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Association Between Vaginal Bacterial Microbiota and Vaginal Yeast Colonization

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Background. Vaginal yeast is frequently found with *Lactobacillus*-dominant microbiota. The relationship between vaginal yeast and other bacteria has not been well characterized.

Methods. These analyses utilized data from the Preventing Vaginal Infections trial. Relative abundance of vaginal bacteria from 16S ribosomal ribonucleic acid gene amplicon sequencing and quantities of 10 vaginal bacteria using taxon-directed polymerase chain reaction assays were compared at visits with and without detection of yeast on microscopy, culture, or both.

Results. Higher relative abundances of *Megasphaera* species type 1 (risk ratio [RR], 0.70; 95% confidence interval [CI], 0.52–0.95), *Megasphaera* species type 2 (RR, 0.81; 95% CI, 0.67–0.98), and *Mageeibacillus indolicus* (RR, 0.46; 95% CI, 0.25–0.83) were associated with lower risk of detecting yeast. In contrast, higher relative abundances of *Bifidobacterium bifidum*, *Aerococcus christensenii*, *Lactobacillus mucosae*, *Streptococcus equinus/infantarius/lutentiensis, Prevotella bivia*, *Dialister propionicifaciens*, and *Lactobacillus crispatus/helveticus* were associated with yeast detection. Taxon-directed assays confirmed that increasing quantities of both *Megasphaera* species and *M indolicus* were associated with lower risk of detecting yeast, whereas increasing quantities of *L crispatus* were associated with higher risk of detecting yeast.

Conclusions. Despite an analysis that examined associations between multiple vaginal bacteria and the presence of yeast, only a small number of vaginal bacteria were strongly and significantly associated with the presence or absence of yeast.

Keywords. vaginal bacterial microbiota; vulvovaginal candidiasis; yeast.

Approximately 75% of women will have vulvovaginal candidiasis (VVC) at least once in their lifetime [1]. Of these, approximately half will suffer at least 1 recurrence, and 5%–8% will have recurrent VVC, defined as 4 or more episodes per year [1]. In addition to uncomfortable symptoms and the potential for recurrences, VVC has been associated with increased risk of human immunodeficiency virus (HIV) acquisition [2].

The role of the vaginal microbiota as a mediator of vaginal yeast colonization, a prerequisite for VVC, is not well understood. In vitro studies suggest that lactobacilli can disrupt pathogenic mechanisms or directly kill *Candida* species [3–5]. In contrast to this in vitro research, studies in women have suggested that yeast colonization may occur more frequently in the setting of a *Lactobacillus*-dominant vaginal microbiota [6–8]. In addition, one study using culture for detection of lactobacilli found that *Lactobacillus crispatus* prevalence was higher in women with VVC compared to women without VVC [9]. In a study of Kenyan women, *Lactobacillus* detection by culture on Rogosa agar was associated with an approximately 4-fold higher risk of symptomatic VVC compared with the absence of cultivable *Lactobacillus* species [10]. Furthermore, many studies have shown an inverse association between the presence of bacterial vaginosis (BV) and the presence of vaginal yeast [10–13].

This analysis examined the relationship between vaginal bacteria and vaginal yeast colonization using both 16S ribosomal ribonucleic acid (rRNA) gene amplicon sequencing to provide relative abundances and taxon-directed quantitative polymerase chain reaction (qPCR) methods to measure absolute concentrations of bacteria in a sample of Kenyan and American women.

MATERIALS AND METHODS

Study Population and Procedures

Data came from the Preventing Vaginal Infections (PVI) trial (ClinicalTrials.gov number NCT01230814), a randomized, placebo-controlled trial evaluating the use of vaginal metronidazole plus miconazole suppositories versus placebo to prevent BV, VVC, and *Trichomonas vaginalis* (TV) [14]. Participants were recruited in Kenya and the United States. Women were

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eligible for inclusion if they were 18–45 years of age, sexually active, HIV-seronegative, and had a documented vaginal infection (with or without symptoms) with BV, VVC, TV, or any combination of these conditions at their screening visit. Women currently using metronidazole, tinidazole, clindamycin, or antifungal agents were excluded from enrolling. All women with TV infection and symptomatic women with BV or VVC at screening were treated with oral or intravaginal metronidazole, oral fluconazole, or both, as indicated. Women were enrolled 7–28 days after their screening visit and could not have a current symptomatic vaginal infection requiring treatment. If a woman had a vaginal infection requiring treatment at the screening visit, then they were eligible to be enrolled 14–28 days after screening.

At enrollment, participants completed a face-to-face interview to ascertain demographic data, contraceptive methods, sexual history, and vaginal washing practices. Women also underwent a vaginal speculum examination with vaginal fluid sample collection. Samples included 1 swab for vaginal saline wet mount, 1 swab for microbiome analyses, and 1 swab for yeast culture. The swab for yeast culture was plated directly on Sabouraud's agar in the clinic. The agar plate was transported to the laboratory for incubation within 4 hours. Follow-up study visits were conducted monthly. A vaginal speculum examination with sample collection was conducted at screening, enrollment, and months 2, 4, 6, 8, 10, and 12. Regardless of study arm, women who presented at follow-up with symptomatic vulvovaginitis, vaginal discharge, or itching were treated with open-label metronidazole and oral fluconazole. In addition, study participants were asked not to self-treat for vaginal symptoms and were encouraged to seek treatment for symptoms by returning to the research clinic. Treatment was provided at no cost in the research clinic, providing an incentive to adhere to this guidance.

This study was approved by the human subjects research committees at all collaborating institutions. 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.

Laboratory Methods

A vaginal saline wet mount was examined at ×40 magnification to identify motile TV organisms, clue cells, and fungal elements. One drop of 10% potassium hydroxide was added to the wet mount slide to identify budding yeast, pseudohyphae, or both. Before 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. Gram stains of vaginal secretions were examined for BV using the criteria of Nugent and Hillier [14].

Deoxyribonucleic Acid Extraction and Broad-Range Polymerase Chain Reaction and Sequencing

Vaginal fluid samples for molecular microbiological assays were stored at −80°C at study sites, then batched and transported on dry ice to the Fred Hutchinson Cancer Research Center in Seattle, Washington for analysis. Deoxyribonucleic acid (DNA) was extracted from vaginal swabs using the QIAamp BiOstic Bacteremia DNA Kit (QIAGEN, Hilden, Germany). Swabs without human contact were also processed in the same manner as test samples to monitor for potential contamination during processing and DNA extraction. Polymerase chain reaction inhibition was monitored using an internal amplification control qPCR assay [15], and bacterial DNA concentrations were measured using a qPCR that targeted the V3-V4 region of the 16S rRNA gene [16]. Relative abundances of bacterial taxa were measured using broad-range PCR targeting the V3-V4 region of the 16S rRNA gene coupled with sequencing on the Illumina MiSeq instrument (Illumina, San Diego, CA) [17]. Raw sequence reads were demultiplexed using Illumina MiSeq's onboard software. Demultiplexed reads were processed using barcodecop v0.4.1 to enforce barcode quality using default settings as well as ensuring exact barcode matches to forward and reverse reads [18]. The DADA2 package version 1.6.0 was used for quality filtering and read trimming (fastqPairedFilter with parameters truncLen = c(280 250), trimLeft = 15, truncQ = 2), error correction and dereplication, paired-end assembly (mergePairs with maxMismatch = 1), and chimera removal resulting in a list of unique sequence variants (default parameters were used unless otherwise noted) [19]. Sequence variants were classified using the phylogenetic placement tool *pplacer* [20] and a curated reference set of vaginal bacteria [16]. Sequence reads have been submitted to the NCBI Short Read Archive (BioProject PRJNA638104).

Quantitative Polymerase Chain Reaction

The qPCR methods have previously been described [21, 22]. In brief, PCR assays targeted specific regions of the 16S rRNA gene for each bacterium. Each qPCR assay was also run with controls that consisted of all reagents required for amplification but without DNA to monitor for bacterial contamination of PCR reagents. Amplification controls used exogenous DNA from a segment of jellyfish aequorin gene to detect any PCR inhibitors, as previously described [15]. Bacterium-specific qPCR assays targeted *L crispatus*, *Lactobacillus jensenii*, *Lactobacillus iners*, BV-associated bacterium 1 (BVAB1), BVAB2, *Mageeibacillus indolicus* (previously BVAB3), *Atopobium vaginae*, *Sneathia* spp, *Megasphaera* spp, and *Gardnerella* species. Quantities were expressed as 16S rRNA gene copies per swab, with 95 copies/swab as the threshold for linear quantitation. For these analyses, quantities below this threshold were considered undetectable.

Data Analysis

These analyses utilized specimens and data from follow-up visits completed by women in the placebo arm of the PVI Trial. Data were analyzed at the visit level with some women contributing data only when yeast was present, only when yeast was absent, or a combination of visits when yeast was present and when yeast was absent. Women in the treatment arm were excluded because of the impact of the intervention on vaginal bacteria and fungi [23]. Enrollment visits were excluded because of the potential for having recently been treated for a symptomatic vaginal infection at the screening visit. Women in the placebo arm of the trial who were treated for symptomatic vulvovaginitis during the study were included in this analysis. Participant demographics, sexual history, yeast morphotypes, and vaginal bacteria are reported as proportions for categorical data or medians with an interquartile range (IQR) for continuous data.

Deep Sequencing Statistical Analysis

Diversity of vaginal microbiota was compared between visits that had vaginal yeast present and visits when vaginal yeast was absent. The Shannon Diversity Index and Chao 1 Richness Estimator were calculated. Generalized estimating equations with a Poisson link and independent correlation structure were used to calculate risk ratios (RRs) and 95% confidence intervals (CIs). Potential confounding factors were identified a priori based on known associations with both vaginal bacteria and vaginal yeast. These included age (continuous) [24] and use of hormonal contraception (categorical; nonhormonal, estrogen-progestogen combinations, and progesterone-only contraceptives) [25]. Mean relative abundance of bacterial taxa was calculated for visits with and without yeast present.

Logistic regression was used to calculate Wald score statistics for all bacterial taxa identified by deep sequencing of 16S rRNA gene amplicons. These were then ranked from largest to smallest score. All taxa that had a Wald score $P \leq .05$ were included in the analysis comparing the relative abundance of bacteria for visits with and without yeast present. Generalized estimating equations with Poisson link and independent correlation structure were used to calculate RRs for a 1-standard deviation (SD) increase in relative abundance of each bacterial taxon for visits with and without yeast. This analytic approach accounts for multiple visits per participant and for variations in the number of visits between participants. Adjusted relative risks controlled for age and use of hormonal contraception. A Benjamini-Hochberg false discovery rate of 0.05 was applied to account for multiple testing. Due to model instability, only the models for taxa with a prevalence ≥1% were included in the final results.

Quantitative Polymerase Chain Reaction Statistical Analysis

Quantities of bacteria were categorized as absent and evenly distributed tertiles of increasing concentration. Generalized estimating equations with a log link, independent correlation structure, and robust standard errors were used to calculate relative RRs for the association between individual bacteria and the presence of yeast by culture, wet mount, or both. Prior studies informed the selection of potential confounding factors. Adjusted analyses controlled for age [24, 26, 27] and use of hormonal contraception [25, 28].

Effect modification by country (United States versus Kenya) was examined for each type of bacteria, based on PVI trial results showing different vaginal bacterial quantities in Kenyan versus US women [23]. Stratified estimates were presented if the interaction term *P* value was ≤.10. Analyses were conducted using Stata software version 14 (StataCorp, College Station, TX).

RESULTS

In the PVI trial, 111 women randomized to the placebo arm contributed 631 follow-up visits that were included in the present analysis. Participants had a median age of 29 years (IQR, 23–34) (Table 1). The majority of women (n = 95, 86%) used some type of contraceptive including 12 (11%) that contained estrogen combined with a progestogen and 35 (31%) that contained only a progestogen. Yeast was detected on vaginal saline wet mount alone at 7 (1%) follow-up visits, by culture alone at 86 (14%) follow-up visits, and by both wet mount and culture at 72 (11%) follow-up visits. A total of 74 (67%) of the 111 women had visits with yeast detected. In this subset of 74 women, the median proportion of visits with yeast was 33.3% (IQR, 16.7%–50%).

Lactobacillus iners and *Gardnerella* species were the 2 most abundant vaginal bacteria, regardless of whether yeast was present (Supplementary Material Figure 1). Mean Shannon Diversity Index was not significantly different at visits with yeast present (mean Shannon Diversity Index = 1.04; SD = 0.87) compared with visits when yeast was absent (mean Shannon Diversity Index 1.18; SD = 0.87) (RR, 0.87; 95% CI, 0.71–1.07). Likewise, microbiota richness measured by the Chao 1 Richness Estimator did not differ significantly at visits with yeast present (mean Chao 1 Richness Estimator 13.51; SD = 10.54) compared with visits when yeast was absent (mean Chao 1 Richness Estimator 14.41; SD = 10.29) (RR, 0.99; 95% CI, 0.98–1.01).

The relative abundances of a number of bacterial taxa were associated with a higher risk of detecting yeast in unadjusted analyses. Relative risks are calculated for each 1-SD change in bacterial taxa. *Bifidobacterium bifidum* (RR, 1.14; 95% CI, 1.08–1.20), *Aerococcus christensenii* (RR, 1.13; 95% CI, 1.08–1.18), *Lactobacillus mucosae* (RR, 1.08; 95% CI, 1.05–1.11), *Streptococcus equinus/infantarius/lutetiensis* (RR, 1.08; 95% CI, 1.05–1.11), *Prevotella bivia* (RR, 1.12; 95% CI, 1.02–1.24), and *Dialister propionicifaciens* (RR, 1.09; 95% CI, 1.03–1.15) (Table 2) were associated with a higher risk of yeast detection. In contrast, higher relative abundances of 3 bacterial taxa were associated with a lower risk of detecting yeast. These included *Megasphaera* sp Type 1 (RR, 0.70; 95% CI, 0.52–0.94),

Table 1. Demographics, Sexual History, Vaginal Washing Practices, Yeast Characteristics, and Vaginal Bacterial Microbiota for 111 Women Contributing 631 Visits Included in This Analysis

Variable	Median (IQR) or Number (%)
Number of visits	6 (6–6)
Age (years)	29 (23–34)
Country	
Kenya	85 (77)
United States	26 (23)
Education >8 years	80 (72)
Marital Status at Time of Enrollment	
Married	30 (27)
Separated/divorced	43 (39)
Never married	33 (30)
Widow	5 (5)
Parity (number of live births)	2 (1–3)
Contraceptive use reported at time of enrollment	95 (86)
Oral contraceptive pill	12 (11)
Depot medroxyprogesterone acetate	25 (23)
Progestin implant	10 (9)
IUD	10 (9)
Tubal ligation	5 (5)
Condoms alone	30 (32)
Other	3 (3)
Unless Indicated, the Following Variables Are Calculated as Proportion of the Total 631 Visits Contributed by the 111 Women	
Any unprotected vaginal sex in the past week	213 (34)
Abstinent in the past week	142 (23)
100% condom use in the past week	276 (56)
Number of sex partners in the past week	1 (1–3)
Number of vaginal sex acts in the past week	3 (2–5)
Reports vaginal washing	167 (27)
Detection of Vaginal Microbiota	
Lactobacillus crispatus	174 (28)
Lactobacillus jensenii	161 (26)
Lactobacillus iners	559 (89)
BVAB1	150 (24)
BVAB2	268 (43)
Mageeibacillus indolicus (formerly BVAB3)	199 (32)
Atopobium vaginae	459 (73)
Sneathia spp	385 (61)
Megasphaera spp	277 (44)
Gardnerella species	573 (91)
Wet-prep positive for yeast	79 (13)
Budding yeast only[a]	51 (65)
Pseudohyphae only[a]	4 (5)
Budding yeast and pseudohyphae[a]	24 (30)
Positive Culture for Yeast	158 (25)
Germ tube positive indicative of presumptive *Candida albicans* [41]	86 (54)

Abbreviations: BVAB, bacterial vaginosis-associated bacterium; IUD, intrauterine device; IQR, interquartile range.

[a]Denominator is 79 visits with any yeast present on wet prep.

Megasphaera sp Type 2 (RR, 0.81; 95% CI, 0.67–0.97), and *M indolicus* (RR, 0.46; 95% CI, 0.25–0.83). All of these associations remained statistically significant, and with similar magnitude, after adjustment for age and use of hormonal contraception. In addition, in adjusted analyses, the association between the relative abundance of *L crispatus/helveticus* and yeast was statistically significant (RR, 1.14; 95% CI, 1.02–1.27).

In analyses of qPCR data, compared with women without detectable *L crispatus*, women with the highest concentration of this *Lactobacillus* species had a higher risk of yeast detection (1st tertile RR = 0.94, 95% CI = 0.56–1.60; 2nd tertile RR = 0.96, 95% CI = 0.54–1.70; 3rd tertile RR = 1.81, 95% CI = 1.24–2.63) (Table 3). The association was similar and remained statistically significant when adjusted for age and contraceptive use (1st tertile adjusted RR [aRR] = 0.95, 95% CI = 0.56–1.61; 2nd tertile aRR = 1.02, 95% CI = 0.58–1.79; 3rd tertile aRR = 1.89, 95% CI = 1.32–2.71). Among the BV-associated bacteria evaluated, the risk of detecting vaginal yeast was significantly lower with increasing quantities of *M indolicus* when compared with absent (1st tertile aRR = 0.72, 95% CI = 0.44–1.18; 2nd tertile aRR = 0.64, 95% CI = 0.32–1.26; 3rd tertile aRR = 0.38, 95% CI = 0.15–0.93).

There was evidence of effect modification by country for the association between *Megasphaera* and yeast ($P = .03$) and for the association between *L iners* and yeast ($P = .07$). Increasing quantities of *Megasphaera*, when compared with no *Megasphaera*, were significantly associated with the absence of yeast in Kenyan women (1st tertile aRR = 0.75, 95% CI = 0.43–1.29; 2nd tertile aRR = 0.33, 95% CI = 0.15–0.72; 3rd tertile aRR = 0.50, 95% CI = 0.25–1.03) but not in US women (1st tertile aRR = 0.67, 95% CI = 0.30–1.51; 2nd tertile aRR = 1.44, 95% CI = 0.54–3.82; 3rd tertile aRR = 0.81, 95% CI = 0.32–2.05). In contrast, increasing quantities of *L iners*, compared with no *L iners*, were associated with the absence of yeast in Kenyan women (1st tertile aRR = 0.53, 95% CI = 0.31–0.92; 2nd tertile aRR = 0.61, 95% CI = 0.37–1.03; 3rd tertile aRR = 1.04, 95% CI = 0.61–1.78) but not in US women (1st tertile aRR = 1.23, 95% CI = 0.72–2.21; 2nd tertile aRR = 1.29, 95% CI = 0.72–2.33; 3rd tertile aRR = 1.62, 95% CI = 0.85–3.09).

DISCUSSION

This study makes a unique contribution to the literature by demonstrating associations between vaginal bacteria and detection of yeast using 2 different techniques for molecular evaluation of the vaginal microbiota. Results from deep sequencing of the 16S rRNA gene highlighted a number of vaginal bacteria for which higher relative abundance was associated with a higher risk of yeast including *B bifidum*, *A christensenii*, *L mucosae*, *S equinus/infantarius/lutetiensis*, *P bivia*, *D propionicifaciens*, and *L crispatus/helveticus*. In contrast, higher relative abundances of *Megasphaera* species and *M indolicus*, bacteria frequently detected in BV, were associated with a lower risk of detecting yeast. Additional analyses using taxon-directed PCR assays confirmed that increasing quantities of both vaginal *Megasphaera* species and *M indolicus* were associated with lower risk of

Table 2. Association Between Relative Abundances of Specific Bacterial Taxa Identified by Deep Sequencing of 16S rRNA Gene Amplicons With a Prevalence ≥1% and the Presence of Yeast on Saline Wet Mount, Culture, or Both, at 163 Visits With Yeast Present and 450 Visits Without Yeast Present

Bacterial Taxa	Overall Mean Relative Abundance % (SD)	Prevalence (%)	Mean Relative Abundance in % (SD), and Range (%) in Visits With Yeast (n = 163)	Mean Relative Abundance in % (SD), and Range (%) in Visits Without Yeast (n = 459)	Relative Risk (95% CI) per 1-SD Change	*P* Value	Adjusted[a] Relative Risk (95% CI) per 1-SD Change	Adjusted *P* Value
Bifidobacterium bifidum	**0.003 (0.0002)**	**3.1%**	**0.009 (0.0004), (0–0.3)**	**0.001 (0.0001), (0–0.2)**	**1.14 (1.08–1.20)**	**<.001**	**1.13 (1.08–1.19)**	**<.001**
Megasphaera sp type 1	**1.53 (0.035)**	**29.6%**	**0.73 (0.020), (0–12.2)**	**1.81 (0.039), (0–27.3)**	**0.70 (0.52–0.94)**	**.02**	**0.70 (0.52–0.95)**	**.02**
Aerococcus christensenii	**0.043 (0.01)**	**41.9%**	**0.69 (0.02), (0–17.7)**	**0.34 (0.007), (0–7.4)**	**1.13 (1.08–1.18)**	**<.001**	**1.13 (1.07–1.18)**	**<.001**
Lactobacillus mucosae	**0.015 (0.002)**	**2.9%**	**0.05 (0.003), (0–3.8)**	**0.004 (0.0005), (0–0.7)**	**1.08 (1.05–1.11)**	**<.001**	**1.09 (1.05–1.12)**	**<.001**
Streptococcus equinus/ infantarius/ lutetiensis	**0.035 (0.005)**	**1.1%**	**0.12 (0.009), (0–10.1)**	**0.004 (0.001), (0–1.6)**	**1.08 (1.05–1.11)**	**<.001**	**1.08 (1.04–1.11)**	**<.001**
Atopobium parvulum	0.006 (0.001)	1.1%	0.02 (0.002), (0–1.9)	<0.0001 (<0.0001), (0–0.2)	1.08 (1.06–1.09)	<1.01E-320[b]	1.07 (1.04–1.09)	<.001
Dialister sp type 2	0.81 (0.02)	40.4%	0.51 (0.012), (0–8.7)	0.91 (0.019), (0–16.8)	0.78 (0.61–1.00)	.05	0.79 (0.62–1.01)	.06
Prevotella bivia	**0.74 (0.03)**	**19.9%**	**1.23 (0.034), (0–18.3)**	**0.56 (0.027), (0–28.7)**	**1.12 (1.02–1.24)**	**.02**	**1.13 (1.03–1.23)**	**.009**
Bifidobacterium breve	0.031 (0.004)	2.1%	0.11 (0.01), (0–10.0)	0.004 (0.001), (0–1.7)	1.08 (1.07–1.09)	<1.001E-320[b]	1.06 (1.04–1.09)	9.361E-9[b]
Prevotella amnii	12.28 (0.031)	27.6%	0.72 (0.024), (0–17.7)	1.41 (0.033), (0–31.6)	0.78 (0.60–1.01)	.06	0.79 (0.61–1.03)	.08
Mageeibacillus indolicus	**0.24 (0.012)**	**19.5%**	**0.06 (0.002), (0–1.6)**	**0.31 (0.014), (0–21.6)**	**0.46 (0.25–0.83)**	**.01**	**0.46 (0.25–0.83)**	**.009**
Lactobacillus crispatus/ helveticus	6.04 (0.211)	16.2%	9.23 (0.26), (0–100)	4.91 (0.19), (0–99.8)	1.13 (0.99–1.29)	.07	**1.14 (1.02–1.27)**	**.02**
Eggerthella sp type 1 (species)	0.91 (0.014)	43.3%	0.70 (0.013), (0–5.9)	0.98 (0.014), (0–7.9)	0.84 (0.68–1.04)	.1	0.85 (0.69–1.04)	.1
Prevotella genogroup 4	0.21 (0.007)	17.3%	0.11 (0.005), (0–4.7)	0.24 (0.007), (0–6.4)	0.79 (0.55–1.12)	.2	0.80 (0.57–1.12)	.2
Dialister propionicifaciens	**0.29 (0.002)**	**5.5%**	**0.05 (0.003), (0–2.8)**	**0.02 (0.001), (0–1.7)**	**1.09 (1.03–1.15)**	**.002**	**1.12 (1.05–1.19)**	**<.001**
Megasphaera sp type 2	**0.75 (0.025)**	**19.1%**	**0.41 (0.014), (0–9.2)**	**0.88 (0.028), (0–21.5)**	**0.81 (0.67–0.97)**	**.02**	**0.81 (0.67–0.98)**	**.03**
Prevotella genogroup 3	0.49 (0.015)	17.7%	0.28 (0.010), (0–10.7)	0.56 (0.017), (0–19.5)	0.81 (0.62–1.07)	.1	0.81 (0.62–1.06)	.1

Abbreviations: CI, confidence interval; rRNA, ribosomal ribonucleic acid; SD, standard deviation.

NOTES: Relative risks and adjusted relative risks for the detection of yeast are calculated for each 1-SD change in relative abundance of bacteria. Bold values are those statistically significant in both univariate and multivariate analyses after applying a Benjamin-Hochberg false discovery rate of 0.05.

[a]Adjusted for age and use of hormonal contraception.

[b]These models are very unstable due to very few (or no) visits in either the cases or controls having any of this particular taxa. They are not included in the adjustment for multiple comparisons.

detecting yeast. Although there was no significant difference in Chao1 Richness Estimator or Shannon Diversity Index between women with and without yeast detected, this does not preclude significant differences in relative abundances or absolute concentrations of individual bacterial taxa.

Previous literature has shown that BV diagnosed by Nugent score is associated with the absence of vaginal yeast [10–13]. In this context, it is interesting that only 2 bacterial species frequently detected in BV were significantly associated with the absence of yeast in this study. There are several potential mechanisms through which these 2 bacteria could inhibit *Candida* growth in the vagina. *Mageeibacillus indolicus* produces indole [29], an aromatic heterocyclic organic compound that inhibits *Candida* in vitro [30]. *Megasphaera* spp and *M indolicus* could also reduce yeast colonization by out-competing *Candida* for essential nutrients [31]. Understanding the mechanisms that underpin the associations between vaginal bacteria and yeast at a species and molecular level may provide useful insights into the pathogenesis and prevention of VVC.

It is interesting to note that *Lactobacillus* species have been shown to inhibit *Candida* growth, or directly kill *Candida*, in vitro [3, 4, 32, 33] and in vivo [4]. However, this conflicts with the results of this study and of other published work that suggest that lactobacilli and yeast are frequently present in the vaginal microbiota at the same time [6, 7, 9, 14]. Moreover, a study published in 2019 found a 2-times higher odds of detecting *Candida albicans* with *L crispatus*-dominant community state types (CSTs), compared with CSTs with less *Lactobacillus* [8]. In the present study, higher relative abundances of *L crispatus/helveticus* and *L mucosae* were associated with the presence of yeast. Furthermore, using taxon-directed qPCR, increasing quantities of *L crispatus* were associated with higher risk of yeast detection. One potential hypothesis to explain this finding is that lactic acid produced by *L crispatus* and *L mucosae* suppresses the body's immune response to *C albicans*, allowing the yeast to persist [34, 35]. Alternatively, *Candida* and *Lactobacillus* species may both thrive in an environment replete with glycogen or other suitable substrates that support their growth.

In the qPCR analysis, the association between *Megasphaera* and the absence of yeast was restricted to the subset of women in Kenya. The reason for the strong effect modification observed in the present study is not clear but could be due to biological or behavioral differences. The vaginal microbiota is known to differ in women of different ethnicities and from different geographic areas [14, 16, 36]. Furthermore, there were examples of behavioral differences between US women and Kenyan women in the original trial, such as the prevalence of vaginal washing practices [37, 38]. Alternatively, the difference in findings in Kenya compared with the United States could be due to chance, particularly in light of the small number of US women. In addition, despite statistical differences seen in these 2 populations of women, there may not be clinical significance to the effect modification seen.

This study had some notable strengths. The data are unique in their ability to examine the effects of vaginal bacteria on yeast using complementary methods of relative abundance data from deep sequencing of the 16S rRNA gene and absolute concentrations of individual types of bacteria using taxon-directed qPCR assays. Use of these complementary methods is helpful for validating the findings base on the relative abundance data, because amplicon sequencing does not always correlate well with qPCR for bacteria present at <10% relative abundance [39]. An additional strength was the use of both culture and vaginal saline wet mount for identification of yeast [40], because the combination of methods provides a higher diagnostic yield than either method alone.

There were also limitations in this study. First, the 16S rRNA gene amplicon analyses and the qPCR analyses included multiple comparisons, increasing the likelihood of identifying an association by chance. This limitation was partially addressed in the 16S rRNA gene amplicon analyses by using the Benjamini-Hochberg false discovery rate to adjust for multiple testing. Nonetheless, it will be important to confirm the presented findings in other populations. Second, in this secondary analysis, qPCR data were available only for a limited number of bacterial taxa that were selected in the parent study based on their associations with BV or vaginal health in US women [23]. It is possible that other vaginal bacteria have important concentration-dependent interactions with yeast, as evidenced by the amplicon sequencing results. Further work with taxon-directed assays in parallel with amplicon sequencing will be important to clarify these associations. This study included a relatively small number of cases of vaginal yeast. To utilize all available data, this analysis included both women who had yeast at 1 or more visits and women who never had yeast. Future studies in larger populations could examine whether similar associations are present when comparing visits with versus without yeast in individual women. In addition to the relatively small number of vaginal yeast cases, vaginal yeast was classified as detected or not detected, and data for yeast quantities was not available. Future studies should explore associations between vaginal bacteria and the concentration of vaginal yeast. In addition, it is possible that the associations identified in these analyses were affected by confounding factors that were not included. A larger study would be required to allow for more comprehensive multivariable modeling and adjustment for additional confounding factors. Finally, although the results of germ tube testing allow for identification of *C albicans*, further species level identification of yeast is not available.

Table 3. Association Between Increasing Quantities of Vaginal Bacteria and the Presence of Yeast on Saline Wet Mount, Culture, or Both in 111 Women at 631 Visits

Species (log_{10} min, log_{10} max)	Visits With Yeast Present n = 165 n (%)	Visits With Yeast Absent n = 465 n (%)	RR (95% CI)	*P* Value	Adjusted RR[a] (95% CI)	*P* Value
Lactobacillus crispatus				.007		.002
Absent	111 (67.3)	345 (74.2)	Reference		Reference	
1st tertile (2.01, 3.66)	11 (6.7)	37 (8.0)	0.94 (0.56–1.60)		0.95 (0.56–1.61)	
2nd tertile (3.97, 7.69)	14 (8.5)	46 (9.9)	0.96 (0.54–1.70)		1.02 (0.58–1.79)	
3rd tertile (7.77, 9.95)	29 (17.6)	37 (8.0)	1.81 (1.24–2.63)		1.89 (1.32–2.71)	
Lactobacillus jensenii				.8		.9
Absent	120 (72.7)	349 (75.1)	Reference		Reference	
1st tertile (2.15, 5.52)	14 (8.5)	42 (9.0)	0.98 (0.59–1.61)		0.95 (0.57–1.59)	
2nd tertile (5.57, 7.01)	16 (9.7)	42 (9.0)	1.08 (0.63–1.84)		1.08 (0.64–1.81)	
3rd tertile (7.13, 8.51)	15 (9.1)	32 (6.9)	1.25 (0.77–2.02)		1.20 (0.74–1.96)	
Lactobacillus iners[b]				.006		.01
Absent	24 (14.5)	48 (10.3)	Reference		Reference	
1st tertile (2.33, 7.98)	44 (26.7)	180 (38.7)	0.59 (0.35–0.99)		0.59 (0.35–0.98)	
2nd tertile (8.00, 8.91)	42 (25.5)	139 (29.9)	0.70 (0.42–1.15)		0.68 (0.42–1.11)	
3rd tertile (8.92, 9.98)	55 (33.3)	98 (21.1)	1.08 (0.66–1.77)		1.02 (0.63–1.66)	
Kenyan Women	n = 127	n = 355				
Lactobacillus iners				.002		.003
Absent	23 (18.1)	42 (11.8)	Reference		Reference	
1st tertile (2.33, 7.98)	34 (26.8)	143 (40.3)	0.54 (0.31–0.94)		0.53 (0.31– 0.92)	
2nd tertile (8.00, 8.91)	31 (24.4)	108 (30.4)	0.63 (0.37–1.07)		0.61 (0.37–1.03)	
3rd tertile (8.91, 9.94)	39 (30.7)	62 (17.5)	1.09 (0.65–1.83)		1.04 (0.61–1.78)	
US Women	n = 38	n = 110				
Lactobacillus iners				.07		.5
Absent	1 (2.6)	6 (5.5)	Reference		Reference	
1st tertile (2.33, 7.94)	10 (26.3)	37 (33.6)	1.49 (0.70–3.16)		1.23 (0.72–2.21)	
2nd tertile (8.04, 8.90)	11 (28.9)	31 (28.2)	1.83 (0.96–3.51)		1.29 (0.72–2.33)	
3rd tertile (8.93, 9.98)	16 (42.1)	36 (32.7)	2.15 (1.19–3.90)		1.62 (0.85–3.09)	
BVAB1				.7		.8
Absent	133 (80.6)	348 (74.8)	Reference		Reference	
1st tertile (2.18, 7.57)	16 (9.7)	51 (11.0)	0.86 (0.46–1.64)		0.91 (0.50–1.68)	
2nd tertile (7.61, 8.90)	7 (4.2)	32 (6.9)	0.65 (0.29–1.47)		0.65 (0.29–1.48)	
3rd tertile (8.93, 10.67)	9 (5.5)	34 (7.3)	0.76 (0.33–1.71)		0.76 (0.34–1.66)	
BVAB2				.1		.2
Absent	110 (66.7)	253 (54.4)	Reference		Reference	
1st tertile (2.30, 6.38)	28 (17.0)	74 (15.9)	0.48 (0.22–1.06)		0.88 (0.60–1.31)	
2nd tertile (6.39, 7.24)	16 (9.7)	74 (15.9)	0.59 (0.31–1.11)		0.59 (0.31–1.12)	
3rd tertile (7.28, 8.80)	11 (6.7)	64 (13.8)	0.91 (0.60–1.36)		0.50 (0.23–1.09)	

Table 3. Continued

Species (log_{10} min, log_{10} max)	Visits With Yeast Present n = 165 n (%)	Visits With Yeast Absent n = 465 n (%)	RR (95% CI)	*P* Value	Adjusted RR[a] (95% CI)	*P* Value
Mageeibacillus indolicus				.2		.1
Absent	128 (77.6)	305 (65.4)	Reference		Reference	
1st tertile (2.15, 6.21)	20 (12.1)	71 (15.3)	0.74 (0.45–1.23)		0.72 (0.44–1.18)	
2nd tertile (6.30, 7.39)	12 (7.3)	49 (10.5)	0.66 (0.34–1.31)		0.64 (0.32–1.26)	
3rd tertile (7.40, 9.19)	5 (3.0)	41 (8.8)	0.37 (0.14–0.96)		0.38 (0.15–0.93)	
Atopobium vaginae				.4		.4
Absent	46 (27.9)	126 (27.1)	Reference		Reference	
1st tertile (2.23, 6.65)	47 (28.5)	97 (20.9)	1.22 (0.76–1.97)		1.18 (0.74–1.87)	
2nd tertile (6.69, 8.18)	38 (23.0)	126 (27.1)	0.87 (0.51–1.47)		0.86 (0.51–1.43)	
3rd tertile (8.20, 9.57)	34 (20.6)	116 (24.9)	0.85 (0.50–1.44)		0.85 (0.51–1.42)	
Leptotrichia/Sneathia				.5		.5
Absent	69 (41.8)	177 (38.1)	Reference		Reference	
1st tertile (2.03, 6.78)	50 (30.3)	121 (26.0)	1.04 (0.73–1.49)		1.01 (0.71–1.45)	
2nd tertile (6.78, 7.89)	20 (12.1)	86 (18.5)	0.67 (0.38–1.18)		0.66 (0.37–1.18)	
3rd tertile (7.91, 9.41)	26 (15.8)	81 (17.4)	0.87 (0.54–1.38)		0.88 (0.56–1.40)	
Megasphaera[c]				.1		.1
Absent	112 (67.9)	242 (52.0)	Reference		Reference	
1st tertile (2.11, 7.06)	22 (13.3)	78 (16.8)	0.70 (0.44–1.11)		0.72 (0.46–1.13)	
2nd tertile (7.13, 7.87)	10 (6.1)	48 (10.3)	0.55 (0.28–1.05)		0.54 (0.28–1.05)	
3rd tertile (7.89, 9.81)	21 (12.7)	97 (20.9)	0.56 (0.31–1.02)		0.57 (0.32–1.03)	
Kenyan Women	n = 127	n = 355				
Megasphaera				.2		.2
Absent	94 (74.0)	196 (55.2)	Reference		Reference	
1st tertile (2.11, 7.06)	15 (11.8)	47 (13.2)	0.75 (0.43–1.30)		0.75 (0.43–1.29)	
2nd tertile (7.13, 7.87)	5 (3.9)	41 (11.5)	0.34 (0.15–0.73)		0.33 (0.15–0.72)	
3rd tertile (7.89, 9.81)	13 (10.2)	71 (20.0)	0.48 (0.23–0.98)		0.50 (0.25–1.03)	
US Women	n = 38	n = 110				
Megasphaera				.2		.2
Absent	18 (47.4)	46 (41.8)	Reference		Reference	
1st tertile (2.46, 7.01)	7 (18.4)	31 (28.2)	0.66 (0.28–1.54)		0.67 (0.30–1.51)	
2nd tertile (7.42, 7.87)	5 (13.2)	7 (6.4)	1.48 (0.59–3.74)		1.44 (0.54–3.82)	
3rd tertile (7.89, 9.65)	8 (21.1)	26 (23.6)	0.84 (0.30–2.35)		0.81 (0.32–2.05)	
Gardnerella vaginalis				.4		.3
Absent	17 (10.3)	41 (8.8)	Reference		Reference	
1st tertile (2.19, 7.87)	66 (40.0)	155 (33.3)	1.02 (0.61–1.70)		0.97 (0.59–1.60)	
2nd tertile (7.87, 9.16)	46 (27.9)	173 (37.2)	0.72 (0.40–1.28)		0.67 (0.39–1.17)	
3rd tertile (9.16, 10.49)	36 (21.8)	96 (20.6)	0.93 (0.52–1.67)		0.89 (0.51–1.56)	

Abbreviations: BVAB, bacterial vaginosis-associated bacterium; CI, confidence interval; OR, odds ratio; RR, risk ratio.

[a]Adjusted for age and use of hormonal contraceptives.

[b]Overall association presented and then stratified by country study site because of significant effect modification ($P = .07$).

[c]Overall association presented and then stratified by country study site because of significant effect modification ($P = .03$).

CONCLUSIONS

In conclusion, despite an analysis that examined associations between multiple vaginal bacteria and the presence of yeast, only a small number of vaginal bacteria were strongly and significantly associated with the presence or absence of yeast. Understanding the mechanisms through which vaginal bacteria may inhibit or promote *Candida* colonization could open new avenues for prevention and treatment of vaginal candidiasis.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases online*. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Supplementary Material Figure 1. Vaginal bacterial composition by relative abundance in cases versus controls. Taxa with a relative abundance ≥1% were included.

Supplementary Digital Content that has the list of sequences used.

Notes

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