroides or Porphyromonas
- No singular cause of CP/CPPS has been identified and it is most likely a syndrome with multifactorial factors
Microbiome in BPH/LUTS

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sample Size (n)</th>
<th>Sample Type</th>
<th>Analysis Method</th>
<th>Relevant Microbiota</th>
<th>Primary Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis et al.</td>
<td>6</td>
<td>MSU</td>
<td>16S rRNA gene sequencing</td>
<td>Firmicutes, Proteobacteria, Actinobacteria,</td>
<td>• Diminish in numbers and increase in diversity with age</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fusobacteria, Bacteroidetes</td>
<td></td>
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<tr>
<td>Bajie et al.</td>
<td>49</td>
<td>MSU, TUC</td>
<td>EQUC, 16S rRNA gene sequencing</td>
<td>Streptococcus, Veillonella, Gardnerella,</td>
<td>• An increase in IPSS was associated with significantly high odds of detectable</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Staphylococcus, Candida</td>
<td>bacteria in TUC</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• TUC is adequate to sample the bladder microbiome</td>
</tr>
<tr>
<td>Yu et al.</td>
<td>21 BFH, 13 Pc</td>
<td>Voided urine, EPS/semenal fluid</td>
<td>16S rRNA gene sequencing with PCR-DGGE analysis</td>
<td>Eubacterium, Deflectillicoccus</td>
<td>• Bacterial flora in the EPS of patients with BPH differ from those with PC</td>
</tr>
<tr>
<td>Holland et al.</td>
<td>30 men</td>
<td>Urine &amp; fecal samples</td>
<td>16S rRNA gene sequencing</td>
<td>Lachnospiraceae, Bacteroidaceae,</td>
<td>• The abundance of L. blautii continued to have a protective correlation with</td>
</tr>
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<td></td>
<td></td>
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<td></td>
<td>Erysipelotrichiaceae, Ruminococcaceae,</td>
<td>LUTS when looking at various levels of IPSS severity</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Prevotellaceae, Clostridiales, Veillonellaceae,</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Enterococcaceae, Corynebacteriaceae, Incertae</td>
<td></td>
</tr>
<tr>
<td>Jain et al.</td>
<td>36 men</td>
<td>Resected tissue</td>
<td>EQUC, 16S rRNA gene sequencing,</td>
<td>Coagulase-positive Staphylococcus, L. coli,</td>
<td>• The presence of live bacteria in 55.5% of patient tissues</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>immunohistological staining</td>
<td>Micrococcus species, Proteobacteria,</td>
<td>• Staining confirmed the presence of cells with damaged DNA lesions in BPH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Actinobacteria, Firmicutes, Bacteroidetes</td>
<td>tissues and correlated with the severity of inflammation</td>
</tr>
<tr>
<td>Lee et al.</td>
<td>77 men with BPH, 30 controls</td>
<td>MSU</td>
<td>16S Metagenomic-Sequencing</td>
<td>Haemophilus, Staphylococcus, Dolosigranulum,</td>
<td>• Some bacterial genera correlated with a high IPSS as well as severe storage</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Lactaria, Plasmodactylaribacterium, Enhydrobacter, Bacillus, [Ruminococcus] torques,</td>
<td>and voiding symptoms</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Faecalibacterium, Finegoldia</td>
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</tbody>
</table>

- **Total number of bacteria** in the distal urethra **decreased with age**, while **number of genera increased** (increased ‘diversity’)
- **Change in the urethra and bladder microbiota with age** might be associated with increasing LUTS in older males, which is typically due to BPH
- **Infection with certain bacteria** may induce a **chronic inflammatory state** in the prostate via the enhanced production of proinflammatory cytokines
Research in Korea?

Fig. 2. Count of microbiome research funding and publications in Korea.

**Primarily Gut microbiome**
Similar to NA & EU
Recurrent cystitis vs. acute uncomplicated cystitis

- Cath urine with 16S rRNA amplicon sequencing in acute uncomplicated cystitis (AUC) & recurrent cystitis (RC)
- 42 pts.

**Table:**

<table>
<thead>
<tr>
<th>Positive culture rates</th>
<th>Number of Patients</th>
<th>Conventional Urine Culture (+)</th>
<th>Urine NGS (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute uncomplicated cystitis</td>
<td>11</td>
<td>4 (36.4%)</td>
<td>8 (72.7%)</td>
</tr>
<tr>
<td>Recurrent cystitis</td>
<td>32</td>
<td>3 (9.3%)</td>
<td>21 (67.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>7 (16.7%)</td>
<td>29 (69.0%)</td>
</tr>
</tbody>
</table>

Urine NGS is much more sensitive than conventional urine culture (69.0% vs. 16.7%, p < 0.05)

Microbiome diversity was significantly higher in RC compared to AUC, and microbiome composition was significantly different.

- *Pseudomonas, Acinetobacter,* and Enterobacteriaceae were found in AUC
- *Sphingomonas, Staphylococcus, Streptococcus,* and *Rothia* spp. were detected in RC

Difference in bacterial diversity and pattern → recurrent cystitis as a different entity
Gardnerella vaginalis in recurrent UTI

No difference in Gardnerella (+) detection in healthy control vs. rUTI (22.2% vs. 18.0%, p=0.677)

Gardnerella-positive group could be divided into three urotypes: (1) Escherichia-dominant group, (2) Gardnerella-dominant group, and (3) Lactobacillus-dominant group.

- All of the Escherichia-dominant groups were associated with rUTI.
- Atopobium, Megasphaera, and Ureaplasma, known to be associated with bacterial vaginosis, were detected only in rUTI.

Gardnerella does not cause UTI and is often detected in asymptomatic normal individuals but may be associated with vaginitis-induced dysbiosis causing UTI

Inconclusive
Semen microbiota in CPPS

- 17 CP/CPPS vs. 4 control
- Semen 16s rRNA seq.

- None of the semen samples showed colony formation in conventional bacterial cultures.
- Pyrosequencing revealed multiple bacterial genera in all samples, including an abundance of fastidious bacteria.
- Corynebacterium, Pseudomonas, Sphingomonas, Staphylococcus, and Streptococcus were frequently detected nonspecifically in both the patient and control groups.
- However, Achromobacter, Stenotrophomonas, and Brevibacillus were more frequently found in the CP/CPPS patients.
Different cancer types have different urine microbiome

Urine 16s rRNA sequencing
- 85 pts., including 30 BCa, 27 PCa, 12 RCC, 16 control
- Showed different urinary microbiome composition depending on type of GU malignancy

Urinary microbiome profile in men with genitourinary malignancies

6 genera of Cutibacterium, Peptoniphilus, Sphingomonas, Staphylococcus, Micrococcus, and Moraxella showed significantly different abundance between the 4 groups:
- Micrococcus sp. was significantly increased in BCa
- Cutibacterium acnes, Cutibacterium granulosum, Peptoniphilus lacydonensis, and Tessaracoccus were significantly increased in both PCa and RCC.

*first void urine

Ahn HK et al. (2022) Investig Clin Urol
Limitations & considerations

- Catheterized urine > voided urine for accurate representation of “bladder” microbiota (risk of contamination, esp. in women)
- Standardize collection of specimen
- Store and transport under bacteriostatic conditions (e.g. oxygen exposure → increase aerobes but induce death in anaerobes)
Low biomass – a fundamental problem

- \(<10^5\) colony-forming units per milliliter, approximately
  - For instance, female urinary microbiota is estimated to contain \(10^4 – 10^5\) (CFU)/ml vs. \(10^{12}\) CFU/g in feces
- Proximity to other bacterial niche with higher microbial biomass (vagina, gut) makes isolation of target samples and avoidance of contamination extremely difficult

Contamination even by laboratory agents

Salter SJ et al. (2014) BMC Urology

Critical Mis-interpretation of results
A MUST CONCERN
Future directions

1. Clarification of role as clinical biomarker
   → Is it replicable?

<table>
<thead>
<tr>
<th>Sex</th>
<th>Study</th>
<th>No. of patients</th>
<th>Urine collection method</th>
<th>Urobiome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Siddiqui et al. [65] (2011)</td>
<td>8</td>
<td>CCU</td>
<td><em>Lactobacillus</em>, <em>Prevotella</em>, <em>Gardnerella</em>, <em>Peptostreptococcus</em>, <em>Dialister</em>, <em>Finegoldia</em>, <em>Anaerococcus</em>, <em>Allisonella</em>, <em>Streptococcus</em>, <em>Staphylococcus</em></td>
</tr>
<tr>
<td></td>
<td>Wolfe et al. [66] (2012)</td>
<td>12</td>
<td>CCU, TUC, SP</td>
<td><em>Lactobacillus</em>, <em>Actinobaculum</em>, <em>Aerococcus</em>, <em>Anaerococcus</em>, <em>Atopobium</em>, <em>Burkholderia</em>, <em>Coronabacterium</em>, <em>Gardnerella</em>, <em>Prevotella</em>, <em>Balstonia</em>, <em>Streptococcus</em>, <em>Staphylococcus</em>, <em>Veillonella</em></td>
</tr>
<tr>
<td></td>
<td>Feins et al. [14] (2012)</td>
<td>15</td>
<td>MSU</td>
<td><em>Lactobacillus</em>, <em>Corynebacterium</em>, <em>Staphylococcus</em>, <em>Streptococcus</em>, <em>Prevotella</em></td>
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<td></td>
<td>Lewis et al. [67] (2013)</td>
<td>10</td>
<td>OCU</td>
<td><em>Firmicutes</em>, <em>Actinobacteria</em>, <em>Bacteroidetes</em></td>
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<tr>
<td></td>
<td>Hilk et al. [68] (2014)</td>
<td>24</td>
<td>TUC</td>
<td><em>Lactobacillus</em>, <em>Corynebacterium</em>, <em>Streptococcus</em>, <em>Actinomyces</em>, <em>Staphylococcus</em>, <em>Anaerococcus</em>, <em>Gardnerella</em>, <em>Bifidobacterium</em>, <em>Actinobaculum</em></td>
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<td></td>
<td>Pearce et al. [29] (2014)</td>
<td>58</td>
<td>TUC</td>
<td><em>Lactobacillus</em>, <em>Gardnerella</em>, <em>Corynebacterium</em>, <em>Enterobactieracae</em>, <em>Anaerococcus</em>, <em>Bifidobacterium</em>, <em>Streptococcus</em>, <em>Staphylococcus</em>, <em>Streptococcus</em>, <em>Peptostreptococcus</em>, <em>Porphyromonas</em>, <em>Prevotella</em>, <em>Peptostreptococcus</em>, <em>Atopobium</em>, <em>Ruminobacter</em>, <em>Trucrellia</em>, <em>Allanscardia</em>, <em>Veillonella</em></td>
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<td></td>
<td>Karstens et al. [56] (2016)</td>
<td>10</td>
<td>TUC</td>
<td><em>Anoxybacillus</em>, <em>Lactobacillus</em>, <em>Prevotella</em>, <em>Gardnerella</em>, <em>Arthrobacter</em>, <em>Escherichia</em>, <em>Shigella</em></td>
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<td></td>
<td>Thomas-White et al. [30] (2016)</td>
<td>60</td>
<td>TUC</td>
<td><em>Lactobacillus</em>, <em>Gardnerella</em>, <em>Staphylococcus</em>, <em>Streptococcus</em>, <em>Enterococcus</em>, <em>Bifidobacterium</em>, <em>Atopobium</em>, <em>Enterobacteriaceae</em></td>
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<tr>
<td></td>
<td>Wu et al. [69] (2017)</td>
<td>25</td>
<td>TUC</td>
<td><em>Lactobacillus</em>, <em>Prevotellaceae</em>, <em>Enterobacteriaceae</em>, <em>Veillonellaceae</em>, <em>Tissierellaceae</em>, <em>Bifidobacteriales</em></td>
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<tr>
<td></td>
<td>Gottschick et al. [70] (2017)</td>
<td>49</td>
<td>MSU</td>
<td><em>Lactobacillus</em>, <em>equisitatus</em></td>
</tr>
<tr>
<td></td>
<td>Abernethy et al. [71] (2017)</td>
<td>20</td>
<td>TUC</td>
<td><em>Lactobacillus</em>, <em>Ureaphilus</em>, <em>Porphyromonas</em>, <em>Prevotella</em>, <em>Bacteroides</em></td>
</tr>
<tr>
<td></td>
<td>Wang et al. [72] (2017)</td>
<td>21</td>
<td>MSU</td>
<td><em>Lactobacillus</em>, <em>Vaginatranslum</em>, <em>Porphyromonas</em>, <em>Prevotella</em>, <em>Bacteroides</em></td>
</tr>
<tr>
<td></td>
<td>Rani et al. [73] (2017)</td>
<td>5</td>
<td>MSU</td>
<td><em>Proteobacteria</em>, <em>Firmicutes</em>, <em>Actinobacteria</em>, <em>Bacteroidetes</em></td>
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<td></td>
<td>Komoski et al. [28] (2018)</td>
<td>84</td>
<td>TUC</td>
<td><em>Lactobacillus</em>, <em>Gardnerella</em>, <em>Tepidimonas</em>, <em>Prevotella</em></td>
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<td>Meriweather et al. [74] (2019)</td>
<td>18</td>
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<td><em>Lactobacillus</em>, <em>Prevotella</em></td>
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<td>Bredler et al. [75] (2019)</td>
<td>20</td>
<td>MSU</td>
<td><em>Lactobacillus</em></td>
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<tr>
<td></td>
<td>Liu et al. [76] (2020)</td>
<td>3</td>
<td>TUC</td>
<td><em>Gardnerella</em>, <em>Porphyromonas</em>, <em>Prevotella</em>, <em>Propionibacterium</em></td>
</tr>
</tbody>
</table>

Different bacteria identified with varying results depending on collection methods

Large, controlled studies required
Future directions

2. Functional role of microbiomes - multidisciplinary

- **Shotgun Metagenomic approach**
  - High bacterial coverage, but cannot distinguish live vs. dead bacteria
  - No information on function
  - Can provide information on host-bacteria interaction via functional profiling
  - Cost...

- **16S rRNA gene sequencing**
  - Taxonomic profile
  - Functional profile
  - Prediction

Microbiome
Dysbiosis & immunity interaction in disease

Environmental factors (e.g., antibiotic, diet)

Microbiome disturbance
e.g., microbial richness↓
- Ruminococcaceae↓, Lactobacillus↓, Proteobacteria↑
- metabolite change

Genetic susceptibility (e.g., NOD2, ATG16L1)

Immune dysregulation
e.g., Th17, Th2, Th1↑
- Treg↓
- IgA↓

Disease (e.g., IBD)
3. **Urobiome-targeted therapy**

→ restoration of microbiota to its healthy state: probiotics, FMT

**Fecal Microbiota Transplantation for Recurrent Clostridium difficile Infection Reduces Recurrent Urinary Tract Infection Frequency**

Raseen Tariq, Darrell S Pardi, Pratish K Tosh, Randall C Walker, Raymund R Razonable, Sahil Khanna

*Clinical Infectious Diseases, Volume 65, Issue 10, 15 November 2017, Pages 1745–1747, https://doi.org/10.1093/cid/cix618*

- FMT for Recurrent C. difficile infection
- Restore gut microbiota and reduce frequency of recurrent UTI by decolonizing MDR organisms

**Figure 1.** Frequency of urinary tract infections. Graph shows the number of infections 1 year before and 1 year after fecal microbiota transplantation and 1 year before and 1 year after the third Clostridium difficile infection episode in the control group. Each square and line represent 1 patient.
Microbiome for Precision medicine

Conventional therapy for UTI and rUTI

- Prolonged antibiotic exposure
- Multi-drug resistant uropathogens
- Microbiota dysbiosis

Host-microbiome modulation

- Probiotics e.g. Lactobacillus spp.
- Colonization with asymptomatic E. coli
- E. coli coated catheters
- Bioengineered E. coli
- Bacterial inhibitors e.g. Mannosides

Health-associated commensals

- Uropathogens
- Immunomodulation targeting host factors
- Damaged urothelium
- Immune cells
- Pro- and anti-inflammatory cytokines e.g. IL-6, IL-22
- Host innate immune receptors PRRs e.g. NLRs, TLRs
- Vaccines
- Anti-microbial peptides

Microbiota transplantation e.g. FMT, VMT

Microbiome restoration

Urinary tract

Female reproductive tract

Gastrointestinal tract
3.9. Urinary biomarkers and microbiome

Interest in the role of urinary biomarkers for the diagnosis of LUT dysfunction has increased in recent years. Nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), prostaglandin E2, adenosine triphosphate (ATP) and purinergic receptors (P2X) in bladder tissue have been studied as biomarkers for OAB. Serum beta natriuretic peptide (BNP), urinary 6-sulfouracil, melatonin and C-reactive protein (CRP), melatonin, vasopressin levels have been studied in relation to nocturia. For SUI, urinary IL 12-70, urinary (ungf) N-telopeptide of type I collagen (NTx) and urinary microbiota have been studied. Currently, studies investigating urinary biomarkers are methodologically limited often due to failing to control for confounding variables and results are conflicting [111].

Another area of discovery is the role of urinary microbiota in identifying and differentiating various types of UI and other LUT disease in women. A SR described studies showing differences in the types and relative proportions of bacteria such as Lactobacillus, Gardnerella, and Atopobium vaginae, among women with different types of UI compared with healthy controls. Urinary microbiota has also been shown to differ depending on women’s response to anticholinergic treatment response [112]. Further research is needed before the place of urine microbiota assessment in the clinical pathway for women with LUTS is fully defined.

Further information on the diagnostic efficacy of biomarkers in OAB can be found in Section 4.1.3.

### Summary of evidence and recommendation for urinary biomarkers

<table>
<thead>
<tr>
<th>Summary of evidence</th>
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<tr>
<td>There is insufficient evidence on the diagnostic accuracy and validity of urinary biomarkers for LUT disease in women.</td>
<td>3</td>
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<tr>
<td>Differences in the urinary microbiota have been found to be associated with different types of LUT dysfunction in women, including UI, and with different responses to treatment.</td>
<td>3</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Strength rating</th>
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<tbody>
<tr>
<td>Do not routinely use urinary biomarkers or estimation of the urinary microbiota in the diagnosis and management of LUT disease in women.</td>
<td>Strong</td>
</tr>
</tbody>
</table>
Changes in urinary and/or gut, vaginal microbiome can modulate host response to anti-microbial therapy
Take Home Message

- Explosive growth of microbiome researches (urobiome ~2%)
- Dysbiosis is associated with disease (UTI, bladder ca., IC)
- Urinary microbiome research is still in its early stages
- Limitations: low biomass, contamination, replication
- More studies on MOA, functional profile

MORE DATA NEEDED
Emerging trends and challenges in microbiome research

Seong Jin Jeong
Department of Urology, SNUBH
urojsj@snubh.org

Thank you for attention!