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The Urinary Microbiome

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Overview

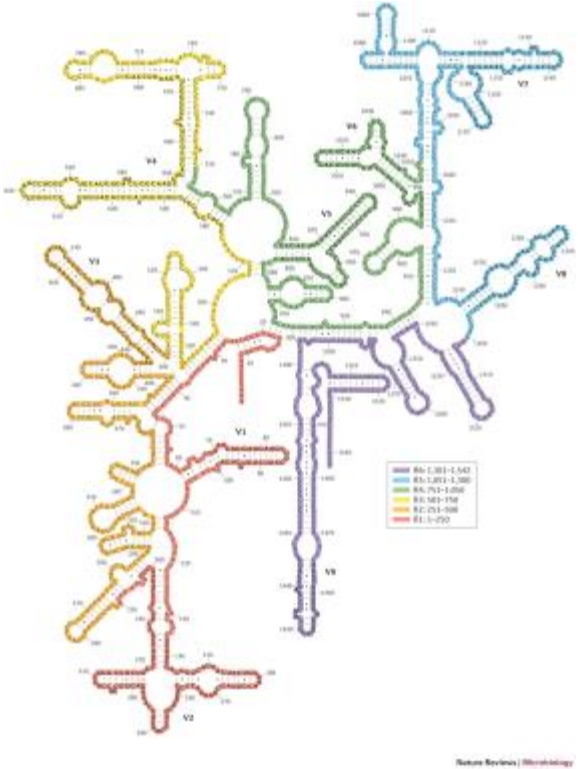
- Existence of a Female Urinary Microbiome & Microbiota
- Implications for Urinary Tract Infections
- Current and Future Projects

Overview

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

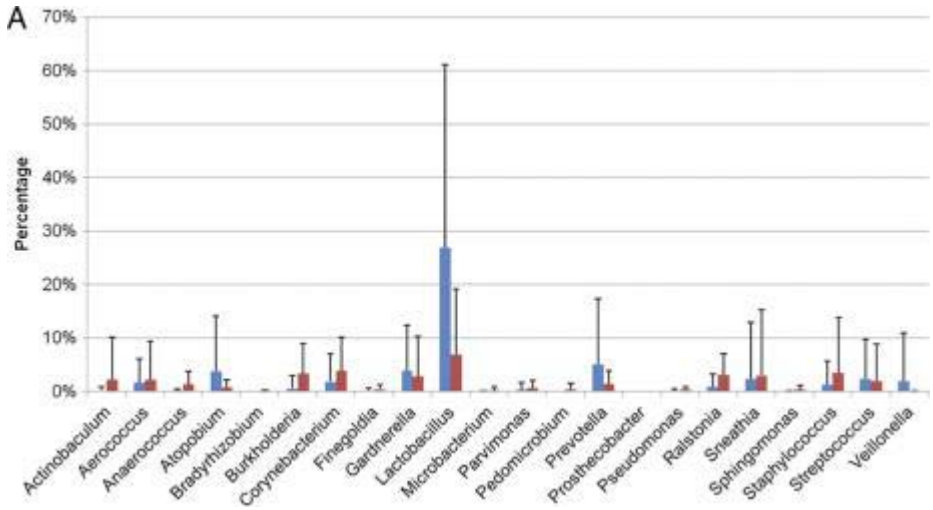
A Female Urinary Microbiome exists

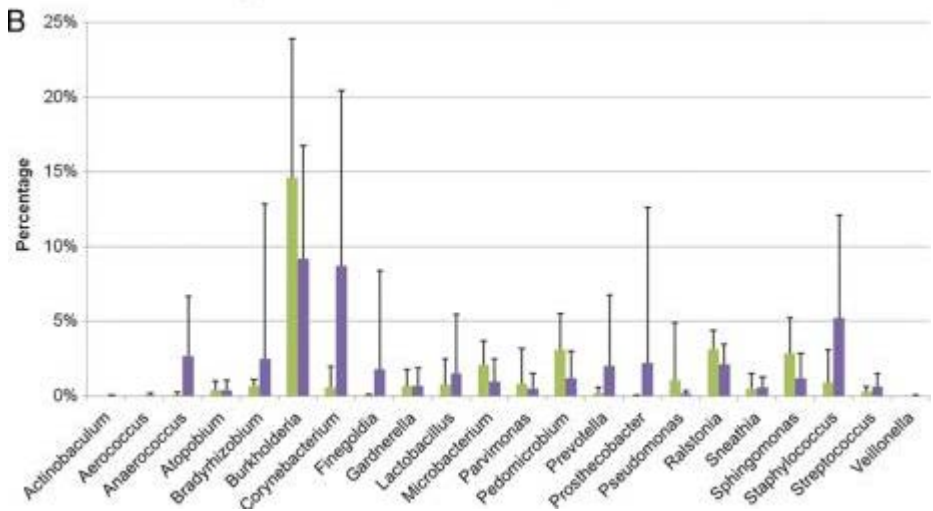
- 16S sequencing – V4 region
- Illumina MiSeq
- Mothur bioinformatics



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

Protocol (Volume)	Media	Condition	Identification
SUC (1µL)	Blood, MacConkey	Aerobic 35C	24 hr
EQUC (100µL)	Blood, Chocolate, CNA	5% CO_2 35C	48 hr
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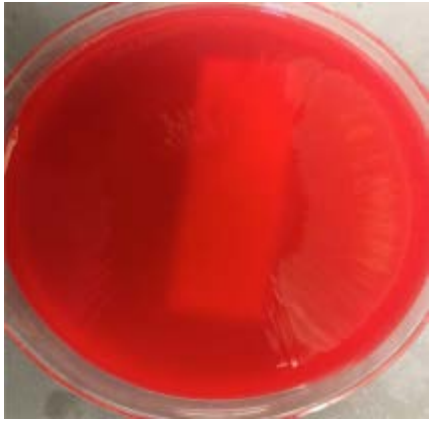
Standard Urine Culture (SUC)



1 µL, Aerobic at 35C, 24hrs

Enhanced Quantitative Urine Culture (EQUC)

Hilt *et al.*, 2014

Urine is not sterile – Female Urinary Microbiota (FUM)

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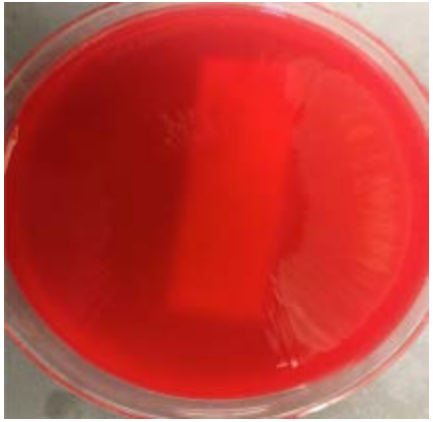

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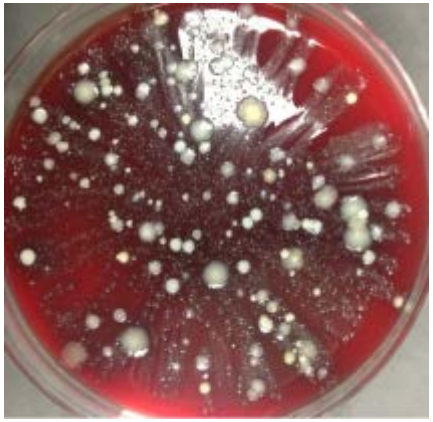

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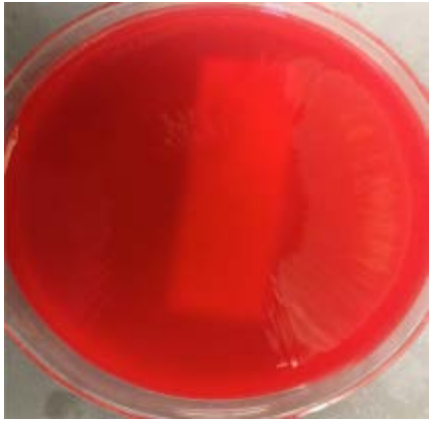

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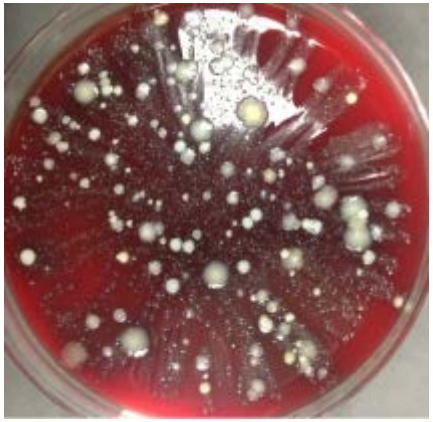
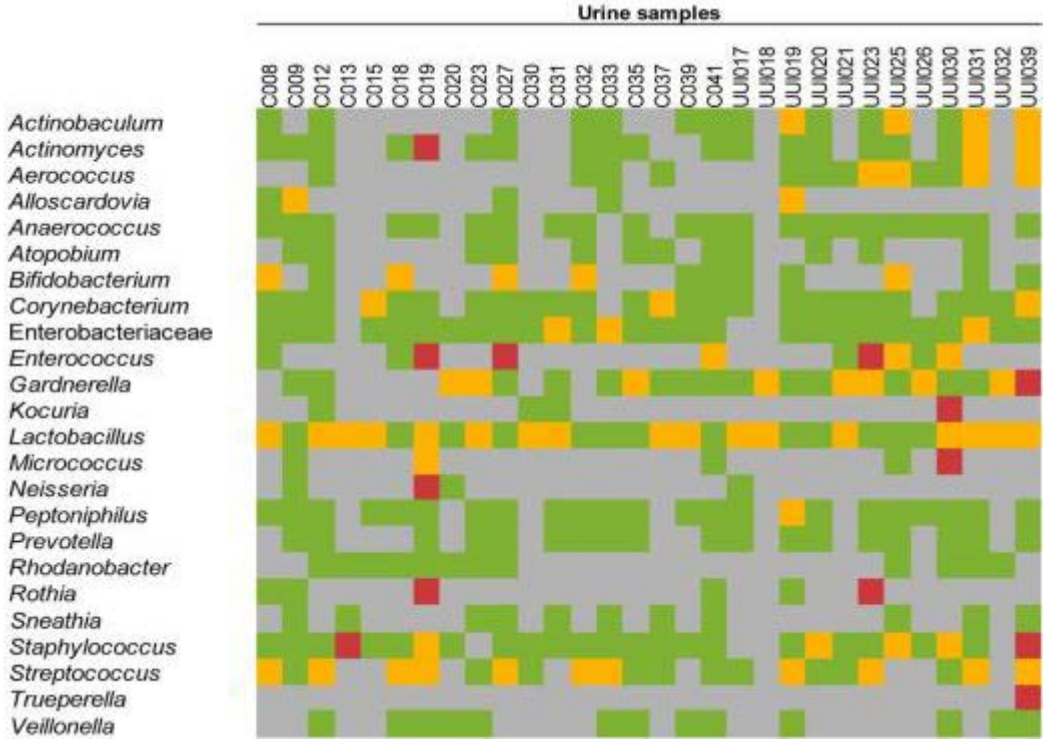


100 µL, Aerobic at 35C, 48hrs

SUC has a 90% false-negative rate

Hilt *et al.*, 2014

Sequencing & EQUC are complementary



Sequence only

EQUC only

Sequence & EQUC

Not Detected

Pearce *et al.*, 2014

Is the FUM involved in Lower Urinary Tract Disorders?

Adult women seeking clinical care

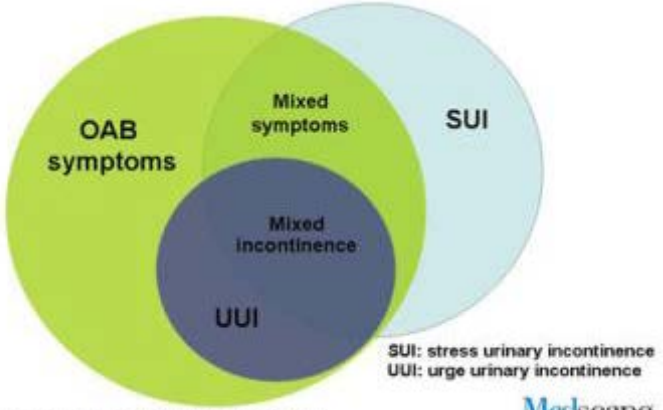
Pelvic Floor Distress Inventory (PFDI) Questionnaire

Transurethral catheterized urine (TUC) specimen

UUI **(N=60)**

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

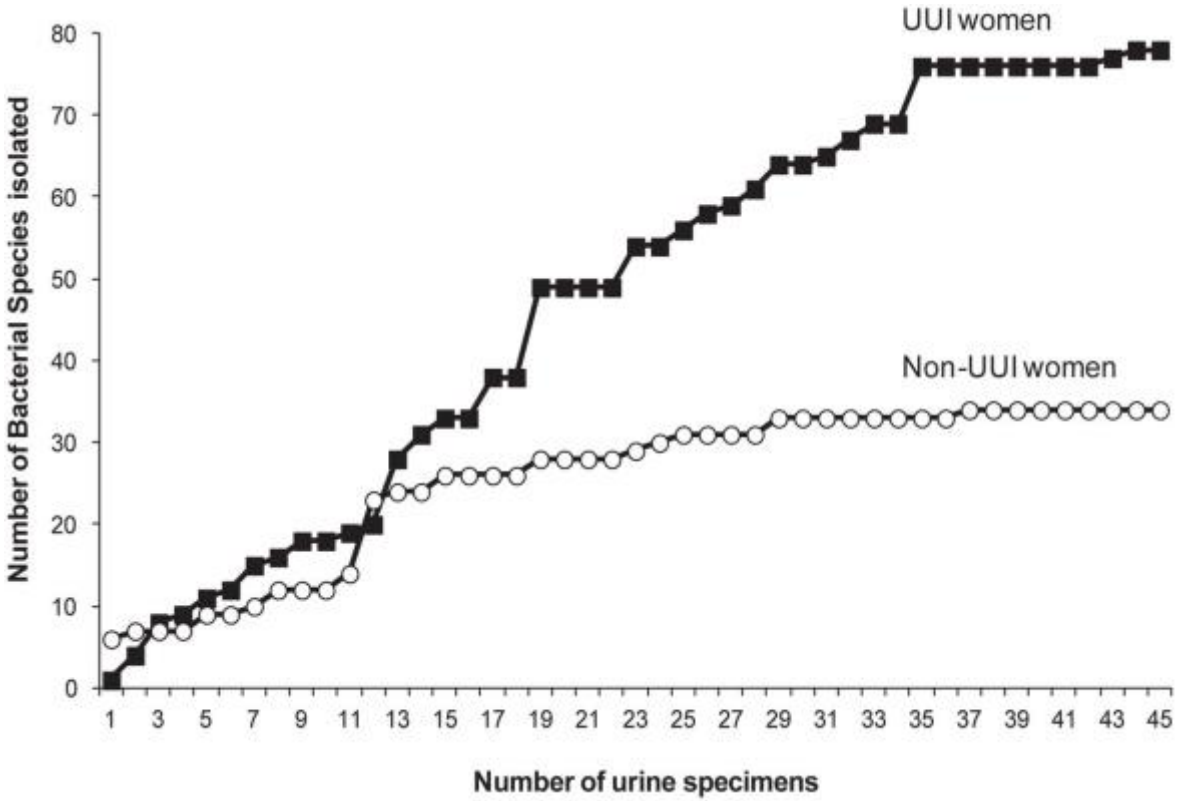
EQUC &
16S rRNA sequencing



Adapted from Wein AJ. *J Urol.* 2006;175(3 pt 2):S5-S10.

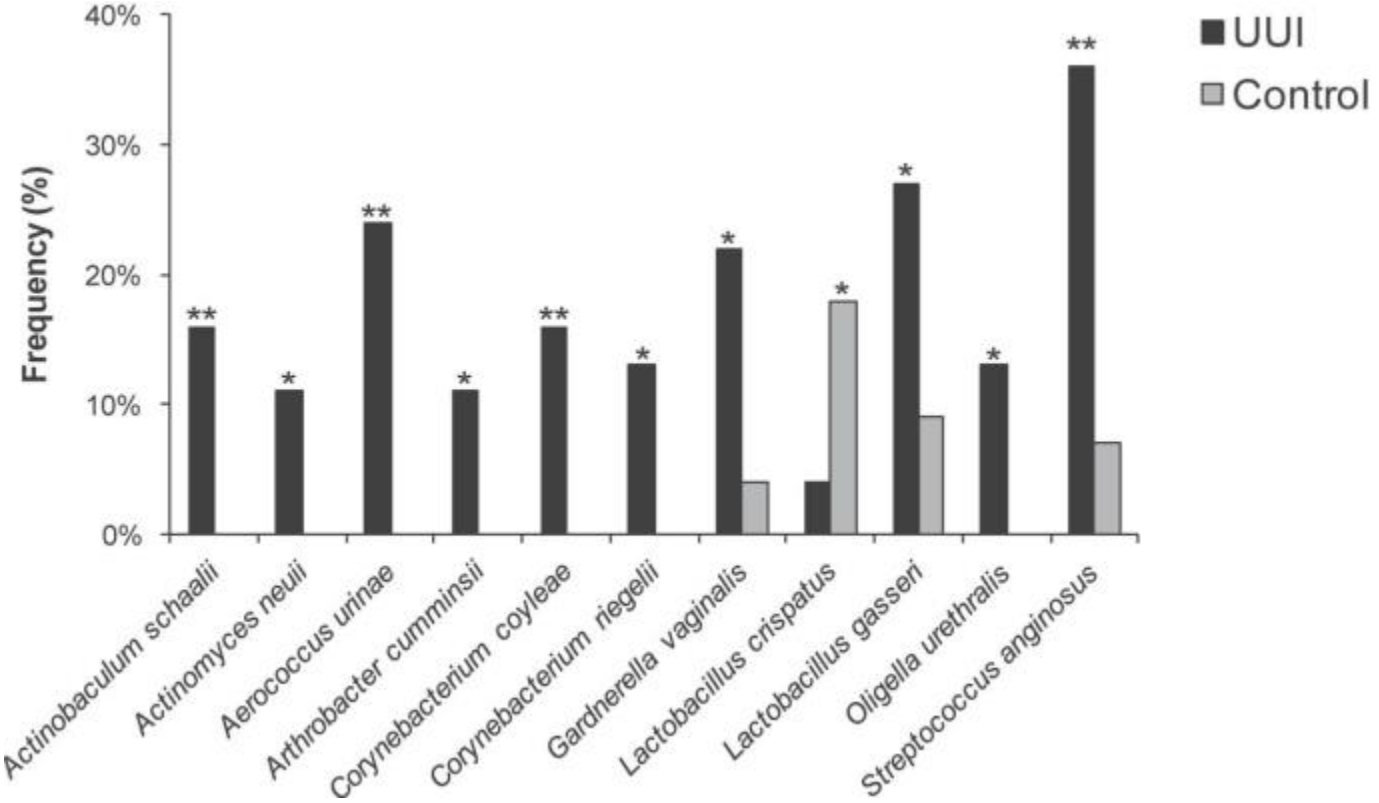
Pearce *et al.*, 2014

FUM diversity is higher in women with UUI



Pearce *et al.*, 2014

FUM composition differs between UUI and non-UUI women



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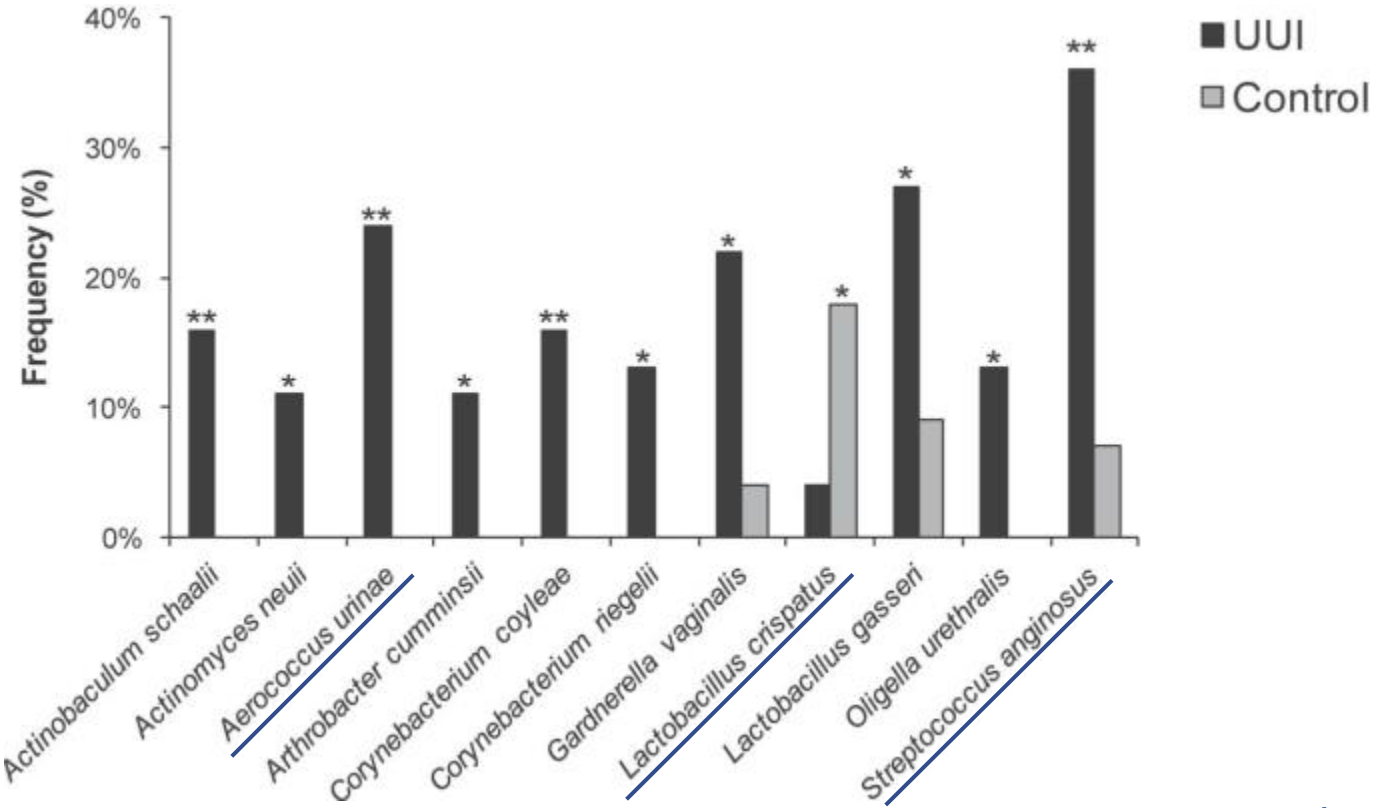

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- Current and Future Projects

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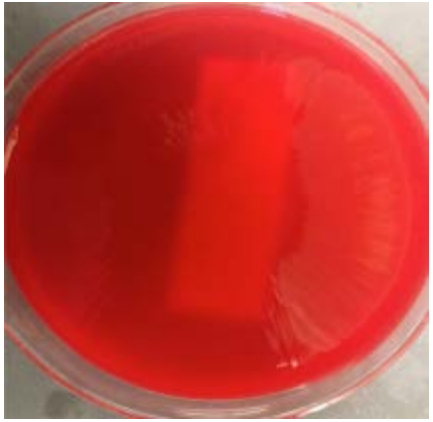
Standard Urine Culture (SUC)



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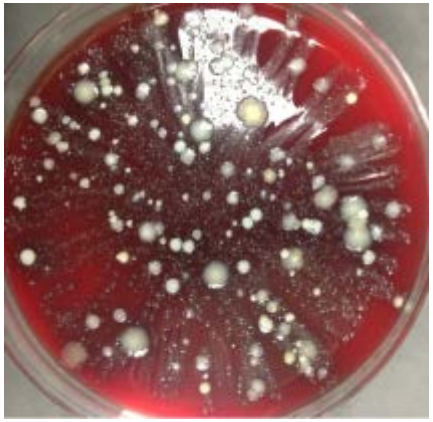
Enhanced Quantitative Urine Culture (EQUC)



100 µL, Aerobic at 35C, 48hrs

Hilt *et al.*, 2014

Hypothesis

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

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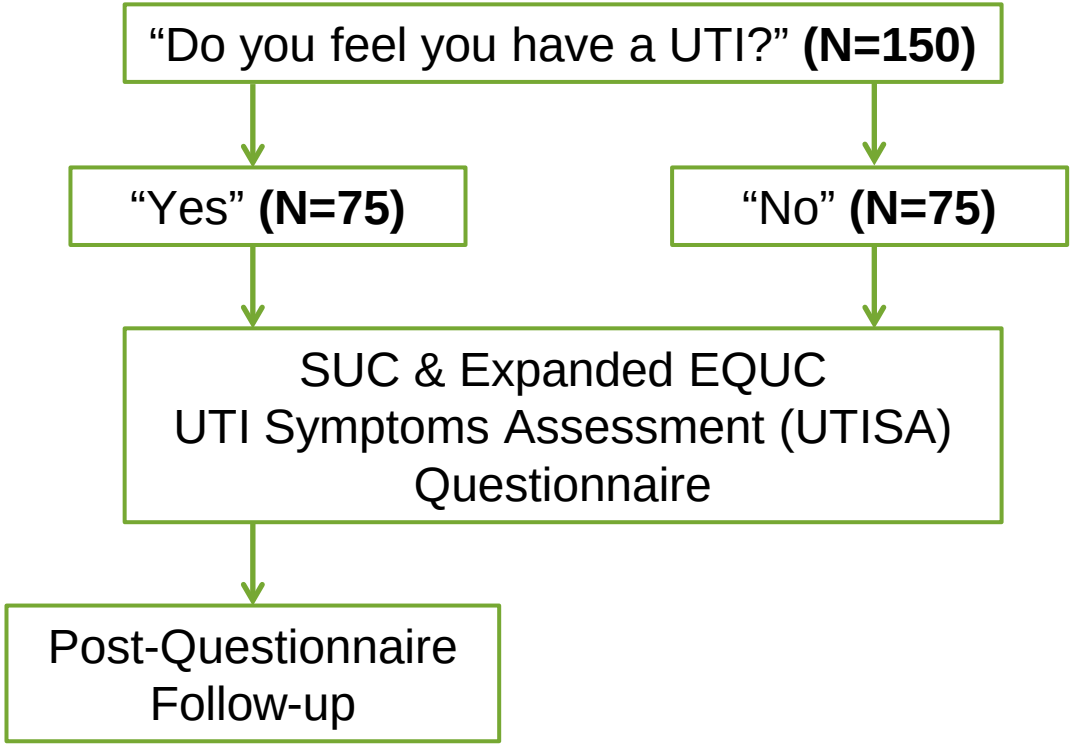
- Standard Urine Culture (SUC) protocol
- Urinary Symptoms
- ≥10^5 CFU/mL for diagnosis

Study design



UTISA Questionnaire

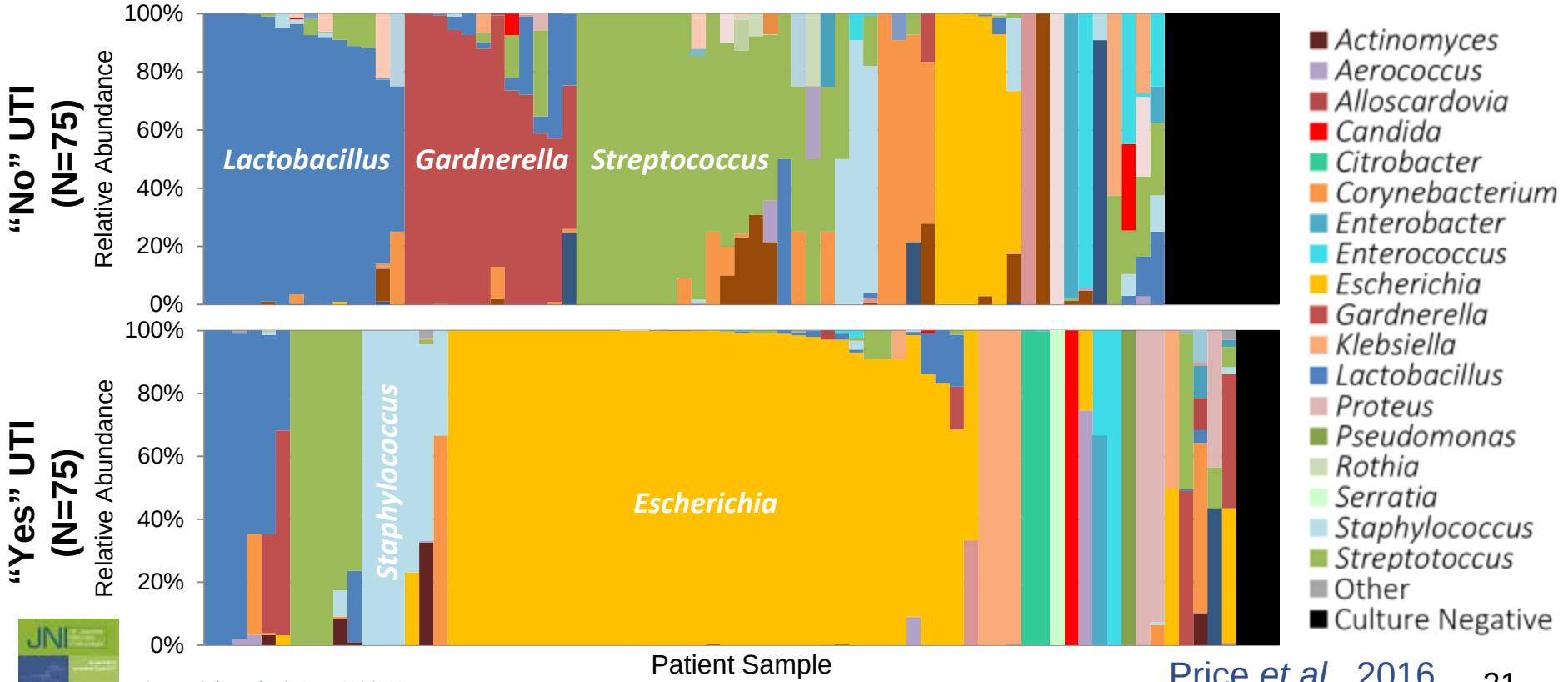
- Urgency
- Frequency
- Dysuria
- Difficulty Urinating
- Abdominal/Pelvic Pain/Pressure
- Low Back Pain
- Blood in Urine

Price *et al.*, 2016
Clayson *et al.*, 2005

Study design

Protocol (Volume)	Media	Condition	Identification
SUC (1µL)	Blood, MacConkey	Aerobic 35C	24 hr
Expanded EQUC (1, 10, 100µL)	Blood, Chocolate, CNA	5% CO_2 35C	24 hr 48 hr
	Blood, MacConkey	Aerobic 35C	24 hr 48 hr
	Anaerobic	Anaerobic 35C	48 hr
	Anaerobic	Microaerophilic (5% O_2, 10% CO_2, 85% N) 35C	48 hr

Composition differs at the genus level



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
  - ≥10^5 CFU/mL for diagnosis

SUC fails to detect 67% of uropathogens

Culturing Protocol	**Uropathogens** (N=182)
100ul Expanded EQUC	96% (N=174)
10ul Expanded EQUC	65% (N=118)
1ul Expanded EQUC	52% (N=95)
Standard Urine Culture (SUC)	**33% (N=60)**

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-*E. coli* uropathogens

Culturing Protocol	**Detection of *E. coli*** (N=50)	**Non-*E. coli* Uropathogens** (N=132)
100ul Expanded EQUC	100% (N=50)	94% (N=124)
10ul Expanded EQUC	94% (N=47)	55% (N=72)
1ul Expanded EQUC	92% (N=46)	36% (N=48)
Standard Urine Culture (SUC)	89%(N=44)	**12% (N=16)**

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

Hypothesis

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

- Standard Urine Culture (SUC) protocol
  - **Urinary Symptoms**
  - ≥10^5 CFU/mL for diagnosis

Urinary symptoms and UTI diagnosis

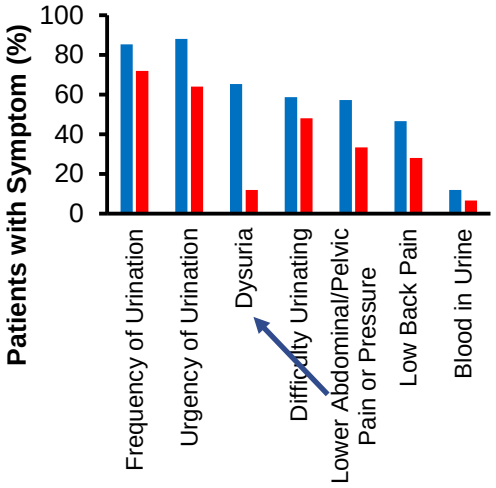
UTI Diagnosis:
Self-Report
UTI: N=75
No UTI: N=75

UTI Diagnosis:
SUC (≥10^5 CFU/mL)
UTI: N=57
No UTI: N=93

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

Dysuria is a strong indicator of UTI

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



UTI Diagnosis:
SUC (≥10^5 CFU/mL)
UTI: N=57
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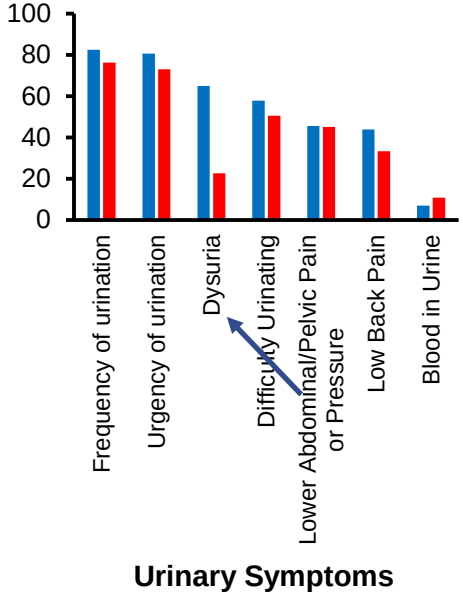
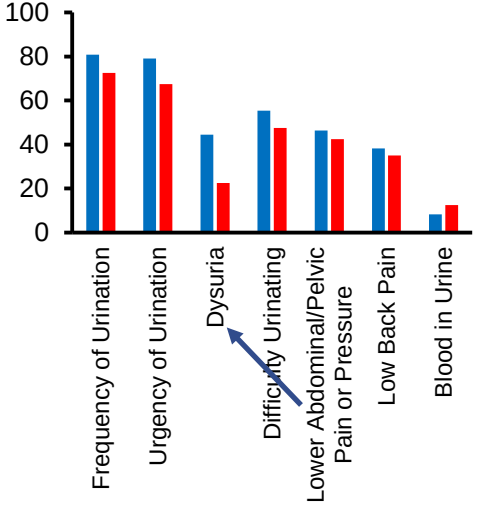


UTI Diagnosis:
EQUC (≥10 CFU/mL)
UTI: N=110
No UTI: N=40



UTI
No UTI

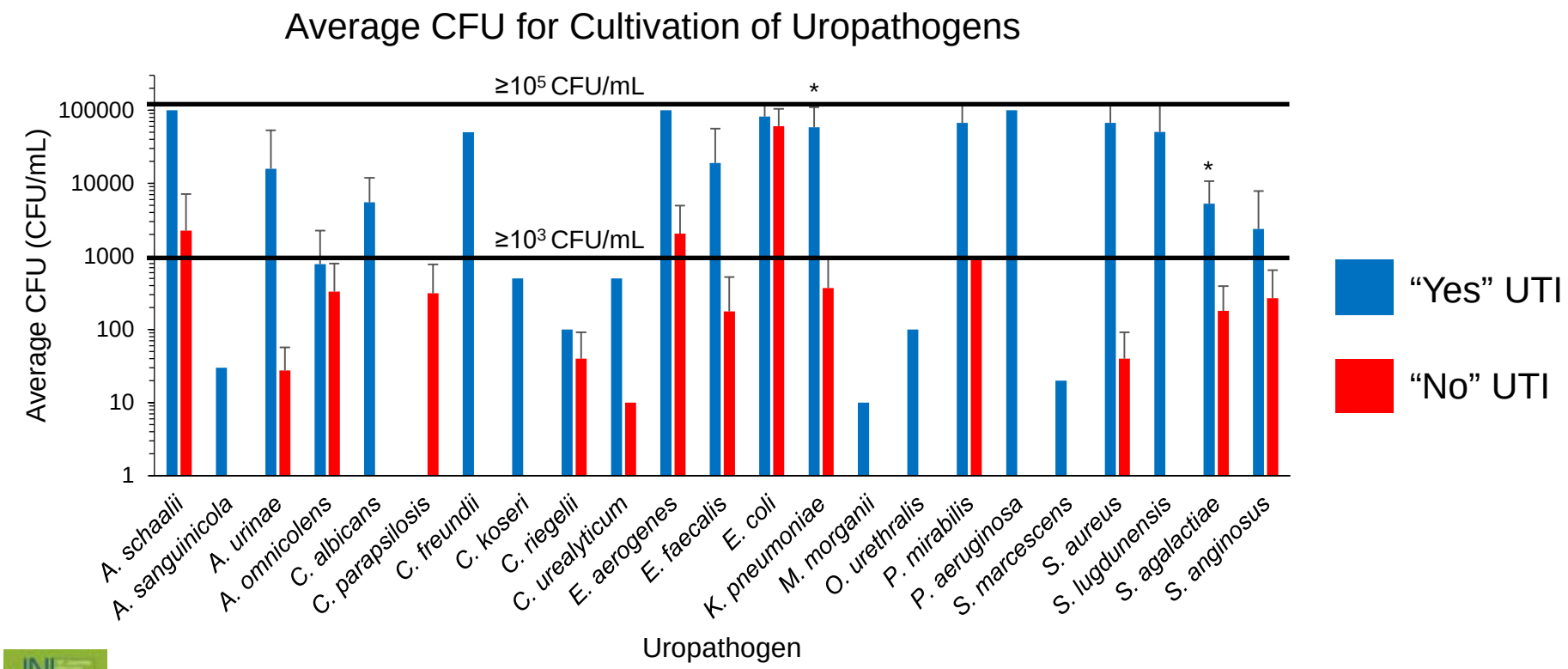
Dune *et al.*, 2017

Hypothesis

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

- Standard Urine Culture (SUC) protocol
- Urinary Symptoms
- **≥10^5 CFU/mL for diagnosis**

≥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% CO_2; 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 ≥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**

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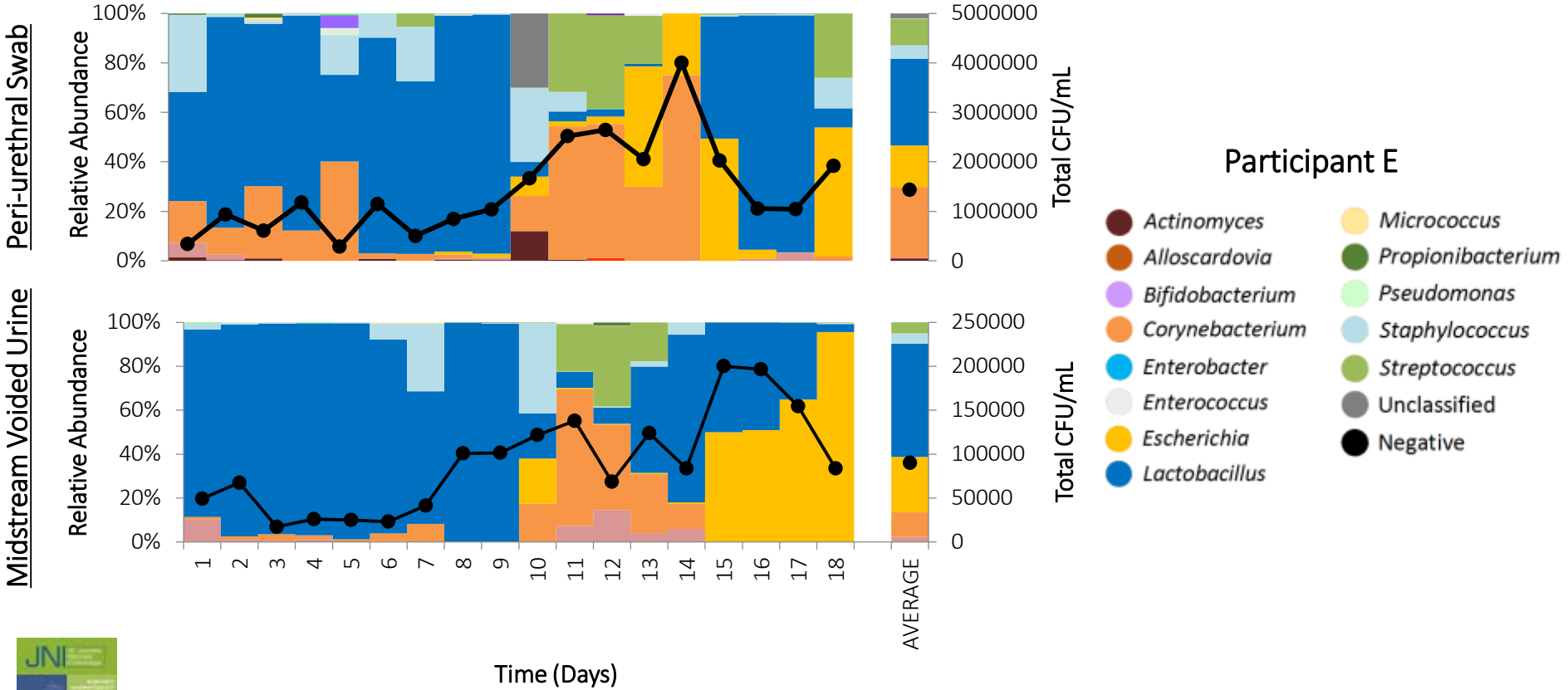
Current projects

- 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



Acknowledgments

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