

THE URINARY MICROBIOME IN WOMEN WITH MIXED URINARY INCONTINENCE

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INTRODUCTION AND OBJECTIVE

Mixed urinary incontinence (MUI), involuntary leakage of urine associated with both significant urgency difficult to defer and stress provocations such as coughing, laughing, and sneezing, significantly impacts quality of life. MUI pathophysiology is the least understood, and therapies are the least targeted, with treatment of stress symptoms potentially exacerbating urgency UI, while treatment of urgency UI symptoms potentially exacerbate stress UI symptoms. (Minassian, 2017) In the search for underlying causes of incontinence, the female urinary microbiome has emerged as a possible contributor to MUI symptoms. We present results from a supplementary study of the parent study, Effects of Surgical Treatment Enhanced with Exercise for Mixed Urinary Incontinence (ESTEEM) trial which recruited women with MUI scheduled to undergo a midurethral sling with perioperative behavioral/pelvic floor exercise versus sling alone (NCT01959347). The objective of this study was to characterize the urinary microbiome in women with MUI compared to asymptomatic age-matched controls. The primary aim examined the difference in *Lactobacillus* predominance between MUI and controls; the secondary aim compared the bacterial taxa in MUI and controls. The hypothesis was that these groups differed by *Lactobacillus* predominance and by other bacterial communities between groups

METHODS

The methodology for this study (inclusion/exclusion criteria, participant flow, laboratory methods and the analysis plan) has been previously published. (Komesu, 2017) Briefly, this case-control study recruited MUI participants from a parent study conducted at 8 US sites who had moderate to severe urinary incontinence symptoms. Two hundred and ten women (126 MUI/84 Controls) were required to distinguish MUI and Control urinary microbiomes. DNA was extracted from catheterized urine, regions v4-6 of the 16S ribosomal RNA gene were amplified by polymerase chain reaction and sequenced. Urinary taxa were evaluated at the genus level and categorized into community types using Dirichlet multinomial mixture (DMM) methods (Holmes, 2012). The study defined predominance as a sample in which a specific genus constituted > 50% of an individual's taxonomic community. Univariate and multivariable analyses identified differences between MUI and Control groups. Significance was set at P<.05.

RESULTS

Mean age of all 207 subjects for whom microbiome analysis was successful (123 MUI, 84 controls) was 53±11 years. Compared to controls MUI women had a greater Body Mass Index (BMI), were more commonly Hispanic, had a history of recurrent UTIs and used vaginal estrogen (Table 1). DMM identified 6 community types with significant differences in proportion of taxa between communities (P=.032) (Figure). These communities differed by age (P=.001) and smoking history (P<.001) (Table 2) and consisted of a *High-Lactobacillus* (HLac) community (>80% *Lactobacillus*) which had the largest proportion of Controls (Controls 63.3%, MUI 36.7%), a *Moderate-Lactobacillus* (MLac) community, and a *Mixed* community (Figure 1). The HLac community (see Figure, Community 1) served as the comparator for multivariable analyses. Multivariable analysis of all women (Table 3) revealed BMI (aOR 1.09, 95% CI, 1.04-1.15) and specific DMM communities were associated with MUI. Compared to the HLac community, the MLac and Mixed communities were more strongly associated with MUI, aOR 3.51 (95% CI, 1.29-9.59) and aOR 2.99 (95% CI, 1.06-8.47), respectively.

Due to DMM community differences in age (Table 2), separate multivariable analyses were performed for age <51 (pre-menopausal) and ≥51 (post-menopausal) years. In women <51 years, BMI (aOR 1.09, 95% CI, 1.02-1.17) and specific DMM communities were again associated with MUI (Table 4). Specifically compared to the HLac community, the MLac and Mixed communities were associated with MUI, aOR 8.46, 95% CI, 1.89-37.77 and aOR 7.48, 95% CI, 1.32-42.47, respectively. In women ≥51 years, BMI (aOR 1.11, 95% CI 1.03-1.20) was associated with MUI but DMM communities were not.

Table 1. Bivariate Comparison of MUI/Control Groups – All Women

| Variable | Category | Study Group | | p-value |
|---|----------------------------------|---------------|------------------|----------|
| | | MUI (N = 123) | Control (N = 84) | |
| DMM Community - N (%) | Community 1 | 11 (8.9) | 19 (22.6) | 0.0315 * |
| | Community 2 | 34 (27.6) | 16 (19.0) | |
| | Community 3 | 16 (13.0) | 16 (19.0) | |
| | Community 4 | 17 (13.8) | 13 (15.5) | |
| | Community 5 | 6 (4.9) | 4 (4.8) | |
| | Community 6 | 39 (31.7) | 16 (19.0) | |
| Age - Mean (SD) | | 53.0 (10.8) | 53.0 (11.7) | 0.9834 |
| BMI - Mean (SD) | | 32.7 (7.1) | 28.4 (6.6) | <.0001 * |
| Race - N (%) | American Indian or Alaska Native | 2 (1.6) | 1 (1.2) | 0.7968 |
| | Asian | 1 (0.8) | 0 (0.0) | 0.4074 |
| | Black or African American | 16 (13.0) | 19 (22.6) | 0.0701 |
| | White | 91 (74.0) | 64 (76.2) | 0.7192 |
| | Other | 14 (11.4) | 2 (2.4) | 0.0173 * |
| Ethnicity - N (%) | Hispanic or Latina | 28 (22.8) | 6 (7.1) | 0.0108 |
| | Not Hispanic or Not Latina | 93 (75.6) | 77 (91.7) | |
| | Unknown | 2 (1.6) | 1 (1.2) | |
| | | | | |
| Primary Language - N (%) | English | 112 (91.1) | 83 (98.8) | 0.0096 * |
| | Spanish | 11 (8.9) | 0 (0.0) | |
| | Other | 0 (0.0) | 1 (1.2) | |
| Currently smoking - N (%) | | 15 (12.2) | 6 (7.1) | 0.2371 |
| Smoking status - N (%) | Never smoked | 71 (57.7) | 55 (65.5) | 0.2617 |
| | History of smoking | 52 (42.3) | 29 (34.5) | |
| 3 or more UTI in past year - N (%) | | 12 (9.8) | 0 (0.0) | 0.0032 * |
| Positive SUI diagnosis in past year - N (%) | | 87 (70.7) | 3 (3.6) | <.0001 * |
| Ever Pregnant - N (%) | | 116 (94.3) | 73 (86.9) | 0.0634 |
| Menstrual Status - N (%) | Pre-menopausal | 36 (29.3) | 25 (29.8) | 0.8302 |
| | Post-menopausal | 64 (52.0) | 46 (54.8) | |
| | Not sure | 23 (18.7) | 13 (15.5) | |
| Estrogen by prescription - N (%) | Oral | 10 (8.1) | 6 (7.1) | 0.7940 |
| | Skin Patch | 5 (4.1) | 4 (4.8) | 0.8092 |
| | Vaginal Cream/Tablets | 21 (17.1) | 3 (3.6) | 0.0029 * |
| | None | 89 (72.4) | 69 (82.1) | 0.1039 |

* Comparison significant at 0.05 level of significance

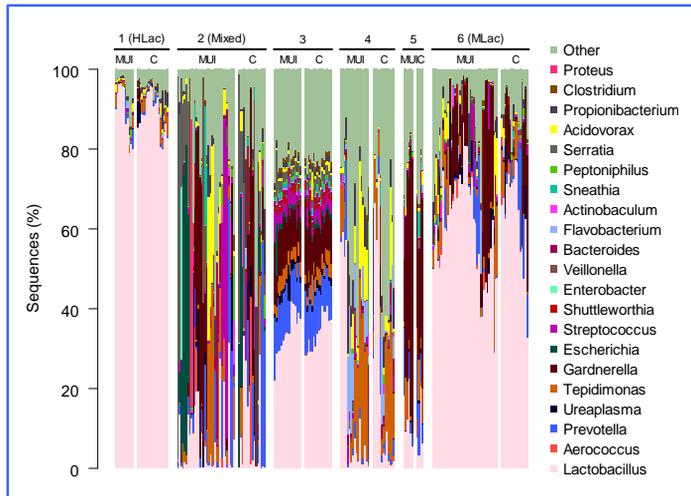


Figure 1. Dirichlet Multinomial Mixture (DMM) Microbiota Communities 1-6
Columns: Community Number/Labels. MUI, C=Controls. Bacterial Genus: Color-coded on right

Table 2. Bivariate Comparison of DMM Community Types - All Women

| Variable | Category | DMM Community | | | | | | P value |
|------------------------------------|----------------------------------|------------------------|------------------------|------------------------|------------------------|----------------------|------------------------|----------|
| | | Comty 1 (N = 30) | Comty 2 (N = 50) | Comty 3 (N = 32) | Comty 4 (N = 30) | Comty 5 (N = 10) | Comty 6 (N = 55) | |
| Study Group - N (%) | MUI Control | 11 (36.7) 19 (63.3) | 34 (68.0) 16 (32.0) | 16 (50.0) 16 (50.0) | 17 (56.7) 13 (43.3) | 6 (60.0) 4 (40.0) | 39 (70.9) 16 (29.1) | 0.0315 * |
| Age - Mean (SD) | | 49.8 (9.4) | 56.8 (11.7) | 55.5 (12.0) | 55.3 (12.7) | 51.8 (9.3) | 48.8 (8.8) | 0.0014 * |
| BMI - Mean (SD) | | 29.7 (6.1) | 30.9 (7.5) | 29.6 (6.5) | 29.3 (7.3) | 31.2 (7.1) | 33.3 (7.4) | 0.0795 |
| Race - N (%) | American Indian or Alaska Native | 0 (0.0) | 1 (2.0) | 1 (3.1) | 0 (0.0) | 0 (0.0) | 1 (1.8) | 0.8738 |
| | Asian | 1 (3.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0.3132 |
| | Black or African American | 6 (20.0) | 9 (18.0) | 5 (15.6) | 4 (13.3) | 1 (10.0) | 10 (18.2) | 0.9657 |
| | White | 23 (76.7) | 33 (66.0) | 25 (78.4) | 23 (76.7) | 8 (80.0) | 43 (78.2) | 0.7254 |
| | Other | 0 (0.0) | 7 (14.0) | 3 (9.4) | 3 (10.0) | 1 (10.0) | 2 (3.6) | 0.2227 |
| Ethnicity - N (%) | Hispanic or Latina | 2 (6.7) | 12 (24.0) | 4 (12.5) | 6 (20.0) | 2 (20.0) | 8 (14.5) | 0.7013 |
| | Not Hispanic or Not Latina | 28 (93.3) | 37 (74.0) | 28 (87.5) | 23 (76.7) | 8 (80.0) | 46 (83.6) | |
| | Unknown | 0 (0.0) | 1 (2.0) | 0 (0.0) | 1 (3.3) | 0 (0.0) | 1 (1.8) | |
| | | | | | | | | |
| Primary Language - N (%) | English | 30 (100.0) | 44 (88.0) | 31 (96.9) | 28 (93.3) | 10 (100.0) | 52 (94.5) | 0.1739 |
| | Spanish | 0 (0.0) | 6 (12.0) | 0 (0.0) | 2 (6.7) | 0 (0.0) | 3 (5.5) | |
| | Other | 0 (0.0) | 0 (0.0) | 1 (3.1) | 0 (0.0) | 0 (0.0) | 0 (0.0) | |
| Currently smoking - N (%) | | 3 (10.0) | 1 (2.0) | 1 (3.1) | 3 (10.0) | 3 (30.0) | 10 (18.2) | 0.0184 * |
| Smoking status - N (%) | Never smoked | 15 (50.0) | 30 (60.0) | 25 (78.1) | 24 (80.0) | 4 (40.0) | 28 (50.9) | <.0001 * |
| | Quit smoking <6 mo. | 1 (3.3) | 1 (2.0) | 0 (0.0) | 0 (0.0) | 2 (20.0) | 0 (0.0) | |
| | Quit smoking >6 mo. | 11 (36.7) | 18 (36.0) | 6 (18.8) | 3 (10.0) | 1 (10.0) | 17 (30.9) | |
| | Currently smoking | 3 (10.0) | 1 (2.0) | 1 (3.1) | 3 (10.0) | 3 (30.0) | 10 (18.2) | |
| 3 or more UTI in past year - N (%) | | 1 (3.3) | 5 (10.0) | 1 (3.1) | 0 (0.0) | 0 (0.0) | 5 (9.1) | 0.3137 |
| Ever Pregnant - N (%) | | 26 (86.7) | 48 (96.0) | 28 (87.5) | 26 (86.7) | 9 (90.0) | 52 (94.5) | 0.5007 |
| Menstrual Status - N (%) | Pre-menopausal | 10 (33.3) | 10 (20.0) | 8 (25.0) | 7 (23.3) | 2 (20.0) | 24 (43.6) | 0.0668 |
| | Post-menopausal | 13 (43.3) | 34 (68.0) | 20 (62.5) | 19 (63.3) | 5 (50.0) | 19 (34.5) | |
| | Not sure | 7 (23.3) | 6 (12.0) | 4 (12.5) | 4 (13.3) | 3 (30.0) | 12 (21.8) | |
| Estrogen by prescription - N (%) | Oral | 2 (6.7) | 4 (8.0) | 4 (12.5) | 1 (3.3) | 0 (0.0) | 5 (9.1) | 0.7204 |
| | Skin Patch | 1 (3.3) | 2 (4.0) | 2 (6.3) | 2 (6.7) | 0 (0.0) | 2 (3.6) | 0.9373 |
| | Vaginal Cream/Tablets | 4 (13.3) | 9 (18.0) | 2 (6.3) | 3 (10.0) | 1 (10.0) | 5 (9.1) | 0.6359 |
| | None | 23 (76.7) | 36 (72.0) | 23 (71.9) | 23 (76.7) | 9 (90.0) | 44 (80.0) | 0.8036 |

* Comparison significant at 0.05 level of significance

Table 3. Multivariable Analysis of MUI/Control Groups – All Women

| Model Term | P-value for Effect | Comparison | Estimated Odds Ratio | 95% Confidence Interval | P-value for Comparison |
|----------------|--------------------|---|----------------------|-------------------------|------------------------|
| Age | 0.5554 | Age | 1.01 | (0.97, 1.05) | 0.5554 |
| BMI | 0.0004 * | BMI | 1.09 | (1.04, 1.15) | 0.0004 * |
| DMM Community | 0.2011 | DMM Community 2 vs. DMM Community 1 | 2.99 | (1.06, 8.47) | 0.0388 * |
| | | DMM Community 3 vs. DMM Community 1 | 1.73 | (0.57, 5.26) | 0.3342 |
| | | DMM Community 4 vs. DMM Community 1 | 2.15 | (0.69, 6.75) | 0.1884 |
| | | DMM Community 5 vs. DMM Community 1 | 2.19 | (0.46, 10.43) | 0.3228 |
| | | DMM Community 6 vs. DMM Community 1 | 3.51 | (1.29, 9.59) | 0.0144 * |
| Smoking status | 0.7544 | Never Smoked vs. Ever Smoked | 1.11 | (0.57, 2.18) | 0.7544 |
| Ethnicity | 0.0131 * | Hispanic/Latina vs. Not Hispanic/Latina | 3.59 | (1.31, 9.83) | 0.0131 * |
| | | Menopause: Pre vs. Post | 1.24 | (0.47, 3.26) | 0.6669 |
| | | Menopause: Pre vs. Not Sure | 1.23 | (0.46, 3.28) | 0.6809 |
| | | Menopause: Post vs. Not Sure | 1.00 | (0.37, 2.66) | 0.9886 |

* Unknown ethnicity combined with Not Hispanic/Latina

* Comparison significant at 0.05 level of significance

Table 4. Multivariable Analysis of MUI/Control Groups - Women <51 Years of Age

| Model Term | P-value for Effect | Comparison | Estimated Odds Ratio | 95% Confidence Interval | P-value for Comparison |
|------------------------|--------------------|---|----------------------|-------------------------|------------------------|
| Age | 0.6775 | Age | 0.98 | (0.89, 1.08) | 0.6775 |
| BMI | 0.0146 * | BMI | 1.09 | (1.02, 1.17) | 0.0146 * |
| DMM Community | 0.0428 * | DMM Community 2 vs. DMM Community 1 | 7.48 | (1.32, 42.47) | 0.0237 * |
| | | DMM Community 3 vs. DMM Community 1 | 1.90 | (0.32, 11.17) | 0.4744 |
| | | DMM Community 4 vs. DMM Community 1 | 1.33 | (0.21, 8.36) | 0.7576 |
| | | DMM Community 5 vs. DMM Community 1 | 5.95 | (0.45, 78.18) | 0.1724 |
| | | DMM Community 6 vs. DMM Community 1 | 8.46 | (1.89, 37.77) | 0.0057 * |
| Smoking status | 0.4456 | Never Smoked vs. Ever Smoked | 1.51 | (0.52, 4.44) | 0.4456 |
| Ethnicity ^a | 0.0869 | Hispanic/Latina vs. Not Hispanic/Latina | 3.51 | (0.83, 14.79) | 0.0869 |
| | | Menopause: Pre vs. Post | 1.08 | (0.23, 5.02) | 0.9244 |
| Menstrual status | 0.7847 | Menopause: Pre vs. Not Sure | 1.53 | (0.44, 5.60) | 0.5198 |
| | | Menopause: Post vs. Not Sure | 1.42 | (0.34, 5.97) | 0.6305 |
| | | | | | |

* Unknown ethnicity combined with Not Hispanic/Latina

* Comparison significant at 0.05 level of significance

CONCLUSION

Differences were identified in the urinary microbiome of the MUI group compared to the asymptomatic control group among pre-menopausal but not post-menopausal women. This difference in the pre- and post-menopausal MUI microbiome may contribute to the phenotypic differences in treatment response and disease severity in these 2 populations. Future work may further characterize age-dependent differences in the MUI microbiome.

References

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