The Urinary Microbiome

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Loyola University Chicago
Overview

• Existence of a Female Urinary Microbiome & Microbiota
• Implications for Urinary Tract Infections
• Current and Future Projects
Overview

• Existence of a Female Urinary Microbiome & Microbiota
• Implications for Urinary Tract Infections
• Current and Future Projects
A Female Urinary Microbiome exists

- 16S sequencing – V4 region
- Illumina MiSeq
- Mothur bioinformatics

Wolfe et al., 2012
A Female Urinary Microbiome exists

Transurethral Catheter (TUC)

Suprapubic Aspirate (SPA)

Needle Stick

Skin Swab

Wolfe et al., 2012
Urine is not sterile

<table>
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<tr>
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Standard Urine Culture (SUC)

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Enhanced Quantitative Urine Culture (EQUC)

1 μL, Aerobic at 35C, 24hrs

Hilt et al., 2014
**Urine is not sterile – Female Urinary Microbiota (FUM)**

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**Standard Urine Culture (SUC)**

- 1 µL, Aerobic at 35°C, 24hrs

**Enhanced Quantitative Urine Culture (EQUC)**

- 100 µL, Aerobic at 35°C, 48hrs

Hilt *et al.*, 2014
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**Standard Urine Culture (SUC)**
- 1 μL, Aerobic at 35°C, 24hrs

**Enhanced Quantitative Urine Culture (EQUC)**
- 100 μL, Aerobic at 35°C, 48hrs

SUC has a 90% false-negative rate

Hilt et al., 2014
Sequencing & EQUC are complementary

Pearce et al., 2014
Is the FUM involved in Lower Urinary Tract Disorders?

Adult women seeking clinical care

Pelvic Floor Distress Inventory (PFDI) Questionnaire

Transurethral catheterized urine (TUC) specimen

UUI (N=60)  Non-UUI (N=58)

EQUC & 16S rRNA sequencing

Pearce et al., 2014
FUM diversity is higher in women with UUI

Pearce et al., 2014
FUM composition differs between UUI and non-UUI women

Pearce et al., 2014
FUM composition differs between UUI and non-UUI women

Pearce et al., 2014
Overview

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SUC has a 90% false-negative rate

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Standard Urine Culture (SUC)

1 μL, Aerobic at 35°C, 24hrs

Enhanced Quantitative Urine Culture (EQUC)

100 μL, Aerobic at 35°C, 48hrs

Hilt et al., 2014
Hypothesis

The standard **definitions** and **measures** of UTIs are inefficient at detecting clinically relevant infections.
Hypothesis

The standard definitions and measures of UTIs are inefficient at detecting clinically relevant infections.

- Standard Urine Culture (SUC) protocol
  - Urinary Symptoms
  - \( \geq 10^5 \) CFU/mL for diagnosis
Study design

“Do you feel you have a UTI?” (N=150)

“Yes” (N=75)  “No” (N=75)

SUC & Expanded EQUC
UTI Symptoms Assessment (UTISA) Questionnaire

Post-Questionnaire Follow-up

UTISA Questionnaire

- Urgency
- Frequency
- Dysuria
- Difficulty Urinating
- Abdominal/Pelvic Pain/Pressure
- Low Back Pain
- Blood in Urine

Price et al., 2016
Clayson et al., 2005
## Study design

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<tr>
<td>Anaerobic</td>
<td>Microaerophilic (5% O₂, 10% CO₂, 85% N) 35C</td>
<td>48 hr</td>
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</table>
Composition differs at the genus level

**No** UTI (N=75)

- Lactobacillus
- Gardnerella
- Streptococcus

**Yes** UTI (N=75)

- Staphylococcus
- Escherichia

Price et al., 2016
Hypothesis

The standard definitions and measures of UTIs are inefficient at detecting clinically relevant infections.

- **Standard Urine Culture (SUC) protocol**
  - Urinary Symptoms
  - \( \geq 10^5 \) CFU/mL for diagnosis
**SUC fails to detect 67% of uropathogens**

<table>
<thead>
<tr>
<th>Culturing Protocol</th>
<th>Uropathogens (N=182)</th>
</tr>
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<tbody>
<tr>
<td>100ul Expanded EQUC</td>
<td>96% (N=174)</td>
</tr>
<tr>
<td>10ul Expanded EQUC</td>
<td>65% (N=118)</td>
</tr>
<tr>
<td>1ul Expanded EQUC</td>
<td>52% (N=95)</td>
</tr>
<tr>
<td>Standard Urine Culture (SUC)</td>
<td>33% (N=60)</td>
</tr>
</tbody>
</table>

**Uropathogens (SUC/EQUC):**

Actinobaculum schaalii (0/6), Aerococcus sanguinicola (0/1), Aerococcus urinae (1/15), Alloscardovia omnicolens (0/8), Candida albicans (0/2), Candida parapsilosis (0/4), Citrobacter freundii (1/1), Citrobacter koseri (0/1), Corynebacterium riegelii (0/4), Corynebacterium urealyticum (0/2), Enterobacter aerogenes (1/3), Enterococcus faecalis (1/16), Escherichia coli (44/50), Klebsiella pneumoniae (4/10), Morganella morganii (0/1), Oligella urethralis (0/1), Proteus mirabilis (2/4), Pseudomonas aeruginosa (1/1), Serratia marcescens (0/1), Staphylococcus aureus (3/7), Staphylococcus lugdunensis (1/2), Streptococcus agalactiae (1/10), Streptococcus anginosus (0/32)

*Price et al., 2016*
SUC fails to detect 88% of non-\textit{E. coli} uropathogens

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<thead>
<tr>
<th>Culturing Protocol</th>
<th>Detection of \textit{E. coli} (N=50)</th>
<th>Non-\textit{E. coli} Uropathogens (N=132)</th>
</tr>
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<tbody>
<tr>
<td>100ul Expanded EQUC</td>
<td>100% (N=50)</td>
<td>94% (N=124)</td>
</tr>
<tr>
<td>10ul Expanded EQUC</td>
<td>94% (N=47)</td>
<td>55% (N=72)</td>
</tr>
<tr>
<td>1ul Expanded EQUC</td>
<td>92% (N=46)</td>
<td>36% (N=48)</td>
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<tr>
<td>Standard Urine Culture (SUC)</td>
<td>89% (N=44)</td>
<td>12% (N=16)</td>
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\textbf{Uropathogens (SUC/EQUC):}  
\textit{Actinobaculum schaalii} (0/6), \textit{Aerococcus sanguinicola} (0/1), \textit{Aerococcus urinae} (1/15), \textit{Alloscardovia omnicolens} (0/8), \textit{Candida albicans} (0/2), \textit{Candida parapsilosis} (0/4), \textit{Citrobacter freundii} (1/1), \textit{Citrobacter koseri} (0/1), \textit{Corynebacterium riegliei} (0/4), \textit{Corynebacterium urealyticum} (0/2), \textit{Enterobacter aerogenes} (1/3), \textit{Enterococcus faecalis} (1/16), \textit{Escherichia coli} (44/50), \textit{Klebsiella pneumoniae} (4/10), \textit{Morganella morganii} (0/1), \textit{Oligella urethralis} (0/1), \textit{Proteus mirabilis} (2/4), \textit{Pseudomonas aeruginosa} (1/1), \textit{Serratia marcescens} (0/1), \textit{Staphylococcus aureus} (3/7), \textit{Staphylococcus lugdunensis} (1/2), \textit{Streptococcus agalactiae} (1/10), \textit{Streptococcus anginosus} (0/32)
Hypothesis

The standard definitions and measures of UTIs are inefficient at detecting clinically relevant infections.

- Standard Urine Culture (SUC) protocol
- Urinary Symptoms
- \( \geq 10^5 \) CFU/mL for diagnosis
## Urinary symptoms and UTI diagnosis

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<tr>
<td><strong>Self-Report</strong></td>
<td></td>
</tr>
<tr>
<td>UTI: N=75</td>
<td></td>
</tr>
<tr>
<td>No UTI: N=75</td>
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<tr>
<th>UTI Diagnosis:</th>
<th>SUC ($\geq 10^5$ CFU/mL)</th>
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<tr>
<td>UTI: N=57</td>
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<tr>
<td>No UTI: N=93</td>
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<th>UTI Diagnosis:</th>
<th>EQUC ($\geq 10$ CFU/mL)</th>
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<tr>
<td>UTI: N=110</td>
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</tr>
<tr>
<td>No UTI: N=40</td>
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Dysuria is a strong indicator of UTI

**UTI Diagnosis: Self-Report**
- UTI: N=75
- No UTI: N=75

**UTI Diagnosis: SUC (≥10⁵ CFU/mL)**
- UTI: N=57
- No UTI: N=93

**UTI Diagnosis: EQUC (≥10 CFU/mL)**
- UTI: N=110
- No UTI: N=40

![Graphs showing frequency of symptoms and UTI diagnosis](Image)

Dune et al., 2017
Hypothesis

The standard definitions and measures of UTIs are inefficient at detecting clinically relevant infections.

- Standard Urine Culture (SUC) protocol
  - Urinary Symptoms
  - $\geq 10^5$ CFU/mL for diagnosis
$\geq 10^5$ CFU/mL fails to detect most uropathogens

Price et al., 2016
Conclusions

• Use of Standard Urine Culture (SUC) is not a good indicator of UTI
  – Optimal protocol: 100µL; Blood, MacConkey, CNA; 5% CO₂; 48 hrs

• Symptoms of Frequency and Urgency or urination are not good indicators of UTI
  – Presence of Pain and Burning during urination (Dysuria) is associated with multiple UTI definitions

• Use of a $\geq 10^5$ CFU/mL threshold is not a good indicator of UTI
  – Lower/No threshold better detects possible Gram-positive and polymicrobial UTIs
  – Failure to treat infections $<10^5$ CFU/mL results in poor clinical outcome
Overview

• Existence of a Female Urinary Microbiome & Microbiota
• Implications for Urinary Tract Infections
• Current and Future Projects
Current projects

• Clinical trail to determine if treatment based off EQUC leads to improved patient outcome
• Measuring the effects of Estrogen on the FUM
• Determining if the FUM contributes to Interstitial Cystitis (IC/PBS)
• Measuring the stability of the FUM
Natural development of a UTI

Participant E

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<tr>
<th>Time (Days)</th>
<th>Total CFU/mL</th>
<th>Relative Abundance</th>
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<tr>
<td>1-18</td>
<td>0-5000000</td>
<td>0-100%</td>
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**Peri-urethral Swab**

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**Midstream Voided Urine**

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**Organisms**

- Actinomyces
- Alloscardovia
- Propionibacterium
- Bifidobacterium
- Pseudomonas
- Staphylococcus
- Enterobacter
- Enterococcus
- Escherichia
- Lactobacillus
Acknowledgments

Loyola Urinary Education and Research Collaboration (LUEREC)

- Linda Brubaker, MD
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- Michelle VanKuiken, MD
- Krystal Thomas-White, PhD
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- Gina Kuffel, BSc
- Kathleen McKinley, BSc
- Mary Tulke, RN