

THE URINARY MICROBIOME OF WOMEN WITH URINARY URGENCY INCONTINENCE

Hypothesis/Aims of Study:

Using the quantitative polymerase chain reaction, we recently detected bacterial DNA in pre-treatment catheterized urine samples of female participants with urinary urgency incontinence (UUI) in the NICHD Anticholinergic versus Botulinum Toxin Comparison (ABC) Trial. The presence of bacterial DNA was associated with decreased risk of post-treatment urinary tract infection (UTI) (1,2). The objective of this study was to use DNA sequencing to characterize bacterial communities in the urine of women without clinical infection before UUI study treatment and to explore associations with patient demographics, symptoms and clinical outcomes, especially post-treatment development of UTI.

Study Design, Materials and Methods:

Bacterial DNA was extracted from catheterized urine samples and amplified with universal primers that target the V4 region of the 16S ribosomal RNA gene. Samples were sequenced at least twice independently with Illumina's MiSeq technology to assure data quality and reproducibility. Sequence reads were classified to bacterial genus. Sequence status was compared to clinical variables.

Results:

Bacterial DNA was successfully amplified and sequenced in about half (51.1%, n=93/182, SEQ POS) of the urines. Race, ethnicity, prior anticholinergic use, or treatment did not differ between SEQ POS and SEQ NEG cohorts. However, women in the SEQ POS cohort were younger ($p=0.0007$), heavier ($p=0.0009$), had more baseline UUI episodes ($p<0.0001$), responded better to UUI treatment ($p=0.0013$), and were less likely to develop UTI after initiation of UUI treatment ($p=0.0011$).

Table 1. Clinical Outcomes by Sequence Status

Characteristic	Sequence Positive	Sequence Negative	p-value
UTI, N (%)			0.0011
Yes	8 (9%)	24 (27%)	
No	85 (91%)	65 (73%)	
Change in UUIE, N	90	89	0.0013
Mean (SD)	-4.36 (2.7)	-3.32 (1.9)	
Median (Min-Max)	-4.03 (-11.7 – 0.7)	-3.17 (-9.3 - 2.5)	
OABq Symptom Severity Change, N	90	89	0.37
Mean (SD)	-46.8 (23.8)	-43.7 (22.7)	
Median (Min-Max)	-48.1 (-86.7 – 11.1)	-44.4 (-93.3 – 9.2)	
OABq HRQL Change, N	90	89	0.60
Mean (SD)	39.2 (21.9)	37.3 (25.2)	
Median (Min-Max)	37.5 (-9.0 – 96.7)	36.9 (-28.8 – 93.8)	

In SEQ POS urines, 8 major groups (termed urotypes) were identified. Most were dominated by a single bacterial genus (>50% total sequence reads). Nearly half of the SEQ POS urines (45%) were dominated by *Lactobacillus*, followed by *Gardnerella* (17%), *Gardnerella* and *Prevotella* (9%), Enterobacteriaceae (9%), *Staphylococcus* (3%), *Aerococcus* (2%), and *Bifidobacterium* (2%). The rest (13%) were diverse without a dominant family (Fig. 1). Race and ethnicity were similar across urotypes, but age differed ($p=0.0094$); urotypes dominated by Enterobacteriaceae, *Staphylococcus* & *Aerococcus* were only in post-menopausal women. Relative to SEQ POS women who did not develop a post-treatment UTI, those who did develop a UTI had, on average, fewer *Lactobacillus* (20.6 vs 47.3% of total sequences per sample) and less diversity, but more *Aerococcus* (9.6 vs 1.2%), *Bifidobacterium* (9.8 vs 0.6%), Enterobacteriaceae (20.0 vs 6.9%), and *Staphylococcus* (9.1 vs 2.5%) (Fig. 2).

Interpretation of Results: Over half of female ABC trial participants with UUI, without evidence of clinical infection, contained sequenceable bacterial DNA in pre-treatment urines. The urotypes were typically dominated by a single genus, most often *Lactobacillus* or *Gardnerella*. Pre-treatment urotypes may be associated with risk of post-treatment UTI.

Concluding Message:

The female urinary microbiome is related to clinical treatment outcomes of interest in women with UUI.

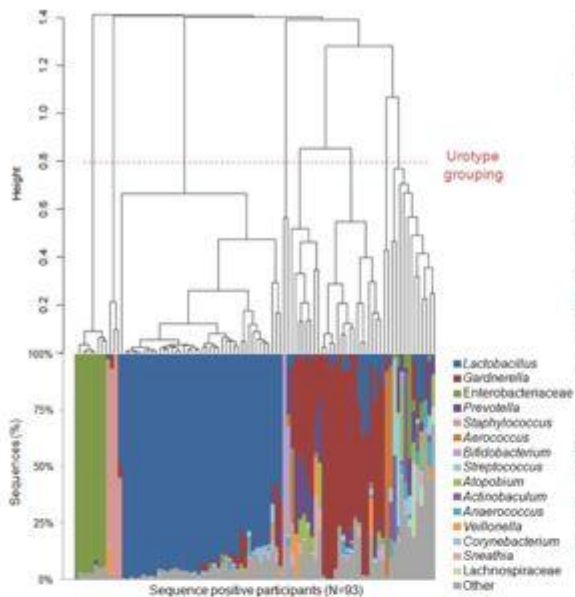
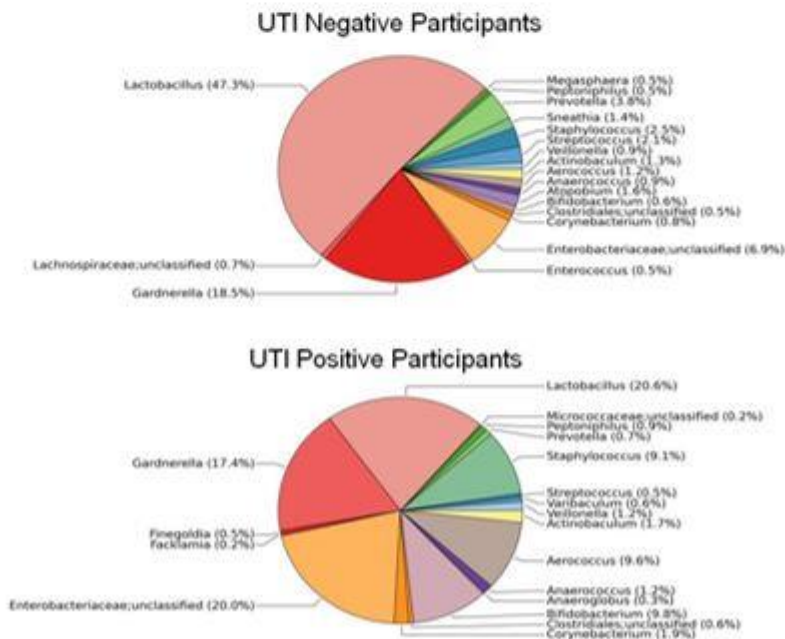


Figure 1. The urinary microbiome profile of sequence positive participants. The urinary microbiome profiles of sequence positive participants cluster together, as demonstrated in the dendrogram (top), and by the dominant bacterial taxa present, as depicted in the histogram (bottom). The dendrogram was based on the Euclidean distance between the sequence profile of each urine. The histogram displays the bacterial taxa detected by sequencing as the percentage of sequences per urine by sequence positive participants (N=93). Bacteria were classified to the genus level with the exception of Enterobacteriaceae and Lachnospiraceae, which could only be classified to the family level. The 15 most sequence abundant bacterial taxa were displayed and the remainder of the taxa, including unclassified sequences, were grouped into the category "Other". Urine samples consisting of the same dominant bacterial taxa grouped together in the dendrogram and were classified into the following urotypes, as shown by the dashed horizontal line: Lactobacillus, Gardnerella, Gardnerella/Prevotella, Enterobacteriaceae, Staphylococcus, Aerococcus and Diverse.

Fig 2. Comparison of average bacterial sequence abundance by post-treatment clinical UTI occurrence



References

1. Brubaker L, Nager CW, Richter HE, Visco A, Nygaard I, Barber M, Schaffer J, Meikle S, Wallace D, Shibata N, Wolfe AJ. Urinary Bacteria in Adult Women with Urgency Urinary Incontinence. *Int Urogynecol J*. 2014 Feb 11 Epub ahead of print. PMID: 24515544.
2. Visco A, Brubaker L, Richter H, Nygaard I, Paraiso MF, Menefee S, Schaffer J, Lowder J, Khandwala S, Sirls L, Spino C, Nolen T, Wallace D, Meikle S. Anticholinergics Therapy vs OnabotulinumtoxinA for Urgency Urinary Incontinence. *New Engl J Med*. 2012 Nov; 8;367(19):1803-1813. PMID:23036134.

Disclosures

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