The role and restoration of the microbiome in urology

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Department of Surgery/ Microbiology & Immunology

Canadian Centre for Human Microbiome and Probiotics
Improved Understanding of the Bacterial Vaginal Microbiota of Women before and after Probiotic Instillation
Jeremy P. Burton,1,2* Peter A. Cadieux,2 and Gregor Reid1,2

Evaluation of the Bacterial Vaginal Flora of 20 Postmenopausal Women by Direct (Nugent Score) and Molecular (Polymerase Chain Reaction and Denaturing Gradient Gel Electrophoresis) Techniques
Jeremy P. Burton and Gregor Reid

Detection of Atopobium vaginae in Postmenopausal Women by Cultivation-Independent Methods Warrants Further Investigation
Jeremy P. Burton,1,2* Estelle Devillard,1,2 Peter A. Cadieux,2 Jo-Anne Hammond,3 and Gregor Reid1,2,4
Today

• What is known about the female urinary microbiome and how do they differ from other niches?
• What might the urinary microbiome do? (UPEC competition, immunity, neurotransmitters)
• Effects of external factors (antibiotics, medication)
• Microbiome and the upper tract (kidneys and stones)
• Urology and the gut microbiome (stone and androgens)
Gender and Urology

Female prevalent
- Prolapse
- Incontinence/ Urgency etc. (up to 17% vs 11% age dependent)
- Urinary tract infection (30X)
- Interstitial cystitis/ PBS (up to 6% vs 3%)

Male prevalent
- Kidney stones (13 vs 7%)
- Bladder cancer (4X)
- Kidney cancer (2X)
- Enlarged Prostate/ Prostatitis/ Cancer
- Dysfunction/ andrology
Microbiome and Urology?

• Until recently, considered sterile in healthy individuals distal to the urethra

• Not just the urinary microbiome important, but the gut and even the partners
Characterising the urinary microbiota

• **2007** - HMP- MSU sterile

• **2011** – First description of bacterial communities in midstream urine.

• **2012** – Midstream urine acceptable for urinary microbiota studies. MSU=Bladder

• **2014** – Urine is not sterile; the bacteria are alive!
Evidence of Uncultivated Bacteria in the Adult Female Bladder

Alan J. Wolfe, Evelyn Toh, Noriko Shibata, Ruichen Rong, Kimberly Kenton, MaryPat FitzGerald, Elizabeth R. Mueller, Paul Schreckenberger, Qunfeng Dong, David E. Nelson and Linda Brubaker

• 65 urine specimens examined by standard and enhanced culture. 80% grew bacterial species using enhanced, while 92% were reported as no growth ($10^3$ CFU/ml) by the clinical laboratory.

• Thirty-five different genera and 85 different species—*Lactobacillus* (15%), followed by *Corynebacterium* (14.2%), *Streptococcus* (11.9%), *Actinomyces* (6.9%), and *Staphylococcus* (6.9%)

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**TABLE 2** Comparison of cultured isolates to genera detected by 16S rRNA sequence in urine samples

<table>
<thead>
<tr>
<th>Urine</th>
<th>Isolate cultured</th>
<th>CFU/ml</th>
<th>% of sequences per sample (genus level classification)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OAB18</td>
<td><em>Lactobacillus jensenii</em></td>
<td>&gt;1,000</td>
<td>86.6</td>
</tr>
<tr>
<td></td>
<td><em>Gardnerella vaginalis</em></td>
<td>&gt;1,000</td>
<td>13.0</td>
</tr>
<tr>
<td>OAB21</td>
<td><em>Lactobacillus jensenii</em></td>
<td>140</td>
<td>92.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus iners</em></td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Gardnerella vaginalis</em></td>
<td>40</td>
<td>4.9</td>
</tr>
<tr>
<td>OAB23</td>
<td><em>Gardnerella vaginalis</em></td>
<td>&gt;1,000</td>
<td>80.0</td>
</tr>
<tr>
<td></td>
<td><em>Rothia dentocariosa</em></td>
<td>Broth&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Not detected</td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus anginosus</em></td>
<td>60</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td><em>Aerococcus urinae</em></td>
<td>50, 60</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td><em>Enterococcus faecalis</em></td>
<td>Broth&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
<tr>
<td>OAB26</td>
<td><em>Gardnerella vaginalis</em></td>
<td>300</td>
<td>97.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sequence data cannot distinguish between species.

<sup>b</sup> Cultured in thioglycolate broth; therefore, unable to determine starting CFU/ml.
How does the female urinary microbiome differ from other niches?

First-void urine, MSU and vaginal swab samples

rUTI

Control

<table>
<thead>
<tr>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
<th>A7</th>
<th>A8</th>
<th>A9</th>
<th>A10</th>
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</thead>
<tbody>
<tr>
<td>C1</td>
<td>C2</td>
<td>C3</td>
<td>C4</td>
<td>C5</td>
<td>C6</td>
<td>C7</td>
<td>C8</td>
<td>C9</td>
<td>C10</td>
</tr>
</tbody>
</table>

**Legend:**
- *Gardnerella* spp.
- *Atopobium* spp.
- *Prevotella* spp.
- *Staphylococcus* spp.
- *Lactobacillus* spp.
- *Escherichia-Shigella* spp.

Note: There is no significant difference in relative bacterial abundance between affected and control participants for any of the 3 sample types. A#= Affected participant ID; C#= Control participant ID.
Is the bladder microbiome different between women and men?
<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (n)</th>
<th>Notable taxa*</th>
<th>Sample collection method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dong et al. (2011)</td>
<td>Men with STI (10) Men without STI (22)</td>
<td><em>Lactobacillus, Sneathia, Veillonella, Corynebacterium, Prevotella, Streptococcus, Ureaplasma, Mycoplasma, Anaerococcus, Atopobium, Aerococcus, Staphylococcus, Gemella, Enterococcus, Finegoldia, Neisseria, Propionibacterium, Raistonia</em></td>
<td>First-void urine</td>
</tr>
<tr>
<td>Siddiqui et al. (2011)</td>
<td>Healthy women (8)</td>
<td><em>Lactobacillus, Prevotella, Gardnerella, Peptoniphilus, Dialister, Finegoldia, Anaerococcus, Allisonella, Streptococcus, Staphylococcus</em></td>
<td>Clean-catch midstream urine</td>
</tr>
<tr>
<td>Fouts et al. (2012)</td>
<td>Healthy controls (26; 58% women) Patients with NBD (27; 48% women)</td>
<td>Orders: <em>Lactobacillales, Enterobacteriales, Actinomycetales, Bacillales, Clostridiales, Bacteroidales, Burkholderiales, Pseudomonadales, Bif dobacteriales, Coriobacteriales</em></td>
<td>Midstream urine, intermittent catheterization, Foley catheter</td>
</tr>
<tr>
<td>Nelson et al. (2012)</td>
<td>Healthy adolescent men (18)</td>
<td><em>Lactobacillus, Streptococcus, Sneathia, Mycoplasma, Ureaplasma</em></td>
<td>First-void urine</td>
</tr>
<tr>
<td>Siddiqui et al. (2012)</td>
<td>Women with IC (8)</td>
<td><em>Lactobacillus, Gardnerella, Corynebacterium, Prevotella, Ureaplasma, Enterococcus, Atopobium, Proteus, Cronobacter</em></td>
<td>Clean-catch midstream urine</td>
</tr>
<tr>
<td>Wolfe et al. (2012)</td>
<td>Healthy women (12) Women with POP or UI (11)</td>
<td><em>Lactobacillus, Actinobaculum, Aerococcus, Anaerococcus, Atopobium, Burkholderia, Corynebacterium, Gardnerella, Prevotella, Raistonia, Sneathia, Staphylococcus, Streptococcus, Veillonella</em></td>
<td>Clean-catch midstream urine, suprapubic aspirate, transurethral catheter</td>
</tr>
<tr>
<td>Lewis et al. (2013)</td>
<td>Healthy men (6) Healthy women (10)</td>
<td><em>Jnquetella, Parvimonas, Proteiniphilum, Saccharofermentans Phyla: Actinobacteria, Bacteroidetes</em></td>
<td>Clean-catch midstream urine</td>
</tr>
<tr>
<td>Fricke et al. (2014)</td>
<td>Patients receiving first renal transplant (60; 37% women)</td>
<td><em>Lactobacillus, Enterococcus, Pseudomonas, Streptococcus Families: Bif dobacteriaeae, Corynebacterinae</em></td>
<td>Not described</td>
</tr>
<tr>
<td>Hilt et al. (2014)</td>
<td>Healthy women (24) Women with OAB (41)</td>
<td><em>Lactobacillus, Corynebacterium, Streptococcus, Actinomycyes, Staphylococcus, Aerococcus, Gardnerella, Bifidobacterium, Actinobaculum</em></td>
<td>Transurethral catheterization</td>
</tr>
<tr>
<td>Pearce et al. (2014)</td>
<td>Healthy women (58) Women with urgency UI (60)</td>
<td><em>Gardnerella, Lactobacillus, Actinobaculum, Actinomycyes, Aerococcus, Arthraborter, Corynebacterium, Oligella, Staphylococcus, Streptococcus</em></td>
<td>Transurethral catheterization</td>
</tr>
<tr>
<td>Willner et al. (2014)</td>
<td>Patients with acute uncomplicated UTI (50; 76% women)</td>
<td><em>Anaerococcus, Peptoniphilus, Streptococcus, Lactobacillus, Staphylococcus, Escherichia, Pseudomonas</em></td>
<td>Midstream urine</td>
</tr>
</tbody>
</table>

* Identified by the authors of the original studies as predominant or of significantly more prevalent than other populations; listed as genera, unless otherwise noted. Abbreviations: IC, interstitial cystitis; NBD, neurogenic bladder dysfunction; OAB, overactive bladder; POP, pelvic organ prolapse; STI, sexually transmitted infection; UI, urinary incontinence.
No studies completely agree

- Still in the discovery phase, snapshot studies
- Patient cohort (sex, disease status)
- Sample type, processing and extraction methodology
- PCR primers, 16S region, PCR cycle number, sequencing platform
- Bioinformatics pipelines and cut offs...lack of standardisation
Impact of urine processing on 16S rRNA
What could the microbiome be doing for us in the urinary tract?

- Immunity
- Barrier
- Homeostasis
Lactobacilli compete with pathogens: Ethanolamine

Table 2. Role of UTI-specific genes in fitness

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Function</th>
<th>Competitive indices</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bladder</td>
<td>Kidney</td>
</tr>
<tr>
<td>cus</td>
<td>Copper resistance</td>
<td>0.04</td>
<td>0.39</td>
</tr>
<tr>
<td>cys</td>
<td>Thiosulfate uptake</td>
<td>1.83</td>
<td>1.83</td>
</tr>
<tr>
<td>eutR</td>
<td>Ethanolamine uptake</td>
<td>0.049</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>and metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fdHF</td>
<td>Formate dehydrogenase</td>
<td>0.42</td>
<td>0.42</td>
</tr>
<tr>
<td>kdp</td>
<td>Potassium uptake</td>
<td>0.33</td>
<td>0.26</td>
</tr>
<tr>
<td>nik</td>
<td>Nickel uptake</td>
<td>0.29</td>
<td>0.43</td>
</tr>
<tr>
<td>phnR</td>
<td>Phosphonate uptake</td>
<td>1.47</td>
<td>2.64</td>
</tr>
<tr>
<td></td>
<td>and metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tau</td>
<td>Taurine uptake</td>
<td>2.18</td>
<td>1.33</td>
</tr>
<tr>
<td>wcALM</td>
<td>Colanic acid biosynthesis</td>
<td>2.23</td>
<td>2.23</td>
</tr>
</tbody>
</table>

Statistically significant fitness defect ($P < 0.05$, Wilcoxon signed rank test) is denoted in boldface.
Fibronectin adhesion

Adhesion of Lactobacillus iners AB-1 to Human Fibronectin: A Key Mediator for Persistence in the Vagina?

Amy McMillan, BSc\textsuperscript{1,2}, Jean M. Macklaim, BSc\textsuperscript{1,3}, Jeremy P. Burton, PhD\textsuperscript{2}, and Gregor Reid, PhD, MBA\textsuperscript{1,2}
The mechanism of action of BCG therapy for bladder cancer—a current perspective

GI Redelman-Sidi, Michael S. Glickman and Bernard H. Bochner
Bacterial neuroactive abilities

- Could bacterial production of neurotransmitters inhibit signals to the brain for frequency? urgency? pain?

- Does it play a role in urinary tract infections or urinary urgency and incontinence?

Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve

Javier A. Bravo\textsuperscript{a,1}, Paul Forsythe\textsuperscript{b,c,1}, Marianne V. Chew\textsuperscript{b}, Emily Escaravage\textsuperscript{b}, Hélène M. Savignac\textsuperscript{d,4}, Timothy G. Dinan\textsuperscript{b,6}, John Bienenstock\textsuperscript{b,1,2}, and John F. Cryan\textsuperscript{a,4,9,2}

\textsuperscript{a}Laboratory of NeuroGastroenterology, Alimentary Pharmacology Centre, \textsuperscript{b}School of Pharmacy, and Departments of \textsuperscript{c}Psychiatry and \textsuperscript{d}Anatomy, University College Cork, Cork, Ireland; \textsuperscript{e}McMaster Brain-Body Institute, St. Joseph’s Healthcare, Hamilton, ON, Canada L8N 4A6; and Departments of \textsuperscript{f}Medicine and \textsuperscript{g}Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada L8S 4L8

Bacteria activate sensory neurons that modulate pain and inflammation

Isaac M. Chiu\textsuperscript{1}, Balthasar A. Heesters\textsuperscript{2,3}, Nader Ghasemlou\textsuperscript{1}, Christian A. Von Hehn\textsuperscript{1}, Fan Zhao\textsuperscript{1}, Johnathan Tran\textsuperscript{1}, Brian Wainger\textsuperscript{1}, Amanda Strominger\textsuperscript{1}, Sriya Muralitharan\textsuperscript{1}, Alexander R. Horsswill\textsuperscript{2,6}, Juliane Bubeck Wardenburg\textsuperscript{4,6}, Sun Wook Hwang\textsuperscript{1,7}, Michael C. Carroll\textsuperscript{1} & Clifford J. Woolf\textsuperscript{1}

*Enterococcus faecalis* Subverts and Invades the Host Urothelium in Patients with Chronic Urinary Tract Infection

Harry Horsley\textsuperscript{1}, James Malone-Lee\textsuperscript{1}, David Holland\textsuperscript{1}, Madeleine Tuz\textsuperscript{1}, Andrew Hibbert\textsuperscript{1}, Michael Kelsey\textsuperscript{1}, Anthony Kupelian\textsuperscript{1}, Jennifer L. Rohn\textsuperscript{1}

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Bacterial Strain</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin</td>
<td><em>Lactococcus lactis</em> subsp. <em>cremoris</em> (MG 1363)</td>
<td>201</td>
</tr>
<tr>
<td></td>
<td><em>L. lactis</em> subsp. <em>lactis</em> (IL1403)</td>
<td>201</td>
</tr>
<tr>
<td></td>
<td><em>Lactobacillus plantarum</em> (FI8595)</td>
<td>201</td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus thermophilus</em> (NCFB2392)</td>
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</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em> K-12</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td><em>Morganella morganii</em> (NCIMB, 10466)</td>
<td>202</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em> (NCIMB, 673)</td>
<td>202</td>
</tr>
<tr>
<td></td>
<td><em>Hafnia alvei</em> (NCIMB, 11999)</td>
<td>202</td>
</tr>
<tr>
<td>Dopamine</td>
<td><em>Bacillus cereus</em></td>
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<tr>
<td></td>
<td><em>B. mycoides</em></td>
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<td><em>B. subtilis</em></td>
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<td><em>Proteus vulgaris</em></td>
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<td><em>Serratia marcescens</em></td>
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<td><em>S. aureus</em></td>
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<tr>
<td></td>
<td><em>E.coli</em></td>
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<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
<td><em>H. alvei</em> (NCIMB, 11999)</td>
<td>202</td>
</tr>
</tbody>
</table>
Metabolites found in the urine of humans or shown to interact with bladder tissue.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-arachidonylglycerol</td>
<td>Enkephalin L</td>
</tr>
<tr>
<td>3-Methoxytyramine, (3-MT)</td>
<td>Epinephrine</td>
</tr>
<tr>
<td>5a-Pregnane-3,20-dione</td>
<td>Estradiol</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Etiocholanolone</td>
</tr>
<tr>
<td>Agmatine</td>
<td>Gamma hydroxybutyric acid (GHB)</td>
</tr>
<tr>
<td>Allopregnanolone (THP)</td>
<td>Gamma-aminobutyric acid (GABA)</td>
</tr>
<tr>
<td>Anandamide</td>
<td>Glycine</td>
</tr>
<tr>
<td>Androstanediol</td>
<td>Histamine</td>
</tr>
<tr>
<td>Androsterone</td>
<td>L-aspartic acid (Aspartate)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>L-glutamic acid (Glutamate)</td>
</tr>
<tr>
<td>D-serine</td>
<td>Melatonin</td>
</tr>
<tr>
<td>Dehydroepiandrosterone (DHEA)</td>
<td>N-Methyl-D-aspartic acid (NMDA)</td>
</tr>
<tr>
<td>Dehydroepiandrosterone sulfate (DHEA-S)</td>
<td>N-methyltryptamine</td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td>Dopamine</td>
<td>O-Arachidonoyl ethanolamide</td>
</tr>
<tr>
<td>D-aspartic acid (Aspartate)</td>
<td>p-Octopamine</td>
</tr>
<tr>
<td>L-glutamic acid (Glutamate)</td>
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</tr>
<tr>
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<td>Phenethylamine</td>
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<td>N-Methyl-D-aspartic acid (NMDA)</td>
<td>Pregnenolone</td>
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<tr>
<td>N-methyltryptamine</td>
<td>Pregnenolone sulfate (PS)</td>
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<tr>
<td>Norepinephrine</td>
<td>Progesterone</td>
</tr>
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<td>O-Arachidonoyl ethanolamide</td>
<td>Serotonin (5-HT)</td>
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<td>O-Arachidinoyl ethanolamide</td>
<td>Substance P</td>
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<tr>
<td>Palmitoylethanolamide (PEA)</td>
<td>Synephrine</td>
</tr>
<tr>
<td>Phenethylamine</td>
<td>Taurine</td>
</tr>
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<td>Pregnenolone</td>
<td>Testosterone</td>
</tr>
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<td>Pregnenolone sulfate (PS)</td>
<td>Tetrahydrodeoxycorticosterone (THDOC)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Tryptamine</td>
</tr>
<tr>
<td>Serotonin (5-HT)</td>
<td>Tyramine</td>
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<td>Tetrahydrodeoxycorticosterone (THDOC)</td>
<td>Tyramine</td>
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</tbody>
</table>
Targeted metabolomics

Solid-phase microextraction (SPME) LC-MS

Does *Enterococcus* produce neuroactive substances in vitro?

*E. faecalis 29212*
The Female Urinary Microbiome: a Comparison of Women with and without Urgency Urinary Incontinence

qPCR screens of exposed human cells

**Receptors**
- TRPV (TRPV1, TRPV4)
  - CDH1, JUP, CTNNA1, CTNNA3, CTNNB1
- Trace amine associated (TAAR1)
- Dopamine (DRD1)
- Adrenergic (ADRA2A, ADRB3)
- Glutamate (GRIN1)
- Nicotinic Acetylcholine (CHRNA3, CHRNA7)
- Muscarinic Acetylcholine (CHRM1-4)
- Purinoceptor (P2RX1)

**Other**
- Monoamine oxidases (MAOA, MAOB)
- Substance P (TAC1)
- Vasoactive Intestinal Peptide (VIP)
- CGRP (CALCA, CALCB)
**Fold-Change in Gene Expression Relative to GAPDH**

- **GRIN 1**-Causes cell depolarization due to influx of Ca^{2+} ions

- Mono amine oxidase A and B Catalyze oxidative deamination of biogenic amine neurotransmitters: Norepinephrine, Serotonin, Tyramine, Norepinephrine, Dopamine, Phenethylamine
Escherichia coli 83972 Bacteriuria Protects Against Recurrent Lower Urinary Tract Infections in Patients With Incomplete Bladder Emptying

Fredrik Sundén, Lars Håkansson, Eva Ljunggren and Björn Wullt*

From the Department of Urology (FS, EL, BW), Spinal Injuries Unit Lund/Orup, Department of Rehabilitation (LH), and Department of Microbiology, Immunology and Glycobiology (BW), Lund University Hospital, Lund, Sweden

Asymptomatic Bacteriuria *Escherichia coli* Are Live Biotherapeutics for UTI

Charles N. Rudick1*, Aisha K. Taylor1,2*, Ryan E. Yaggio1, Anthony J. Schaeffer1, David J. Klumpp1,2*

1 Department of Urology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, United States of America, 2 Microbiology-Immunology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, United States of America
External influences on the urinary environment?

- Many factors (diet eg. mannose, sex, systemic inflammation etc.)
- Early stage
- Medications eg AbX and others (small No of studies)
- Other microbiomes (eg gut, vagina- UPEC reservoir).
  - Neuroactive substances and possible bladder cross talk (UUI)
In continence medication response relates to the female urinary microbiota

Krystal J. Thomas-White¹ & Evann E. Hilt¹ & Cynthia Fok² & Meghan M. Pearce¹ & Elizabeth R. Mueller³,¹⁰ & Stephanie Kliethermes⁴ & Kristin Jacobs⁵ & Michael J. Zilliox⁶ & Cynthia Brincat³,¹⁰ & Travis K. Price¹ & Gina Kuffel⁷ & Paul Schreckenberger⁸ & Xiaowu Gai⁹ & Linda Brubaker³,¹⁰ & Alan J. Wolfe¹

5-10 mg Solifenacin (antimuscarinic)
Taking Antibiotics Can Change the Gut Microbiome for Up to a Year

A new study illuminates the problems antibiotic overuse could cause for individual patients.

Sub inhibitory Abx prime uropathogens
Sub therapeutic ciprofloxacin increases bacterial burden and recurrence frequency.

Goneau et al. mBio 2015; doi:10.1128/mBio.00356-15
AbX priming increases invasive capacity of UPEC.
Bacteria in the upper tract and kidneys?
Bacteria in the kidney?

- During reflux and infection (pyelonephritis), flagellated bacteria
- Systemic source and translocation in chronic disease (CKD)
- In kidney stones?

RESEARCH ARTICLE

The Interaction between Enterobacteriaceae and Calcium Oxalate Deposits

Evan Barr-Beare¹, Vijay Saxena¹, Evann E. Hilt², Krystal Thomas-White², Megan Schober³, Birong Li¹, Brian Becknell¹,⁴, David S. Hains⁵, Alan J. Wolfe², Andrew L. Schwaderer⁶,⁷,⁸
Kidney stones

- Painful and need to be removed to prevent damage
- In Canada, kidney stones affect ~5% of the population, with a lifetime risk of ~10%, but increasing in prevalence
- Recurrence within 5-7 years is 50%, within 20 years is 75%
Before and after shock wave lithotripsy (ESWL)

Stone (2F, 8M-no stents)

Healthy (3F, 3M)

Clinic Visit

OR

Follow Up

21 Days

3 Months

1

2

3

4

Patient

Lactobacillus

Gardnerella

Escherichia/Shigella

Corynebacterium

Streptococcus

Pseudomonas

Bifidobacterium

Prevotella

Cloacibacterium

Staphylococcus

Campylobacter

Anaerococcus

Actinobaculum

Sphingomonas

Propionimicrobium

Luteococcus
ESWL Lithotripsy

- Male and female microbiome compositions are different
- Urinary microbiome changes after ESWL.
- May facilitate infection as 10-39.8% of lithotripsy develop post-operative infection. 0.3-9.3% develop sepsis.
- Others have reported kidney stone microbiome but did these come from there?
- Samples required directly from kidney
Getting the right sample

• Kidney stones contaminated through stents and other devices through UGT.
Next generation sequencing (NGS) of the 16S rRNA gene

Vulnerable to carry over and contamination at the time point of sample collection and laboratory processing especially if low abundance

It only sees the DNA sequences, it cannot differentiate live and dead cells
Kidney samples for microbiome

- Ontario Tumor Bank: renal cell carcinoma, transitional cell carcinoma, various histology etc. All patients received nephrectomy

- 56 patients, 112 samples, 36 males and 20 females. Tumor tissue sample + adjacent normal tissue

**Extended culture/ PMA-qPCR**

- RED-detected in sequencing and culture
- BLUE-detected only by enhanced culture
- GREEN-detected only by sequencing
Sequencing results

Tumour

Adjacent

PCOA biplot
Bacteria in the kidney?

- Limited, **but bacteria present** and some cultivable
- No apparent different between gender
- Where are they from? Some casual similarities between nasal, bronchiole, blood and ESWL microbiome
- What’s it doing? Nidus for inflammation? transients?
- Undertaking further confirmation
Gut microbiome and urology
Gut microbiome’s importance?

- Kidney stones, uremic toxins, UTI reservoir, carcinogens
- Cross talk-neuroactive substances
- Regulation of inflammation (transplant, CKD)

Clinical investigation

Chronic kidney disease alters intestinal microbial flora

Nosratola D. Vaziri¹, Jakk Wong², Madeleine Pahl¹, Yvette M. Piceno², Jun Yuan¹, Todd Z. DeSantis³, Zhenmin Ni¹, Tien-Hung Nguyen² and Gary L. Andersen²
Dietary oxalate
Oxalobacter formigenes May Reduce the Risk of Calcium Oxalate Kidney Stones

David W. Kaufman,* Judith P. Kelly,* Gary C. Curhan,† Theresa E. Anderson,* Stephen P. Dretler,‡ Glenn M. Preminger,§ and David R. Cave‖

Table 1.  *O. formigenes* in stool among patients with recurrent CaOx kidney stones and control subjects

| *O. formigenes* Status | Case Patients  
| (n = 247) | Control Subjects  
| (n = 259) | Crude OR | Multivariate OR (95% CI)* |
| --- | --- | --- | --- | --- | --- |
| Positive | 42 | 17 | 99 | 38 | 0.3 | 0.3 (0.2 to 0.5) |
| Negative | 205 | 83 | 160 | 62 | 1.0b | 1.0b |

*OR based on unconditional logistic regression with the following factors in the model: *O. formigenes*, age, gender, region, education, race, dietary oxalate, antibiotic use, and family history of stones. OR calculated using conditional logistic regression: 0.3 (0.1 to 0.5).

bReference category.
A Human Strain of *Oxalobacter* (HC-1) Promotes Enteric Oxalate Secretion in the Small Intestine of Mice and Reduces Urinary Oxalate Excretion

Marguerite Hatch, Ph.D. and Robert W. Freel, Ph.D.
Microbiota of stone-formers?

- 220 patients with a kidney stone undergoing planned percutaneous nephrolithotomy (PCNL) or ureteroscopy (URS), 20+ non-stone-forming control participants

- Oral swab, mid-stream urine sample, fecal sample, stones collected from patients after surgery

- Stone-free status is confirmed with ultrasound in control participants
Transfer of Intestinal Microbiota From Lean Donors Increases Insulin Sensitivity in Individuals With Metabolic Syndrome
• Treat *Clostridium difficile*-associated diarrhea with ~90% success
• FMTs are currently being investigated to treat other diseases, such as metabolic syndrome
Establishing a donor pool

- Medical histories and physical examinations undertaken by a physician.
- Low Hgb A1C, low fasting lipids, normal liver function tests, normal HOMA, negative anti-TTG antibodies
- **Exclusions:**
  - BMI > 25
  - Any underlying health conditions
  - Positive for any infectious diseases (screened for 31 viral, bacterial, fungal and protozoan agents)
  - High risk behaviours
  - Family history of diabetes, coronary disease, obesity, gastrointestinal disease, liver disease and colon cancer
Potential Donors Pre-Screened (n=46)  

Passed History/Examination (n=23)  

Passed Blood/Stool Tests (n=5)  

Donor Accepted (n=1)  

Total Cost = $15,180
Microbiome reconditioning

- How do we restore *Oxalobacter* or oxalate-utilizing bacteria?
Can we repair microbiomes?

16S rRNA

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</table>

O. formigenes (No oxalate)  
O. formigenes (60 mM oxalate)  
Lactobacillus (No oxalate)  
Lactobacillus (60 mM oxalate)

Fold-change in abundance

Time (h)
Gut microbiome and androgen biogenesis?
• Bacteria may modify, activate or inactivate ingested compounds prior to being able to act upon its’ intended target; this has huge implications for the host.

• Microbes likely play a role in other cancer therapies including conventional and immunotherapy agents.

• Could the microbiome have a role in the regulation of androgen deprivation therapy in prostate cancer? Is there a microbiome difference between those who are sensitive to therapy and those who are not?

• At “high level” the gut microbiome looks similar between sex, but is it?
Sex Differences in the Gut Microbiome Drive Hormone-Dependent Regulation of Autoimmunity

Janet G. M. Markle,1,2 Daniel N. Frank,3 Steven Mortin-Toth,2 Charles E. Robertson,4 Leah M. Feazel,3 Ulrike Rolle-Kampczyk,5 Martin von Bergen,5,6,7 Kathy D. McCoy,8 Andrew J. Macpherson,8 Jayne S. Danska1,2,9*

A

Cumulative TID (%) vs. Age (weeks)

Females SPF
Males SPF

***

B

Cumulative TID (%) vs. Age (weeks)

Females GF
Males GF

n.s.

C

Testosterone (pg/mL) vs. Sex Hygiene

F SPF
F GF
M SPF
M GF

*

D

PC1 (37.8%) vs. PC2 (18.0%)

F SPF
M SPF
F GF
M GF
Transfer of gut microbiota from adult males to immature females altered the recipient's microbiota, resulting in elevated testosterone and metabolomic changes, reduced islet inflammation and autoantibody production, and robust T1D protection.

Production of testosterone by the microbiome may have significant implications in treatment of prostate cancer and other conditions (not just in males).

Could the microbiota subvert ADT?

- Testosterone Standard
- Castrate-Resistant Prostate Cancer Patient Sample
- Media Control
**Clostridium scindens**: a human gut microbe with a high potential to convert glucocorticoids into androgens

- RNA-Seq data suggests that the two-carbon side chain of glucocorticoids may feed into the pentose-phosphate pathway and are used as a carbon source.
- Did not look at testosterone

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Jason M. Ridlon, *† Shigeo Ikegawa, § João M. P. Alves, **** Biao Zhou, †† Akiko Kobayashi, †† Takashi Iida, †† Kuniko Mitamura, § Genzoh Tanabe, § Myrna Serrano, *† Ainee De Guzman, §§ Patsy Cooper, † Gregory A. Buck, *† and Phillip B. Hylemon †,*†
**Clostridium scindens** as a model producer

The administration of glucocorticoids with abiraterone acetate is necessary to manage adverse events related to mineralocorticoid excess, such as hypokalemia, hypertension, and fluid retention, which can occur as a result of CYP17A1 inhibition.
Hydrocortisone → DesC → Hydroxyandrostenedione → Testosterone

Fold Change

Steroid-17,20-Desmolase
Is the microbiome trying to replenish host’s metabolic capacity?

- Early results. Functional? *In vitro* studies looking LNCaP cells and androgen receptor activation
- Carriage and expression of *des* genes in the microbiomes of 50 CRPC patients, but other patients get corticosteroids
- If bacteria involved can we alter by AbX or F-FMT?
- Role of bacteria in sex hormone regulation-implications for both men and women
Summary

- Bacteria are almost found ubiquitously, even in sites once considered sterile. What are they doing?
- Differences between male and female microbiomes will help us determine their relative importance.
- Bacteria in the intestinal tract play an important role in urological health, the metabolism of drugs and biogenesis of other substances.