Association between vaginal bacterial microbiota and the presence and clinical presentation of vulvovaginal candidiasis

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Abstract

Association between vaginal bacterial microbiota and the presence and clinical presentation of vulvovaginal candidiasis

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Introduction: Vulvovaginal candidiasis (VVC) is among the most common reasons for women to seek medical care. Annual costs related to VVC in the United States are estimated at over $1 billion. Vaginal yeast exists in an environment with a diverse and changing vaginal bacterial microbiota. Little is known about how the vaginal bacterial community influences the presence, phenotypic expression and symptoms associated with vaginal yeast.

Methods: In a secondary analysis of data from the Preventing Vaginal Infections trial (ClinicalTrials.gov number, NCT01230814), this study explored the associations between the quantities of ten vaginal bacteria (using categories of undetectable and three evenly distributed tertiles above the threshold of detection with PCR) and the presence of yeast on vaginal wet mount, culture, or both. In women with yeast on saline wet mount, the association between bacterial concentrations and the presence of pseudohyphae was examined. Finally, the association between detection of pseudohyphae on wet mount and symptomatic VVC was characterized.

Results: Among 221 women in this analysis, bacterial concentrations of *Leptotrichia/Sneathia* (1st tertile adjusted odds ratio (aOR) 0.47, 95% confidence interval [CI] 0.20-1.09, 2nd tertile aOR 0.27, 95% CI 0.11-0.64, 3rd tertile aOR 0.23, 95% CI 0.09-0.56, p<0.001)*, Atopobium vaginae* (1st tertile aOR 0.55, 95% CI 0.23-1.30, 2nd tertile

0.55, 95% CI 0.24-1.24, 3rd tertile aOR 0.26, 95% CI 0.11-0.66, p=0.006), *Megasphaera* (1st tertile aOR 0.31, 95% CI 0.12-0.84, 2nd tertile aOR 0.44, 95% CI 0.18-1.13, 3rd tertile 0.28, 95% CI 0.10-0.81, p=0.005), *Mageeibacillus indolicus* (1st tertile aOR 0.58, 95% CI 0.21-1.62, 2nd tertile aOR 0.45, 95% CI 0.14-1.46, 3rd tertile aOR 0.29, 95% CI 0.08-1.07, p=0.02), and BVAB2 (1st tertile adjusted odds ratio (aOR) 0.49, 95% CI 0.20-1.23, 2nd tertile aOR 0.43, 95% CI 0.17-1.06, 3rd tertile aOR 0.44, 95% CI 0.17-1.16, p=0.03) were strongly associated with the presence of vaginal yeast. In the subset of women with yeast on saline wet mount, detection of *L. jensenii* was associated with the presence of pseudohyphae (aOR 4.51, 95% 0.96-21.28, p=0.06). Finally, the detection of pseudohyphae on vaginal wet mount was strongly associated with symptomatic VVC (OR 4.90, 95% CI 0.99-24.21, p=0.05). This association became stronger in an analysis restricted to germ tube positive (presumptive *Candida albicans)* cases ((OR 8.25, 95% CI 1.15-59.00, p=0.04).

Discussion: Vaginal bacteria may play an important role in mediating the presence, phenotypic expression, and clinical outcome of vaginal yeast. Understanding the complex relationship between vaginal bacteria and yeast could inform future studies of novel treatment and prevention interventions for VVC based on therapies directed at the vaginal bacterial microbiota.

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I would like to thank, in particular, my family for supporting me on my research endeavors.

Introduction

Approximately 75% of women will have vulvovaginal candidiasis (VVC) at least once in their lifetime [1]. Of these, approximately half will suffer at least one recurrence and 5-8% will have recurrent vulvovaginal candidiasis, defined as four or more episodes per year [1]. In addition to bothersome symptoms and recurrences, vulvovaginal candidiasis has been associated with increased risk of acquiring HIV infection [2]. *Candida albicans* and *Candida glabrata* are the primary species implicated in vulvovaginal candidiasis [1]. Symptomatic VVC may be related to the presence of pseudohyphae in *C. albicans*, but primary data demonstrating this association are scarce [3, 4]. *Candida glabrata* can form biofilms that may contribute to recurrent vulvovaginal candidiasis, but does not have pseudohyphae like most other *Candida* species [5, 6].

The role of vaginal *Lactobacillus* species as mediators of yeast colonization, phenotypic expression, and pathogenesis of VVC is not well understood [7-10]. Several *in vitro* observations suggest that *Lactobacilli* can suppress or directly kill *Candida* species. First, *L. rhamnosus* and *L. reuteri* suppress growth of *Candida albicans* [11]. Second, *Lactobacillus* species impair biofilm production in *C. glabrata* [5]. Third, *L. crispatus*, *L. vaginalis*, and *L. gasseri* have demonstrated direct killing or interruption of adherence of *C. albicans* to vaginal epithelium [12]. In contrast to the *in vitro* research described above, studies in women have suggested that yeast colonization may occur more frequently in the setting of a *Lactobacillus*-dominated vaginal microbiota [13, 14]. Additionally, one study using culture for detection of lactobacilli found that *L. crispatus* prevalence was higher in women with VVC compared to women without VVC [15]. In a study of Kenyan women, *Lactobacillus* colonization was associated with a nearly 4-fold higher risk of symptomatic VVC compared to the absence of *Lactobacillus* species by culture [16]. Few studies have investigated the relationships between bacterial vaginosis (BV)-associated bacteria in the vaginal microbiota, the presence of vaginal yeast, and identification of pseudohyphae. This exploratory analysis sought to examine the

relationship between vaginal bacteria, vaginal yeast morphotypes (buds, pseudohyphae or both), and symptomatic VVC [17].

Methods

Study Population and Procedures

Data for this analysis were from the Preventing Vaginal Infections (PVI) trial (ClinicalTrials.gov Number, NCT01230814), a randomized clinical trial evaluating the use of vaginal metronidazole plus miconazole suppositories versus matching placebo to prevent vaginal infections including BV, VVC, and *Trichomonas vaginalis* (TV) [18]. Participants were recruited in four research sites, three in Kenya and one in the United States. Women were eligible for inclusion if they were between 18 and 45 years of age, sexually active, HIV-seronegative, and had a documented vaginal infection (with or without symptoms) with BV, VVC, or TV at their screening visit. All women with TV infection and symptomatic women with BV or VVC at screening were treated with oral or intravaginal metronidazole, intravaginal miconazole, or both, as indicated. Women were enrolled for the study 7-28 days following their screening visit. If a woman was treated for a vaginal infection at her screening visit, a minimum of two weeks were required before enrollment. Women could not enroll if they had a current symptomatic vaginal infection requiring treatment.

At enrollment, participants completed a detailed face-to-face interview with a study nurse to ascertain demographic data, parity, contraceptive methods, sexual history, and vaginal washing practices. Women also underwent a physical examination including vaginal speculum examination with sample collection.

Laboratory Methods

A vaginal saline wet mount of collected secretions was examined at 40x magnification to identify motile TV organisms, clue cells, and fungal elements. One drop of 10% potassium

hydroxide was added to the wet mount slide and re-examined for the presence of budding yeast, hyphae, or both. Prior to conducting laboratory analyses for the PVI trial, all laboratory staff passed the Microbicide Trials Network vaginal wet preparation proficiency test. Culture for vaginal yeast was performed on Sabouraud agar. A germ tube test was performed to identify presumptive *Candida albicans*. Serological testing for HIV infection was performed using either two parallel rapid tests or two parallel laboratory-based enzyme-linked immunoassays (ELISAs).

Vaginal samples for quantitative PCR (qPCR) were stored at -80˚C at the study sites and then batched and transported on dry ice to the Fred Hutchinson Cancer Research Center in Seattle, Washington for analysis. The qPCR methods have previously been described [19]. In brief, PCR probes targeted the 16S rRNA gene for each bacterium. Bacterial RNA was extracted and amplified alongside controls. Each qPCR assay was run with controls that consisted of all reagents required for amplification but without DNA to monitor for bacterial contamination of PCR reagents. Amplification controls also used exogenous DNA from a segment of jellyfish aequorin gene to detect any PCR inhibitors. Bacterium-specific qPCR assays targeted *Lactobacillus crispatus, L. jensenii, L. iners,* bacterial vaginosis-associated bacterium 1 (BVAB1), BVAB2, *Mageeibacillus indolicus* (previously BVAB3), *Atopobium vaginae, Leptotrichia/Sneathia, Megasphaera,* and *Gardnerella vaginalis.* Quantities are expressed as gene copies per swab with the threshold of detection of 95 copies/swab.

Data Analysis

This analysis utilized specimens and data at each participant's enrollment visit. Participant demographics, sexual history, yeast morphotypes and vaginal bacterial microbiota were calculated as proportions of the total for categorical data or medians with an interquartile range for continuous data.

Quantities of the different bacterial taxa were grouped into undetectable (defined as ≤95 copies/swab) and then evenly distributed tertiles for levels above the threshold for quantitation.

Unadjusted odds ratios were calculated using logistic regression, with the quantity of vaginal species as exposure and the presence of yeast by wet mount, culture, or both as outcome. Potential confounding factors for inclusion in adjusted analyses were defined *a priori.* These included age, because both vaginal microbiota and risk of VVC change through a woman's life [20-22], and hormonal contraception, because hormonal contraceptives may influence both the vaginal bacterial microbiota and the presence of yeast [23, 24]. Adjusted odds ratios were estimated with age as a continuous variable and hormonal contraceptive method as a categorical variable of non-hormonal, estrogen-progestogen combinations and progesterone-only contraceptives.

In the subset of women with yeast on saline wet mount, logistic regression was used to calculate odds ratios for the association between the presence of vaginal bacterial taxa as the exposure and the presence of pseudohyphae on the vaginal saline wet mount as the outcome. Again, this analysis was adjusted for age and hormonal contraceptives. A sensitivity analysis was performed that included only women with a positive germ tube test (indicating presumptive *C. albicans*), because the other main species of yeast causing VVC, *C. glabrata,* is germ-tube negative and does not form pseudohyphae [1, 6].

Lastly, in the subset of women with yeast on vaginal saline wet mount, we examined the association between pseudohyphae as exposure and symptomatic VVC as the outcome. Symptomatic VVC was defined as having one or more self-reported symptoms or clinically-identified signs. These could include one or more of self-report of vaginal itching, self-report of vaginal discharge, and clinicians' observation of abnormal vulvovaginal erythema or edema on physical examination [17]. Unadjusted odds ratios were calculated using logistic regression. A sensitivity analysis was repeated after restricting to the subset of samples with germ-tube positive yeast.

Correlations between detection of the vaginal bacterial taxa included in this analysis were examined using Spearman's rank correlation coefficients.

Some women may have been treated with metronidazole, miconazole, or both at the screening visit 2-4 weeks prior to enrollment. Because antimicrobial treatment could have changed the vaginal microbiome and influenced the presence, phenotype, and symptoms associated with yeast, we conducted additional sensitivity analyses. Women who had documented TV infection (regardless of symptoms) or a diagnosis of BV or VVC with symptoms of abnormal vaginal discharge, vaginal itching, or both, which may have led to treatment at the screening visit, were excluded. The association between the vaginal microbiota as exposure and the presence of yeast as the outcome was then re-examined.

This study was approved by the human subjects research committees at Kenyatta National Hospital (Nairobi, Kenya), the University of Washington (Seattle, WA), the Fred Hutchison Cancer Research Center (Seattle, WA), and the University of Alabama at Birmingham (Birmingham, AL). All participants completed written informed consent for participation. In addition, all women included in the present analysis provided written informed consent for storage and future testing of their specimens. These analyses were conducted using Stata software, release 14 [25].

Results

In the PVI trial, 234 women were enrolled. Of these, 221 (94.4%) returned for at least one follow-up visit and consented to storage and future testing of vaginal samples. These women form the population for the present analysis. Baseline characteristics are presented in Table 1. Participants had a median age of 29 years (interquartile range [IQR] 24-34). The majority of women (N=182, 82.4%) used some type of contraceptive, including 22 (10.0%) that contained estrogen combined with a progestogen and 78 (35.3%) that contained only progesterone. The majority of women reported that they had been sexually active in the past week (n=179, 81.0%), and a third reported unprotected vaginal sex in the past week (n=61, 34.1%).

The number and proportion of samples that were PCR positive for vaginal bacteria ranged from 46 (20.8%) for *L. jensenii* to 205 (92.8%) for *G. vaginalis.* Seventy-nine (35.7%) women had yeast. This total included 30 (13.6%) positive by both wet-prep and culture and 49 (22.2%) positive on culture alone. There were no cases where yeast was observed on wet-prep but not detected in culture. Of 30 samples with a positive wet prep, 2 (6.7%) had pseudohyphae only, 19 (63.3%) had budding yeast only, and 9 (30.0%) had both pseudohyphae and budding yeast. Sixty-nine (31.2%) women met criteria for symptomatic vulvovaginal candidiasis based on at least one sign or symptom of vulvar edema, vulvar erythema, vaginal itching or vaginal discharge.

In unadjusted analyses, increasing concentrations of six bacterial taxa, *Leptotrichia/Sneathia, A. vaginae, Megasphaera, M. indolicus, BVAB2*, and *G. vaginalis*, were significantly associated with lower odds of detecting yeast when compared to women in whom the bacteria were not detected (Table 2). These associations remained significant after adjusting for age and use of hormonal contraception with the exception of one, *G. vaginalis* (Table 2 and Figure 1). In the adjusted analyses, *Leptotrichia/Sneathia* (1st tertile adjusted odds ratio (aOR) 0.47, 95% confidence interval [CI] 0.20-1.09, 2nd tertile aOR 0.27, 95% CI 0.11-0.64, 3rd tertile aOR 0.23, 95% CI 0.09-0.56), p<0.001 and *A. vaginae* (1st tertile aOR 0.55, 95% CI 0.23-1.30, 2nd tertile 0.55, 95% CI 0.24-1.24, 3rd tertile aOR 0.26, 95% CI 0.11-0.66, p=0.006) were most strongly associated with the absence of yeast followed by *Megasphaera* (1st tertile aOR 0.31, 95% CI 0.12-0.84, 2nd tertile aOR 0.44, 95% CI 0.18-1.13, 3rd tertile 0.28, 95% CI 0.10-0.81, p=0.005)*, M. indolicus* (1st tertile aOR 0.58, 95% CI 0.21-1.62, 2nd tertile aOR 0.45, 95% CI 0.14-1.46, 3rd tertile aOR 0.29, 95% CI 0.08-1.07, p=0.02) and BVAB2 (1st tertile adjusted odds ratio (aOR) 0.49, 95% CI 0.20-1.23, 2nd tertile aOR 0.43, 95% CI 0.17-1.06, 3rd tertile aOR 0.44, 95% CI 0.17-1.16, p=0.03). *Lactobacillus* species were associated with a higher likelihood of detecting yeast, though none of these findings were statistically significant. Associations between vaginal bacterial concentrations and the presence of yeast were similar in

a sensitivity analysis that excluded 33 women who may have received metronidazole, antifungals, or both, based on the presence of *T. vaginalis* or symptomatic BV or VVC at screening (Table 3).

Among 30 women with yeast on saline wet mount, there was a strong association between the presence of *L. jensenii* and pseudohyphae (OR 3.72, 95% CI 0.99-13.90, p=0.05), which was of borderline statistical significance (Figure 2). This borderline association remained with adjustment for age and hormonal contraceptive use (aOR 4.51, 95% 0.96-21.28, p=0.06), (Figure 2). There were no strong associations between the other bacterial taxa and the presence of pseudohyphae. In the subset of 23 (10.4%) women with germ-tube positive yeast isolates, the odds ratios remained similar, but with wider confidence intervals reflecting the smaller sample size (Table 5).

Of the 30 women with yeast detected on saline wet mount, pseudohyphae were detected in 7 (63.6%) of 11 women with symptomatic VVC compared to 5 (26.3%) of 19 women without symptoms (OR 4.90, 95% CI 0.99-24.21, p=0.05). In the subset of women that had vaginal samples that were germ tube positive, the association between pseudohyphae and symptomatic VVC became even stronger (OR 8.25, 95% CI 1.15-59.00, p=0.04).

Some of the bacterial taxa in this analysis were significantly correlated with one another (Figure 2). In general, *L. crispatus* was negatively correlated with the bacterial taxa that are associated with BV, whereas BV-related species were moderately to highly correlated with one another.

Discussion

These data suggest that vaginal bacterial microbiota influences the presence, phenotypic expression, and clinical outcome of vaginal yeast. This study makes a unique contribution to the literature by demonstrating that several bacterial taxa that are frequently found in the vaginal microbiota of women with BV are associated with the absence of vaginal

yeast. When yeast was identified on vaginal wet mount, the presence of lactobacilli, particularly *L. jensenii*, was associated with the presence of pseudohyphae. There was a strong association between the presence of pseudohyphae and symptomatic VVC, particularly for presumptive *C. albicans.* .

Prior studies have provided conflicting results regarding the associations between vaginal *Lactobacillus* species and yeast. *In-vitro* studies suggest antagonism between yeast and lactobacilli, whereas some i*n-vivo* data suggest a positive association between the presence of lactobacilli and VVC [5, 7-16]. In the present study, *Leptotrichia/Sneathia, A. vaginae, Megasphaera, M. indolicus* and *BVAB2,* bacteria that have typically been associated with BV, were associated with the absence of yeast. This extends our understanding from prior studies that have shown that BV diagnosed by Nugent score is inversely associated with the presence of vaginal yeast [26, 27]. These earlier studies have led to the hypothesis that amines produced by BV-associated bacteria may inhibit yeast. Similarly, differences in vaginal pH between BV and VVC might impact *C. albicans* ability to adhere to the epithelium [1, 27].

Clinical observation and textbook chapters on VVC have suggested that symptomatic VVC is related to the presence of pseudohyphae, a more invasive morphology of *Candida albicans* [3, 4]. However, published primary data on this association are scarce. The results presented here show a nearly five-fold greater odds of having symptomatic VVC if pseudohyphae are present, confirming this association. On the other hand, over one-third of women with symptomatic VVC lacked pseudohyphae, implicating other pathways to the development of symptoms. Extracellular proteases and phospholipase from yeast may lead to symptoms, particularly if there are not phagocytic cells in vaginal discharge to clear the yeast [1]. Additionally, allergy and atopic conditions are associated with recurrent VVC and may also contribute to symptomatic VVC with activation of the host response [1, 28].

This study has some notable strengths. The data are unique in their ability to correlate different quantities of bacteria with the presence and morphology of yeast. Both culture with

germ tube analysis of positive isolates and vaginal saline wet mount were used for diagnosis of yeast based on current guidelines [17, 29]. Additionally, detailed questions regarding specific symptoms and signs allowed for more accurate characterization of symptomatic VVC. Lastly, the collection of detailed information about women's methods of contraception, together with age, permitted adjustment for these potentially important confounding factors.

This study has some limitations. First, because only 30 women had yeast on vaginal wet mount Aims 2 and 3 had limited statistical power. Despite this, given the strength of some of these associations, a potentially important association between the presence of *L. jensenii* and pseudohyphae on the vaginal wet mount was observed. Second, this exploratory analysis included multiple comparisons, increasing the likelihood of identifying an association by chance (Type I error). As such, it will be important to confirm these findings in other populations. Third, the screening and enrollment criteria of the PVI trial required that women had a vaginal infection at screening. Screened women were then treated if they had TV (regardless of symptoms), or symptomatic BV or VVC. At enrollment women could not have a symptomatic vaginal infection requiring treatment. To address this, we conducted a sensitivity analysis that excluded women who had symptoms at the screening visit that would have been treated. This analysis confirmed the findings of the primary analysis. Nonetheless, the screening procedures and enrollment criteria of the PVI trial could limit generalizability of these findings. Further analyses using follow-up visits from women in the placebo arm of the trial will be important for exploring the extent to which the screening and enrollment procedures influenced the outcomes observed in the cross-sectional baseline analysis. Fourth, selection of bacteria for this secondary analysis was somewhat arbitrary, and included a group of taxa that were tested in the PVI trials based on their associations with BV or vaginal health. It is likely that other vaginal bacteria also have important interactions with yeast. Finally, the assumption was made that germ tube positivity was indicative of presumptive *C. albicans,* while a negative germ tube test was considered to be presumptive *Candida glabrata.* These assumptions are reasonable, but may results in a small

amount of misclassification. For example, *C. tropicalis* and *C. dubliniensis* can also be germ-tube positive, but these species are much less common in vaginal isolates [30]. These potential limitations necessitate future studies that can confirm the relationships identified in this analysis and explore additional vaginal bacteria.

In conclusion, this study demonstrates that the vaginal bacterial microbiota may influence both the presence and phenotypic expression of yeast. These effects may, in turn, contribute to a women's risk of symptomatic VVC. Understanding the complex relationship between vaginal bacteria and yeast could inform future studies of novel treatment and prevention interventions for VVC based on therapies directed at the vaginal bacterial microbiota.

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Table 1. Participant demographics, contraceptive methods, sexual history, vaginal washing practices, yeast characteristics and vaginal bacterial microbiota of 221 women.

Variable	Median (IQR) or Number (percent)
Age	29 years (24-34 years)
Research site	
Kenya	169 (76.5%)
University of Alabama-Birmingham	52 (23.5%)
Education >8 years	149 (67.4%)
Marital status	
Married	62 (28.1%)
Separated/divorced	78 (35.3%)
Never married	72 (32.6%)
Widow	9 (4.1%)
Parity (number of live births)	2 (2-3)
Type of contraceptive use (n=182)	
Oral contraceptive pill	22 (12.1%)
Depot medroxyprogesterone acetate	51 (28.0%)
Progestin implant	13 (7.1%)
IUD	14 (7.7%)
Tubal ligation	15 (8.2%)
Condoms alone	61 (33.5%)
Other	6 (3.3%)
Abstinent in the past week	42 (19.0%)
Any unprotected vaginal sex in the past week	61 (34.1%)
Among women not abstinent in the past week (N=179):	
100% condom use in the past week	95 (53.1%)
Number of sex partners in the past week	1 (1-3)
Number of vaginal sex acts in the past week	3 (2-4)
Reports vaginal washing	110 (49.8%)
Proportion of women with positive qPCR	
L. crispatus	54 (24.4%)
L. jensenii	46 (20.8%)
L. iners	196 (88.7%)
BVAB1	47 (21.3%)
BVAB2	112 (50.7%)
Mageeibacillus indolicus (formally BVAB3)	68 (30.8%)
Atopobium vaginae	167 (75.6%)
Leptotrichia/Sneathia	157 (71.0%)
Megasphaera	105 (47.5%)
Gardnerella vaginalis	205 (92.8%)
Wet-prep positive for yeast	30 (13.6%)
Budding yeast only[1]	19 (63.3%)
Pseudohyphae only[1]	2 (6.7%)
Budding yeast and pseudohyphae[1]	9 (30.0%)
Positive culture for yeast:	79 (35.7%)
Germ tube positive (n=79 culture positive)	53 (67.1%)
Number of women with symptomatic VVC[2]	69 (31.2%)

[1] Denominator is 30 women with any yeast present on wet prep.

[2] Symptomatic VVC was defined as a saline wet mount positive for yeast and/or positive culture for yeast *and* vulvar erythema, vulvar edema, vaginal itching or vaginal discharge

Table 2. Association between quantity of vaginal bacteria and the presence of yeast on saline went mount, culture, or both in 221 women.

Species	**OR (95% CI)**	**p-value**	**Adjusted OR[1] (95% CI)**	**p-value**
L. crispatus		0.4		0.5
Undetectable	Reference		Reference	
1st tertile	2.80 (1.07, 7.36)		1.95 (0.70, 5.47)	
2nd tertile	1.02 (0.36, 2.86)		1.17 (0.36, 3.76)	
3rd tertile	1.43 (0.51, 3.95)		1.20 (0.40, 3.63)	
L. jensenii		0.6		0.3
Undetectable	Reference		Reference	
1st tertile	1.49 (0.53, 4.20)		1.70 (0.53, 5.39)	
2nd tertile	1.68 (0.58, 4.85)		1.74 (0.55, 5.57)	
3rd tertile	0.96 (0.31, 2.93)		1.39 (0.39, 4.94)	
L. iners		0.6		0.4
Undetectable	Reference		Reference	
1st tertile	0.51 (0.20, 1.33)		0.66 (0.23, 1.86)	
2nd tertile	0.68 (0.27, 1.74)		1.08 (0.39, 2.98)	
3rd tertile	0.87 (0.34, 2.22)		1.10 (0.41, 3.12)	
BVAB1		0.2		0.4
Undetectable	Reference		Reference	
1st tertile	0.58 (0.18, 1.90)		1.04 (0.29, 3.80)	
2nd tertile	0.23 (0.05, 1.04)		0.16 (0.02, 1.30)	
3rd tertile	0.96 (0.33, 2.76)		1.07 (0.32, 3.58)	
BVAB2		0.01		0.03
Undetectable	Reference		Reference	
1st tertile	0.38 (0.16, 0.88)		0.49 (0.20, 1.23)	
2nd tertile	0.42 (0.19, 0.95)		0.43 (0.17, 1.06)	
3rd tertile	0.44 (0.19, 0.99)		0.44 (0.17, 1.16)	
M. indolicus		0.05		0.02
Undetectable	Reference		Reference	
1st tertile	0.55 (0.20, 1.49)		0.58 (0.21, 1.62)	
2nd tertile	0.41 (0.14, 1.16)		0.45 (0.14, 1.46)	
3rd tertile	0.52 (0.19, 1.39)		0.29 (0.08, 1.07)	
A. vaginae		0.001		0.006
Undetectable	Reference		Reference	
1st tertile	0.42 (0.19, 0.91)		0.55 (0.23, 1.30)	
2nd tertile	0.48 (0.22, 1.03)		0.55 (0.24, 1.24)	
3rd tertile	0.24 (0.10, 0.54)		0.26 (0.11, 0.66)	
Leptotrichia/ Sneathia		<0.001		<0.001
Undetectable	Reference		Reference	
1st tertile	0.44 (0.21, 0.93)		0.47 (0.20, 1.09)	
2nd tertile	0.30 (0.14, 0.65)		0.27 (0.11, 0.64)	
3rd tertile	0.25 (0.11, 0.56)		0.23 (0.09, 0.56)	
Megasphaera		0.001		0.005
Undetectable	Reference		Reference	
1st tertile	0.22 (0.09, 0.57)		0.31 (0.12, 0.84)	
2nd tertile	0.37 (0.16, 0.86)		0.44 (0.18, 1.13)	
3rd tertile	0.32 (0.13, 0.76)		0.28 (0.10, 0.81)	
G. vaginalis		0.05		0.08
Undetectable	Reference		Reference	
1st tertile	0.82 (0.27, 2.42)		0.94 (0.31, 2.91)	
2nd tertile	0.36 (0.12, 1.01)		0.33 (0.10, 1.05)	
3rd tertile	0.48 (0.16, 1.44)		0.55 (0.18, 1.76)	

[1] Adjusted for age and use of hormonal contraceptives

Table 3. Sensitivity analysis examining the associations between vaginal bacterial concentrations and the presence of yeast, excluding women who may have received metronidazole, antifungals, or both, prior to study entry (n=188)

Species	**OR (95% CI)**	**p-value**	**Adjusted OR[1] (95% CI)**	**p-value**
L. crispatus		0.3		0.5
Undetectable	Reference		Reference	
1st tertile	1.68 (0.58, 4.90)		0.99 (0.30, 3.26)	
2nd tertile	0.85 (0.25, 2.91)		1.11 (0.30, 4.07)	
3rd tertile	1.92 (0.64, 5.78)		1.66 (0.50, 5.54)	
L. jensenii		0.05		0.08
Undetectable	Reference		Reference	
1st tertile	2.06 (0.63, 6.70)		0.99 (0.30, 3.26)	
2nd tertile	3.09 (0.83, 11.43)		1.11 (0.30, 4.07)	
3rd tertile	2.06 (0.57, 7.43)		1.66 (0.50, 5.54)	
L. iners		0.5		0.3
Undetectable	Reference		Reference	
1st tertile	0.50 (0.19, 1.32)		0.60 (0.20, 1.79)	
2nd tertile	0.67 (0.26, 1.76)		1.03 (0.36, 2.95)	
3rd tertile	0.97 (0.37, 2.53)		1.24 (0.44, 3.48)	
BVAB1		0.3		0.5
Undetectable	Reference		Reference	
1st tertile	0.39 (0.08, 1.90)		0.58 (0.11, 3.08)	
2nd tertile	0.22 (0.05, 1.01)		0.16 (0.02, 1.26)	
3rd tertile	1.16 (0.38, 3.53)		1.42 (0.39, 5.14)	
BVAB2		0.01		0.03
Undetectable	Reference		Reference	
1st tertile	0.26 (0.10, 0.70)		0.28 (0.09, 0.82)	
2nd tertile	0.39 (0.17, 0.90)		0.37 (0.15, 0.96)	
3rd tertile	0.42 (0.18, 0.98)		0.44 (0.16, 1.20)	
M. indolicus		0.05		0.02
Undetectable	Reference		Reference	
1st tertile	0.43 (0.15, 1.24)		0.45 (0.15, 1.35)	
2nd tertile	0.40 (0.14, 1.16)		0.44 (0.13, 1.48)	
3rd tertile	0.51 (0.19, 1.40)		0.30 (0.08, 1.13)	
A. vaginae		<0.001		0.002
Undetectable	Reference		Reference	
1st tertile	0.33 (0.13, 0.82)		0.43 (0.16, 1.13)	
2nd tertile	0.31 (0.13, 0.73)		0.35 (0.14, 0.87)	
3rd tertile	0.18 (0.07, 0.45)		0.21 (0.08, 0.57)	
Leptotrichia/ Sneathia		<0.001		<0.001
Undetectable	Reference		Reference	
1st tertile	0.37 (0.16, 0.88)		0.32 (0.12, 0.83)	
2nd tertile	0.24 (0.10, 0.56)		0.19 (0.07, 0.50)	
3rd tertile	0.19 (0.08, 0.46)		0.16 (0.06, 0.43)	
Megasphaera		0.001		0.004
Undetectable	Reference		Reference	
1st tertile	0.22 (0.08, 0.57)		0.28 (0.10, 0.77)	
2nd tertile	0.30 (0.12, 0.73)		0.34 (0.12, 0.92)	
3rd tertile	0.29 (0.12, 0.70)		0.26 (0.09, 0.88)	
G. vaginalis		0.03		0.03
Undetectable	Reference		Reference	
1st tertile	0.51 (0.13, 1.95)		0.57 (0.14, 2.27)	
2nd tertile	0.21 (0.05, 0.80)		0.17 (0.04, 0.69)	
3rd tertile	0.28 (0.07, 1.06)		0.31 (0.08, 1.21)	

[1] Adjusted for age and use of hormonal contraceptives

Table 4. Association between the presence of vaginal bacterial taxa and pseudohyphae in 30 women with yeast on saline wet mount.

Bacterial Taxon	OR (95% CI)	p-value	Adjusted OR[1] (95% CI)	p-value
L. crispatus	1.34 (0.61, 2.96)	0.5	1.74 (0.59, 5.13)	0.3
L. jensenii	3.72 (0.99, 13.90)	0.05	4.51 (0.96, 21.28)	0.06
L. iners	1.19 (0.57, 2.51)	0.6	2.18 (0.76, 6.28)	0.1
BVAB1	0.32 (0.05, 1.89)	0.2	N/A	-
BVAB2	0.96 (0.47, 1.96)	0.9	N/A	-
M. indolicus	0.98 (0.37, 2.61)	1.0	N/A	-
A. vaginae	0.71 (0.34, 1.50)	0.4	0.41 (0.14, 1.25)	0.1
Leptotrichia/Sneathia	0.62 (0.26, 1.48)	0.3	0.32 (0.07, 1.37)	0.1
Megasphaera	0.88 (0.42, 1.87)	0.7	0.46 (0.14, 1.54)	0.2
G. vaginalis	0.85 (0.40, 1.80)	0.7	0.55 (0.21, 1.45)	0.5

[1] Adjusted for age and use of hormonal contraceptives

N/A=Not applicable. Odds ratios presented as N/A could not be calculated given too few observations.

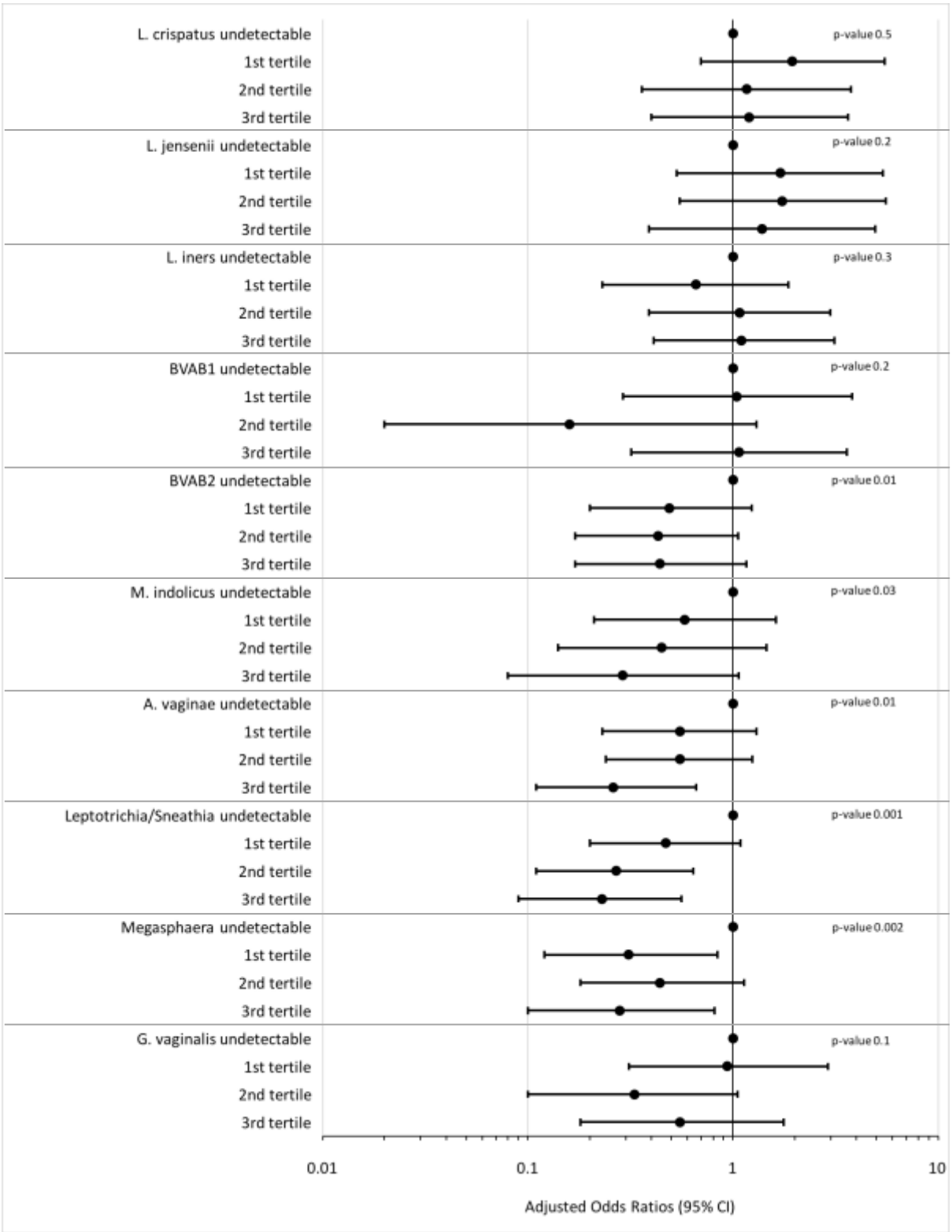
Table 5. Sensitivity analysis examining the associations between vaginal bacterial concentrations and the presence of pseudohyphae among women with yeast on wet mount, excluding women with a negative germ tube test (n=23)

Bacterial Taxon	**OR (95% CI)**	**p-value**	**Adjusted OR[1] (95% CI)**	**p-value**
L. crispatus	1.17 (0.49, 2.81)	0.7	1.46 (0.33, 6.47)	0.6
L. jensenii	4.58 (0.79, 26.5)	0.09	4.15 (0.60, 28.6)	0.1
L. iners	1.06 (0.45, 2.48)	0.9	1.86 (0.54, 6.41)	0.3
BVAB1	N/A	-	N/A	-
BVAB2	0.67 (0.25, 1.79)	0.4	N/A	-
M. indolicus	0.96 (0.33, 2.77)	0.9	N/A	-
A. vaginae	0.84 (0.37, 1.93)	0.7	0.48 (0.14, 1.65)	0.2
Leptotrichia/Sneathia	0.60 (0.22, 1.62)	0.3	0.29 (0.06, 1.34)	0.1
Megasphaera	0.92 (0.42, 2.00)	0.8	0.40 (0.10, 1.52)	0.2
G. vaginalis	0.64 (0.24, 1.72)	0.4	0.48 (0.14, 1.63)	0.2

[1] Adjusted for age and use of hormonal contraceptives

N/A= Not applicable. Odds ratios presented as N/A could not be calculated given too few observations.

Figure 1. Association between quantity of vaginal bacteria and the presence of yeast on saline went mount, culture, or both in 221 women.



Reference category is defined as undetectable for each particular bacterial taxon. Point estimates of adjusted odds ratios are represented by black circles for different evenly distributed quantities of taxa above the threshold of detection (>95 copies/swab), with horizontal bars representing 95% confidence intervals. P-values are for overall association, comparing the three tertiles to the undetectable category.

Figure 2. Heat map showing Spearman's rank correlation coefficients between vaginal bacterial taxa.

	L. crispatus	*L. jensenii*	*L. iners*	*BVAB1*	*BVAB2*	*M. indolicus*	*Atopobium vaginae*	*Leptotrichia/ Sneathia*	*Megasphaera*	*Gardnerella vaginalis*
L. crispatus	1.000									
L. jensenii	-0.7826	1.000								
L. iners	-0.7826	0.7000	1.000							
BVAB1	0.2236	0.1000	0.1000	1.000						
BVAB2	-0.1118	-0.3000	-0.3000	0.0000	1.000					
M. indolicus	0.2236	0.1000	0.1000	1.0000*	0.0000	1.000				
Atopobium vaginae	0.1118	-0.1000	-0.1000	0.8000	0.6000	0.8000	1.000			
Leptotrichia/Sneathia	-0.2236	0.0000	0.2000	0.6000	0.7000	0.6000	0.9000*	1.000		
Megasphaera	-0.1118	0.3000	0.2000	0.9000*	0.3000	0.9000*	0.9000*	0.8000	1.000	
Gardnerella vaginalis	-0.4472	0.3000	0.7000	0.6000	0.2000	0.6000	0.6000	0.8000	0.7000	1.000

*P-value<0.05

Correlation Coefficients

< -0.6	
-0.4 - 0.6	
-0.3 < 0	
<0 - 0.3	
0.4 - 0.6	
> 0.6	

BV-associated bacteria are positively correlated with each other. *L. crispatus* is often negatively associated with the other bacteria though not statistically significant.