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Urinary Fungi Associated with Urinary Symptom Severity among Women with Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS)

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Ethical Responsibilities of the Authors

The authors agree to the conditions outlined in the submission instructions

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CM is an employee of the NIH/NIDDK. The authors report no other potential conflict of interest.

2. Research involving Human Participants

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

3. Research involving Animals

This article does not contain any studies with animals performed by any of the authors.

4. Informed consent

Written informed general consent for specimen procurement and data analysis was obtained from all individual participants included in the study.

Abstract

Purpose: To correlate the presence of fungi with symptom flares, pain and urinary severity in a prospective, longitudinal study of women with IC/BPS enrolled in the MAPP Research Network.

Methods: Flare status, pelvic pain, urinary severity, and midstream urine were collected at baseline, 6, 12 months from female IC/BPS participants with at least one flare and age-matched participants with no reported flares. Multilocus PCR coupled with electrospray ionization/mass spectrometry was used for identification of fungal species and genus. Associations between “mycobiome” (species/genus presence, relative abundance, Shannon’s/Chao1 diversity indices) and current flare status, pain, urinary severity were evaluated using generalized linear mixed models, permutational multivariate analysis of variance, Wilcoxon’s Rank Sum test.

Results: The most specific analysis detected 13 fungal species from 8 genera in 504 urine samples from 202 females. A more sensitive analysis detected 43 genera. No overall differences were observed in fungal species/genus composition or diversity by flare status or pain severity. Longitudinal analyses suggested greater fungal diversity (Chao1 Mean Ratio 3.8, 95% CI 1.3–11.2, $p=0.02$) and a significantly greater likelihood of detecting any fungal species ($OR=5.26$, 95% CI 1.1–25.8, $p=0.04$) in high versus low urinary severity participants. Individual taxa analysis showed a trend toward increased presence and relative abundance of *Candida* ($OR=6.63$, 95% CI 0.8–58.5, $p=0.088$) and *Malassezia* (only identified in ‘high’ urinary severity phenotype) for high vs. low urinary symptoms.

Conclusion: This analysis suggests the possibility that greater urinary symptom severity is associated the urinary mycobiome urine in some females with IC/BPS.

INTRODUCTION:

Many patients diagnosed with IC/BPS experience waxing and waning urinary and bladder pain symptoms with exacerbations often called “flares” [1]. A diverse fungal community has been found in preliminary studies of urine of healthy females using next generation sequencing [2]. A preliminary report using baseline data from females with IC/BPS subjects enrolled in the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network Epidemiological/Phenotyping (EP) [3] Study suggested that fungi, rather than bacteria, were associated with symptom flares [4]. That specific study evaluated only baseline enrollment specimens and employed a technique that only identified *Candida* and *Saccharomyces* sp. To our knowledge there is no data or evidence in literature examining the impact of the mycobiome in either symptom patterns or flares in patients with IC/BPS patients.

Evaluation of female IC/BPS participants enrolled in MAPP-EP Study [3], with and without flare at 6 and 12 months follow-up allowed for a more comprehensive fungal identification to examine the role of fungi in symptom patterns experienced in IC/BPS. We hypothesize that symptom flares, as well as symptom patterns (pain and urinary severity), may be linked with changes in the urinary mycobiome.

METHODS:

The MAPP Study.

As part of the MAPP-EP Study female participants with a diagnosis of IC/BPS were recruited for baseline phenotyping and a 12 month longitudinal follow-up of the treated history of symptoms [3]. Details of the study design, including study population eligibility criteria, data acquisition and biological sample collection, and descriptions of the disease specific questionnaires are available [3,5]. A midstream urine (VB2) specimen was collected at baseline and cultured at the investigator study site to identify active infection. Participants with a positive urine culture (traditional uropathogen colony forming units $10^5/\text{mL}$) at baseline were excluded from the EP study, since subjects with a defined UTI do not by definition, IC/BPS.

Participants, Symptom Assessment, and Specimen

At the time of enrollment (baseline – Visit 1), and at the 6 (visit 13) and 12 month (visit 25) study visits, each participant was asked the following question, “Are you currently experiencing a flare of your urologic or pelvic pain symptoms (possible responses “yes” or “no”)? By this we mean, are you currently experiencing symptoms that are much worse than usual?” [2]. All available urine samples of all participants who reported a flare at any of the 3 time points were included in our sample, as well as all available urine samples of a randomly selected subset of patients who did not report a flare at any of the 3 time points. Participants who responded with “yes” at a given time were included in the flare group at that time. Pain and urinary symptom severity were evaluated at each time point using two derived scales based on the Genitourinary Pain Index (GUPI) and the Interstitial Cystitis Symptom Score (ICSI)[9]. Each participant was asked to provide a midstream clean catch (saline wipe only) urine specimen (VB2, ~20mL) at the baseline, 6 month and 12 month clinic visits.

Specimen handling

Urine specimens were collected using standardized collection kits that contained all the components needed for collection and storage of the urine specimens. After collection at the MAPP Network Discovery sites [3], specimens were transferred from urine cups to 50 mL conical tubes and frozen (as soon as possible) at -80°C until shipment to the central MAPP Network Tissue Analysis and Technology Core (TATC). Upon receipt at the TATC specimens were thawed, thoroughly mixed, and aliquoted into 1 and 3 mL aliquots. Specimens were frozen at -80°C until used.

DNA extraction and fungal domain assays:

Specimens were evaluated with a commercial molecular diagnostic, the Broad Fungal Screen assay [6,7] performed on the PLEX-ID (Abbott Molecular). In brief, total DNA was extracted (see Appendix 1) using 3 mL from each urine sample as described. This is a single plex assay with 16 unique broad-range PCR primer sets, including 9 broad-range primer sets, 6 short-range primer sets, and 1 control primer set. A synthetic DNA construct was also included within each reaction mixture (20 copies per reaction) as an internal positive

amplification control as well as a quantitative calibrant. This synthetic construct (approximately 1,800 nucleotides) contained all 16 primer-binding regions; however, these regions each contained either insertions or deletions, causing a mass shift to allow product differentiation from the actual sample. By comparing the signal obtained from the synthetic construct amplicons and the target amplicons, an approximate template copy number could be determined. The fungal DNAs were amplified by polymerase chain reaction (PCR) using the 16 primer pair Fungal detection systems developed by Ibis⁶. The individual amplicons were “weighed” using the Ibis instrumentation by electrospray ionization (ESI) time-of-flight (TOF) mass spectrometry (MS), which reports molecular mass. The molecular mass of each amplicon was then translated into a unique base sequence composition (the number of A’s, T’s, C’s & G’s) and the corresponding species identities of each amplicon were then revealed by comparing each result with a database containing base composition data on virtually all known pathogenic and commensal fungal genus and species that have been sequenced to date. Details of this methodology are available in [4,6,7,8] and Appendix 1. This analysis resulted in two levels of results, a ‘specific’ set that identified fewer species with high certainty and a ‘sensitive’ set that identified more species with less certainty.

Statistical Analysis

Analyses of ‘specific’ and ‘sensitive’ results were conducted separately with ‘specific’ results analyzed at the species, genus, and ‘any fungal taxa’ level, and ‘sensitive’ results analyzed at the genus and ‘any fungal taxa’ level.

Separate analyses evaluated the association of fungal species composition with concurrent flare status, high versus low pain severity tertile, and high versus low urinary tertile, where symptom severity tertiles at each time were defined by the 33rd and 66th percentile of symptom severity measures at that time in our sample of MAPP Network IC/BPS participants. Across all visits, pain and urinary analyses excluded observations with respective symptom severity falling in the middle tertile such that our comparison was of the highest versus lowest symptom severity tertiles. Log-linear mixed effects models were used to evaluate the association of current flare compared to nonflare, high compared to low pain tertile, and high compared to low urinary tertile as time-varying covariates with Chao1[10] (measure of diversity), simultaneously utilizing data from all 3 visits across all participants. Logistic mixed effects models were similarly used to longitudinally assess associations of flare status and pain and urinary symptom tertiles with the presence or absence of individual taxa. Models included fixed effects for flare status or symptom tertile and visit (baseline, 6 months, or 12 months), and a random intercept for participant. Permutational multivariate analysis of variance (PERMANOVA) and comparisons of Shannon’s Diversity Index [11] and relative abundance were performed separately at baseline, 6 months, and 12 months in cross-sectional analysis since longitudinal methods were either inappropriate due to unsatisfied modeling assumptions or unavailable. PERMANOVA is an extension of standard Analysis of Variance (ANOVA) to compare overall taxa composition between groups using a resampling permutation approach rather than a normality assumption. Shannon’s Index and relative abundance were compared by flare status or symptom tertile using Wilcoxon’s Rank-Sum Test. Tests of cross-sectional associations at each time were not adjusted for multiple comparisons since the correlation of each test across time would make such

adjustments overly conservative. Tests of the presence or relative abundance of individual taxa within a comparison (flare status, pain tertile or urinary tertile) were adjusted for multiple comparisons by controlling the False Discovery Rate [12].

RESULTS:

Subject characteristics: Data were available for analysis from 507 visits across 202 female participants of whom 103 reported a flare during at least one visit out of the baseline, 6-month, and 12-month visits, and 99 did not report a flare at any of those times. Table 1a–c describes the characteristics of the subjects identified with flare/no flare (1a) and low and high tertile pain (1b) and urinary (1c) symptoms. Comparing flare and non-flare at each time, those who were in a flare were younger, and more likely to have widespread pain. Comparing low and high pain tertiles, low pain tertile subjects were older and less likely to have chronic fatigue, fibromyalgia, and bladder pain syndrome. Comparing low and high urinary tertiles, there were no significant age differences, but high urinary tertile subjects were more likely to have IBS, chronic fatigue, and bladder pain syndrome.

Fungal Detection: The specific IBIS analysis detected 13 fungal species from 8 genera (Figure 2 a,b,c) across 504 urine specimens. There were no species detected in 466 (92.4%) specimens, 1 species in 36 (7.1%) specimens, and 2 species in 2 specimens (0.4%). Analogously, the sensitive analysis resulted in the detection of 43 genera across 507 specimens, with fungi detected in 108 specimens, and no fungi detected in 399.

Specific IBIS detection level analysis

Species Level Differences: Chao1 species diversity was not associated with flare status (1.5 (0.7,3.2), $p=0.28$) (Table 2, Figure 1) and was non-significantly elevated (2.4 (1.0–6.0), $p=0.06$) (Table 2, Figure 1) among participants with high vs low pain severity. There was greater diversity among high urinary severity compared to low urinary severity (Chao1 Mean Ratio 3.8 (95% CI 1.3–11.2), $p=0.02$) (Table 2, Figure 1). There were no significant differences in the presence of individual species by flare status, high vs low pain severity, or high vs low urinary severity, but of the 4 species (*Candida albicans*, *Candida dubliniensis*, *Candida glabrata*, and *Malassezia pachydermatis*) present for at least 3 person-times, 3 were present exclusively in high urinary tertile participants (Table 3 a–c). Multivariate analysis by PERMANOVA and comparison of Shannon's Index in cross-sectional analyses at each visit did not detect differences in fungal composition by flare status or pain or urinary tertile at the species level. Notably, Shannon's Index was nearly uniformly zero across all person times due to the small number of species detected. Similarly, mean relative abundance across participants for each taxon were also low, and did not differ significantly by flare status or pain or urinary tertile.

Genus Level Differences: Results of analysis at the genus-level (Tables 4 and 5a–c) were largely similar but revealed a trend toward increased presence and relative abundance of *Candida* for high versus low urinary status (OR=6.63, $p=0.088$ for *Candida* presence).

Differences were detected in high versus low urinary severity status in multivariate analysis ($p=0.015$ month 6, $p=0.026$ month 12), and relative abundance (*Candida* $p=0.0089$ month 6).

Any Fungal Taxa Level Differences: The likelihood of detecting any fungal species was greater for high versus low urinary severity patients (OR=5.26, $p=0.04$) in longitudinal analysis, but not comparing flare status ($p=0.38$) or pain tertile ($p=0.11$).

Sensitive IBIS detection level analysis

The sensitive Ibis technique detected species across 43 genera but did not show any significant associations between fungal composition and flare status, pain, or urinary symptoms severity (Appendix 2).

Discussion:

Until recently, “healthy” urine has been considered to be sterile, with the presence of culturable microorganisms considered an indicator of disease. Using high-throughput next generation sequencing (NGS) methods that exploit conserved regions in bacterial genes (specifically the 16S ribosomal RNA locus), diverse bacterial communities have been identified in urine from healthy asymptomatic subjects. A considerable body of literature describes shifts in patterns of bacterial communities in the urine associated with bladder diseases, including urinary incontinence [13–18], urinary symptoms in urogynecology surgical patients [19], neurogenic voiding dysfunction [20], and interstitial cystitis [21].

There is growing evidence of a diverse fungal microbiome, or “mycobiome”, that also exists in the human body. Alterations in the mycobiome occurring in the absence of large alterations in fungal species perceived to be classical infections have been implicated in chronic Hepatitis B [22], atopic dermatitis [23], dandruff [24], inflammatory bowel disease [25], cystic fibrosis [26], and asthma [27]. The specific alterations differ in each pathology; in disease states, there can be either a decreased or increased diversity from that seen in the healthy state. A previously published comparison, employing similar non-culture technology, did not identify a difference in the “bacterial” microbiota of women with IC/BPS who reported flares compared to those who did not report a flare [4]. However, the study did indicate a significant difference in the prevalence of fungi in the midstream urine specimen between flare and non-flare groups. A significantly greater prevalence of fungi (*Candida* and *Saccharomyces sp.*) was detected in the flare group (15.7%) compared to the non-flare group in VB2 (3.9%) ($p=0.006$). Despite our use of more specific fungal identification than in our previous studies (in which we did not identify abundance differences and the technique used only identified *Candida* and *Saccharomyces sp.*), we observed no overall differences in fungal species or genus composition or diversity for self-reported flare status or pain severity. However, longitudinal analyses revealed a significantly greater likelihood of detecting any fungal species in high versus low urinary severity patients. Individual taxa analysis showed a trend toward increased presence and relative abundance of *Candida* and *Malassezia* for high versus low urinary patients in longitudinal analysis. These two genera have been implicated in a number of other inflammatory diseases, such as IBD where the presence of these fungi are associated with higher symptom severity and a worse prognosis [28]. In our study, both species and genus-level longitudinal and cross sectional analyses suggested greater fungal diversity among high urinary severity compared to low urinary severity at 6 and 12 months.

This study further adds some credence to our hypothesis that urinary fungi appear to be involved in symptoms, but not necessarily in the way we had predicted. The absence of differences in IC/BPS participants with self-reported flares compared to those with no flares as well as the participants with high vs low pain severity, would seem to negate the importance of fungi in IC/BPS. However, the observation that increased fungal presence, diversity and/or abundance were associated with high urinary symptom severity is very intriguing. The flare question posed to participants queried exacerbations of either pain or urinary symptoms. We have since learned that pain and urinary symptoms in IC/BPS patients should be considered separately [9], so it is very possible that the flare construct we employed (combining the two domains) affected our findings. We may have been able to report a more powerful association if we had independently asked about exacerbations in pain and urinary symptoms.

The results must be interpreted with caution because of the small number of participants identified with fungal species and its impact on the analyses employed. While multivariate analysis by PERMANOVA did detect differences in fungal composition by urinary severity level at the genera level, it did not do so at the species level. Similarly, it was difficult to examine differences in fungal diversity in this population, as Shannon's Index was nearly uniformly zero across all person times due to the small number of species detected. In the more sensitive analyses for which more genera were identified, no significant associations between fungal composition and patient flare status, pain or urinary severity phenotypes were noted.

CONCLUSION:

This comprehensive analysis of fungi identified in female IC/BPS urine specimens suggests that increased prevalence of fungi is associated with more severe urinary symptoms in some patients but not pain or self-reported flare states.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figures 1a-c:

Mean (95%CI) Chao 1 by (a) flare, (b) high or low pain tertile or (c) high or low urinary tertile by month.

Both:
Candida
Malassezia
Saccharomyces

High Pain:
Cladosporium
Penecillium

Low Pain:
Pichia
Yarrowia

Figures 2 a-c:

Venn diagram of overlapping fungal genera by (a) flare, (b) high or low pain tertile or (c) high or low urinary tertile (c).

Table 1:

Characteristics of the subjects identified with flare/no flare (1a) and low and high tertile pain (1b) and urinary (1c) symptoms

Table 1a		Flare		Non-flare		
Variable	Visit #	Total N	Mean (SD) or N (%)	Total N	Mean (SD) or N (%)	P-value
Age	1	70	40.7(15.11)	123	41.8(14.28)	0.0340
	13	28	38.5 (15.10)	135	43.0 (14.38)	
	25	32	36.9(15.00)	119	44.2 (14.34)	
Duration	1	70	8.8 (10.46)	120	9.5 (10.67)	0.2751
	13	27	7.5 (9.68)	132	9.4 (10.49)	
	25	31	8.2 (9.77)	117	10.2(11.47)	
IBS	1	70	29 (41.4%)	123	35 (28.5%)	0.2246
	13	28	11 (39.3%)	135	42 (31.1%)	
	25	32	9 (28.1%)	119	42 (35.3%)	
CFS	1	70	16 (22.9%)	123	18 (14.6%)	0.0701
	13	28	7 (25.0%)	135	19 (14.1%)	
	25	32	7 (21.9%)	119	18 (15.1%)	
FM	1	70	11 (15.7%)	123	13 (10.6%)	0.3757
	13	28	2 (7.1%)	135	18 (13.3%)	
	25	32	6 (18.8%)	119	11 (9.2%)	
PP and Beyond	1	70	60 (85.7%)	121	88 (72.7%)	0.0561
	13	28	22 (78.6%)	133	98 (73.7%)	
	25	32	26 (81.3%)	118	90 (76.3%)	
BPS	1	70	59 (84.3%)	123	112 (91.1%)	0.7942
	13	28	25 (89.3%)	135	117 (86.7%)	
	25	32	30 (93.8%)	119	105 (88.2%)	
Table 1b		Low Pain Tertile		High Pain Tertile		
Variable	Vist#	Total N	Mean (SD) or N (%)	Total N	Mean (SD) or N (%)	P-value
Age	1	63	46.2 (14.47)	69	37.1 (13.23)	0.0002
	13	52	46.1(14.14)	55	37.0 (12.59)	
	25	47	46.9 (13.48)	47	40.1 (16.21)	
Duration	1	63	9.8 (11.65)	68	10.3 (10.60)	0.8521
	13	52	8.5 (9.26)	53	8.9 (10.54)	
	25	47	9.7 (11.13)	46	9.5 (11.36)	
IBS	1	63	21 (33.3%)	69	28 (40.6%)	0.2978
	13	52	12 (23.1%)	55	23 (41.8%)	
	25	47	18 (38.3%)	47	16 (34.0%)	
CFS	1	63	7 (11.1%)	69	22 (31.9%)	0.0003
	13	52	1 (1.9%)	55	16 (29.1%)	
	25	47	7 (14.9%)	47	13 (27.7%)	

Table 1a		Flare		Non-flare		
Variable	Visit #	Total N	Mean (SD) or N (%)	Total N	Mean (SD) or N (%)	P-value
FM	1	63	4 (6.3%)	69	15 (21.7%)	0.0075
	13	52	2 (3.8%)	55	10 (18.2%)	
	25	47	3 (6.4%)	47	5 (10.6%)	
PP and Beyond	1	62	47 (75.8%)	69	55 (79.7%)	0.6313
	13	52	39 (75.0%)	55	43 (78.2%)	
	25	47	36 (76.6%)	47	36 (76.6%)	
BPS	1	63	47 (74.6%)	69	67 (97.1%)	0.0002
	13	52	38 (73.1%)	55	52 (94.5%)	
	25	47	37 (78.7%)	47	46 (97.9%)	
Table 1c		Low Urinary Tertile		High Urinary Tertile		
Variable	Vist#	Total N	Mean (SD) or N (%)	Total N	Mean (SD) or N (%)	P-value
Age	1	59	43.5 (15.27)	66	39.2 (14.69)	0.4749
	13	52	40.6 (14.10)	56	42.6 (15.45)	
	25	48	43.4(13.99)	49	40.9 (15.31)	
Duration	1	58	9.6 (11.26)	64	10.3 (11.00)	0.2924
	13	52	7.5 (8.44)	54	10.2 (11.18)	
	25	48	9.4(11.10)	48	12.1 (12.57)	
IBS	1	59	18 (30.5%)	66	30 (45.5%)	0.0354
	13	52	13 (25.0%)	56	25 (44.6%)	
	25	48	15 (31.3%)	49	23 (46.9%)	
CFS	1	59	6 (10.2%)	66	19 (28.8%)	0.0061
	13	52	5 (9.6%)	56	16 (28.6%)	
	25	48	5 (10.4%)	49	14 (28.6%)	
FM	1	59	6 (10.2%)	66	13 (19.7%)	0.2667
	13	52	6 (11.5%)	56	9 (16.1%)	
	25	48	5 (10.4%)	49	7 (14.3%)	
PP and Beyond	1	59	47 (79.7%)	66	50 (75.8%)	0.6066
	13	51	36 (70.6%)	55	44 (80.0%)	
	25	48	37 (77.1%)	48	39 (81.3%)	
BPS	1	59	45 (76.3%)	66	63 (95.5%)	<.0001
	13	52	40 (76.9%)	56	53 (94.6%)	
	25	48	36 (75.0%)	49	49 (100.0%)	

(IBS= irritable bowel syndrome; CFS= chronic fatigue syndrome; FM=fibromyalgia; PP=pelvic floor; BPS=bladder pain syndrome)

Table 2:

Longitudinal Analysis of Chao at the Species Level – Specific Detection Level

	Nonflare			Flare		Association	
	Visit	n	Mean (sd)	n	Mean (sd)	Mean Ratio ^I	p
Flare Status	1	123	0.049 (0.22)	68	0.059 (0.24)	1.52 (0.71,3.22)	0.2796
	13	135	0.081 (0.3)	28	0.107 (0.31)	-	-
	25	118	0.085 (0.28)	32	0.188 (0.47)	-	-
	Low Pain Tertile			High Pain Tertile		Association	
	Visit	n	Mean (sd)	n	Mean (sd)	Mean Ratio ^I	p
Pain Tertile	1	63	0.016 (0.13)	67	0.075 (0.26)	2.43 (0.98,6.04)	0.056
	13	52	0.077 (0.27)	55	0.164 (0.42)	-	-
	25	46	0.065 (0.25)	47	0.17 (0.43)	-	-
	Low Urinary Tertile			High Urinary Tertile		Association	
	Visit	n	Mean (sd)	n	Mean (sd)	Mean Ratio ^I	p
Urinary Tertile	1	59	0.051 (0.22)	64	0.062 (0.24)	3.75 (1.26,11.19)	0.0177 ²
	13	52	0.019 (0.14)	56	0.161 (0.42)	-	-
	25	48	0.021 (0.14)	49	0.163 (0.43)	-	-

^IDetermined by Poisson Generalized Linear Mixed Model a with random intercept for participant²Significant at the 0.05 level

Table 3:

Longitudinal Analysis of Individual Taxa at the Species Level – Specific Detection Level

Table 3a		Nonflare				Flare		Association: Presence vs. Absence ^I		Association: Relative Abundance ²	
Flare Status	Taxa	Visit	N Present/Total	Mean Relative Abundance	N Present/Total	Mean Relative Abundance	Odds Ratio (95% CI)	p			p
	Candida albicans	1	2/123 (1.6%)	0.0163	0/68 (0%)	0	0.67 (0.08,5.62)	0.71			0.2953
		13	3/135 (2.2%)	0.0222	1/28 (3.6%)	0.0357	-	-			0.6815
		25	2/118 (1.7%)	0.0169	1/32 (3.1%)	0.0312	-	-			0.6161
	Candida dubliniensis	1	0/123 (0%)	0	2/68 (2.9%)	0.0294	*				0.0575
		13	2/135 (1.5%)	0.0148	0/28 (0%)	0	-	-			0.5257
		25	0/118 (0%)	0	0/32 (0%)	0	-	-			-
	Candida glabrata	1	2/123 (1.6%)	0.0163	0/68 (0%)	0	0.04 (0.527,45)	0.4973			0.2953
		13	1/135 (0.7%)	0.0071	1/28 (3.6%)	0.0357	-	-			0.2171
		25	1/118 (0.8%)	0.0085	1/32 (3.1%)	0.0312	-	-			0.3264
	Malassezia pachydermatis	1	1/123 (0.8%)	0.0081	0/68 (0%)	0	1.91 (0.18,19.72)	0.5871			0.4638
		13	1/135 (0.7%)	0.0074	0/28 (0%)	0	-	-			0.6605
		25	2/118 (1.7%)	0.0169	2/32 (6.2%)	0.0511	-	-			0.1648
	Saccharomyce es cerevisiae	1	0/123 (0%)	0	0/68 (0%)	0	1.51 (0.15,14.87)	0.7247			0.4611
		13	2/135 (1.5%)	0.0148	1/28 (3.6%)	0.0357	-	-			-
		25	1/118 (0.8%)	0.0085	0/32 (0%)	0	-	-			0.6139
Table 3b		Low Pain Tertile				High Pain Tertile		Association: Presence vs. Absence ^I		Association: Relative Abundance ²	
Pain Tertile	Taxa	Visit	N Present/Total	Mean Relative Abundance	N Present/Total	Mean Relative Abundance	Odds Ratio (95% CI)	p			p
	Candida albicans	1	0/63 (0%)	0	0/67 (0%)	0	0.65 (0.01,40.03)	0.8401			-
		13	2/52 (3.8%)	0.0385	1/55 (1.8%)	0.0182	-	-			0.5344
		25	1/46 (2.2%)	0.0217	2/47 (4.3%)	0.0426	-	-			0.5807
	Candida dubliniensis	1	0/63 (0%)	0	2/67 (3%)	0.0299	-	-			0.172
		13	0/52 (0%)	0	2/55 (3.6%)	0.0364	-	-			0.1712
		25	0/46 (0%)	0	0/47 (0%)	0	-	-			-
	Candida glabrata	1	0/63 (0%)	0	0/67 (0%)	0	-	-			-
		13	0/52 (0%)	0	2/55 (3.6%)	0.0357	-	-			0.1712
		25	0/46 (0%)	0	2/47 (4.3%)	0.0426	-	-			0.1641
	Malassezia pachydermatis	1	1/63 (1.6%)	0.0159	0/67 (0%)	0	0.99 (0.03,36.42)	0.9956			0.3097

Table 3a		Nonflare				Flare		Association: Presence vs. Absence ¹		Association: Relative Abundance ²	
	Taxa	Visit	N	Present/Total	Mean Relative Abundance	N	Present/Total	Mean Relative Abundance	Odds Ratio (95% CI)	p	p
	Saccharomyces cerevisiae	13	0/52 (0%)	0	0.0182	1/55 (1.8%)	0.0182	-	-	-	0.3403
		25	1/46 (2.2%)	0.0217	0.0348	2/47 (4.3%)	0.0348	-	-	-	0.5894
		1	0/63 (0%)	0	0	0/67 (0%)	0	0.95 (0.13,6.92)	0.9617		
		13	1/52 (1.9%)	0.0192	0.0364	2/55 (3.6%)	0.0364	-	-	-	0.6009
		25	1/46 (2.2%)	0.0217	0	0/47 (0%)	0	-	-	-	0.3225
Table 3c		Low Urinary Tertile				High Urinary Tertile		Association: Presence vs. Absence ¹		Association: Relative Abundance ²	
	Taxa	Visit	N	Present/Total	Mean Relative Abundance	N	Present/Total	Mean Relative Abundance	Odds Ratio (95% CI)	p	p
Urinary Tertile	Candida albicans	1	2/59 (3.4%)	0.0339	0	0/64 (0%)	0	1.06 [*] (0.226,6.2)	0.983	0.1423	
		13	0/52 (0%)	0	0.0357	2/56 (3.6%)	0.0357	-	-	-	0.1751
		25	0/48 (0%)	0	0.0204	1/49 (2%)	0.0204	-	-	-	0.3325
	Candida dubliniensis	1	0/59 (0%)	0	0.0312	2/64 (3.1%)	0.0312	-	-	-	0.1764
		13	0/52 (0%)	0	0.0357	2/56 (3.6%)	0.0357	-	-	-	0.1751
	Candida glabrata	25	0/48 (0%)	0	0	0/49 (0%)	0	-	-	-	-
		1	1/59 (1.7%)	0.0169	0	0/64 (0%)	0	*2.38 (0,Inf)	0.9999	0.3053	
		13	0/52 (0%)	0	0.0351	2/56 (3.6%)	0.0351	-	-	-	0.1751
	Malassezia pachydermatis	25	0/48 (0%)	0	0.0204	1/49 (2%)	0.0204	-	-	-	0.3325
		1	0/59 (0%)	0	0	0/64 (0%)	0	-	-	-	
		13	0/52 (0%)	0	0.0179	1/56 (1.8%)	0.0179	-	-	-	0.3446
		25	0/48 (0%)	0	0.0537	3/49 (6.1%)	0.0537	-	-	-	0.0854

¹ Determined by logistic mixed model with a random intercept for participant

² Determined by Wilcoxon Rank-Sum test separately at each time

^{*} Regression analysis unstable

Table 4:

Longitudinal Analysis of Chao at the Genus Level – Specific Detection Level Analysis

	Nonflare			Flare		Association	
	Visit	n	Mean (sd)	n	Mean (sd)	Mean Ratio ^I	p
Flare Status	1	123	0.049 (0.22)	68	0.059 (0.24)	1.54 (0.73,3.25)	0.2574
	13	135	0.074 (0.26)	28	0.107 (0.31)	-	-
	25	118	0.085 (0.28)	32	0.188 (0.47)	-	-
	Low Pain Tertile			High Pain Tertile		Association	
	Visit	n	Mean (sd)	n	Mean (sd)	Mean Ratio ^I	p
Pain Tertile	1	63	0.016(0.13)	67	0.075 (0.26)	2.37 (0.97,5.79)	0.0587
	13	52	0.077 (0.27)	55	0.145 (0.36)	-	-
	25	46	0.065 (0.25)	47	0.17(0.43)	-	-
	Low Urinary Tertile			High Urinary Tertile		Association	
	Visit	n	Mean (sd)	n	Mean (sd)	Mean Ratio ^I	p
Urinary Tertile	1	59	0.051 (0.22)	64	0.062 (0.24)	3.57 (1.22,10.43)	0.0198
	13	52	0.019(0.14)	56	0.143 (0.35)	-	-
	25	48	0.021 (0.14)	49	0.163 (0.43)	-	-

^IDetermined by Poisson Generalized Linear Mixed Model a with random intercept for participant

Table 5;

Longitudinal Analysis of Individual Taxa at the Genus Level – Specific Detection Level

Table 5a		Nonflare			Flare		Association: Presence vs. Absence ¹		Association: Relative Abundance ²	
	Taxa	Visit	N Present/Total	Mean Relative Abundance	N Present/Total	Mean Relative Abundance	Odds Ratio (95% CI)	p		p
Flare Status	Candida	1	5/123 (4.1%)	0.0407	3/68 (4.4%)	0.0441	0.96 (0.24,3.81)	0.9487		0.9122
	Candida	13	7/135 (5.2%)	0.0519	2/28 (7.1%)	0.0714	-	-		0.6848
	Candida	25	4/118 (3.4%)	0.0339	2/32 (6.2%)	0.0625	-	-		0.4696
	Malassezia	1	1/123 (0.8%)	0.0081	0/68 (0%)	0	3.21 (0.4,25.56)	0.2707		0.4638
	Malassezia	13	1/135 (0.7%)	0.0074	0/28 (0%)	0	-	-		0.6605
	Malassezia	25	2/118 (1.7%)	0.0169	3/32 (9.4%)	0.0823	-	-		0.0342
	Saccharomyces	1	0/123 (0%)	0	0/68 (0%)	0	1.51 (0.15,14.87)	0.7247		-
	Saccharomyces	13	2/135 (1.5%)	0.0148	1/28 (3.6%)	0.0357	-	-		0.4611
	Saccharomyces	25	1/118 (0.8%)	0.0085	0/32 (0%)	0	-	-		0.6139
Table 5b		Low Pain Tertile			High Pain Tertile		Association: Presence vs. Absence ¹		Association: Relative Abundance ²	
	Taxa	Visit	N Present/Total	Mean Relative Abundance	N Present/Total	Mean Relative Abundance	Odds Ratio (95% CI)	p		p
Pain Tertile	Candida	1	0/63 (0%)	0	4/67 (6%)	0.0597	3.91 (0.43,35.83)	0.2271		0.0506
	Candida	13	3/52 (5.8%)	0.0577	5/55 (9.1%)	0.0909	-	-		0.5202
	Candida	25	1/46 (2.2%)	0.0217	4/47 (8.5%)	0.0851	-	-		0.1811
	Malassezia	1	1/63 (1.6%)	0.0159	0/67 (0%)	0	1.76 (0.09,35.16)	0.7102		0.3097
	Malassezia	13	0/52 (0%)	0	1/55 (1.8%)	0.0182	-	-		0.3403
	Malassezia	25	1/46 (2.2%)	0.0217	3/47 (6.4%)	0.056	-	-		0.3306
	Saccharomyces	1	0/63 (0%)	0	0/67 (0%)	0	0.95 (0.13,6.92)	0.9617		-
	Saccharomyces	13	1/52 (1.9%)	0.0192	2/55 (3.6%)	0.0364	-	-		0.6009
	Saccharomyces	25	1/46 (2.2%)	0.0217	0/47 (0%)	0	-	-		0.3225
Table 5c		Low Urinary Tertile			High Urinary Tertile		Association: Presence vs. Absence ¹		Association: Relative Abundance ²	
	Taxa	Visit	N Present/Total	Mean Relative Abundance	N Present/Total	Mean Relative Abundance	Odds Ratio (95% CI)	p		p
Urinary Tertile	Candida	1	3/59 (5.1%)	0.0508	3/64 (4.7%)	0.0469	6.63 (0.75,58.48)	0.0884		0.9243
	Candida	13	0/52 (0%)	0	7/56 (12.5%)	0.125	-	-		0.0089 ³
	Candida	25	0/48 (0%)	0	3/49 (6.1%)	0.0612	-	-		0.0854
	Malassezia	1	0/59 (0%)	0	0/64 (0%)	0	-	-		-

Table 5a		Nonflare				Flare				Association: Presence vs. Absence ¹		Association: Relative Abundance ²	
Taxa	Visit	N	Present/Total	Mean Relative Abundance	N	Present/Total	Mean Relative Abundance	Odds Ratio (95% CI)	p				
Malassezia	13	0/52	(0%)	0	1/56	(1.8%)	0.0179	-	-		0.3446		
Malassezia	25	0/48	(0%)	0	4/49	(8.2%)	0.0742	-	-		0.0455		

¹ Determined by logistic mixed model with a random intercept for participant

² Determined by Wilcoxon Rank-Sum test separately at each time

³ Significant at the 0.01 level following adjustment for n=5 tests controlling the False Discovery Rate