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Associations between the vaginal microbiome and *Candida* colonization in women of reproductive age

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Abstract

Background: The composition of bacteria within the vaginal microbiome has garnered a lot of recent attention and has been associated with reproductive health and disease. Despite the common occurrence of yeast (primarily *Candida*) within the vaginal microbiome, there is still an incomplete picture of relationships between yeast and bacteria (especially lactobacilli), as well as how such

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Condensation: *Lactobacillus iners*-dominant vaginal microbiomes are more likely to harbor vaginal *Candida* than *Lactobacillus crispatus*-dominant vaginal microbiomes.

associations are governed. Such relationships could be important to a more holistic understanding of the vaginal microbiome and its connection to reproductive health.

Objective: To perform molecular characterization of clinical specimens to define associations between vaginal bacteria (especially *Lactobacillus* species) and *Candida* colonization. *In vitro* studies were conducted to test the two most common dominant *Lactobacillus* species (*Lactobacillus crispatus* and *Lactobacillus iners*) in their ability to inhibit *Candida* growth and to examine the basis for such inhibition.

Study Design: A nested cross-sectional study of reproductive age women from the Contraceptive CHOICE Project was conducted. Vaginal swabs from 299 women were selected to balance race and BV status, resulting in similar representation of black and white women in each of the three Nugent score categories [normal (0–3), intermediate (4–6), and bacterial vaginosis (7–10)]. Sequencing of the 16S ribosomal gene (V4 region) was used to determine the dominant *Lactobacillus* species present (primarily *L. iners* and *L. crispatus*), defined as >50% of the community. Subjects without dominance by a single *Lactobacillus* species were classified as Diverse. A *Candida*-specific qPCR targeting the internally transcribed spacer 1 (ITS1) was validated using vaginal samples collected from a second cohort of women and used to assess *Candida* colonization. 255 nonpregnant women with sufficient bacterial biomass for analysis were included in the final analysis. Generalized linear models were employed to evaluate associations between *Lactobacillus* dominance, sociodemographic and risk characteristics and vaginal *Candida* colonization. In separate *in vitro* studies, the potential of cell-free supernatants from *L. crispatus* and *L. iners* cultures to inhibit *Candida* growth was evaluated.

Results: Forty-two women (16%) were vaginally colonized with *Candida*. Microbiomes characterized as Diverse (38%), *L. iners*-dominant (39%), and *L. crispatus*-dominant (20%) were the most common. The microbiome, race and *Candida* colonization co-varied with a higher prevalence of *Candida* among black women and *L. iners*-dominant communities compared to white women and *L. crispatus*-dominant communities. *L. iners*-dominant communities were more likely to harbor *Candida* than *L. crispatus*-dominant communities (OR = 2.85, 95% CI: 1.03 to 7.21; Fisher's Exact, p = 0.048). *In vitro, L. crispatus* produced greater concentrations of lactic acid and exhibited significantly more pH-dependent growth inhibition of *C. albicans*, suggesting a potential mechanism for the clinical observations.

Conclusion: In nonpregnant women, *L. iners*-dominant communities were significantly more likely to harbor *Candida* than *L. crispatus*-dominant communities, suggesting that *Lactobacillus* species have different relationships with *Candida*. *In vitro* experiments indicate that *L. crispatus* may impede *Candida* colonization more effectively than *L. iners* through a greater production of lactic acid.

Keywords

vaginal microbiome; *Candida*; *Lactobacillus*; race; pH

Introduction

The human vagina is a dynamic ecosystem that hosts microbes from diverse taxa. Profiling 16S ribosomal gene diversity has expanded our understanding of the vaginal microbiome,

allowing exploration of links between bacterial composition and reproductive outcomes. Vaginal microbial communities can be clustered into five common community types.[1] Four of these are dominated by a single *Lactobacillus* species: *L. crispatus*, *L. gasseri*, *L. iners*, or *L. jensenii*. The final community type (often described as "Diverse") has few lactobacilli and exhibits greater representation of anaerobic bacteria such as *Gardnerella vaginalis, Atopobium vaginae* and *Prevotella spp*.[1] The prevalence of these community types varies with race and ethnicity; black and Hispanic women more frequently host *L. iners*-dominant and Diverse communities than white women, who more frequently host *L. crispatus*-dominant communities.[1,2] Diverse communities often harbor bacterial taxa that are abundant during bacterial vaginosis (BV), a condition diagnosed by clinical (Amsel) criteria or by Nugent scoring,[3] a 0–10 scale generated by scoring bacterial morphotypes in Gram-stained vaginal smears (0–3, normal; 4–6, intermediate; 7–10, BV). BV is associated with increased risks of sexually transmitted infections and adverse reproductive outcomes.[4]

Candida (most commonly *C. albicans*) is a common member of the vaginal microbiome (found in ~30% of women[5]). The prevalence of non-*albicans* species among women with vaginal *Candida* varies, ranging from ~10–30%.[5–9] Vaginal *Candida* colonization may lead to vulvovaginal candidiasis (VVC), characterized by an aggressive host response to *Candida* overgrowth.[10] However, *Candida* colonization is frequently asymptomatic and not all women colonized with *Candida* go on to experience VVC.[5] Vaginal *Candida* colonization has also been linked to other adverse reproductive outcomes.[8,11–16]

Several prior studies have examined relationships between vaginal bacteria and *Candida*. A few of these studies implicate an abundance of lactobacilli with a greater likelihood of harboring *Candida*.[5,6,17] Other studies suggest there may be co-occurrence of *Candida* with some BV-associated bacteria,[18–21] and specifically that *Candida* may be correlated with the simultaneous presence of both lactobacilli and BV-associated bacteria.[19–21] An important limitation is that prior studies, whether using molecular or culture-based techniques, have not distinguished between lactobacilli at the species level. This is a significant limitation, which if resolved, may shed light on why some women are so prone to *Candida* colonization and candidiasis.

Taken together with the prior studies above, several considerations led us to hypothesize that *L. iners* in particular may support the co-occurrence of *Candida, especially compared to L. crispatus*. *L. iners* is unique among the lactobacilli in being prevalent within less stable Nugent intermediate and BV communities[1,22,23] and in producing a cytolytic toxin.[24,25] Furthermore, *L. iners* dominance has been associated with other negative health outcomes such as increased risks of *Chlamydia trachomatis* infection,[26] incident BV,[27] defects in vaginal mucus that compromise antiviral barrier function,[28] and cytokine signatures linked with HIV risk.[29] We performed two types of studies to test our hypothesis that *L. iners* may preferentially support *Candida* colonization 1) a molecular evaluation of clinical specimens, and 2) *in vitro* growth inhibition studies.

Methods

Study design:

This nested cross-sectional study uses samples and questionnaire data collected by the Contraceptive CHOICE Project (CHOICE)[30] according to Washington University IRB-approved protocol 201108155. In total, 9256 women from the St. Louis-area gave informed consent from August 2007 through September 2011. For this nested study, 299 women enrolled from 08/2008–06/2009 were selected based on power calculations made from preliminary data. Women enrolled in the CHOICE study were between the ages of 14 and 45, reported sexual activity in the past six months or anticipated sexual activity with a male partner and were seeking contraception. Women with a history of tubal ligation or hysterectomy were excluded. All women underwent a pregnancy test. Vaginal swab specimens were self-collected in the vast majority of cases, then stored at −80℃ until analysis. Of the swabs used in the final analysis, one was collected by a clinician and the collection method was missing for five samples.

Women who completed a baseline survey (including Sociodemographic data) and had a vaginal swab available were eligible for inclusion. Samples from all participants underwent Nugent scoring to determine BV status.[3,31,32] Unfortunately, vaginal pH and data regarding menstrual cycle and recent sexual activity was only available for a subset of women and were inadequate for analysis. Overall, the distribution of self-reported race/ethnicity of women in the CHOICE study were representative of the St. Louis region; few women reported a race other than “black or African-American” (hereafter referred to as “black”) or “white.” Due to small numbers of other groups, only women who reported “black” or “white” race were eligible for inclusion in this sub-study.

Composition of the vaginal microbiota has been previously associated with race.[1] To test whether *Candida* was associated with vaginal niches occupied by particular bacterial communities, we sought a strategy to avoid inadequate representation of less common community types in the different demographic groups so that we would be powered to ask whether *Candida* is associated with particular microbial patterns. We used frequency matching to similarly represent black and white women in each of the three Nugent categories. We used a normal:intermediate:BV ratio of 2:2:1 to ensure that we had samples represented across the Nugent spectrum, while balancing the practical reality that relatively few BV specimens were available from white women. Of the 299 subjects selected, 35 were pregnant at the time of swab collection and excluded from final analysis. Additionally, 9 specimens were excluded due to low bacterial biomass. See Supplemental Methods.

Microbiome analysis and *Candida* colonization status:

DNA was extracted from eluted vaginal swabs and 16S ribosomal profiling of the V4 hypervariable region was performed as described in the Supplemental Methods. The microbiome was classified based on the dominant *Lactobacillus* species present, defined as 50% relative abundance or greater and referred to as, “*L. crispatus*-, *L. iners*-, *L. gasseri*-, or *L. jensenii*-dominant” microbiomes. Communities without a single *Lactobacillus* species reaching 50% were referred to as Diverse communities. A pan-*Candida* qRT-PCR[33] that

amplifies the internally transcribed spacer 1 (ITS1) was used to determine *Candida* colonization status using isolated DNA as template. Prior to analysis we validated this assay among vaginal specimens collected from a second cohort of women enrolled at a different site. See Supplemental Methods for details.

Candida growth inhibition:

Candida strains were grown in yeast extract-peptone-dextrose (YPD) media. *C. albicans* strain SC5314 was obtained from the American Type Culture Collection. Vaginal strains of *Candida* (*C. albicans*: BAT8133, BAT8135, BAT8143, BAT8152, BAT8154, BAT3353A; *C. glabrata*: BAT8139, BAT3353B) were isolated from women as described in the Supplemental Methods. *L. crispatus* (MV-1A-US, JV-V01, MV-3A-US, 125–2-CHN) and *L. iners* (UP II 143-D, Lactin V09V1-C, LEAF 2032-Ad, LEAF 3008-A) strains were obtained from BEI resources and cultured in De Man, Rogosa and Sharpe (MRS) media for 48 hours to make cell free supernatants (CFS). All *Candida* growth inhibition experiments were conducted in 96-well plates. Each well contained a 1:1 ratio of CFS and YPD inoculated with ~10^6 *C. albicans* colony-forming units (CFU)/mL. YPD was buffered with 300 mM sodium bicarbonate and 300 mM HEPES sodium salt for neutralization assays. For lactic acid growth inhibition assays, fresh MRS was supplemented with racemic lactic acid. A micro pH electrode was used to measure pH of each mixture and lactate was measured with a colorimetric assay. Protonated lactic acid concentrations were calculated using lactate molarity and pH using the Henderson-Hasselbalch equation (pKa = 3.9). See Supplemental Methods for more details about *Candida* growth inhibition experiments.

Statistical analysis:

Statistical analyses and data representation were completed in R (v3.5.1) and Prism (v7). Fisher's Exact Tests (Fisher) were used to assess for associations between cohort characteristics and race, with odds ratios (OR) determined by a conditional maximum likelihood estimate. Unless otherwise noted, we used an extension of the generalized linear model (GLM) method that included race as a potentially confounding covariate to test for associations between cohort characteristics and *Candida* colonization status, using the exponent of the coefficient from the logistic regression to calculate ORs. Note that because *Candida* colonization incidence is >10% the odds ratios may not be an accurate approximation of the relative risk; see[34] for conversion between the two.

We used type-II analysis of variance (ANOVA-II) with Wald test and Tukey's Honestly Significant Different Test (Tukey) to evaluate significance in these models. In instances where multiple statistical tests were performed, we relied on GLM accounting for race. Mann-Whitney tests were used to test for associations with *Candida* abundance and effect size (r) was calculated from the Z value. Statistical tests for *in vitro* experiments included one-way ANOVA with Tukey's correction for multiple comparisons and Mann-Whitney tests as appropriate. Regardless of the statistical method used, P-values < 0.05 were considered significant.

Results

Description of the clinical cohort:

Two-hundred fifty-five non-pregnant women of reproductive age were included in our analysis. In this cohort, 53% of women identified as “white” and 47% identified as “black”. Forty-four (17%) women had BV, while 109 (43%) and 102 (40%) had intermediate and normal vaginal flora respectively. About half of the women (54%) reported using public assistance or having trouble meeting daily needs and were classified as having low socioeconomic status. Body mass index (BMI) was calculated and categorized using standard methods and definitions. Most women (64.3%) reported at least one prior pregnancy. Seventy-two women (28.2%) reported vaginal douching in the last 180 days. Race was found to be associated with socioeconomic status ($p < 0.0001$), BMI ($p = 0.003$), gravidity ($p < 0.0001$) and vaginal douching ($p < 0.0001$). A summary of demographic data and cohort characteristics by race is presented in Supplemental Table 1.

Forty-two (16%) women were vaginally colonized with *Candida*. Of these, most (90%) were colonized by *C. albicans*. *C. glabrata* was less common (~10%). Sequencing of the vaginal microbiome revealed that fifty-two women (20%) had *L. crispatus*-dominant microbiomes, 99 (39%) had *L. iners*-dominant microbiomes and 98 (38%) had microbiomes that were not dominated by a single *Lactobacillus species* (Diverse). We were not powered to test associations between *Candida* and microbiomes dominated by *Lactobacillus jensenii* or *gasseri since* few women (n=6) exhibited these microbiomes. Black women were more likely than white women to have *L. iners*-dominant communities (46.7% vs 31.9% Fisher’s Exact; OR = 1.87, 95% CI: 1.10 to 3.14, $p = 0.020$) and less likely to have *L. crispatus*-dominant communities (11.9% vs. 22.1% Fisher’s Exact; OR = 0.380, 95% CI: 0.185 to 0.747, $p = 0.003$).

Associations between *Candida* and cohort characteristics:

Forty-two (16%) women were vaginally colonized with *Candida*. Of these, most (90%) were colonized by *C. albicans*. *C. glabrata* colonization was less common (~10%). Table 1 contains a summary of *Candida* status by sociodemographic and other cohort characteristics. Only race was significantly correlated with vaginal *Candida*; black women were more likely to be colonized compared to white women (OR =2.05, 95% CI: 1.03 to 4.25, Fisher’s Exact, $p = 0.042$). Based on these findings, race was considered to be a potential confounder and incorporated into subsequent analyses using generalized linear models (GLM) to evaluate factors associated with *Candida* colonization.

Associations between *Candida* and cohort characteristics

Candida colonization rates did not differ based on Nugent-defined BV status (GLM; ANOVA-II, $p = 0.897$). We did not find any association between a woman’s socioeconomic status and vaginal *Candida* colonization. *Candida* colonization did not differ significantly among underweight (20% *Candida*), normal weight (18%) and overweight (23%) women. However, obese women were less likely to be colonized compared to non-obese women (GLM; OR = 0.322, 95% CI: 0.123 to 0.744; Tukey’s HSD, $p = 0.013$, see Supplement for

comment). Women reporting current use of hormonal contraceptives containing estrogen and progestin were *Candida*-colonized at higher rates than women reporting non-hormonal methods, although this did not reach statistical significance (GLM; OR = 1.77, 95% CI: 0.858 to 3.58; Tukey's HSD, p = 0.237, see Supplement for details). Women who reported vaginal douching in the last 180 days were less likely to be *Candida* positive compared to women who reported no vaginal douching (GLM; OR = 0.364, 95% CI: 0.143 to 0.838; Tukey's HSD, p = 0.047).

Relationships between *Candida* colonization and the vaginal microbiome:

Next, we investigated relationships between *Candida* colonization and dominant members of the vaginal microbiome based on 16S ribosomal gene profiling. *Candida* prevalence did not differ between *Lactobacillus* dominated (50% or greater *Lactobacillus*) and non-*Lactobacillus* dominated microbiomes (GLM; ANOVA-II, p = 0.327). Although the absolute abundance of *Candida* as measured by qPCR did not differ within *L. iners*-dominant communities compared to other community types (Mann-Whitney, r = 0.046, p = 0.617), *L. iners*-dominant communities were more likely to harbor *Candida* than non-*L. iners*-dominant communities (GLM; OR = 2.00, 95% CI: 1.02 to 3.98; Tukey's HSD, p = 0.045; see supplemental Table 2). Further analysis specifically showed that *L. iners*-dominant communities were more likely to be colonized than *L. crispatus*-dominant communities (OR = 2.85, 95% CI: 1.03 to 7.21; Fisher's Exact, p = 0.048). Among *Candida* positive women, higher levels of *Candida* (by qRT-PCR) were observed among black women compared to white women, although not statistically significant (Mann-Whitney test, r = 0.173, p = 0.131).

In vitro studies: inhibition of *Candida* growth by lactobacilli:

Both *L. crispatus* and lactic acid have been shown to thwart the growth of *C. albicans*.[35–37] Next, we compared the inhibitory potential of *L. crispatus* and *L. iners* on *Candida* growth *in vitro*. *C. albicans* was cultured together with cell free supernatants (CFS) from *L. crispatus* and *L. iners* (8 strains total), followed by *Candida* CFU enumeration. Compared to *L. iners* CFS, *L. crispatus* CFS resulted in lower pH (pH = 4.0 vs. pH = 4.6, p < 0.0001) and correspondingly higher levels of protonated lactic acid in CFS-YPD (55 mM vs. 11 mM, p < 0.0001) (Figure 2). Buffering CFS-YPD to a neutral pH reduced levels of protonated lactic acid to below appreciable levels, ablated *Candida* growth inhibition, and eliminated the difference in *C. albicans* growth observed between *L. crispatus* and *L. iners* (Figure 2). Further, lactic acid was sufficient to inhibit *Candida* growth. In particular, significantly more growth inhibition was observed at 49 mM protonated lactic acid compared to 11 mM, levels comparable to the *L. crispatus* and *L. iners* CFS-YPD respectively. Similar findings were seen using *vaginal* isolates of *C. albicans*. In contrast, *C. glabrata* exhibited only modest growth inhibition (Figure 2). Together, these data suggest that lactic acid is both necessary and sufficient for growth inhibition of *C. albicans in vitro*.

Comment

Principal Findings:

We demonstrate that *Candida* colonization is associated with characteristics of the vaginal microbiome (dominance of *L. iners* compared to *L. crispatus*). Results in clinical specimens are consistent with *in vitro* data, which show that *L. crispatus* produces a pH-dependent factor that inhibits *C. albicans* growth more effectively compared to secreted factors of *L. iners* grown under the same conditions.

Results:

As a relatively common vaginal microbial community member, *Candida* may influence reproductive health. Previous studies suggested vaginal *Lactobacillus* colonization as a risk factor for *Candida* colonization or VVC,[5,6,17] but seem inconsistent with other reports of *Candida*-bacteria associations.[18–21] Here we provide more taxonomic resolution, showing that that not all *Lactobacillus*-dominant communities are equally associated with *Candida* colonization.

Clinical Implications:

Clinicians often group all lactobacilli together. This study adds to the growing body of evidence suggesting that *L. iners*-dominant communities are more permissive to vaginal colonization with potential pathogens, including *Candida*.

Research Implications:

Of interest, black race was associated with obesity and vaginal douching as in prior studies. But surprisingly, the correlation between *Candida* and black race cannot be accounted for by obesity or douching because obese women and those who douche were actually ***less*** likely to be colonized with *Candida* (OR = 0.322 and 0.364 respectively). The literature contains inconsistent reports regarding the role of *Lactobacillus* colonization as a risk factor for *Candida* colonization or VVC.[5,6,17,18–21] We show that that not all *Lactobacillus*-dominant communities are equally associated with *Candida*. *In vitro* data provide one possible explanation, showing that *L. iners* strains do not produce the same magnitude of lactic acid compared to *L. crispatus* strains. An alternative, albeit not mutually exclusive explanation, is that vaginal *Candida* colonization may shift the microbiome to favor *L. iners*.

Interestingly, we observed similar rates of *Candida* colonization in *L. crispatus*-dominant and Diverse communities. With fewer lactic acid producing bacteria present, the vaginal pH of women with Diverse microbiome is less acidic.[1] These findings indicate that Diverse communities resist *Candida* by lactic acid-independent mechanisms.

Additional studies are needed to evaluate potential mechanisms governing these relationships and apply these findings in clinical settings.

Strengths and Limitations:

Key strengths of our study design were the validation of a *Candida*-specific qPCR assay[33] for laboratory testing for *Candida* colonization, offering flexibility in settings where archived

frozen vaginal swabs are more practical. We acknowledge that the specimens selected for this study are not a naturalistic representation of vaginal microbiomes. Rather, the frequency matching of black and white women across the Nugent spectrum is a strength that enabled power to test associations between yeast and bacteria in different racial groups. Limitations include: 1) the sample size and number of *Candida*-positive women were relatively small, limiting power to model multiple potential confounders, 2) this cohort may not be representative of the U.S. population, 3) clinical data were not available to examine the relationship between *Candida* colonization and VVC, and 4) our *in vitro* findings may not be representative of *in vivo* relationships.

Conclusion:

These data suggest that *L. iners*-dominant vaginal communities may support the co-occurrence of *Candida*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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AJOG at a Glance:

A. The purpose of the study was to characterize the relationship between the composition of the vaginal microbiome and *Candida* colonization among non-pregnant women.

B. Women with *Lactobacillus iners*-dominant microbiomes were more likely to harbor *Candida* than women with *Lactobacillus crispatus*-dominant microbiomes. *In vitro* data suggests higher production of lactic acid by *Lactobacillus crispatus* compared to *Lactobacillus iners* may contribute to differential anti-*Candida* activity. Neutralization of pH eliminated the anti-*Candida* activity secreted by lactobacilli.

C. Consideration of *Candida* as part of the vaginal microbiome may have utility for understanding different relationships between vaginal microbiome and adverse outcomes.

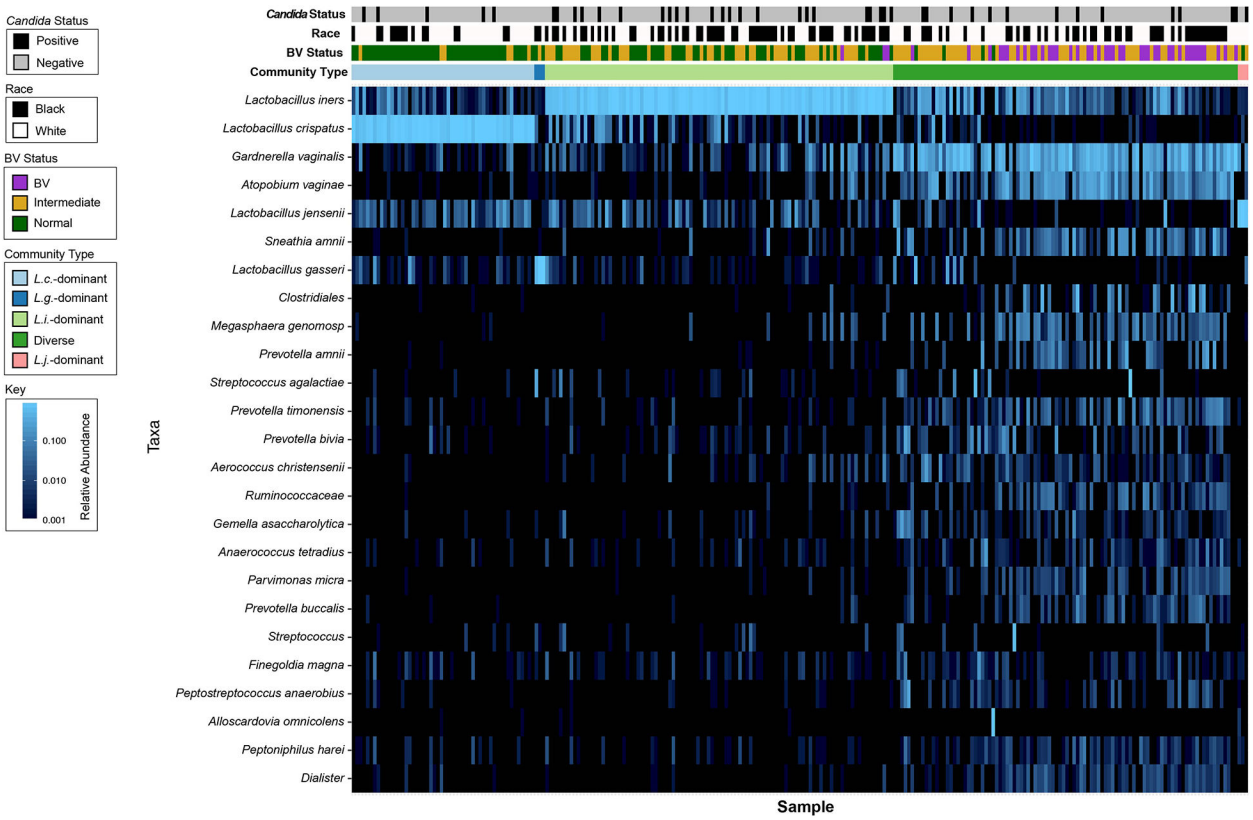

Figure 1: Heatmap of all samples in the cohort clustered by community type.

Heat map of samples clustered by community type showing the top 25 taxa observed across the cohort. The bars above the heatmap indicate community type, BV status by Nugent score, race and *Candida* status. In the heat map, *light blue* indicates the highest abundance, *darker blues* indicate lower abundance and *black* indicates very low abundance or not present. Black race ($p = 0.037$) and *L. iners*-dominant communities ($p = 0.045$) were associated with *Candida* colonization.

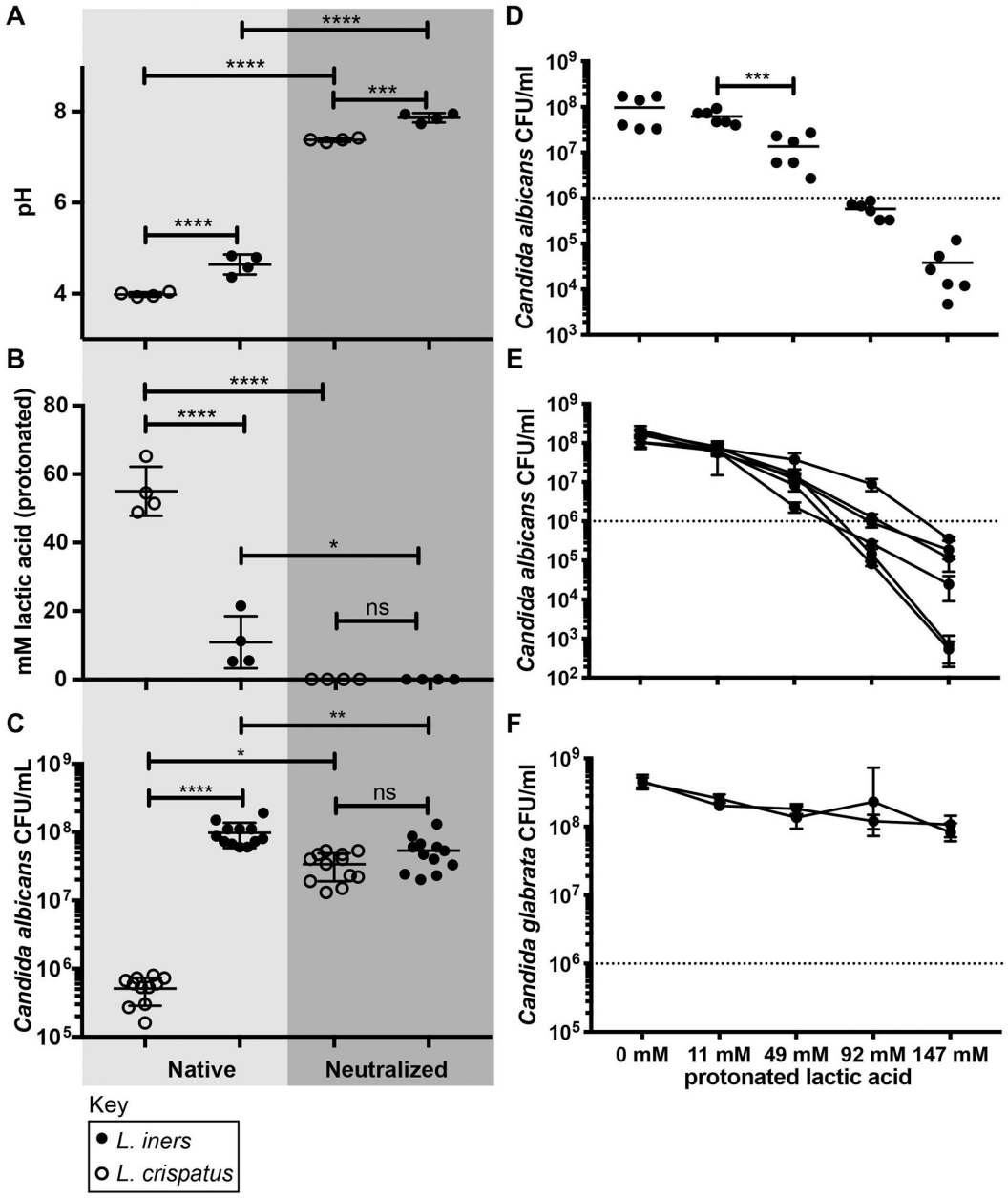

Figure 2: *In vitro* inhibition of *Candida* by *Lactobacillus* CFS and lactic acid.

A-B, Characterization of *Candida* growth medium supplemented with *Lactobacillus* CFS (YPD-CFS) in native and buffered states from four *L. crispatus* and four *L. iners* strains, prior to *Candida* inoculation. **A**, pH of YPD-CFS; **B**, Concentration of protonated lactic acid in YPD-CFS; **C**, Growth inhibition of *Candida* laboratory strain SC5314, showing three technical replicates for each *Lactobacillus* YPD-CFS. Analysis by one-way ANOVA with Tukey's correction for multiple comparisons. **D-F**, Characterization of the inhibitory effect of lactic acid supplemented medium on *Candida* growth. Three technical replicates from two biological experiments are shown. **D**, Growth inhibition of SC5314 by lactic acid showing Mann-Whitney test comparison of 11 mM to 49 mM protonated lactic acid; **E**, Lactic acid growth inhibition of 6 vaginal *C. albicans* isolates; **F**, Lactic acid growth inhibition of 2

vaginal *C. glabrata* isolates. Data points in panel D reflect 6 replicates from two experiments for each condition. Error bars in E-F show the standard deviation from the mean of three replicates for each isolate. Approximate starting inoculum for growth assays is indicated by a dashed line. Statistical significance: ns (not significant), *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.0001$.

Table 1:

Characteristics of subjects with vaginal *Candida* compared with those without vaginal *Candida*.

Characteristics	Total Cohort	*Candida* Positive	*Candida* Negative	P-value
Total Number of Subjects	255	42 (16.5)	213 (83.5)	
Age				0.811
< 20	28 (11.0)	6 (14.3)	22 (10.3)	
20 to 29	178 (69.8)	29 (69.0)	149 (70.0)	
30 to 39	44 (17.3)	7 (16.7)	37 (17.4)	
40 +	5 (2.0)	0 (0.0)	5 (2.3)	
Race				0.042
Black	120 (47.1)	26 (61.9)	94 (44.1)	
White	135 (52.9)	16 (38.1)	119 (55.9)	
Nugent-defined Vaginal Flora				0.833
Normal	102 (40.0)	15 (35.7)	87 (40.8)	
Intermediate	109 (42.7)	19 (45.2)	90 (42.3)	
BV	44 (17.3)	8 (19.0)	36 (16.9)	
Socioeconomic Status (SES)				1
Low SES	138 (54.1)	23 (54.8)	115 (54.0)	
Not Low SES	117 (45.9)	19 (45.2)	98 (46.0)	
Body Mass Index (kg/m2)				0.127
Underweight (< 18.5)	15 (5.9)	3 (7.1)	12 (5.6)	
Normal Weight (18.5 – 24.9)	103 (40.4)	19 (45.2)	84 (39.4)	
Overweight (25 – 30)	48 (18.8)	11 (26.2)	37 (17.4)	
Obese (> 30)	78 (30.6)	7 (16.7)	71 (33.3)	
Not Documented	11 (4.3)	2 (4.8)	9 (4.2)	
Current Birth Control Method				0.320
Estrogen + Progestin[a]	72 (28.2)	16 (38.1)	56 (26.3)	
Progestin[b]	12 (4.7)	1(2.4)	11 (5.2)	
Non-Hormonal[c]	171 (67.1)	25 (59.5)	146 (68.5)	
Vaginal Douching in Last 180 Days				0.323
Yes	72 (28.2)	8 (19.0)	64 (30.0)	
No	182 (71.4)	34 (81.0)	148 (69.5)	
Don’t Know	1 (0.4)	0 (0.0)	1 (0.5)	
Gravidity				0.160
None	91 (35.7)	15 (35.7)	76 (35.7)	
1	58 (22.7)	6 (14.3)	52 (24.4)	
2	47 (18.4)	6 (14.3)	41 (19.2)	
3+	59 (23.1)	15 (35.7)	44 (20.7)	
Community Type				0.113

Characteristics	Total Cohort	*Candida* Positive	*Candida* Negative	P-value
L. crispatus-dominant	52 (20.4)	5 (11.9)	47 (22.1)	
L. iners-dominant	99 (38.8)	23 (54.8)	76 (35.7)	
L. jensenii-dominant	3 (1.2)	1 (2.4)	2 (0.9)	
L. gasseri-dominant	3 (1.2)	0 (0.0)	3 (1.4)	
Diverse	98 (38.4)	13 (31.0)	85 (39.9)	

Values are n (%). Fisher's Exact Tests were used to determine p-values for each set of variables without adjusting for race. Note that p-values given in the text use GLM (accounting for race as a potential confounder).

[a]Women who reported the oral contraceptive pill or the birth control ring;

[b]Women who reported the levonorgestrel-containing intrauterine device or depot medroxyprogesterone acetate;

[c]Women who reported condoms, rhythm/natural family planning, abstinence, withdrawal or nothing.