Bacterial Vaginosis and Vaginal Yeast, But Not Vaginal Cleansing, Increase HIV-1 Acquisition in African Women

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Objective: To evaluate interrelationships between bacterial vaginosis (BV), vaginal yeast, vaginal practices (cleansing and drying/tightening), mucosal inflammation, and HIV acquisition.

Methods: A multicenter, prospective, observational cohort study was conducted, enrolling 4531 HIV-negative women aged 18 to 35 years attending family planning clinics in Zimbabwe and Uganda. Participants were tested for HIV and reproductive tract infections and were interviewed about vaginal practices every 3 months for 15 to 24 months. BV was measured by Gram stain Nugent scoring, vaginal yeast by wet mount, and mucosal inflammation by white blood cells on Gram stain.

Results: HIV incidence was 4.12 and 1.53 per 100 woman-years of follow-up in Zimbabwe and Uganda, respectively (a total of 213 incident infections). Women with BV or vaginal yeast were more likely to acquire HIV, especially if the condition was present at the same visit as the new HIV infection and the visit preceding it (hazard ratio [HR] = 2.50, 95% confidence interval [CI]: 1.68 to 3.72 and HR = 2.97, 95% CI: 1.67 to 5.28 for BV and yeast, respectively). These relationships did not seem to be mediated by mucosal inflammation. Vaginal drying/tightening was associated with HIV acquisition in univariate (HR = 1.49, 95% CI: 1.03 to 2.15) but not multivariate models. Vaginal cleansing was not associated with HIV acquisition.

Conclusions: BV and yeast may contribute more to the HIV epidemic than previously thought.

Key Words: Africa, bacterial vaginosis, candidiasis, heterosexual transmission, HIV-1, vaginal practices

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Approximately 60% of all infections in sub-Saharan Africa occur among women.[1] Although the probability of male-to-female HIV transmission during vaginal intercourse under normal circumstances is considered low, cofactors may substantially increase acquisition risk.[2] These cofactors include, among others, reproductive tract infections (RTIs) and possibly "traditional" vaginal practices for hygiene, sexual satisfaction, pregnancy, and disease prevention.

Many studies have shown that sexually transmitted infections (STIs) facilitate HIV transmission.[3–5] The evidence for vaginal infections that are not necessarily sexually transmitted (eg, bacterial vaginosis [BV], candidiasis) as cofactors in HIV transmission is less clear but is mounting.[6–9] Many of the studies showing a link between BV and HIV were cross-sectional, however, making it difficult to draw conclusions about a temporal relation. Few prospective studies have investigated BV or candidiasis as a risk factor for HIV, and these showed positive associations.[7–9] The prevalence of vaginal infections, particularly BV, is high in many countries. For example, 20% to 50% of women of reproductive age are affected in Zimbabwe.[10] Therefore, even a small increased HIV risk per individual may have a large impact at the population level.

Vaginal practices are widespread throughout the world, particularly in sub-Saharan Africa.[11] They include cleansing the vagina with water, soap, antiseptic solutions, or household cleaning products (usually using a finger or piece of cloth); wiping the vagina to remove vaginal fluids; and inserting herbal or nonherbal preparations to constrict or tighten the vagina. Vaginal practices have been suggested as risk factors

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that may increase women's vulnerability to HIV.[6] Most studies in Africa have documented prevalences of vaginal practices of 30% to 50%, with higher levels usually shown among sex workers.[6] Nevertheless, there is little epidemiologic evidence to support a temporal association between vaginal practices and HIV acquisition. Of studies including data on HIV, most were cross-sectional, several were inadequately powered, and few adjusted for potential confounding variables.[6] In 2006, results of 2 large prospective studies were published, and they showed conflicting results.[12,13]

Some of the proposed mechanisms by which BV and vaginal yeast could increase HIV acquisition are by changing the cervicovaginal epithelial integrity and/or permeability and by causing inflammation and/or immune activation in the female genital tract.[14,15] BV was initially considered a non-inflammatory disorder because of a lack of polymorphonuclear cells in the vaginal discharge and a lack of signs of inflammation in the vaginal epithelium. Recent evidence suggests that BV may induce less visible inflammatory markers, however, such as various cytokines.[14] Candidiasis is known to cause vaginal irritation, inflammation, and immune activation.[15] Vaginal practices could alter the vaginal flora or could have a direct effect on the cervicovaginal mucosa.[6] Data on vaginal practices disrupting the vaginal flora are not conclusive (because of a possible reverse temporality [ie, the presence of a vaginal infection could prompt vaginal cleansing]) but are supported by epidemiologic findings from Europe and the United States showing associations between douching and incident and prevalent BV.[6] The possibility that vaginal practices may disrupt the cervicovaginal epithelium has been investigated in 3 small studies, 2 of which observed epithelial damage that was repaired within 2 to 7 days.[6] Thus far, no studies of vaginal practices and HIV have included markers of genital inflammation and/or immune activation.

We hypothesized that BV, vaginal yeast, vaginal practices, and mucosal inflammation may be associated with each other and with HIV acquisition. These hypotheses were evaluated within a large prospective cohort study of women in Zimbabwe and Uganda, of which the primary objective was to evaluate the relation between hormonal contraception and HIV acquisition. Our study is the first to focus on the interrelationships between several vaginal factors that may increase women's risk of HIV acquisition and to include a marker of mucosal inflammation.

METHODS

The study was conducted between November 1999 and January 2004. Women were recruited from family planning and general health care services at 4 sites in Harare and Chitungwiza (Zimbabwe) and 3 sites in Kampala (Uganda). Participants were aged 18 to 35 years, were HIV-negative, were sexually active, were not pregnant, and had not injected drugs or had a blood transfusion in the prior 3 months. Women were ineligible if they had a hysterectomy, had used an intrauterine device, or had a spontaneous or induced abortion in the previous month. Each site attempted to enroll equal numbers of women into 3 contraceptive groups: combined oral contraceptive (COC; low-dose pills containing 30 μg of ethinylestradiol and 150 μg of levonorgestrel), depot-medroxyprogesterone acetate (DMPA; 150 mg administered every 12 weeks), or no hormonal method. Women must have been using the method for at least 3 months and intending to continue use for an additional year. The study was approved by ethical review committees of collaborating institutions in the United States, Uganda, and Zimbabwe. All women provided written informed consent before participating in the study.

Study procedures have been reported elsewhere.[16] Briefly, at screening, consenting women were counseled and tested for HIV, syphilis, and herpes simplex virus type 2 (HSV-2). Women returned within 15 days for their test results and possible enrollment. At enrollment, eligible women were interviewed in their local language about demographics, sexual and contraceptive behavior, and reproductive health. Questions about vaginal practices included: "In the last 3 months, did you ever use anything to dry or tighten your vagina for sex?" and "In the last 3 months, did you ever use anything to clean the inside of your vagina, for instance, when you're bathing?" If they answered positively, respondents were prompted about the types of products and liquids used and about the frequency of the practice in a typical month. Women were counseled on HIV risk reduction and contraception and received condoms and contraceptives free of charge. A standardized physical and pelvic examination was conducted, and specimens were collected to test for various RTIs. Follow-up visits were conducted every 12 weeks for 15 to 24 months. Follow-up procedures were similar to those at enrollment and included testing for HIV, HSV-2, and other RTIs; women were tested for syphilis biannually.

Vaginal infections were treated on-site, whereas women diagnosed with *Chlamydia*, gonorrhea, or syphilis were recalled for treatment if they had not already been treated based on symptoms. *Chlamydia* was usually treated with doxycycline (100 mg twice daily for 7 days), although azithromycin (1 g) was also used occasionally in Uganda; pregnant women received erythromycin (500 mg 4 times daily for 7 days). Gonorrhea was treated with intramuscular kanamycin (2 g) or norfloxacin (800 mg) in Zimbabwe and with ciprofloxacin (400 mg) in Uganda. Trichomoniasis was treated with metronidazole (2 g) in both countries. BV was also treated with metronidazole but using different doses: 400 mg 3 times daily for 7 days in Zimbabwe and 2 g once, 200 mg 3 times per day for 7 days, or 400 mg 2 times per day for 7 days in Uganda. Candidiasis was treated with a single application of clotrimazole or gynodaktarin cream per day for 3 to 7 days in Zimbabwe and with clotrimazole pessaries (100 mg for 3 to 7 days) or oral nystatin (100,000 U once or twice daily for 14 days) in Uganda.

On-site wet mount microscopy was used to diagnose yeast and trichomoniasis. Yeast was defined as yeast hyphae or buds seen on wet mount (including but not limited to *Candida albicans*; yeast species were not differentiated) regardless of clinical signs and symptoms. In addition, vaginal smears were air-dried and stored for Gram staining and subsequent Nugent scoring (with BV defined as a Nugent score of 7 to 10) and identification of white blood cells (WBCs) as a marker for mucosal inflammation (scored as 0, 1 to 5, or 5+ WBCs per high-power field).[17] Microscopists in each country were trained and had their reading validated before large-scale

reading. Batches of 25 to 100 Gram-stained slides from each country were shipped to the University of California, San Francisco (UCSF) *Chlamydia*/Virology Research Laboratory regularly throughout the slide-reading period for external quality control; in almost all cases, discrepancies in Nugent score categories between local and UCSF microscopists remained <20% in both countries. Vaginal pH was measured by wetting a pH strip with vaginal fluid.

HIV diagnosis was based on serology using an enzyme immunoassay (EIA) with confirmation by at least 1 rapid test and a positive Western blot test. Timing of HIV infection was determined by testing stored dried blood spot samples by HIV DNA polymerase chain reaction (PCR) using Amplicor v1.5 (Roche Diagnostics, Branchburg, NJ). After serologic confirmation of infection, the previous visit specimens were tested serially until a negative HIV DNA PCR result was obtained. The HIV infection visit was defined as the earliest HIV DNA PCR-positive visit. HSV-2 serology was performed by an EIA (Focus Diagnostics, Cypress, CA). Syphilis was diagnosed by rapid plasma reagin (RPR) testing with *Treponema pallidum* hemagglutination test (TPHA)/*Treponema pallidum* particle agglutination (TPPA) assay confirmation (Serodia, Fujirebo, Tokyo, Japan). *Neisseria gonorrhoeae* and *Chlamydia trachomatis* were identified by Amplicor PCR (Roche Diagnostics, Branchburg, NJ).

Analyses were conducted for the 2 countries combined and for each country separately. When results are shown for the 2 countries combined, the results for each country were similar unless specifically stated otherwise. Analyses with incident HIV as the endpoint included participants with at least 1 follow-up visit with valid HIV results. Comparisons of baseline characteristics among groups were assessed using χ^2 tests. Predictors of BV, yeast, and vaginal practices at baseline were determined by backward stepwise logistic regression with $P > 0.05$ as the significance level for removal from the model. The start date of an incident infection was defined as the date the first positive specimen was collected or the clinician observed the finding. Any persistent positive results were excluded from the model. Generalized estimating equation (GEE) models were used to model prevalence over time, and stepwise Cox proportional hazards regression analysis (with $P > 0.05$ as the significance level for removal from the model) was used to model incidence. Temporal relationships between BV, yeast, vaginal practices, and HIV were assessed by determining if the effect measures changed if not only infections at the same visit as the HIV seroconversion were included (current visit) but infections at the visit before that (previous visit) or a combination of visits (previous or current visit and previous and current visit). Potential confounding factors were selected a priori. All analyses were controlled for country (if multiple countries were included in model) and hormonal contraception (taking method switches during the study into account). Analyses were not controlled for treatment because of collinearity with the presence of infections.

RESULTS

A total of 2296 women in Zimbabwe and 2235 women in Uganda were enrolled in the study. The 24-month retention rate was 88% in Zimbabwe and 96% in Uganda. Mean follow-up was 21.9 months; median time between study visits was 81 days. The Ugandan cohort included a higher proportion of women who engaged in risk behaviors or had partners who engaged in risk behaviors (Table 1). Two thirds of women in Zimbabwe (66%) and Uganda (69%) reported vaginal practices at baseline. The most common practice was vaginal cleansing with water (63% and 68%, respectively) or with water and soap (11% and 45%, respectively), using a finger (57% and 64%, respectively) or a piece of cloth (7% and 5%, respectively). Vaginal drying or tightening was common in Zimbabwe only (12%). Women in Zimbabwe cleansed an average of once per day, and women in Uganda cleansed an average of 1.7 times per day.

HIV prevalence at screening and HIV incidence during the study were 38% and 4.1 per 100 woman-years (wy) in Zimbabwe and 16% and 1.5 per 100 wy in Uganda (see Table 1; Table 2). For HSV-2, baseline prevalence and incidence were 53% and 8.6 per 100 wy for Zimbabwe and 50% and 10.6 per 100 wy for Uganda. The prevalence of BV was high at enrollment and at all follow-up visits, ranging from 19% to 29% based on Nugent scoring for both countries combined, with only minor differences between countries (data not shown). Yeast and WBCs were also commonly seen at baseline and during follow-up (see Tables 1, 2). Nonviral STIs were found in 10% of Zimbabwean women and 8% of Ugandan women at baseline; incidence rates for individual nonviral STIs are shown in Table 2.

In this study, strong negative associations were found between BV and yeast, and positive associations were found between yeast and WBCs (Table 3). BV was not consistently associated with WBCs. BV was associated with a high vaginal pH, and yeast was associated with a low vaginal pH. At baseline, WBCs were also positively associated with clinician-observed vaginal inflammation (odds ratio [OR] = 1.99, 95% confidence interval [CI]: 1.25 to 3.18), with clinician-observed mucopurulent cervicitis (OR = 2.87, 95% CI: 1.15 to 7.15), and with trichomonads on wet mount (OR = 2.03, 95% CI: 1.43 to 2.89) and were negatively associated with the presence of any asymptomatic vaginal or cervical infection (OR = 0.60, 95% CI: 0.41 to 0.87), defined as having *N. gonorrhoeae*, *C. trachomatis*, *Trichomonas vaginalis*, BV and/or yeast but not reporting any genital symptoms.

In univariate Cox models using data from both countries, vaginal practices (any practice in the past 3 months) were associated with a decreased incidence of yeast (hazard ratio [HR] = 0.93, 95% CI: 0.84 to 1.02) and WBCs (HR = 0.93, 95% CI: 0.88 to 0.98), but they were not associated with BV. Vaginal drying or tightening (in the past 3 months) was associated with increased incidence of BV (HR = 1.19, 95% CI: 1.07 to 1.32) and yeast (HR = 1.44, 95% CI: 1.28 to 1.63) and decreased incidence of WBCs (HR = 0.81, 95% CI: 0.74 to 0.88). None of these associations persisted in the multivariate models, however.

In multivariate GEE and Cox models using data from both countries, other statistically significant predictors for BV included lack of hormonal contraception (COC and DMPA use were negatively associated with BV prevalence and incidence), older age, younger age at first sex, current breast-feeding, lack

TABLE 1. Participant Characteristics at Enrollment by Country

Characteristic	Zimbabwe % (N = 2296)	Uganda % (N = 2235)	Total % (N = 4531)
COC cohort	37.6	32.2	34.9
DMPA	34.5	33.2	33.9
No hormonal method	27.9	34.5	31.2
Mean age, y (range)	25.9 (18 to 35)	24.9 (18 to 35)	25.4 (18 to 35)
Mean education, y (range)*	9.7 (0 to 17)	8.6 (0 to 19)	9.1 (0 to 19)
Living with partner*	92.8	73.4	83.2
Mean age at first sex, y (range)*	18.5 (12 to 32)	16.6 (7 to 28)	17.5 (7 to 32)
Mean no. live births (range)*	2.2 (0 to 9)	2.1 (0 to 10)	2.16 (0 to 10)
Pregnancy in past 12 mo	20.6	20.6	20.6
Currently breast-feeding*	29.9	25.9	27.9
Median no. sex partners past 12 mo*	1.0 (1 to 63)	1.0 (1 to 800)	1.0 (1 to 800)
Condom use in past 3 mo; all partners combined*			
Consistent use or no sex	24.3	22.7	23.5
Inconsistent condom use	20.6	16.6	18.6
No condom use	55.1	60.7	57.9
Risk attributable to women's own behavior*†	0.7	8.6	4.6
Risk from primary partner*‡	36.2	55.1	45.4
Any vaginal practice in past 3 mo*§	65.8	68.7	67.2
Cleansed vagina in past 3 mo*‖	64.1	68.4	66.2
Dried/tightened vagina in past 3 mo*¶	12.4	1.7	7.1
BV by Nugent scoring (range: 7 to 10)*	29.2	21.4	25.3
Yeast on wet mount*	14.7	4.5	9.7
WBCs on Gram stain (1+)*	16.3	24.4	20.3
Any nonviral STI*#	10.0	7.8	8.9
HSV-2–positive at baseline*	53.4	50.3	51.9
Any STI history***	10.9	34.3	22.5
Any self-reported RTI symptoms in past 12 mo*††	28.2	57.1	42.5
Antibiotic or antifungal prescribed at baseline*	28.0	16.9	22.5

*Difference is statistically significantly different at the $P < 0.05$ level.
†Includes new sex partner, multiple sex partners, or commercial sex in past 3 months.
‡Includes partner who is known to be HIV-positive, has abnormal discharge from penis, has weight loss, and has commercial sex or spent nights away from home.
§Includes women who said "yes" to at least 1 of the following 2 questions: "In the last 3 months, did you ever use anything to dry or tighten your vagina for sex?" and "In the last 3 months, did you ever use anything to clean the inside of your vagina, for instance, when you're bathing?"
‖Includes all women who said "yes" to the following question: "In the last 3 months, did you ever use anything to clean the inside of your vagina, for instance, when you're bathing?"
¶Includes all women who said "yes" to the following question: "In the last 3 months, did you ever use anything to dry or tighten your vagina for sex?"
#Includes syphilis, gonorrhea, *Chlamydia*, and trichomoniasis.
**Includes women who ever had genital sores/ulcers, genital warts, pelvic inflammatory disease, or self-reported gonorrhea or *Chlamydia* in their lifetime.
††Includes women who reported having abnormal vaginal discharge, genital itching, lower abdominal pain not related to menstruation, pain during sex, or bleeding between periods in the past 12 months.

of condom use, any STI history, the woman's own risk behavior, and the primary partner's risk behavior (data not shown). Predictors for yeast included lack of hormonal contraception, higher parity, currently pregnant, currently breast-feeding, lack of condom use, and any STI history (data not shown).

BV was independently associated with HIV incidence (HR = 1.67, 95% CI: 1.24 to 2.26; Table 4). When the Gram stain Nugent score was considered categorically, scores of 7 to 10 (BV) and 4 to 6 (intermediate flora) compared with a score of 0 to 3 were significantly associated with HIV acquisition (HR = 2.29, 95% CI: 1.61 to 3.27 and HR = 2.16, 95% CI: 1.48 to 3.16, respectively). Vaginal yeast was associated with HIV acquisition in Zimbabwe (HR = 1.68, 95% CI: 1.07 to 2.66) but not in Uganda. Vaginal pH was associated with HIV incidence in univariate models (HR = 1.65, 95% CI: 1.30 to 2.10) but dropped out of the multivariate model, probably because of its strong associations with BV and yeast. Neither WBCs on Gram stain nor vaginal practices were associated with HIV acquisition. Vaginal drying and/or tightening was significantly associated with HIV acquisition in the univariate model (HR = 1.49, 95% CI: 1.03 to 2.15), however. In addition to these predictors of interest, the HIV incidence model in Table 4 includes several other risk factors for HIV incidence as described in the table and footnotes. HSV-2 and nonviral STIs were the strongest independent risk factors for HIV incidence. Trichomoniasis (diagnosed by wet mount) was included in the composite nonviral STI variable but was not entered in the model as a separate covariate because it was not associated with HIV incidence in this study.

We also considered the association between BV and HIV among some important subgroups. For example, among women who also had vaginal yeast, the multivariate HR for BV was 2.18 (95% CI: 0.95 to 5.00), whereas among women without vaginal yeast, the multivariate HR for BV was 1.70 (95% CI: 1.23 to 2.34). For women with vaginal yeast only (and not BV),

TABLE 2. Incidence of Infections and WBCs per 100 wy of Follow-Up by Country

	Zimbabwe Incidence (95% CI)	Uganda Incidence (95% CI)	Total Incidence (95% CI)
HIV*	4.12 (3.50 to 4.82)	1.53 (1.18 to 1.96)	2.77 (2.41 to 3.16)
BV by Nugent score*	50.91 (48.30 to 53.49)	45.93 (43.65 to 48.27)	48.30 (46.60 to 50.03)
Yeasts on wet mount*	39.13 (37.06 to 41.23)	19.38 (18.00 to 20.83)	28.86 (27.63 to 30.11)
WBC on wet mount	75.12 (72.10 to 78.15)	107.66 (104.12 to 111.28)	91.54 (89.21 to 93.90)
N. gonorrhoeae by PCR*	3.71 (3.10 to 4.39)	5.22 (4.53 to 5.99)	4.49 (4.02 to 5.00)
C. trachomatis by PCR	3.68 (3.08 to 4.36)	4.14 (3.52 to 4.83)	3.92 (3.48 to 4.39)
T. vaginalis by wet mount	5.84 (5.08 to 6.68)	6.03 (5.27 to 6.86)	5.93 (5.39 to 6.51)
Syphilis by RPR and TPHA*	0.87 (0.60 to 1.23)	1.52 (1.16 to 1.95)	1.21 (0.98 to 1.48)
Any nonviral STI*†	12.28 (11.12 to 13.50)	14.60 (13.38 to 15.89)	13.47 (12.63 to 14.36)
HSV-2	8.58 (7.22 to 10.12)	10.63 (9.17 to 12.25)	9.65 (8.63 to 10.74)

*Difference is statistically significantly different at the $P < 0.05$ level.
†Includes syphilis, gonorrhea, *Chlamydia*, and trichomoniasis.

the multivariate HR was 1.50 (95% CI: 0.92 to 2.45). The association between BV and HIV did not change according to contraceptive use group (P value for interaction = 0.64).

A temporal relation between BV and HIV incidence and between vaginal yeast and HIV incidence was found (Table 5). Both associations were stronger when the condition was present at the same visit as the new HIV infection and the visit preceding it. This temporal relation was especially noteworthy for vaginal yeast.

DISCUSSION

In this study, BV and vaginal yeast were independently associated with HIV acquisition; vaginal cleansing, vaginal pH, and the presence of WBCs were not. Vaginal practices were associated with altered vaginal flora in univariate but not multivariate models and were not associated with the presence of WBCs.

The finding that women with BV or yeast are more likely to acquire HIV is in agreement with 4 other studies that

TABLE 3. Vaginal Environment as a Continuum: Relationships Between BV, Yeast, WBCs, and Vaginal pH Among Women in Zimbabwe and Uganda

All Sites				
Outcome	**Predictor**	**OR/HR**	**95% CI**	***P***
Relationships among prevalences at baseline (logistic regression models)*†				
BV	Yeast	0.56	0.43 to 0.73	<0.001
BV	WBCs	0.84	0.79 to 1.00	0.052
BV	Vaginal pH‡	2.63	2.29 to 3.02	<0.001
Yeast	WBCs	1.49	1.17 to 1.90	0.001
Yeast	Vaginal pH	0.74	0.60 to 0.91	0.005
Relationships among prevalences during all follow-up visits (GEE models)*				
BV	Yeast	0.63	0.56 to 0.71	<0.001
BV	WBCs	0.87	0.82 to 0.92	<0.001
BV	Vaginal pH	3.36	3.16 to 3.56	<0.001
Yeast	WBCs	1.63	1.50 to 1.77	<0.001
Yeast	Vaginal pH	0.84	0.77 to 0.91	<0.001
Relationships among incidence rates during follow-up (Cox proportional hazards models)*				
BV	Yeast	0.74	0.65 to 0.85	<0.001
BV	WBCs	0.96	0.89 to 1.04	0.3577
BV	Vaginal pH	2.44	2.30 to 2.59	0.031
Yeast	WBCs	1.60	1.46 to 1.76	<0.001
Yeast	Vaginal pH	0.84	0.77 to 0.92	<0.001

*Each line in the table represents 1 model, including the outcome and main predictor listed in the model and country and contraceptive cohort.
†Wald confidence limits and Fisher exact P value.
‡Vaginal pH was entered in models as a continuous variable.

TABLE 4. Multivariate Associations Between BV, Yeast, WBCs, Vaginal Practices, and Incident HIV Infection Among Women in Zimbabwe and Uganda

Predictors (All Current Visits)	HR	95% CI	P*
BV by Nugent score†	1.67	1.24 to 2.26	<0.001
Yeast on wet mount‡	1.44	0.95 to 2.20	0.094
WBCs on Gram stain	1.05	0.77 to 1.43	0.762
Any vaginal practice in past 3 mo§	0.81	0.59 to 1.10	0.175
Zimbabwe – Uganda	3.11	2.20 to 4.41	<0.001
COC – no hormones	0.86	0.60 to 1.24	0.415
DMPA – no hormones	1.29	0.90 to 1.84	0.169
No. live births (per birth)	0.84	0.74 to 0.95	0.006
Risk attributable to women's own behavior‖	1.97	1.00 to 3.87	0.051
Risk from primary partner¶	1.56	1.17 to 2.09	0.003
Any STI history#	1.82	1.10 to 3.01	0.019
Any other nonviral STI (time-varying)**	3.21	2.23 to 4.63	<0.001
HSV-2 (time-varying)	4.15	2.75 to 6.25	<0.001

*Stepwise Cox proportional hazards models with BV, yeast, WBCs, vaginal practices, and contraception forced into the model. The model includes 27,619 visit segments and 188 HIV infections. The following predictors were also entered into the model but dropped out: dried vagina in past 3 months (significant in univariate model: HR = 1.49; $P = 0.037$), vaginal pH (significant in univariate model: HR = 1.65; $P < 0.001$), age (<25 years, 25+ years; significant in univariate model: HR = 1.42; $P = 0.010$), schooling (<9 years, 9+ years), living with partner, currently pregnant, currently breastfeeding, age at first sex, and condom use in past 3 months for all partners combined. Use of antibiotics or antifungals was not included in the models because of collinearity with several predictors. Results were similar in models for each country separately with the exception of yeast on wet mount (*see below*).

†When Gram stain scores were considered categorically, scores of 7 to 10 (ie, BV) and 4 to 6 (intermediate flora) compared with a score of 0 to 3 were significantly associated with HIV acquisition (HR = 2.29, 95% CI 1.61 to 3.27 and HR = 2.16, 95% CI: 1.48 to 3.16, respectively) when controlling for variables listed previously.

‡Yeast on wet mount was associated with HIV incidence in the univariate Cox model (HR = 1.85; $P = 0.001$) and in the multivariate Cox model for Zimbabwe alone (HR = 1.68; $P = 0.025$).

§Includes women who said "yes" to at least 1 of the following 2 questions: "In the last 3 months, did you ever use anything to dry or tighten your vagina for sex?" and "In the last 3 months, did you ever use anything to clean the inside of your vagina, for instance, when you're bathing?"

‖Includes women who had multiple sex partners or a new sex partner or engaged in sex work in the past 3 months.

¶Includes women whose partner was known to be HIV-positive, had abnormal discharge from penis, had weight loss, visited a sex worker in the past 3 months, or spent nights away from home in the past month.

#Includes women who ever had genital sores/ulcers, genital warts, pelvic inflammatory disease, or self-reported gonorrhea or *Chlamydia* in their lifetime.

**Includes syphilis, gonorrhea, *Chlamydia*, and trichomoniasis. Trichomoniasis diagnosed by wet mount was not associated with HIV incidence in this study, and was therefore not included as a separate covariate.

investigated the temporal relation between BV and HIV and between yeast and HIV, prospectively,[7–9,18] and 2 nested case-control studies comparing women who seroconverted with those who did not.[19,20] Our results are more likely generalizable, however, because the other studies comprised special population groups (antenatal/postnatal women in Malawi, sex workers attending an STI clinic in Kenya, discordant couples in Zambia, and women aged 35 to 65 years participating in a cervical cancer screening program) or had high loss to follow-up.

A strong negative association between BV and yeast was found. This has been found in other studies as well.[21] BV, yeast, and vaginal pH were each associated with HIV incidence in univariate models, but vaginal pH dropped out of the final multivariate model, probably because of its strong associations with BV and yeast. These data suggest that the vaginal environment represents a continuum with normal flora (dominated by lactobacilli) and a normal vaginal pH (ranging from 3.8 to 4.5) as the ideal state and BV (accompanied by high vaginal pH) and yeast (low vaginal pH) as 2 separate unhealthy conditions that do not often overlap. Each of these unhealthy conditions may increase women's risk of HIV acquisition.

The relation between altered vaginal flora and HIV did not seem to be mediated by mucosal inflammation as defined by the presence of WBCs. Although yeast was associated with the presence of WBCs (as opposed to BV, which was not), WBCs were not associated with HIV acquisition. No other potentially relevant inflammatory markers, such as proinflammatory cytokines, were assessed in this study. It is therefore possible that inflammatory processes and/or immune activation plays a role, because the presence of WBCs on Gram stain is only a crude measure of mucosal inflammation. Recent evidence suggests that BV indeed induces various cytokines, and yeasts are known to cause immune activation.[14,15] It is also possible that BV and yeast lead to changes in epithelial integrity and/or permeability, including changes that are not visible to the naked eye during pelvic examinations. Both of these hypotheses need to be investigated further.

In our study, vaginal practices (cleansing as well as drying and tightening combined) were not associated with HIV incidence. Some evidence for vaginal drying and tightening practices increasing the risk of HIV acquisition was found, however. This effect disappeared in multivariate models, possibly because only 12% of women in Zimbabwe and hardly any women in Uganda reported engaging in these practices. Two other longitudinal studies have investigated the relation between vaginal practices and HIV. McClelland and colleagues[12] found an increased risk of HIV acquisition for vaginal washing among 1270 Kenyan female sex workers participating in a 10-year open cohort study (adjusted HR for washing with water alone = 2.64, 95% CI: 1.00 to 6.97 and adjusted HR for washing with water and soap = 3.84, 95% CI: 1.51 to 9.77). In contrast, Meyer and colleagues[13] found no association between vaginal practices and incident HIV (HR = 1.04, 95% CI: 0.65 to 1.68) among 3570 women aged 35 to 65 years participating in a cervical cancer screening trial in Cape Town, South Africa. Our results are in agreement with those from the Cape Town study but not with those from the Mombasa study. In a previous communication in response to the Mombasa study report, we hypothesized that several important differences between the study cohorts might explain the difference in findings.[22] The Kenyan sex workers were exposed to HIV at a higher level (incidence was 7.7 per 100 person-years as opposed to 2.8 per 100 person-years in our study), almost universally engaged in vaginal washing with most using soaps (as opposed to a substantial number of women in our study using nothing or just water), and cleansed an average of 3 times per day (as opposed to 1 to 1.7 times per day in Zimbabwe and Uganda).[23]

Vaginal practices were associated with altered vaginal flora in univariate models but not in multivariate models.

TABLE 5. Temporal Relationships Between BV, Yeast, Vaginal Practices, and HIV Among Women in Zimbabwe and Uganda

Predictor	HIV Events*	HR	95% CI	P†
BV by Nugent score, current visit‡	174	1.76	1.29 to 2.41	<0.001
BV by Nugent score, previous visit‡	174	1.93	1.42 to 2.63	<0.001
BV by Nugent score, current or previous visit	174	2.16	1.54 to 3.03	<0.001
BV by Nugent score, current and previous visit	174	2.50	1.68 to 3.72	<0.001
Yeast on wet mount, current visit	170	1.46	0.95 to 2.26	0.087
Yeast on wet mount, previous visit	170	1.91	1.30 to 2.81	<0.001
Yeast on wet mount, current or previous visit	170	1.40	0.95 to 2.07	0.090
Yeast on wet mount, current and previous visit	170	2.97	1.67 to 5.28	<0.001
Any vaginal practices in past 3 mo, current visit	178	0.77	0.57 to 1.05	0.102
Any vaginal practices in past 3 mo, previous visit	178	0.82	0.60 to 1.12	0.205
Any vaginal practices in past 3 mo, current or previous visit	178	0.99	0.63 to 1.56	0.965
Any vaginal practices in past 3 mo, current and previous visit	178	0.77	0.54 to 1.09	0.138

*A total of 213 HIV seroconversions occurred during the study; however, not all models included all seroconversions because of missing data on the exposure of interest.

†Cox proportional hazards models controlled for country. Restricted to women for whom all visit intervals were 6 months or less. Use of antibiotics or antifungals was not included in the models because of collinearity with the BV and yeast predictors.

‡The "current visit" is the visit during which the predictor of interest and the HIV status were assessed. The "previous visit" is the visit preceding the current visit.

The published literature is by no means conclusive about the association between vaginal practices and altered vaginal flora in sub-Saharan Africa, but studies from Europe and the United States consistently show associations between douching and BV.[6] It seems likely that the relationships between less abrasive practices (eg, washing with water) and more abrasive practices (eg, douching/use of antiseptic solutions, household or laundry soaps, use of traditional drying/tightening substances) and vaginal flora are substantially different and that the frequency of the practices could also play an important role. Some evidence for this was found in our data, with vaginal drying and tightening practices consistently showing stronger associations with altered vaginal flora than vaginal cleansing practices. Taken together, the data suggest that future studies or interventions related to vaginal practices should take the diversity of practices into account.

A few limitations of our study should be noted. Although we adjusted our analyses for several underlying risk behaviors of the woman herself and her main partner, residual confounding cannot be ruled out because we did not have complete risk-taking and exposure information for entire sexual networks and data on risk behaviors are not always reliable. Two thirds of the women in this study used hormonal contraception as determined by the study design, which is higher than in the general population. Yeast was diagnosed by wet mount microscopy and not by culture. Visible yeast on microscopy is likely to represent symptomatic candidiasis, however, whereas a positive culture also represents asymptomatic colonization.[15] Furthermore, external quality control was done on Gram stain microscopy but not on wet mount microscopy. This may explain why the relation between yeast and HIV was stronger in Zimbabwe than in Uganda (Gram stains and wet mounts were read by the same quality-controlled microscopists in Zimbabwe but not in Uganda) and why trichomoniasis was not associated with HIV incidence in this analysis. Subsequent PCR testing on a subset of specimens revealed that wet mount diagnosis of trichomoniasis was not sensitive.[24] Residual confounding by trichomoniasis of the relation between BV, yeast, and HIV may therefore have been present.

Given the high prevalence of BV and yeast in many countries with generalized HIV epidemics (eg, 29.2% and 14.7%, respectively, in Zimbabwe at baseline), a modest increase in the relative risk of HIV transmission could lead to a substantial attributable risk of HIV infection. Controlling BV and yeast should therefore be a public health priority in these countries. Studies have shown that treatment of vaginal infections leads to a reduction of genital shedding of HIV.[25] Nevertheless, the sole experimental study to treat BV for HIV prevention found that mass treatment with a single dose of metronidazole led to a modest reduction in community-based BV prevalence in the intervention arm but had no effect on HIV incidence.[26] Targeted screening for BV may not be feasible because of lack of clarity about who should be screened and technical, logistic, and financial constraints.[27] Furthermore, clinical management results for BV are often poor, which could be attributable to the fact that early recurrences after treatment are common, the drug of choice (clindamycin) is often not available in developing countries, and the less expensive alternative (metronidazole) is often not prescribed when pregnancy is suspected.[27] Interventions aimed at controlling BV and yeast, although posing challenges, could be an important HIV prevention strategy, particularly in sub-Saharan Africa, where most HIV infections among women occur.

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