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## Incontinence Medication Response Relates to the Female Urinary Microbiota

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## Abstract

**Introduction**—Many adult women have resident urinary bacteria (urinary microbiome/microbiota). In adult women affected by urinary urgency incontinence (UUI), the etiologic and/or therapeutic role of the urinary microbiome/microbiota remains unknown.

**Hypothesis**—Microbiome/microbiota characteristics will relate to clinically relevant treatment response to oral UUI medication.

**Methods**—Adult women initiating oral medication treatment for UUI and a comparator group of unaffected women were recruited in a tertiary care health care system. All participants provided baseline clinical data and urine. Women with UUI were given 5mg solifenacin with potential dose escalation to 10mg for inadequate UUI symptoms control at 4 weeks. Additional data and urine samples were collected from women with UUI at 4 and 12 weeks. The samples were assessed by 16S rRNA gene sequencing and enhanced quantitative urine culturing. The primary outcome was treatment response as measured by the validated Patient Global Symptom Control (PGSC) questionnaire. Clinically relevant UUI symptom control was defined as a 4 or 5 score on the PGSC.

**Results**—The diversity and composition of the urinary microbiome/microbiota of women with and without UUI differed at baseline. Women with UUI had more bacteria and a more diverse microbiome/microbiota. The clinical response to solifenacin in UUI participants was related to baseline microbiome/microbiota, with responders more likely to have fewer bacteria and a less diverse community at baseline. Non-responders had a more diverse community that often included bacteria not typically found in responders.

**Conclusions**—Knowledge of an individual's urinary microbiome/microbiota may help refine UUI treatment. Complementary tools, DNA sequencing and expanded urine culture, provide information about bacteria that appear related to UUI incontinence status and UUI treatment response in this population of adult women.

## Brief Summary

Adult womens' individual urinary microbiome/microbiota differ based on presence of UUI. The microbiota relate to UUI treatment response in women treated with oral UUI medication.

## Keywords

Urinary incontinence; clinical microbiology; solifenacin

## INTRODUCTION

Many adult women undergo treatment for urinary urgency incontinence (UUI), often with oral anticholinergic medication, such as solifenacin [1]. This medication class targets M3 muscarinic receptors to decrease smooth muscle contraction, relieving UUI symptoms of urgency, frequency and urgency incontinence [1]. Although solifenacin decreases urgency episodes in approximately 60% of treated patients, symptoms may persist [2–4] with approximately 40% of treated patients reporting bothersome symptoms [5]. Clinicians recognize this lack of response, although the cause remains unclear and studies into the biologic basis for persistent symptoms are lacking. Many UUI-affected patients are considered to have idiopathic etiology; however, this view pre-dates emerging evidence of resident urinary bacterial communities (urinary microbiota), recently reviewed [6–8]. DNA sequencing and expanded urine culture techniques have demonstrated that adult urine is not sterile [9–16,6] and UUI-affected women have different resident bacterial compositions than non-UUI control populations [11].

In this prospective cohort of adult women with UUI, we compared baseline urinary microbiota of women with UUI to those of non-affected controls. For UUI-affected women subsequently undergoing clinically indicated UUI treatment with solifenacin, we also determined urinary microbiota profiles throughout 12 weeks of treatment, using additional longitudinal samples collected at 4 and 12 weeks. We hypothesized that the microbiome/microbiota (e.g. diversity, overall community structure, and/or specific organisms) would differ between women with and without UUI and between those that responded to solifenacin and those that did not. Two complementary bacterial assessments, sequencing and expanded quantitative urine cultures (EQUC) [10], were used to overcome the insensitivity of standard urine cultures. EQUC was not initially available; however, once this technique was available, it was applied to all remaining samples.

## METHODS

### Study Design and Population

This 12-week open label study began following Institutional Review Board approval. Participants gave verbal and written research consent for chart abstraction and urine collection with analysis for research purposes. Between August 2012 and July 2014, two cohorts of adult women were recruited at a tertiary health care setting, Loyola University Medical Center. Potential participants were screened for eligibility using the long version of the validated symptom questionnaire, the Pelvic Floor Distress Inventory (PFDI) [17,18]. Women seeking UUI treatment were recruited from a specialty Female Pelvic Medicine and Reconstructive Surgery center. Comparison controls screened negative for UUI using the PFDI and were recruited from women's health practices within Loyola's system. These controls were derived from a population of gynecologic patients; those who underwent surgical procedures had a variety of benign gynecologic conditions, most commonly abnormal uterine bleeding confirmed to be of a benign nature. Enrolled participants also completed the overactive bladder questionnaire (OAB-Q) [19]. Exclusion criteria for both cohorts included current urinary tract infection (UTI) (based on urine dipstick), history of recurrent UTI, antibiotic exposure in the past four weeks for any reason, immunologic

deficiency, neurological disease known to affect the lower urinary tract, pelvic malignancy or radiation, untreated symptomatic pelvic organ prolapse (POP) greater than POP-Q Stage II (vaginal protrusion more than one centimeter outside of the vaginal hymen) or pregnancy.

Control participants, without overactive bladder symptoms, provided a single catheterized urine sample for research purposes as well as clinical and questionnaire data. As part of normal clinical care, UUI-affected participants provided a catheterized urine sample prior to UUI treatment with solifenacin, which was provided at no cost for 12 weeks. Participants with UUI also agreed to provide 2 additional catheterized urine samples at 4 and 12 weeks during UUI treatment for longitudinal analysis. The primary outcome was treatment response at 12 weeks. Treatment response was assessed (at 4 and 12 weeks) using the validated Patient Global Symptom Control (PGSC) questionnaire [20], which consists of a single question that addresses improvement following treatment. Scores of 4 or 5 were defined as “response”. PGSC scores  $\leq 3$  were considered “non-response”; these participants were offered a dosage increase to 10mg at 4 weeks. Women whose PGSC score  $\leq 4$  continued the solifenacin 5mg dose for 8 more weeks until primary outcome assessment at 12 weeks. Thus, definitions for 3 response groups were determined *a priori*: non-responders, 5mg responders and 10mg responders. Participants who were intolerant of the medication could withdraw at any point during the study. The research team assessed adverse events at normally scheduled visits, and at other intervals during participant-initiated contact. Figure 1 shows the flow of UUI-affected study participants. The primary outcome was symptomatic response, based on PGSC score, at 12 weeks.

### Sample Collection and Analysis

Urine was collected aseptically via transurethral catheter and a portion placed in a BD Vacutainer Plus C&S preservative tube for culture. Urine culture was performed as described [10] (E-Methods 1). A separate aliquot for sequencing was placed at 4°C for 4 h following collection; 10% AssayAssure (Sierra Molecular; Incline Village, NV) was added before storage at -80°C. Our protocols for DNA extraction, library construction, 16S rRNA sequencing and bioinformatics analysis have been described [11]. All genomic samples were processed in duplicate and analysis was performed using mothur software [21]. Relative abundance was calculated by generating the percent of total classified reads for each individual. All samples were processed in duplicate and the percent reads of replicates were averaged for downstream analysis, which included urotype identification and statistical analysis. Urotypes were determined as described [11]. Briefly, a culture urotype is determined by the dominant (>50%) identified organisms within a given sample. A sequencing urotype is determined by first clustering sequences using a dendrogram and grouping based on similarity; this usually correlates with dominance (>50%) of one organism. Those communities without a dominant organism were classified as “Diverse” urotype. Dendrograms were calculated using Bray Curtis dissimilarity between samples, and the complete method was used for hierarchical clustering via R software, version 2.15.1 [22]. To measure the diversity (richness and evenness of species within a sample), the alpha diversity (inverse Simpson’s index) was calculated using mothur software [21].

## Protocol for the Expanded Quantitative Urine Culture (EQUC)

For standard urine culture, 0.001 mL of urine was spread quantitatively onto 5% sheep blood (BAP) and MacConkey agars (BD BBL™ Prepared Plated Media), then incubated aerobically (35°C for 24 hours). Any amount of each separate morphologic colony type was identified and counted using a detection level of 1,000 colony forming units (CFU)/mL (represented by 1 colony of growth on either plate). “No growth” was reported when no growth was observed, indicating no growth of bacteria at lowest dilution, i.e., 1:1000.

The expanded quantitative urine culture (EQUC) technique uses 0.1 mL of urine spread quantitatively onto BAP, Chocolate and Colistin, Naladixic Acid (CNA) agars (BD BBL™ Prepared Plated Media), then incubated in 5% CO<sub>2</sub> (35°C for 48 hours). A second set of BAPs were inoculated with 0.1 mL of urine and incubated in room atmosphere at 35°C and 30°C for 48 hours, respectively. In addition, 0.1 mL of urine was inoculated onto each of two CDC Anaerobe 5% sheep blood agar (ABAP) plates (BD BBL™ Prepared Plated Media), and incubated in either a Campy gas mixture (5% O<sub>2</sub>, 10% CO<sub>2</sub>, 85% N) or in anaerobic conditions at 35°C for 48 hours. The detection level was 10 CFU/mL, represented by 1 colony of growth on any of the plates. Finally, to detect any bacterial species that may be present at quantities lower than 10 CFU/mL, 1.0 mL of urine was placed in Thioglycollate Medium (BD BBL™ Prepared Tubed Media) and incubated aerobically at 35°C for 5 days. If growth was visually detected, the Thioglycollate Medium was mixed and a few drops were plated on BAP and ABAP agars for isolation, and incubated aerobically and anaerobically (35°C for 48 hours). Each morphologically distinct colony type was isolated on a different plate of the same media to prepare a pure culture that was used for organism identification. Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) with the MALDI Biotyper 3.0 software (Bruker Daltonics, Billerica, MA) was used to identify the bacterial isolates [10]. To determine the false-negative rate, the following equation was used: the number of EQUC positive but standard negative samples divided by the total number of EQUC positive samples. To determine the culture diversity within a population, a species accumulation curve was generated. Species accumulation curves illustrate the number of unique species cultured and identified (using EQUC) with each new patient sampled. When the curve plateaus, it indicates that the community is fully sampled and few, if any, new species will be identified. Therefore, it also measures the overall diversity of the population by total number of unique species isolated.

## Statistical Analyses

Standard statistical methods were used to compare participant demographics and symptom data between UUI patients and controls. Continuous variables are reported as means and standard deviations (SD) or medians and interquartile ranges (IQR) and categorical variables are reported as frequencies and percentages. Collection of the primary outcomes at 12 weeks was necessary for assignment to UUI response groups; therefore, participants who did not provide data through 12 weeks were not included in analyses involving response to solifenacin. Fisher’s exact and Kruskal-Wallis tests were used to compare demographic and culture (e.g., abundance and diversity) information among UUI response groups. For cultured organisms, abundance was measured using total CFU/ml; for sequencing, abundance was measured using percent classified reads or classified organisms. Post-hoc

pairwise comparisons were made using Wilcoxon Rank sum tests for continuous variables with significant overall p-values. A kappa statistic was estimated to determine agreement between urotype classification from samples with both EQUUC and sequencing. All statistical analyses were conducted using SPSS software version 19 or SAS software v9.4 (SAS Institute, Cary, NC). Significance was assessed at an alpha level of 0.05. Results were not adjusted for multiple-comparisons since the analyses are considered descriptive.

## RESULTS

### Population Description Over the Course of the Study

Figure 1 displays the flow of UUI subjects through the 12-week follow-up period. The study investigators withdrew participants who had received antibiotics for unrelated clinical indications (2) and subjects who underwent a cystoscopy for clinical indications (2). An additional 17 participants withdrew by the 4-week visit: 7 due to side effects and 6 lost to follow up. Of the participants who provided UUI symptom response data at 4 weeks, 56% (32/57) reported adequate symptom control; all of the remaining 25 accepted dose escalation (10mg daily).

### Comparison of Baseline UUI to Controls

At baseline, participants included 74 UUI-affected women and 60 controls. The cohorts were similar with respect to race/ethnicity, diabetes, and smoking (Table 1). Participants with UUI were more likely to suffer from hypertension [35% versus 18%,  $p=0.02$ ] and coronary artery disease [12% versus 2%,  $p=0.02$ ]. The UUI population also was older [61.5 years (SD:11.5) versus 49 (SD:14.7),  $p<0.001$ ], heavier [BMI 32.7 (SD:8.4) versus 28 (SD: 5.5),  $p<0.001$ ], and more likely to be estrogen-negative (post-menopausal and not on hormone replacement therapy) [88% versus 43%,  $p\text{-value}<0.001$ ]. As expected, UUI symptoms were significantly worse in UUI-than non-UUI participants.

We detected the presence of bacterial DNA in a similar proportion of urine samples for each cohort (Controls: 43%,  $N=26/60$ , UUI: 50%,  $N=37/74$ ,  $p=0.44$  Table 2A). Table 2A and E-Table 2 display the assigned sequence urotypes. In both groups, the majority of samples were dominated by *Lactobacillus* [Controls (61.5%,  $N=16/26$ ), UUI (40.5%,  $N=15/37$ ),  $p=0.13$ ]. Alpha diversity (measured by the inverse Simpson index) of classified sequences between UUI and control was similar ( $p=0.76$ ).

A large subset of baseline samples was processed by EQUUC (UUI  $N=59/74$ , control  $N=52/60$ ). A greater proportion of UUI-affected women had cultivatable bacteria in their urine compared to control women (84.7% versus 63.5%,  $p=0.01$ ). The groups differed in proportion of cultured samples characterized by dominant organisms (culture urotypes) (Table 2A): *Lactobacillus* [Controls (45.5%,  $N=15/33$ ) versus UUI (22%,  $N=11/50$ )  $p=0.03$ ], and diverse [Controls (6%,  $N=2/33$ ) versus UUI (32%,  $N=16/50$ )  $p=0.006$  Table 2A]. Relative to controls, UUI-affected women had significantly more diversity in unique cultivatable organisms, as assessed by the number of unique cultivatable organisms per cohort (UUI= 80, Controls= 36, Figure 2), significantly more cultured bacterial isolates per participant, as measured by the median [UUI (3) versus Controls (1),  $p<0.001$ , Figure 2 inset] and



significantly more cultivatable bacteria, as measured by median number of CFU per mL per urine specimen [UUI: 340 CFU (IQR: 50–1710) versus Controls: 20 CFU (IQR: 0–305),  $p=0.002$ ].

### Characteristics in the Baseline Samples Correlate with Primary Outcome

Primary outcome data were available for 50 participants at 12 weeks, with 25 “5mg responders”, 15 “10mg responders” and 10 “non-responders”, for an overall response rate of 80%. At baseline, compared to the withdrawn population, participants with primary outcome data weighed less [BMI 31.2 (SD:7.7) versus 35.8 (SD:9.2),  $p=0.03$ ] and were more likely to have had previous pharmacologic treatment for OAB (60% versus 29%,  $p=0.01$ ). They also had greater HRQL scores [61 versus 37,  $p=0.001$ ] and larger urinary distress inventory scores [95 versus 131,  $p=0.02$ ] (E-Table 1). We did not detect other significant group differences in baseline variables for participants who completed the study. (E-Table 1).

At baseline, the 3 response groups had similar demographics and symptom severity (Table 1). At 12 weeks, the HRQL scores differed among all response groups ( $p=0.008$ ). The median score for non-responders (70, IQR: 61–86) differed significantly from both responder groups: 5mg group (93, IQR: 86–99) and 10mg group (94, IQR: 88–99) ( $p=0.004$  and  $p=0.008$ , respectively) (Table 1).

The urine samples from all 50 participants with primary outcome data were tested for detectable bacteria. At baseline, a similar proportion of each response group had detectable bacterial DNA [5mg: 56% N=14/25, 10mg: 46.7% N=7/15, non-responders: 40% N=4/10,  $p=0.66$ , Table 2B], suggesting that there is no difference in the frequency of samples with detectable bacteria present in each response group. In contrast, when we compared bacterial community composition by distribution of urotypes (i.e., the number of individuals with microbiomes dominated by one organism), the baseline samples from each response group tended to be different; however, no statistical testing was conducted due to limited sample sizes. Compared to 10mg responders (12.5%, N=1/8), 5mg responders had a larger proportion of individuals with *Lactobacillus*-dominant urine (50%, N=7/14) (Table 2B). The response groups had similar alpha diversities [5mg: 1.6 (IQR: 1.2–3.7), 10mg: 1.9 (IQR: 1.1–6.3), non-responders: 2.8 (IQR: 1.7–4.1),  $p=0.55$ ].

The subset (N=39/50) of baseline urine samples from participants with primary outcome data that were cultured with EQUC included 19 “5mg responders”, 13 “10mg responders”, and 7 “non-responders”. Cultivable organisms (EQUC) were detected in most participants: 5mg responders: 73.7% (N=14/19), 10mg responders: 100% (N=13/13) and non-responders: 85.7% (N=6/7) ( $p=0.13$ , Table 2B). The dominant urotype by response group was: 5mg response -*Lactobacillus*-dominant urotype 35.7% (N=5/14); 10mg - diverse urotype 38.5% (N=5/13) and 30.8% (N=4/13) *Streptococcus*-dominant urotype; and non-responders – diverse urotype 50% (N=3/6). Each response group had a distinct species accumulation curve, with different total number of unique cultured bacterial isolates: 5mg (32), 10mg (54) and non-responders (42) (Figure 2). Overall, there was a difference in the number of unique species isolated by response group ( $p=0.02$ ). The median number of unique species isolated from each individual within each cohort was similar in the 10mg and non-responder groups [5 versus 8,  $p=0.25$  Figure 2 inset]. However, there was a statistically significant difference

between the 5mg and the other responder groups: [5mg: 1 versus 10mg responders: 5  $p=$  0.03, or non-responders: 8,  $p=$  0.02 Figure 2 inset].

Each response group contained several genera that differed from other response groups (Figure 3A), with *Actinomyces* cultivated more commonly from 10mg responders and non-responders than from 5mg responders ( $p=$ 0.01 and 0.04, respectively). *Streptococcus* was cultivated more frequently from the 10mg responders ( $p<$ 0.001), while *Corynebacterium* was cultivated more commonly from non-responders ( $p=$ 0.002). In addition, several bacterial species differed between response groups (Figure 3B).

### Primary Outcome Comparison – 12 weeks

At 12 weeks, 48 of the 50 participants with primary outcome data were sequenced. Two samples were not sequenced due to inconclusive labeling. Of the remaining 48, 23 samples were positive for bacterial DNA (Table 2B). Similar to baseline, the response groups had similar proportions of 12-week samples with detectable bacterial DNA [5mg: 43.5%  $N=$ 10/23, 10mg: 46.7%  $N=$ 7/15, and non-responders: 60%  $N=$ 6/10,  $p=$ 0.70]. Statistical testing was not performed because of limited sample size; however, the 3 response groups trended toward different sequence urotype distributions, with larger proportions of *Lactobacillus* urotypes in 5mg responders (70%,  $N=$ 7/10), compared to 10mg responders (28.6%,  $N=$ 2/7) and non-responders (33.3%,  $N=$ 2/6) (Table 2B). The response groups had similar alpha diversity ( $p=$ 0.18).

At 12 weeks, most (86%,  $N=$ 38/44) EQUC-characterized urines demonstrated cultivatable organisms (Table 2B). At 12 weeks, each response group tended toward different culture urotypes distributions: 5mg response - *Lactobacillus*-dominant urotype 38.9%  $N=$ 7/18; 10mg -diverse urotype 23.1%  $N=$ 3/13 and 23.1%  $N=$ 3/13 *Streptococcus*-dominant urotype; and non-responders – *Lactobacillus* 42.8%  $N=$ 3/7 (Table 2B). The species accumulation analysis of the baseline and 12-week samples show that the 10mg and non-responders decreased in diversity by 12 weeks, from 55 unique isolates to 23 for the 10mg group and from 40 to 29 for the non-responders. In contrast, the 5mg group increased in diversity, from 32 unique isolates to 41 (E-Figure 1). We did not detect a specific single genus that significantly declined in responders (5mg or 10mg) (E-Figure 2). At the conclusion of the specimen collection, we compared the two methods of bacterial assessment. Approximately half (49.8%,  $N=$ 119/239) of all urine specimens sequenced had detectable bacterial DNA, whereas most specimens (80%,  $N=$ 140/177) cultured with EQUC grew organisms. Among the sequence-positive, culture-positive individuals, the urotype classifications were moderately comparable (Kappa = 0.45) [23].

When comparing EQUC to standard culture, we found EQUC detected more organisms overall. Of all the urine samples in the study, 81.3% ( $N=$ 165/203) grew bacteria by EQUC. Of those, 149 had been deemed culture negative (“No Growth”) by the standard culture, resulting in 90.3% false negative rate for standard culture.



## DISCUSSION

The female urinary microbiota in women affected by UUI appears to differ from a comparison group of unaffected women. Using two complementary techniques, we report differences in composition (urotype), diversity (number of unique bacterial species) and identity of certain bacteria at the genus and species levels. These differences, which were detectable prior to UUI treatment, appear related to treatment response. Moreover, following UUI treatment, certain microbiota characteristics appear associated with a clinically significant response to treatment. Overall, less diversity appears associated with fewer UUI symptoms and with treatment response at 5mg. We also found that bacterial diversity in women who required a higher dose to achieve adequate symptom control is more similar to the diversity found in non-responders, indicating that the diversity of cultivatable organisms might be used to predict treatment response.

The mechanistic relationship between bacterial diversity and UUI symptoms is unknown. However, given our findings, important clinical questions regarding UUI etiology or consequences will need careful study. In this research work, we selected 2 complementary study techniques to assess the bacterial milieu, one of which was developed after we started enrollment. Sequencing, which was available at the onset of our study, provided a broad picture of bladder bacteria, allowing a more complete analysis of bacterial diversity. In addition, sequencing could detect bacteria that even EQUC could not cultivate. However, urine samples contain low biomass relative to other anatomical sites (e.g., gut or vagina). Thus, a proportion of the study population did not contribute sequencing data. This lack of information was often resolved by the use of EQUC. This expanded culture technique for urine became available after our study started. EQUC not only confirms that bladder organisms are alive, but it also allows phenotyping of low biomass individuals not measurable by sequencing.

The very low CFUs associated with UUI may prompt consideration of a lower CFU cut-off (perhaps  $>10^2$ ) for a standard urine culture. Others have suggested lowering the culture threshold for UTI [15,24–27] and even for UUI [28]; however, these studies focused on growing known uropathogens. Here, we showed that the bladder can contain a variety of organisms. Some are likely non-pathogenic normal flora. Others might have an impact on UUI and other lower urinary tract symptoms. Some of these might be protective, whereas others may contribute to symptoms. Indeed, we recently reported that detection of urinary microbiota by quantitative PCR is associated with a reduced risk of post-instrumentation UTI [9] and an association between *Lactobacillus crispatus* and the lack of lower urinary tract symptoms [11]. Furthermore, several bacterial species appear to be associated with UUI [11]. Thus, we recommend the use of expanded culture conditions until our understanding of unrecognized uropathogens improves. EQUC is more likely than sequencing to be readily available in the clinical setting, and may prove useful for rapid phenotyping of individual patients, prior to treatment selection. This is especially important as no single clinical demographic variable adequately predicted treatment outcome.

We have considered the implications of the minority of samples that were “negative” for both techniques. We currently consider this group to be “sub-threshold” rather than “sterile”

and fully anticipate that the very low biomass individuals will be more fully described as technologies evolve.

The dominance of certain genera, such as *Lactobacillus* and *Gardnerella*, may prompt thoughts of vaginal contamination. Our use of aseptically collected specimens via transurethral catheter by experienced individuals, and our prior publications demonstrating the presence of *Lactobacillus* and *Gardnerella* in female bladder urine collected through suprapubic aspirates [12], supports the evidence that both genera are capable of residing in the bladder. The role of these organisms as members of the urinary microbiome and their relationships to vaginal microbiota requires further study. Because the bladder and vagina can contain the same species, the use of catheterized samples is recommended whenever possible and careful interpretation is required when using samples obtained by void.

It is possible that baseline group differences contributed to our findings. However, since UUI incidence increases with age and is therefore linked to menopause status, our sample size is not sufficient to further describe the separate effects of estrogen status, age, and UUI symptoms in relation with microbiota diversity. We acknowledge this possibility; yet, we wish to highlight the importance of the findings between treatment response groups (Figure 2). Women with 1–4 bacterial isolates on EQUC were most likely to respond to the initial 5mg dose of solifenacin, whereas women with more diversity (> 5 bacterial isolates) were less likely to respond or needed an increased dose. These simple cut-offs, detectable prior to treatment, will require validation in larger patient populations. Therefore, in future studies, the use of both EQUC and sequencing for all collected urine specimens is recommended. The results are highly complementary, with sequencing measuring a broad picture of the population and the EQUC protocol measuring the cultivatable organisms within that population.

Clinicians can benefit from expanded knowledge about the bacterial communities that are present in patients with UUI. Although specific changes in clinical management cannot yet be recommended, this promising area of research is generating many new hypotheses. In addition, our findings should inform the design of larger clinical studies that have potential to personalize UUI treatment, provide insight into UUI etiology, and offer promise for novel UUI prevention strategies. We believe that related urinary tract disorders may also benefit from this new knowledge.

## CONCLUSIONS

The response to oral UUI medication (solifenacin) may relate to individual urinary microbiota characteristics that are detectable prior to treatment. Our findings, especially the importance of organism diversity, offer promising possibilities for new ideas for prevention and treatment of UUI in women.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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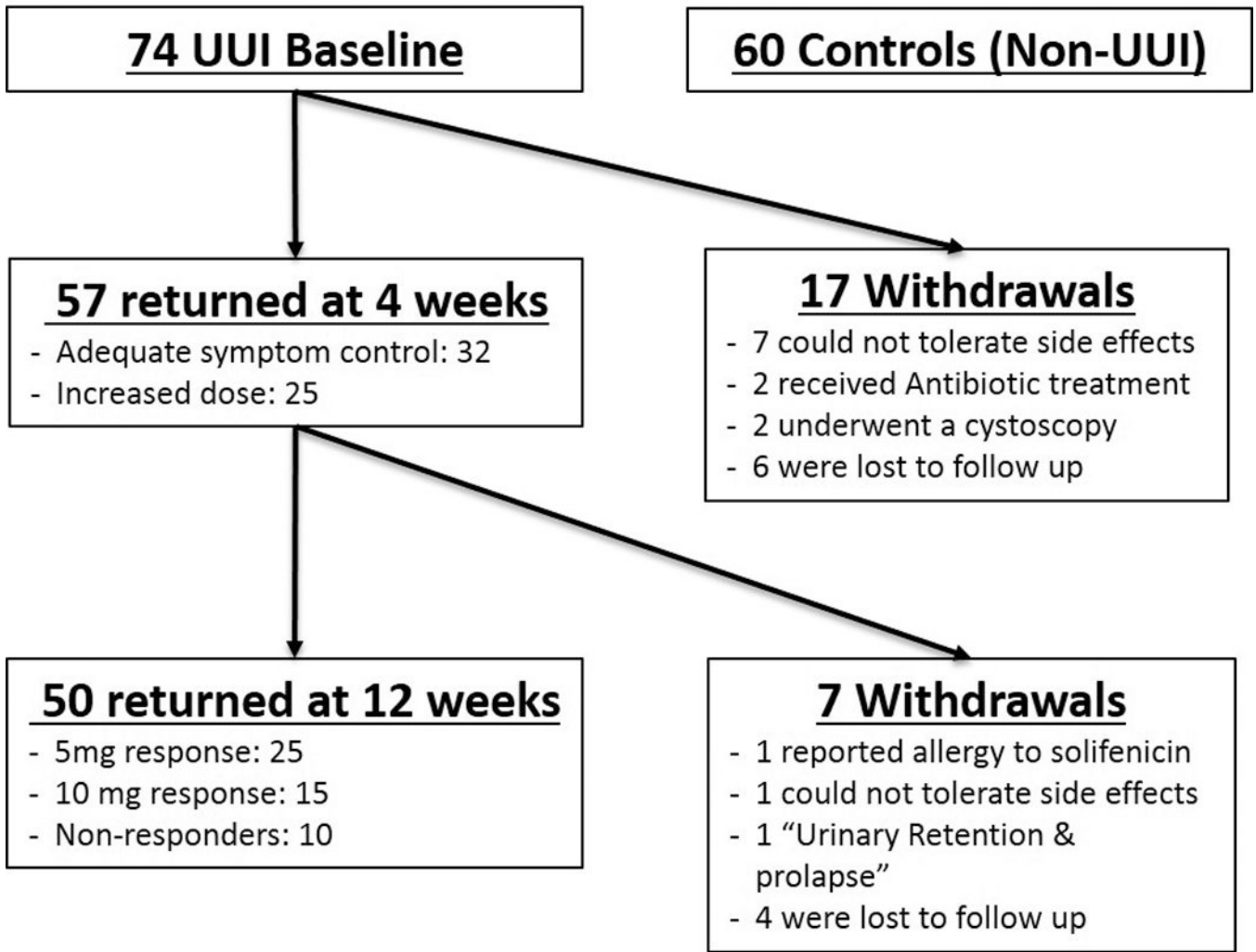
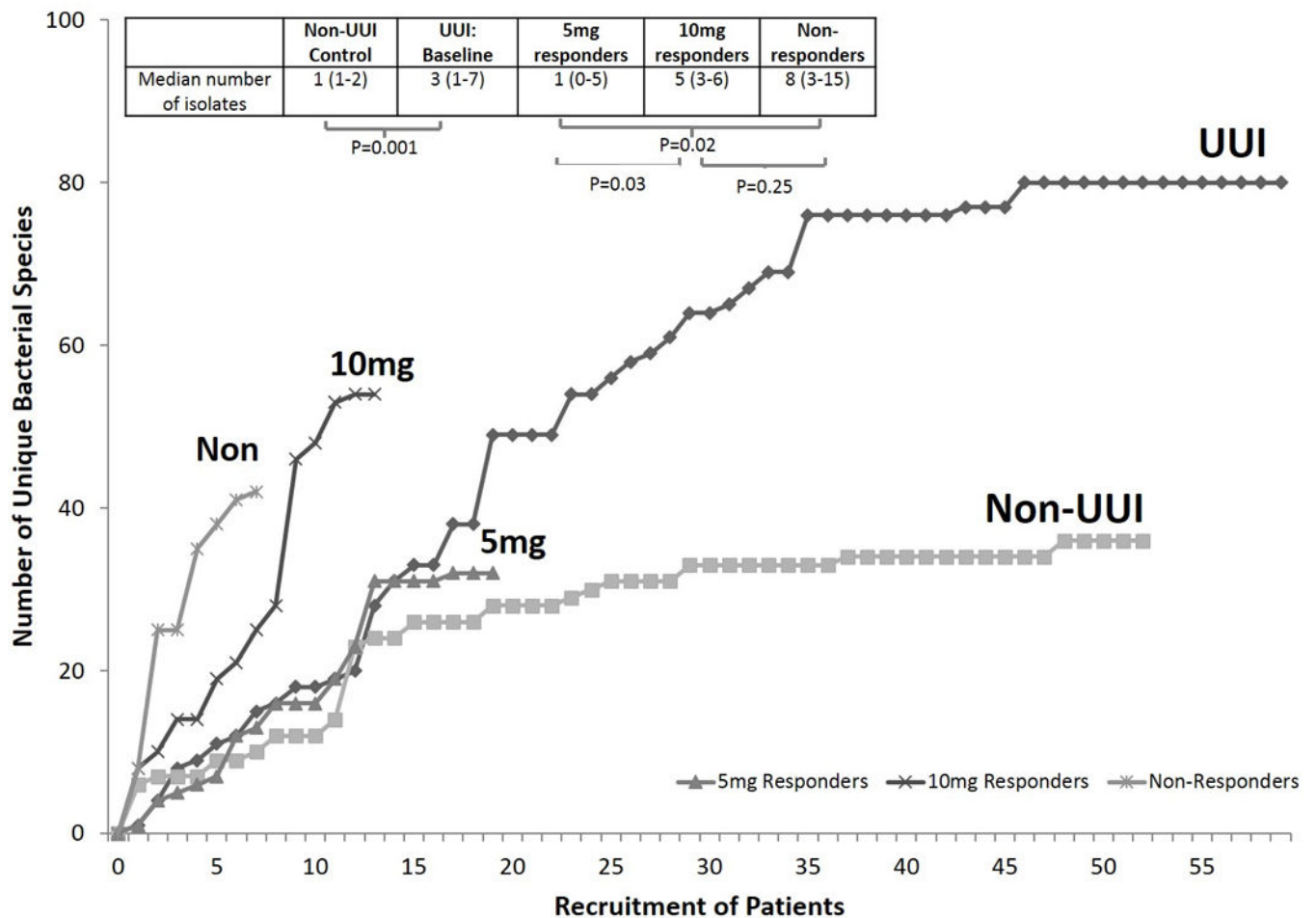


Figure 1. Flow of Study Participants



**Figure 2. Diversity of cultivatable bacteria is distinct between cohorts and response groups at baseline**

Species accumulation analysis illustrates the number of unique species cultured and identified (using EQUIC) with each new patient sampled. When the curve plateaus, it indicates that the community is fully sampled and few, if any, new species will be identified. Therefore, it also measures the overall diversity of the population by total number of unique species isolated. The UII group isolated 80 unique species at baseline and the control cohort isolated 36 unique species. Therefore, the species isolated from UII is far more diverse than the species isolated from controls. There is statistical difference in the median number of isolates collected from each individual in each cohort [ $M=3$  (IQR: 1–7) versus  $M=1$  (IQR: 1–2),  $p<0.001$ ].

The UII curve represents samples at baseline from individuals who will respond or not. When we divide this curve into each stratified by response group we see that the baseline diversity of each group is distinct, suggesting that baseline diversity could be predictive of treatment response. 5mg responders (grey triangles) had low diversity, with a total of 32 unique species isolated. 10mg responders (dark “x”) had high diversity with a total of 54 unique isolates. Non-responders (light “x”) had high diversity with a total of 42 unique isolates. The median number of unique species was not significant between 10mg and non-responders [5 (IQR: 3–6) versus 8 (IQR: 3–15),  $p$ -value= 0.25], but was significant between



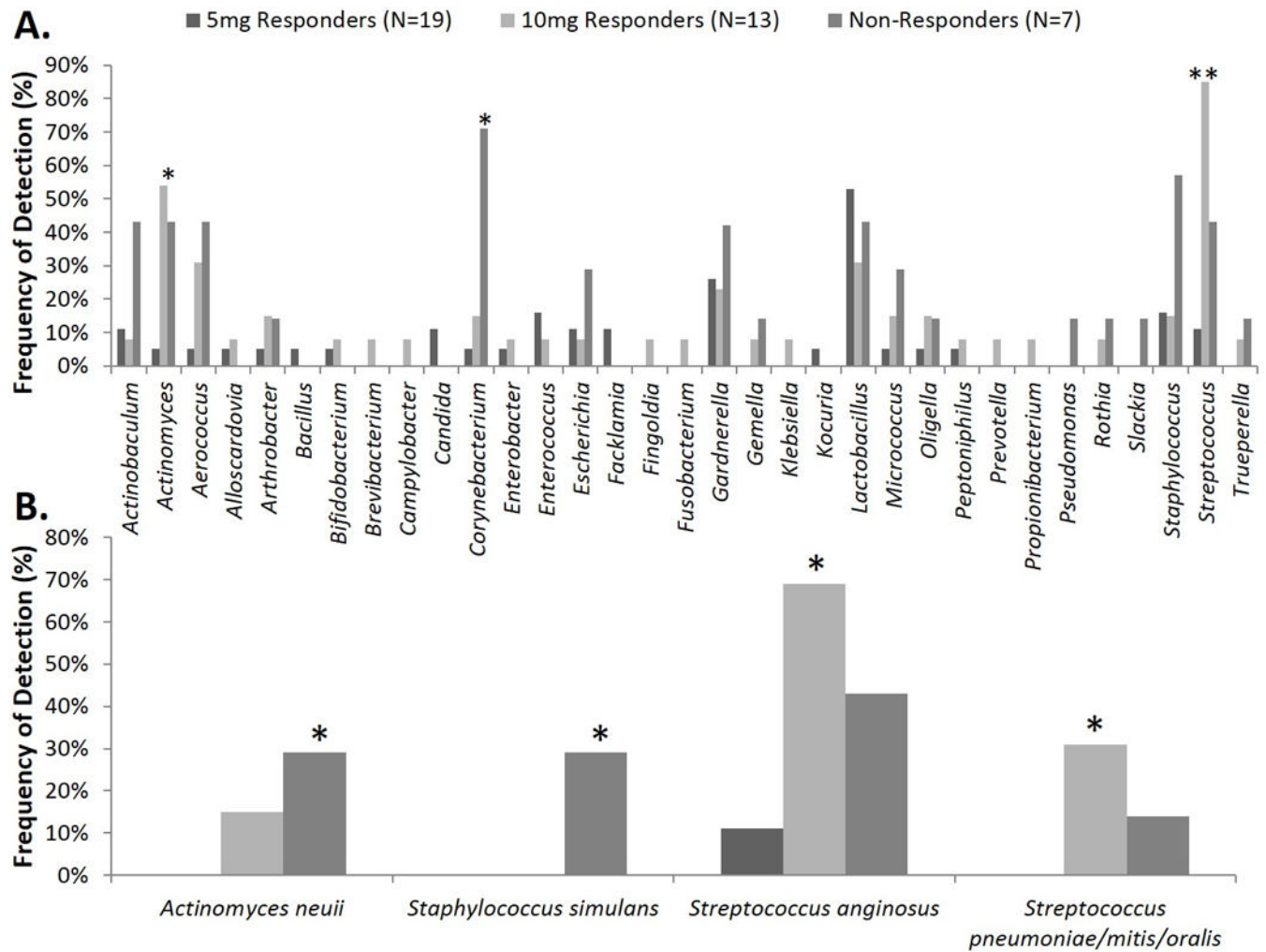
the 5mg and 10mg and between 5mg and non-responders [1 (IQR: 0–5) versus 5 (IQR: 3–6),  $p$ -value= 0.03; and 1 (IQR: 0–5) versus 8 (IQR: 3–15),  $p$ -value= 0.02].

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**Figure 3. Frequency of Detection of Genera and Significant Species Between Response Groups**  
 Comparison of the frequency of culture-based detection at the genus (A) and species level (B) for baseline urines by response group. Fisher’s Exact Test was used to calculate the *p*-values. \* *p*=0.05 \*\* *p*=0.00

**Participant Demographics and Symptom Scores**

**Table 1**

Comparison of baseline demographics between cohorts and response groups. Independent t-tests and Chi-square tests used unless otherwise indicated. Fisher's Exact Test due to small, expected cell counts unless otherwise indicated.

	Baseline		Baseline: 3 Response Groups				p-value
	UUI (N=74)	Non-UUI Control (N=60)	p-value	5mg Responder (N=25)	10mg Responder (N=15)	Non-Responder (N=10)	
<b>Demographics</b>							
Age (years) [Mean (Standard Deviation)]	61.5 (SD:11.5)	49 (SD:14.7)	< <b>0.001<sup>a</sup></b>	60 (SD:12.4)	63 (SD:13.5)	67 (SD:9)	0.36 <sup>b</sup>
Body Mass Index (kg/m <sup>2</sup> ) [Mean (Standard Deviation)]	32.7 (SD:8.4)	28 (SD:5.5)	< <b>0.001<sup>a</sup></b>	31 (SD:7.6)	30 (SD:7)	34 (SD:9)	0.41 <sup>b</sup>
<b>Race/Ethnicity*</b>							
Non Hispanic Caucasian	56 (77%)	43 (72%)		17 (68%)	12 (80%)	9 (90%)	
Hispanic Caucasian	5 (7%)	4 (7%)	0.70	3 (12%)	2 (13%)	0 (0%)	0.70
African-American	13 (18%)	12 (20%)		5 (20%)	1 (7%)	1 (10%)	
Asian	0 (0%)	1 (2%)		0 (0%)	0 (0%)	0 (0%)	
<b>Marital Status</b>							
Married	38 (51%)	37 (62%)		15 (60%)	8 (53%)	5 (50%)	
Separated	3 (4%)	1 (2%)		1 (4%)	0 (0%)	0 (0%)	
Divorced	9 (12%)	6 (10%)	<b>0.01</b>	1 (4%)	3 (20%)	3 (30%)	0.71
Widowed	14 (19%)	1 (2%)		4 (16%)	2 (13%)	1 (10%)	
Single	10 (14%)	15 (25%)		4 (16%)	2 (13%)	1 (10%)	
<b>Estrogen Status**</b>							
Estrogen Positive	7 (9%)	32 (53%)		4 (16%)	2 (13%)	0 (0%)	
Estrogen Negative	65 (88%)	26 (43%)	< <b>0.001</b>	20 (80%)	13 (87%)	10 (100%)	0.68
Unsure	2 (3%)	2 (2%)		1 (4%)	0 (0%)	0 (0%)	
Smoking	6 (8%)	4 (7%)	0.51	1 (4%)	1 (7%)	0 (0%)	0.99
Diabetes	7 (9%)	2 (3%)	0.14	2 (8%)	1 (7%)	1 (10%)	0.99
Hypertension	26 (35%)	11 (18%)	<b>0.02</b>	9 (36%)	5 (33%)	7 (70%)	0.13
Coronary Artery Disease	9 (12%)	1 (2%)	<b>0.02</b>	2 (8%)	2 (13%)	1 (10%)	0.84

	Baseline		Baseline: 3 Response Groups				p-value
	UII (N=74)	Non-UII Control (N=60)	p-value	5mg Responder (N=25)	10mg Responder (N=15)	Non-Responder (N=10)	
Prior Treatment for OAB	37 (50%)	0 (0%)	<0.001	13 (52%)	11 (73%)	6 (60%)	0.45
Vaginal Parity [Median (IQR)]	2 (0-2.5)	2 (1-3)	0.02 <sup>c</sup>	2 (2-3)	2 (0-3)	2 (0-5)	0.88 <sup>c</sup>
<b>Baseline Symptoms [Median (IQR)]</b>							
Symptom Score <sup>d</sup>	70 (55-85)	0 (0-5)	<0.001 <sup>c</sup>	62 (42-88)	75 (65-85)	69 (55-78)	0.61 <sup>c</sup>
HRQL <sup>d</sup>	52 (33-75)	100 (98-100)	<0.001 <sup>c</sup>	68 (50-76)	59 (36-87)	50 (31-70)	0.43 <sup>c</sup>
UDI <sup>e</sup>	110 (61-143)	8 (3-17)	<0.001 <sup>c</sup>	93 (55-131)	95 (52-120)	98 (80-149)	0.63 <sup>c</sup>
POPDI <sup>e</sup>	70 (30-105)	14 (0-42)	<0.001 <sup>c</sup>	68 (42-129)	40 (8-70)	70 (8-101)	0.11 <sup>c</sup>
CRADI <sup>e</sup>	66 (34-112)	17 (2-56)	<0.001 <sup>c</sup>	68 (44-120)	43 (11-81)	75 (20-89)	0.43 <sup>c</sup>
<b>12 Week Symptoms [Median (IQR)]</b>							
Symptom S.score <sup>d</sup>				18 (8-33)	18 (5-28)	54 (18-63)	0.01 <sup>c</sup>
HRQL <sup>d</sup>				93 (86-99)	94 (88-99)	70 (61-86)	0.008 <sup>c</sup>
UDI <sup>e</sup>				22 (14-59)	46 (8-64)	95 (58-113)	0.01 <sup>c</sup>
POPDI <sup>e</sup>				32 (17-68)	13 (4-19)	54 (32-93)	0.002 <sup>c</sup>
CRADI <sup>e</sup>				24 (7-89)	18 (8-39)	53 (32-68)	0.08 <sup>c</sup>

<sup>a</sup> Student's T-Test

<sup>b</sup> One-Way ANOVA

<sup>c</sup> Wilcoxon rank sum test

<sup>d</sup> Symptom Score and Health Related Quality of Life Score (HRQL), Based on OAB questionnaire

<sup>e</sup> Urinary Distress Inventory (UDI), Pelvic Organ Prolapse Distress Inventory (POPDI), Colorectal-Anal Distress Inventory (CRADI), based on Pelvic Floor Disease Inventory

\* Self reported by participant

\*\* Estrogen status is defined as negative if the patient is post-menopausal and not on hormone replacement therapy. Estrogen positive is anyone pre-menopausal and/or on hormone replacement therapy.

**Table 2**

Urotype Proportions

2A: Urotypes proportions of the total sequenced and cultured samples by dominant organisms.				
UII: Baseline Samples				
Sequencing (N=74)		→	Culture (N=59)	
Undetermined *	50% (37/74)	Of the 74 samples collected, 59 were cultured with EQUIC	Undetermined *	15.3% (9/59)
Total Sequenced	50% (37/74)		Total Cultured	84.7% (50/59)
Identified Sequence Urotypes			Identified Culture Urotypes	
<i>Lactobacillus</i>	40.5% (15/37)		<i>Lactobacillus</i>	22% (11/50)
Diverse	21.6% (8/37)		Diverse	32% (16/50)
<i>Gardnerella</i>	21.6% (8/37)		<i>Gardnerella</i>	8% (4/50)
<i>Enterobacteriaceae</i> and <i>Sneathia</i> both Represent 5.4% (2/37)			<i>Streptococcus</i>	18% (9/50)
<i>Staphylococcus</i> and <i>Enterococcus</i> both representd 2.7% (1/37)			Enterobacteriaceae	8% (4/50)
			<i>Staphylococcus, Actinobaculum Alloscardovia, Bifidobacterium, Candida</i> Enterobacteriaceae/ <i>Bacillus</i> each represent 2% (1/50)	

UII: 12 week Samples				
Sequencing (N=50)		→	Culture (N=44)	
Undetermined *	52.1% (25/48)**	Of the 50 samples collected, 44 were cultured with EQUIC	Undetermined *	13.6% (6/44)
Total Sequenced	47.9% (23/48)**		Total Cultured	86.4% (38/44)
Identified Sequence Urotypes			Identified Culture Urotypes	
<i>Lactobacillus</i>	47.8% (11/23)		<i>Lactobacillus</i>	31.6% (12/38)
<i>Gardnerella</i>	26.1% (6/23)		Diverse	18.4% (7/38)
Diverse	13% (3/23)		<i>Streptococcus</i>	13.2% (5/38)
Enterobacteriaceae	8.7% (2/23)		<i>Gardnerella</i>	7.9% (3/38)
Aerococcus	4.3% (1/23)		Enterobacteriaceae	13.2% (5/38)
			<i>Aerococcus, Bifidobacterium Corynebacterium, Lactobacillus/Gardnerella</i> each represent 2.6% (1/38)	
			<i>Streptococcus/Actinomyces</i> represent 5.3% (2/38)	

Control				
Sequencing (N=60)		→	Culture (N=52)	
Undetermined *	56.7% (34/60)	Of the 60 samples collected, 52 were cultured with EQUIC	Undetermined *	36.5% (19/52)
Total Sequenced	43.3% (26/60)		Total Cultured	63.5% (33/52)
Identified Sequence Urotypes			Identified Culture Urotypes	
<i>Lactobacillus</i>	61.5% (16/26)		<i>Lactobacillus</i>	45.5% (15/33)
<i>Gardnerella</i>	15.4% (4/26)		Diverse, <i>Gardnerella, Staphylococcus,</i>	

Control	
Sequencing (N=60)	Culture (N=52)
Diverse 15.4% (4/26)	<i>Streptococcus</i> , <i>Enterococcus</i> , <i>Streptococcus/Bifidobacterium</i> , and Enterobacteriaceae each represent 6% (2/33)
Enterobacteriaceae and <i>Atopobium</i> both represent 3.8% (1/26)	<i>Alloscardovia</i> , <i>Candida</i> , <i>Micrococcus</i> , and Unknown organisms each represent 3% (1/33)

\* Undetermined urotype means the bacterial load in the sample was below the detectable threshold by either sampling method.

\*\* 50 patients returned for the 12-week visit, however, only 48 were sequenced due to unclear specimen labeling. These two samples were processed by

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2B. Urotypes in UII Response Groups	
UII Baseline - 5mg Responders	UII 12 week- 5mg Responders
Sequencing (N=25)	Sequencing (N=23)**
Culture (N=19)	Culture (N=21)
Undetermined* 44% (11/25)	Undetermined* 56.5% (13/23)
Total Sequenced 56% (14/25)	Total Sequenced 43.5% (10/23)
Identified Sequence Urotypes	Identified Sequence Urotypes
<i>Lactobacillus</i> 50% (7/14)	<i>Lactobacillus</i> 70% (7/10)
<i>Gardnerella</i> 21.4% (3/14)	<i>Gardnerella</i> 20% (2/10)
Diverse 14.3% (2/14)	Diverse 10% (1/10)
Enterobacteriaceae and <i>Staphylococcus</i> both represent 7.1% (1/14)	<i>Actinobaculum</i> , <i>Candida</i> , Enterobacteriaceae, Enterobacteriaceae/ <i>Bacillus</i> <i>Staphylococcus</i> , <i>Streptococcus</i> , and Diverse each represent 7.1% (1/14)
Of the samples collected, 19 were cultured with EQUIC	Of the samples collected, 21 were cultured with EQUIC
Undetermined*	Undetermined*
Total Cultured	Total Cultured
Identified Culture Urotypes	Identified Culture Urotypes
<i>Lactobacillus</i>	<i>Lactobacillus</i>
<i>Gardnerella</i>	<i>Gardnerella</i>
Enterobacteriaceae	Enterobacteriaceae
<i>Bifidobacterium</i> , <i>Lactobacillus</i> / <i>Gardnerella</i> , <i>Streptococcus</i> , and <i>Streptococcus/Actinomyces</i> each represent 5.6% (1/18)	<i>Corynebacterium</i> , <i>Gardnerella</i> , and <i>Streptococcus/Actinomyces</i> each represent 7.8% (1/13)
UII Baseline - 10mg Responders	UII 12 week 10mg Responders
Sequencing (N=15)	Sequencing (N=15)
Culture (N=13)	Culture (N=14)
Undetermined* 53.3% (8/15)	Undetermined* 53.3% (8/15)
Total Sequenced 46.7% (7/15)	Total sequenced 46.7% (7/15)
Identified Sequence Urotypes	Identified Sequence Urotypes
<i>Gardnerella</i> 42.8% (3/7)	<i>Lactobacillus</i> 28.6% (2/7)
Diverse 28.6% (2/7)	<i>Gardnerella</i> 28.6% (2/7)
Enterobacteriaceae and <i>Lactobacillus</i> both represent 12.5% (1/8)	Enterobacteriaceae 28.6% (2/7)
Of the samples collected, 13 were cultured with EQUIC	Of the samples collected, 14 were cultured with EQUIC
Undetermined*	Undetermined*
Total Cultured	Total Cultured
Identified Culture Urotypes	Identified Culture Urotypes
Diverse 38.5% (5/13)	Diverse 23.1% (3/13)
<i>Streptococcus</i> 30.8% (4/13)	<i>Streptococcus</i> 23.1% (3/13)
<i>Gardnerella</i> 15.4% (2/13)	Enterobacteriaceae 15.4% (2/13)
Enterobacteriaceae and <i>Lactobacillus</i> both represent 7.8% (1/13)	<i>Lactobacillus</i> 15.4% (2/13)
Of the samples collected, 13 were cultured with EQUIC	Of the samples collected, 14 were cultured with EQUIC
Undetermined*	Undetermined*
Total Cultured	Total Cultured
Identified Culture Urotypes	Identified Culture Urotypes
Diverse 38.5% (5/13)	Diverse 23.1% (3/13)
<i>Streptococcus</i> 30.8% (4/13)	<i>Streptococcus</i> 23.1% (3/13)
<i>Gardnerella</i> 15.4% (2/13)	Enterobacteriaceae 15.4% (2/13)
Enterobacteriaceae and <i>Lactobacillus</i> both represent 7.8% (1/13)	<i>Lactobacillus</i> 15.4% (2/13)
Of the samples collected, 7 were cultured with EQUIC	Of the samples collected, 9 were cultured with EQUIC
Undetermined*	Undetermined*
Total Cultured	Total Cultured
Identified Culture Urotypes	Identified Culture Urotypes
Diverse 38.5% (5/13)	Diverse 23.1% (3/13)
<i>Streptococcus</i> 30.8% (4/13)	<i>Streptococcus</i> 23.1% (3/13)
<i>Gardnerella</i> 15.4% (2/13)	Enterobacteriaceae 15.4% (2/13)
Enterobacteriaceae and <i>Lactobacillus</i> both represent 7.8% (1/13)	<i>Lactobacillus</i> 15.4% (2/13)
UII Baseline - Non-responders	UII 12 week - Non-responders
Sequencing (N=10)	Sequencing (N=10)
Culture (N=7)	Culture (N=9)
Undetermined* 60% (6/10)	Undetermined* 40% (4/10)
Total Sequenced 40% (4/10)	Total sequenced 60% (6/10)
Of the samples collected, 7 were cultured with EQUIC	Of the samples collected, 9 were cultured with EQUIC
Undetermined*	Undetermined*
Total Cultured	Total Cultured
Identified Culture Urotypes	Identified Culture Urotypes
Diverse 38.5% (5/13)	Diverse 23.1% (3/13)
<i>Streptococcus</i> 30.8% (4/13)	<i>Streptococcus</i> 23.1% (3/13)
<i>Gardnerella</i> 15.4% (2/13)	Enterobacteriaceae 15.4% (2/13)
Enterobacteriaceae and <i>Lactobacillus</i> both represent 7.8% (1/13)	<i>Lactobacillus</i> 15.4% (2/13)

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2B. Urotypes in UUI Response Groups			
UUI Baseline - 5mg Responders		UUI 12 week- 5mg Responders	
Sequencing (N=25)	Culture (N=19)	Sequencing (N=23**)	Culture (N=21)
Identified Sequence Urotypes	Identified Culture Urotypes	Identified Sequence Urotypes	Identified Culture Urotypes
<i>Lactobacillus</i> 75% (3/4)	Diverse 50% (3/6)	<i>Lactobacillus</i> 33.3% (2/6)	<i>Lactobacillus</i> 42.8% (3/7)
Diverse 25% (1/4)	<i>Lactobacillus</i> 33.3% (2/6)	<i>Gardnerella</i> 33.3% (2/6)	<i>Aerococcus</i> , <i>Enterobacteriaceae</i> , <i>Streptococcus</i> , and Diverse each
	Enterobacteriaceae 16.7% (1/6)	<i>Aerococcus</i> and Diverse both represent 16.7% (1/6)	

\* Undetermined urotype means the bacterial load in the sample was below the detectable threshold by either sampling method.