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A Prospective Study of Vaginal Bacterial Flora and Other Risk Factors for Vulvovaginal Candidiasis

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Abstract

Background: It has been suggested that vaginal lactobacilli may reduce the risk of vulvovaginal candidiasis (VVC), but supporting data are limited. Our objective was to determine the relationship between vaginal bacterial flora and VVC.

Methods: We conducted a prospective cohort analysis among 151 Kenyan sex workers. At monthly follow-up, VVC was defined as the presence of yeast buds, pseudohyphae, or both on vaginal wet preparation or KOH preparation. Generalized estimating equations were used to identify correlates of VVC.

Results: Participants returned for a median of 12 (interquartile range 11-12) visits. Vulvovaginal candidiasis was present at 162 visits, including 26 with symptomatic VVC. Bacterial vaginosis (BV) was associated with fewer episodes of VVC (adjusted odds ratio [aOR] 0.29, 95% confidence interval [CI] 0.16-0.50). After excluding women with concurrent BV, another possible cause of vaginal symptoms, the likelihood of symptomatic VVC was higher in those with yeast on vaginal wet preparation in the past 60 days (aOR 4.06, 95% CI 1.12-14.74) and those with concurrent vaginal *Lactobacillus* colonization (aOR 3.75, 95% CI 1.30-10.83).

Conclusions: Contrary to a commonly posed hypothesis of a protective effect, we found that vaginal *Lactobacillus* colonization was associated with a >4-fold increase in the likelihood of symptomatic VVC.

Keywords

Vulvovaginal candidiasis; *Lactobacillus*; bacterial vaginosis; women

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INTRODUCTION

Vulvovaginal candidiasis (VVC) affects up to 75% of reproductive age women at least once [1]. Nearly half will experience at least one recurrence, and 5-8% have multiple episodes each year. In addition to discomfort and costs associated with medication and health care visits, several prospective studies have suggested that VVC may increase a woman's risk of becoming infected with HIV-1 [2-5]. While not all studies have found this association [6,7], a meta-analysis published in 2001 also concluded that vaginal yeast infections are associated with a 2-fold increase in the risk for HIV-1 acquisition [8], Because of the high prevalence of this condition, VVC could contribute substantially to the population-level risk of HIV-1 [9].

While numerous risk factors for VVC have been identified, fundamental questions about the pathogenesis of this condition remain unanswered [10]. It has been suggested that normal vaginal flora consisting predominantly of *Lactobacillus* species may protect against the development of VVC, but there are limited data to support this hypothesis [11,12]. The objective of this prospective study, conducted in a population of Kenyan women at increased risk for HIV-1, was to examine correlates of VVC and of the subset of cases of symptomatic VVC. We focused particular attention on the dynamic relationship between vaginal bacterial flora and vaginal yeast.

MATERIALS AND METHODS

Population and Procedures

We recently completed a randomized trial of periodic presumptive treatment for vaginal infections [13]. Women in the study were randomized to receive monthly oral metronidazole plus fluconazole or identical metronidazole placebo and fluconazole placebo capsules. The analyses presented here focus on women who were randomized to receive placebo. Detailed methods have been presented elsewhere [13]. Briefly, 18-45 year old HIV-1-seronegative sex workers attending a municipal clinic in Mombasa, Kenya were eligible to enroll if they were not pregnant and did not currently have symptoms of vulvovaginal pruritis or abnormal vaginal discharge. At baseline and at monthly follow-up visits, women completed an interview covering recent sexual behavior and genital symptoms. Medication use was recorded. To facilitate accurate ascertainment of medication use, women were encouraged to return to the clinic between scheduled visits if they required outpatient evaluation for any reason. Treatment, including medications if indicated, was provided at no cost. Risk reduction counseling was provided at each visit.

At monthly visits, a genital examination including a speculum aided pelvic examination was performed. Trained study clinicians identified vulvitis on the basis of vulvar erythema and edema. Specimens were collected for laboratory diagnosis of genital tract infections, a urine pregnancy test was performed, and blood was collected for HIV-1 serological testing.

Women who reported vulvovaginal pruritis or abnormal vaginal discharge during follow-up were treated syndromically with metronidazole 2 grams as a single dose plus clotrimazole 200 mg vaginal suppositories nightly for three nights. Because of the time required for processing of specimens, participants were invited to return for their laboratory results one week after each examination. These results were used to guide additional treatment for sexually transmitted infections (STIs) including *Neisseria gonorrhoeae*, microscopic cervicitis, and *Trichomonas vaginalis* according to WHO and Kenya Ministry of Health Guidelines [14]. Asymptomatic VVC and asymptomatic BV were not treated, as there is currently no clear indication for treatment of these conditions in non-pregnant women. Participants in the trial were asked to return for a total of 12 monthly follow-up visits. Participation was discontinued if they became pregnant or seroconverted for HIV-1. This study was approved by the ethical review

committees at Kenyatta National Hospital and the University of Washington. All participants provided written informed consent.

Laboratory Methods

Screening for HIV-1 was performed using an enzyme-linked immunosorbent assay (ELISA; Detect-HIV; BioChem ImmunoSystems). Positive samples were confirmed using a second ELISA (Recombigen; Cambridge Biotech or Vironostika; Biomeriux) [15]. Urine pregnancy testing was performed using a rapid β-hCG test (Plasmatec Laboratory Products).

A vaginal saline wet mount was examined microscopically for the presence of budding yeast or pseudohyphae, and for *Trichomonas vaginalis.* A drop of 10% potassium hydroxide was added, and the slide was examined a second time for the presence of budding yeast or pseudohyphae. Women were classified as having VVC if fungal elements were observed on either the wet mount or potassium hydroxide preparation. We defined symptomatic VVC as the presence of fungal elements plus one or more genital signs or symptoms suggestive of vaginitis including vulvitis, vulvovaginal pruritis, or abnormal vaginal discharge.

Gram stained slides of vaginal secretions were evaluated for BV according to microscopic criteria [16]. Endocervical secretions were also Gram stained and examined microscopically. Polymorphonuclear leukocytes (PMN) were enumerated in three non-adjacent high-power fields, and the average cervical PMN count was calculated.

Lactobacillus culture was performed on Rogosa agar (Difco TM, Becton Dickinson), and production of H_2O_2 was assessed by sub-culture on tetramethylbenzadine agar with horseradish peroxidase [17]. Culture for *T. vaginalis* was performed in Diamond's modified medium and culture for *Neisseria gonorrhoeae* was performed on modified Thayer-Martin medium.

Statistical Methods

Analyses were performed using SPSS (version 15; SPSS) and S-Plus 2000 (Mathsoft). All women randomized to the placebo arm of the trial were considered eligible. The primary endpoints were VVC (asymptomatic or symptomatic) and the subset of cases of symptomatic VVC. Separate analyses for each endpoint were performed using Generalized Estimating Equations. This technique allows adjustment for the correlation between multiple events within an individual. Variables associated with the outcome of interest on univariate analysis ($P \leq 0.10$) were included in the multivariate models. Collinearity was evaluated and addressed in all multivariate models. The Wald statistic was used to determine the overall statistical significance for variables with multiple categories (for example different age group strata).

As in prior analyses [18], we assumed a window of 85 days to capture the extended effect following discontinuation of a hormonal contraceptive method (70 days of persistent effect of hormonal contraception after discontinuation + 15 days from acquisition of a vaginal yeast infection to detection at a clinic visit, assuming acquisition at the midpoint between monthly visits). Antimicrobials were modeled to have an effect for 60 days following the date on which they were prescribed. The effect window for previous identification of fungal elements on microscopy as a predictor of symptomatic VVC was also set at 60 days, but excluded the analysis visit because the presence of budding yeast, pseudohyphae, or both was required for the diagnosis of symptomatic VVC. We initially examined the effects of BV and *Lactobacillus* using a 60 day effect window. However, the effect of these exposures on VVC and symptomatic VVC appeared to be related primarily to their presence at the visit on which yeast was detected. Thus, our final analyses were based on the presence BV and *Lactobacillus* at the analysis visit. We also evaluated *Lactobacillus* status stratified by the

ability of isolates to produce H_2O_2. Because the effect did not differ for H_2O_2-producing versus non-H_2O_2-producing strains, we combined *Lactobacillus* culture results in our final analyses.

RESULTS

Between May 2003 and November 2005, 310 women were enrolled in the trial of periodic presumptive treatment for vaginal infections [13]. Of the 155 women randomized to the placebo arm, 151 returned for at least one HIV-1-seronegative follow-up visit. These 151 women are included in the analyses presented here. They contributed a median of 12 (interquartile range [IQR] 11-12) visits, and the median interval between visits was 30 (IQR 28-35) days. Overall, the women accrued 153 person-years of follow-up over 1,570 visits, representing 90% of expected follow-up visits.

Baseline characteristics of the 151 sex workers are presented in Table 1. The median age of participants was 32 (IQR 27-39) years. They reported a relatively low frequency of intercourse (median 1, IQR 0-2) during the preceding week. Only 5 (3.3%) women reported any history of oral-vaginal contact, and none reported a history of anal intercourse. Although the women denied symptoms of vulvovaginal pruritis or abnormal discharge at enrollment, 15 (9.9%) had VVC and 56 (37.1%) had BV by microscopic criteria.

Correlates of Vulvovaginal candidiasis

Vulvovaginal candidiasis was identified at 162 (10.3%) of 1,570 follow-up visits (incidence: 106 visits with VVC per 100 woman-years). This total included 71 women with one or more visits with VVC (median 2, IQR 1-3). Correlates of VVC are presented in Table 2. On univariate analysis, women with BV were significantly less likely to have VVC. This relationship remained statistically significant after adjustment for potential confounding factors. In addition, longer duration of sex work was associated with significantly lower risk for VVC in the multivariate analysis.

Correlates of Symptomatic Vulvovaginal Candidiasis

Symptomatic VVC was identified at 26 (1.7%) out of 1,570 follow-up visits (incidence: 17 visits with symptomatic VVC per 100 woman-years). These 26 episodes occurred in 16 women (median 1, IQR 1-2). One woman had three consecutive visits with symptomatic VVC and another had two. The remainder were either single episodes of symptomatic VVC per woman or two episodes separated by one or more visits without symptomatic VVC.

Of the 26 visits with symptomatic VVC, the signs and symptoms included vulvitis alone at 3 (11.5%) visits, vulvovaginal pruritis alone at 11 (42.3%), abnormal vaginal discharge alone at 6 (23.1%), vulvitis with vulvovaginal pruritis at 3 (11.5%), and vulvovaginal pruritis with abnormal vaginal discharge at 3 (11.5%). Correlates of symptomatic VVC are presented in Table 3. On univariate analysis, women were significantly more likely to develop symptomatic VVC if they had any identification of VVC (regardless of the presence or absence of symptoms) within the past 60 days. There was also an increased risk of symptomatic VVC in women with concurrent vaginal *Lactobacillus* colonization, although this association did not reach statistical significance. These findings were similar following adjustment for potential confounding factors.

Of the 26 women diagnosed with symptomatic VVC on the basis of microscopic detection of budding yeast or pseudohyphae combined with symptoms of vulvovaginal pruritis or abnormal vaginal discharge, 7 (26.9%) had concurrent BV. Because the symptoms could have been related to BV rather than VVC, we repeated the analysis excluding these cases. In this analysis,

prior VVC (aOR 4.06, 95% CI 1.12-14.74) and concurrent *Lactobacillus* colonization (aOR 3.75, 95% CI 1.30-10.83) were both significantly associated with symptomatic VVC.

Yeast Morphology and Symptomatic Vulvovaginal Candidiasis

Compared to visits at which only budding yeast forms were identified on microscopy, there was an increased likelihood of symptomatic VVC at visits where pseudohyphae were seen (OR 2.63, 95% CI 1.24-5.57). Because the symptom of abnormal vaginal discharge may be less specific for the diagnosis of symptomatic VVC [19], we repeated this analysis after excluding the six visits where women reported abnormal vaginal discharge as the only symptom of VVC. Once again, the presence of pseudohyphae was associated with increased risk of symptomatic VVC (OR 2.42, 95% CI 1.09-5.41).

DISCUSSION

This prospective cohort study offers insight into the dynamic interrelationships between vaginal bacterial flora, VVC, and symptomatic VVC. In particular, these data provide strong evidence contradicting the hypothesis that vaginal colonization with *Lactobacillus* reduces the risk of VVC. Indeed, the opposite may be true. We found that BV was associated with a substantial reduction in the risk of VVC. *Lactobacillus* colonization, on the other hand, was associated with as much as a 4-fold increased likelihood of symptomatic VVC.

Two earlier prospective studies have suggested that vaginal *Lactobacillus* is associated with increased risk of vaginal yeast colonization (identified by yeast culture) and VVC (identified by wet preparation or potassium hydroxide preparation) [20,21]. Neither study evaluated whether these associations were independent of the presence of BV. Our present study demonstrated a non-significant positive association between *Lactobacillus* and VVC. However, our results suggest that BV, which is characterized by the presence of a complex polymicrobial bacterial community including high concentrations of anaerobic bacteria [22], may play a more important role in mediating the risk of VVC than does *Lactobacillus.*

While neither of the earlier studies of vaginal yeast colonization and VVC evaluated the association between *Lactobacillus* and symptomatic VVC, one longitudinal study sought to test the hypothesis that women colonized by lactobacilli have a lower rate of vaginal infections [23]. That study, conducted in a U.S. population, demonstrated that women with non-H_2O_2-producing *Lactobacillus* or no *Lactobacillus* (i.e. with or at risk for BV) were at lower risk of symptomatic vaginal yeast infections compared to women with H_2O_2-producing *Lactobacillus*, although these results were not statistically significant. Addressing their primary hypothesis, the authors concluded that the absence of lactobacilli did not increase the risk of VVC. Our present findings go a step further, showing that the likelihood of symptomatic VVC may be significantly increased when lactobacilli are present.

Several mechanisms may contribute to lower rates of VVC in women with BV, including production of bacterial toxins, competition for available energy sources, micronutrients, and mucosal attachment sites. Interestingly, in women who develop VVC despite having concurrent BV, low vaginal interleukin 8 (IL-8) levels could attenuate the aggressive, neutrophil-predominant immune response that appears important in the pathogenesis of symptomatic VVC [24,25]. In contrast, vaginal lactobacilli may produce an immunostimulatory effect, increasing vaginal mucosal IL-8 production [24].

The risk for symptomatic VVC following treatment with metronidazole has been recognized for nearly four decades [26]. In the present study we observed a trend for a >6-fold increased likelihood of symptomatic VVC following treatment with metronidazole. This finding parallels recent studies quantifying this risk in a variety of settings. A randomized trial of suppressive

topical metronidazole in U.S. women with recurrent BV demonstrated a significantly higher rate of vaginal candidiasis in women assigned to the active treatment vs. placebo arm (43% vs. 21%) [27]. Likewise, symptoms of VVC were reported by nearly half of the women treated with oral metronidazole in a prospective cohort study of Australian women [28]. Metronidazole could increase the risk of symptomatic VVC by reducing the population of BV-associated bacteria or by promoting *Lactobacillus* colonization [13]. Systemic administration of metronidazole could also promote vaginal candidiasis by increasing the concentration of *Candida* in the gut [29], although published data addressing this possibility are limited.

In this study, we defined symptomatic VVC according to the presence of yeast forms on vaginal wet mount or KOH preparation plus one or more signs and symptoms including vulvitis, vulvovaginal pruritis, or abnormal vaginal discharge. This definition reflects the signs and symptoms most commonly identified in women with symptomatic VVC [30,31]. While vulvovaginal pruritis without discharge is the most specific clinical presentation, it correctly predicts VVC in only 38% of cases [19]. Thus, we felt that a broader definition of symptoms was preferable for our analyses. We acknowledge that, at visits where both BV and VVC are present, symptoms of abnormal vaginal discharge could be related to either condition. To address this possibility, we repeated our analyses after excluding visits where both BV and VVC were present. The observed relationships between yeast colonization, lactobacillus colonization, and symptomatic VVC were further strengthened in these analyses.

It has been suggested that the presence of pseudohyphae is associated with a higher likelihood of symptomatic VVC. In a rat experimental model of VVC, the production of hyphal elements appeared to be an important but non-essential virulence factor [32]. However, there are limited supporting data from human studies [33]. In our current investigation, we found that among visits where yeast was identified, the likelihood of symptomatic VVC was more than 2-fold higher when pseudohyphae were present, compared to visits where only budding yeast forms were seen. This modest but statistically significant association may reflect the fact that while hyphal forms are more invasive, both blastoconidia and pseudohyphae are capable of destroying superficial epithelial cells by direct invasion [34].

There were limitations to this study. Because yeast culture was not performed, we were not able to identify women with yeast colonization (positive culture with negative microscopy) [12]. Some cases of symptomatic VVC could have been missed by microscopy alone [35]. False positives may also occur when using microscopy to identify yeast, particularly in cases where blastoconidia are observed in the absence of hyphal elements.

While we were able to culture for *Lactobacillus*, there are limitations to this approach. Some species such as *L. iners* may be common in African women [36], but would not be detected by culture on Rogosa agar. It is notable that the overall rate of *Lactobacillus* isolation in this population was much lower than in western populations [17,23]. We have previously shown that vaginal washing, which was highly prevalent among these women, was associated with significantly reduced isolation of *Lactobacillus* species [37]. This practice may have contributed to the low rate of *Lactobacillus* isolation in the present study. Finally, in our analyses of the effects of metronidazole and antifungals on VVC, there is the possibility for confounding by indication. For example, antifungals might be administered for symptomatic VVC, but the condition tends to recur, which could contribute to a positive association between antifungal use and VVC. Nonetheless, these prospectively collected data provide new insights into the relationships between vaginal bacterial flora, VVC, and symptomatic VVC.

Both VVC and BV have been associated with increased HIV-1 risk [2,3,6,38]. In contrast, vaginal colonization with H_2O_2-producing *Lactobacillus* has been associated with lower risk for acquiring HIV-1 [20]. Taken together, the available data suggest that optimal vaginal flora

for minimizing women's HIV-1 risk may include H_2O_2-producing *Lactobacillus* without VVC. Our findings suggest that simply promoting *Lactobacillus* colonization may not reduce VVC. These data substantially advance our understanding of the relationship between vaginal bacterial flora, VVC, and symptomatic VVC. This insight will enhance our ability to improve vaginal health, and potentially to develop strategies for reducing HIV-1 risk through vaginal health interventions.

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Table 1

Enrollment Characteristics of 151 HIV-1-Seronegative Kenyan Female Sex Workers

Variable	Value
Age, median (IQR), years	32 (27-39)
Education, median (IQR), years	8 (7-11)
Duration of sex work, median (IQR), years	4.3 (1.6-8.1)
Vaginal washing in the past week	130 (86.1%)
Frequency, median (IQR), per week[a]	14 (14-21)
Vaginal washing substance[a]	
Water only	43 (33.1%)
Soap or antiseptic[b]	87 (66.9%)
Cloth to clean inside vagina[a]	3 (2.3%)
Bathing frequency, median (IQR), per week	14 (14-14)
Sex partners, median (IQR), in past week	1 (0-1)
Sex frequency, median (IQR), in past week	1 (0-2)
Any unprotected intercourse in past week	32 (21.2%)
New sex partner in past month	22 (14.7)
Any history of anal intercourse	0 (0%)
Any history of oral-vaginal contact	5 (3.3%)
Contraception	
None or tubal ligation	93 (61.6%)
OCP	10 (6.6%)
DMPA	43 (28.5%)
Norplant	3 (2%)
IUD	2 (1.3%)
Vulvovaginal candidiasis	15 (9.9%)
Bacterial vaginosis	56 (37.1%)
Trichomonas vaginalis	2 (1.3%)
Lactobacillus (any)	13 (8.6%)
Lactobacillus (H_2O_2 producing)	3 (2%)
Neisseria gonorrhoeae	0 (0%)
Cervicitis[c]	0 (0%)

Note: Data are no. (%) of subjects unless otherwise specified. DMPA, depot medroxyprogesterone acetate; H_2O_2 hydrogen peroxide; IUD, intrauterine device; OCP, oral contraceptive pill.

[a] N=130 women who reported vaginal washing.

[b] Use of antiseptic was reported by 3 women.

[c] Average polymorphonuclear leukocyte count ≥30 cells per high power field on microscopy of Gram stained cervical secretions.

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Table 2

Risk Factors for Vulvovaginal Candidiasis in HIV-1-Seronegative Kenyan Women

	Visits with VVC	Visits without VVC	Univariate		Multivariate	
Variable	**n = 162**	**n = 1408**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**
Age						
≤20 years	12 (7.4%)	14 (1.0%)	1.0			
21-30 years	64 (39.5%)	514 (36.5%)	0.12 (0.03, 0.49)	0.003		
31-40 years	57 (35.2%)	609 (43.3%)	0.09 (0.02, 0.39)	0.001		
>40 years	29 (17.9%)	271 (19.2%)	0.10 (0.02, 0.47)	0.004		
Overall P-value (Wald Test)				0.02		
Years of sex work[a]						
≤ 5 years	120 (74.5%)	789 (56.5%)	1.0		1.0	
5-10 years	16 (9.9%)	335 (24.0%)	0.38 (0.19, 0.73)	0.004	0.39 (0.19, 0.80)	0.01
10-15 years	16 (9.9%)	145 (10.4%)	0.55 (0.29, 1.91)	0.5	0.67 (0.26, 1.71)	0.4
>15 years	9 (5.6%)	128 (9.2%)	0.46 (0.13, 1.63)	0.2	0.24 (0.05, 1.24)	0.09
Overall P-value (Wald Test)				0.03		
Education ≤8 years	109 (67.3%)	858 (60.9%)	1.39 (0.84, 2.31)	0.2		
Vaginal washing frequency						
None	17 (10.5%)	163 (11.6%)	1.0			
1-14 times/week	82 (50.6%)	744 (52.8%)	1.11 (0.62, 1.98)	0.7		
15-28 times/week	56 (34.6%)	411 (29.2%)	1.19 (0.63, 2.25)	0.6		
>28 times per week	7 (4.3%)	90 (6.4%)	0.83 (0.32, 2.12)	0.7		
Overall P-value (Wald Test)				0.8		
Vaginal washing substance[b]						
None	17 (10.5%)	163 (11.6%)	1.0			
Water	99 (61.1%)	778 (55.3%)	1.17 (0.79, 1.74)	0.4		
Soap or antiseptic	101 (62.3%)	864 (61.4%)	0.97 (0.65, 1.44)	0.9		
Overall P-value (Wald Test)				0.7		
Cloth to clean inside the vagina	6 (3.7%)	37 (2.6%)	1.32 (0.63, 2.76)	0.5		
Bathes <14 times per week	11 (6.8%)	95 (6.7%)	1.34 (0.71, 2.54)	0.4		
Sex partners in last week						
None	74 (45.7%)	522 (37.1%)	1.0		1.0	
One	74 (45.7%)	710 (50.4%)	0.74 (0.55, 1.01)	0.06	0.77 (0.54, 1.09)	0.1
More than one	14 (8.6%)	176 (12.5%)	0.52 (0.26, 1.04)	0.07	0.60 (0.30, 1.24)	0.2

Variable	Visits with VVC n = 162	Visits without VVC n = 1408	Univariate OR (95% CI)	P	Multivariate OR (95% CI)	P
Overall P-value (Wald Test)				0.08		
Unprotected sex in the last week	34 (21.0%)	370 (26.3%)	0.71 (0.45, 1.11)	0.1	0.95 (0.58, 1.55)	0.8
New partner in the past month	17 (10.5%)	187 (13.3%)	0.98 (0.58, 1.64)	0.9		
Contraception[c]						
None or tubal ligation	95 (58.6%)	904 (64.5%)	1.0			
OCP	9 (5.6%)	98 (7.0%)	1.31 (0.40, 4.27)	0.7		
DMPA	53 (32.7%)	352 (25.1%)	1.52 (0.85, 2.74)	0.2		
Norplant	3 (1.9%)	41 (2.9%)	0.95 (0.41, 2.17)	0.9		
IUD	2 (1.2%)	9 (0.6%)	1.18 (0.80, 1.74)	0.4		
Overall P-value (Wald Test)				0.6		
Neisseria gonorrhoeae	0 (0%)	6 (0.4%)	NC			
Cervicitis[d]	4 (2.5%)	17 (1.2%)	2.04 (0.73, 5.68)	0.2		
Bacterial vaginosis[e]	25 (15.4%)	528 (37.5%)	0.27 (0.15, 0.49)	<0.001	0.29 (0.16, 0.50)	<0.001
Trichomonas vaginalis	2 (1.2%)	20 (1.4%)	1.32 (0.48, 3.65)	0.6		
Lactobacillus culture positive[e]	23 (14.2%)	125 (8.9%)	1.34 (0.70, 2.57)	0.4		
Antibiotics[f]						
None	145 (89.5%)	1241 (88.1%)	1.0			
Oral metronidazole[g]	6 (3.7%)	25 (1.8%)	1.24 (0.22, 7.11)	0.8		
Other antibacterials[h]	13 (8.0%)	147 (10.4%)	0.69 (0.33, 1.44)	0.7		
Antifungals[i]	5 (3.1)	17 (1.2%)	1.36 (0.24, 7.75)	0.7		
Overall P-value (Wald Test)				0.5		

Note: DMPA, depot medroxyprogesterone acetate; H_2O_2, hydrogen peroxide; HR, hazard ratio; IUD, intrauterine device; NC, no convergence; OCP, oral contraceptive pill.

[a] Data on years of sex work was missing for 12 visits.

[b] There were 452 visits at vaginal washing with both water and soap/antiseptic were reported.

[c] Data on contraception were missing at 6 visits. Because of the extended window of effect for contraceptives, women who changed methods could contribute to more than one category at some visits.

[d] Average polymorphonuclear leukocyte count ≥30 cells per high power field on microscopy of Gram stained cervical secretions.

[e] Bacterial vaginosis and *Lactobacillus* included analysis visit only.

[f] 60 day effect window for vaginal yeast colonization and antibiotics did not include analysis visit.

[g] Metronidazole was dispensed at 28 visits.

[h] The most common prescriptions for other antibacterials included amoxicillin (55), cloxacillin (31), doxycycline (19), and trimethoprim-sulfamethoxazole (16), ciprofloxacin (7), and norfloxacin (6). Other oral antibacterials, prescribed less than 5 times each, included erythromycin, benzathine penicillin, amoxicillin with clavulanate, and levofloxacin.

[i] There were 20 prescriptions for clotrimazole vaginal pessaries and 2 prescriptions for nystatin vaginal pessaries.

Table 3

Risk Factors for Symptomatic Vulvovaginal Candidiasis in HIV-1-Seronegative Kenyan Women

	Visits with VVC	Visits without VVC	Univariate		Multivariate	
Variable	**n = 26**	**n = 1544**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**
Age						
≤20 years	1 (3.8%)	25 (1.6%)	1.0			
21-30 years	12 (46.2%)	566 (36.7%)	0.56 (0.11, 2.91)	0.5		
31-40 years	10 (38.5%)	656 (42.5%)	0.43 (0.08, 2.37)	0.3		
>40 years	3 (11.5%)	297 (19.2%)	0.26 (0.03, 2.05)	0.2		
Overall P-value (Wald Test)				0.6		
Years of sex work[a]						
≤ 5 years	17 (65.4%)	904 (58.5%)	1.0			
5-10 years	5 (19.2%)	346 (22.6%)	0.96 (0.29, 3.22)	0.9		
10-15 years	2 (7.7%)	159 (10.4%)	0.67 (0.15, 2.88)	0.6		
>15 years	2 (7.7%)	135 (8.8%)	0.78 (0.10, 5.89)	0.8		
Overall P-value (Wald Test)				1.0		
Education ≤8 years	18 (69.2%)	949 (61.5%)	1.40 (0.54, 3.60)	0.5		
Vaginal washing frequency						
None	2 (7.7%)	178 (11.5%)	1.0			
1-14 times/week	15 (57.7%)	811 (52.5%)	1.33 (0.41, 4.26)	0.6		
15-28 times/week	8 (30.8%)	459 (29.7%)	1.45 (0.43, 4.89)	0.5		
>28 times per week	1 (3.8%)	96 (6.2%)	0.98 (0.15, 6.33)	1.0		
Overall P-value (Wald Test)				0.9		
Vaginal washing substance[b]						
None	2 (7.7%)	178 (11.5%)	1.0			
Water	17 (65.4%)	860 (55.7%)	1.37 (0.64, 2.93)	0.8		
Soap or antiseptic	17 (65.4%)	948 (61.4%)	1.39 (0.60, 3.24)	0.8		
Overall P-value (Wald Test)				0.6		
Cloth to clean inside the vagina	1 (3.8%)	42 (2.7%)	1.49 (0.35, 6.32)	0.6		
Bathes <14 times per week	4 (15.4%)	102 (6.6%)	2.41 (0.62, 9.36)	0.2		
Sex partners in last week						
None	13 (50.0%)	583 (37.8%)	1.0			
One	11 (42.3%)	773 (50.1%)	0.76 (0.33, 1.75)	0.5		
More than one	2 (7.7%)	188 (12.2%)	0.55 (0.13, 2.43)	0.4		

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Variable	Visits with VVC n = 26	Visits without VVC n = 1544	Univariate OR (95% CI)	P	Multivariate OR (95% CI)	P
Overall P-value (Wald Test)				0.7		
Unprotected sex in the last week	4 (15.4%)	400 (25.9%)	0.46 (0.13, 1.65)	0.2		
New partner in the past month	3 (11.5%)	201 (13.1%)	1.11 (0.44, 2.80)	0.8		
Contraception[c]						
None or tubal ligation	17 (65.4%)	982 (63.6%)	1.0			
OCP	2 (7.7%)	105 (6.8%)	NC			
DMPA	6 (23.1%)	399 (25.9%)	NC			
Norplant	1 (3.8%)	43 (2.8%)	NC			
IUD	0 (0%)	11 (0.7%)	NC			
Overall P-value (Wald Test)				NC		
Neisseria gonorrhoeae	0 (0%)	6 (0.4%)	NC			
Cervicitis[d]	1 (3.8%)	20 (1.3%)	2.39 (0.30, 18.87)	0.4		
Vaginal yeast[e]	10 (38.5%)	167 (10.8%)	3.40 (1.05, 11.02)	0.04	3.31 (1.09, 10.08)	0.04
Bacterial vaginosis[f]	7 (26.9%)	546 (35.4%)	0.64 (0.27, 1.52)	0.3	0.71 (0.31, 1.61)	0.4
Trichomonas vaginalis	1 (3.8%)	21 (1.4%)	2.54 (0.29, 22.38)	0.4		
Lactobacillus culture positive[f]	6 (23.1%)	142 (9.2%)	2.60 (0.84, 8.01)	0.1	2.27 (0.81, 6.38)	0.1
Antibiotics[e]						
None	23 (88.5%)	1363 (88.3%)	1.0		1.0	
Oral metronidazole[g]	2 (7.7%)	29 (1.9%)	5.92 (0.62, 56.54)	0.1	6.41 (0.68, 60.25)	0.1
Other antibacterials[h]	2 (7.7%)	158 (10.2%)	0.46 (0.12, 1.79)	0.3	0.47 (0.13, 1.69)	0.3
Antifungals[i]	1 (3.8%)	21 (1.4%)	0.34 (0.65, 1.78)	0.2	0.25 (0.04, 1.51)	0.1
Overall P-value (Wald Test)				0.06		

Note: DMPA, depot medroxyprogesterone acetate; H_2O_2, hydrogen peroxide; HR, hazard ratio; IUD, intrauterine device; NC, no convergence; OCP, oral contraceptive pill.

[a] Data on years of sex work was missing for 12 visits.

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[e] 60 day effect window for vaginal yeast colonization and antibiotics did not include analysis visit.

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[h] The most common prescriptions for other antibacterials included amoxicillin (55), cloxacillin (31), doxycycline (19), and trimethoprim-sulfamethoxazole (16), ciprofloxacin (7), and norfloxacin (6). Other oral antibacterials, prescribed less than 5 times each, included erythromycin, benzathine penicillin, amoxicillin with clavulanate, and levofloxacin.

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