Intravaginal Practices, Vaginal Flora Disturbances, and Acquisition of Sexually Transmitted Diseases in Zimbabwean Women

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One hundred sixty-nine Zimbabwean women were studied to determine whether the use of intravaginal practices (cleaning with the fingers, wiping the vagina, and inserting traditional substances) are associated with disturbances of vaginal flora and acquisition of sexually transmitted diseases (STDs). Subjects were interviewed and received counseling and a pelvic examination at enrollment, 1 month, and 6 months, and vaginal specimens were collected at enrollment and at 6 months. Users were more likely than nonusers to have vaginal flora disturbances but were not more likely to acquire an STD (relative risk [RR], 2.15; P = .188).

Certain vaginal flora disturbances were associated with increased STD incidence and HIV prevalence. The absence of lactobacilli from the vaginal flora was associated with being positive for human immunodeficiency virus in baseline (odds ratio [OR], 0.24; P = .001) and 6-month transition multivariate models (OR, 0.39; P = .025). The presence of clue cells at baseline was associated with a higher incidence of STDs (RR, 1.94; P = .025).

Lactobacilli account for >95% of the microorganisms present in the vagina in normal vaginal flora of American and European women of childbearing age and with adequate estrogen levels. [1]. In contrast, studies in Zimbabwe and elsewhere in Africa suggest that only ≈50% healthy African women harbor vaginal lactobacilli [2-4].

Lactobacilli produce a number of compounds that inhibit other microorganisms, including lactic acid, hydrogen peroxide (HPO), lactacin, and acidolin. These compounds likely play an important role in regulating the vaginal flora by providing the HPO necessary for a halide-HPO–myeloperoxidase antimicrobial system and by maintaining an acidic pH in the vagina [5]. It is well documented that the lactobacilli in women with vaginitis are often replaced by less acidophilic microorganisms. In women who have bacterial vaginosis, for instance, HPO-producing lactobacilli are replaced by Gardnerella vaginalis, gram-negative rods, and Peptostreptococcus species [6, 7]. Bacterial vaginosis is therefore often characterized by the presence of clue cells (epithelial cells covered with such microorganisms) on Gram’s stain. Yeast vulvovaginitis (caused by Candida albicans), trichomoniasis (caused by Trichomonas vaginalis), and desquamative inflammatory vaginitis (caused by group B streptococci and unknown factors) may also be caused by a replacement of lactobacilli [1].

Evidence is beginning to accumulate that disturbances in vaginal flora may lead to increased transmission of human immunodeficiency virus (HIV) and other sexually transmitted diseases (STDs). HPO-producing lactobacilli, for instance, have been shown to have a viricidal effect on cell-free HIV-1 and Neisseria gonorrhoeae in vitro [8, 9], and low vaginal pH has been shown to reduce HIV infectivity [10]. Bacterial vaginosis was associated with HIV prevalence in ≥3 cross-sectional studies [11–13] and with trichomoniasis in another cross-sectional study [6]. In a recent study in Malawi, a significant association between bacterial vaginosis and HIV acquisition in both pregnant (adjusted odds ratio [OR], 3.7) and postnatal women (adjusted OR, 2.3) was found [4]. Another recent study in Kenya found that the absence of lactobacilli from the vaginal flora of...
female sex workers at 2 subsequent visits was associated with an increased risk of acquiring HIV (hazard ratio [HR], 2; \( P = 0.01 \)) and gonococcal infection (HR, 1.7; \( P = .02 \)) [14].

In many African countries, the high prevalence of vaginal flora disturbances may therefore be directly correlated with the high prevalence of reproductive tract infections (RTIs) and heterosexual transmitted STDs and HIV. For this reason, it is important to know what causes the vaginal flora disturbances. Some studies carried out in the United States suggest that having multiple sex partners, regular douching, use of spermicides, and use of antibiotics could trigger a shift from the normal vaginal flora to one characteristic of bacterial vaginosis [1, 15–17]. Even though it has been well documented that a variety of intravaginal practices are common in Zimbabwe and several other countries in Africa [18–21], the effect of these practices on the vaginal flora has not been studied to date. Common intravaginal practices in Zimbabwe include finger-cleansing (inserting plain water, water and soap, or other liquids inside the vagina using 1 or 2 fingers), wiping (drying the vagina by inserting cloth, paper, or cotton wool), and inserting traditional substances [18–21].

The primary purpose of this study was to determine whether intravaginal practices of Zimbabwean women are associated with disturbances of the vaginal flora. The secondary purpose was to determine whether these disturbances are associated with the acquisition of an STD.

Subjects and Methods

Overview of study design. The study design consisted of a baseline survey and prospective follow-up at 1 and 6 months after enrollment. Half of the sample recruited was composed of women who engaged in intravaginal practices (users) and the other half of women who did not (nonusers). Study procedures and frequency of follow-up were identical in both groups.

Sample description. Participants were recruited from family planning clinics of the Zimbabwe National Family Planning Council, primary care clinics of the Harare City Health Department, and the postnatal clinic at Harare Central Hospital, all of which are located in high-density suburbs of Harare. Sexually active women between the ages of 18 and 45 years were eligible for the study. Pregnant women, women who had an intrauterine device in place, women who came to the clinic for a severe or chronic gynecological problems (such as infertility), and women who were not willing or able to adhere to the study protocol were excluded. To be included in the user group, the following additional eligibility criteria applied: having finger-cleansed with something other than plain water or wiped inside the vagina \( \geq 12 \) times in the last month or having inserted traditional substances in the vagina \( \geq 4 \) times in the last month. To be included in the nonuser group, a woman should not have finger-cleansed with something other than plain water, wiped, or inserted traditional substances inside the vagina in the last year and not intend to do so within the 6-month duration of the study. Because of the high prevalence of finger-cleansing in Zimbabwe, women who finger-cleansed with plain water only were also eligible for the nonuser group. Finger-cleansing with plain water was thought to be less disruptive to the vaginal flora than finger-cleansing with detergents or drying agents.

Summary of study procedures. After written informed consent was obtained, a physical and pelvic examination (during which microbiology samples and a Pap smear were taken) were performed, a face-to-face interview was conducted, HIV counseling was carried out, blood was drawn, and condoms and travel reimbursements were dispensed. A study gynecologist performed colposcopy on each participant at a separate visit a few days after her enrollment visit. All women received free treatment of STDs and genital infections according to the Zimbabwean Ministry of Health and Child Welfare guidelines [22] and free treatment of suspicious lesions identified by Pap smear or colposcopy [23]. Study procedures during follow-up were identical to those at baseline, but no microbiologic samples were collected at the 1-month follow-up visit.

Evaluation of vaginal flora. Vaginal flora was characterized by using Gram’s stain methods and a confirmatory culture for group B streptococci and \( G. \) vaginalis. A swab was taken from the external cervical os and smeared onto a glass slide for Gram’s stain identification of clue cells, white blood cells, and yeast. A high vaginal swab was smeared onto a glass slide for Gram’s stain identification of lactobacilli. A second high vaginal swab was taken, placed into a tube of Stuart’s transport medium, and inoculated onto blood agar and \( G. \) vaginalis selective agar. Group B streptococci were identified from blood agar on the basis of colony and Gram’s stain morphology and agglutination tests (Streptex; Glaxo Wellcome, Research Triangle Park, NC). Probable \( G. \) vaginalis was identified from \( G. \) vaginalis selective agar by colony and Gram’s stain morphology.

The vaginal pH was measured by smearing vaginal fluid directly onto litmus paper by using a swab. When abnormal vaginal discharge was present, a whiff test was performed. The presence of abnormal vaginal discharge, vaginal pH, and whiff test results were, however, not used as criteria for vaginal flora disturbance in this study. Many participants, particularly users, finger-cleansed and wiped their vaginas just before coming to the clinic, making detection of abnormal discharge and vaginal pH measurement unreliable.

Diagnostic tests. A swab was taken from the external cervical os and immediately inoculated onto Thayer-Martin agar for \( N. \) gonorrhoeae. Another swab was taken from the endocervix and placed into transport medium for detection of chlamydial antigen (Chlamydiazyme; Abbott Laboratories, Abbott Park, IL). A high vaginal swab was placed into Diamond’s medium for maintenance of \( T. \) vaginalis and yeasts. Blood samples were tested for syphilis by using the Rapid Plasma Reagin test (Omega Diagnostics, Alloa, Scotland, UK) and the Treponema pallidum hemaggulination assay (Omega Diagnostics). The presence of HIV antibodies was determined by ELISA (Abbott Laboratories) and a dipstick immunoassay (ImmunoChemical Laboratory, Bangkok, Thailand) [24]. Urine cultures were performed only when urinary tract infection was suspected (which occurred only once during the study).

Statistical methods. Univariate differences in categorical variables between study groups were assessed by use of 2-sided Fisher’s exact tests. Logistic regression was used to investigate predictors for the presence of lactobacilli and clue cells in the baseline survey.
The regression model included selected independent variables describing demographic and sexual behavior characteristics, the presence of STD (as determined by laboratory tests or syndromic diagnoses), HIV serostatus, and treatment characteristics. Because of the large number of additional candidate independent variables and the lack of a priori knowledge about their relative importance, backward stepwise selection was used to aid in model construction (with 0.200 and 0.150 as the significance levels for removal and addition, respectively). The main predictor was engagement in intravaginal practices, and this variable was therefore forced to stay in each model. Self-reported gynecological complaints, physical examination findings, and laboratory results were added to the model one at a time, because several clinical findings were strongly correlated with each other. Models were further investigated for potential nonlinearities (for variables modeled as continuous) and interactions.

Logistic regression models for the presence of lactobacilli and clue cells during follow-up were also constructed. These models were based on independent variables similar to those described earlier but also controlled for within-individual dependence between longitudinally measured outcomes by using a transition model formulation [25]. This allowed us to investigate the effects of covariates on vaginal flora transitions (e.g., depletion of lactobacilli given a previous normal result, or presence of lactobacilli given a previous abnormal result). Dependence on past values of vaginal flora characteristics is affected by controlling for these in the regression models. Significant interactions between previous outcomes and independent variables indicate that their effects differ depending on the value of the previous outcome.

Cumulative incidence was calculated as the number of women who experienced a new event during the 6-month follow-up period, divided by the total number of women followed up. McNemar’s matched pair analyses were carried out by matching each participant’s vaginal flora characteristics at baseline with those at 6-month follow-up, thus taking the transient nature of vaginal flora into account.

Questionnaire data at 1 month were similar to those at baseline, and microbiologic samples were not collected at the 1-month visit. Consequently, data from the 1-month follow-up visit are not discussed in this paper. Data were analyzed by using Stata 5.0 software [26].

Results

Demographics and intravaginal practices. Between April and November 1995, we enrolled a total of 169 women: 99 users and 70 nonusers. Cohort retention at 6 months was 76% for the users and 86% for the nonusers (P = .643). The mean follow-up periods for users (184 days; median, 182 days) and nonusers (180 days; median, 176 days) were also similar (P = .626).

The mean age of the cohort was 30 years. Nearly all women were married (87%) and had ≥1 child (99%). Users were slightly younger than nonusers and were of a lower socioeconomic class (as determined by educational level and type of accommodation). There were no differences in sexual and contraceptive behaviors between the 2 study groups. The women had an average of 3 sex partners in their lifetimes, and only 1 partner in the last 3 months (range, 1–6 partners). Eighty percent of the women were using hormonal contraception (low-dose combined oral contraceptive pills, Ovrette progestin-only pills [Wyeth Laboratories, Philadelphia], Depo Provera injections [Upjohn Pharmaceuticals, Kalamazoo, MI], or Norplant insertions [Leira Laboratories, Turku, Finland]) at baseline.

All users reported that they finger-cleanse an average of once or twice per day with a variety of liquids (water and soap, Dettol or Betadine liquid germicidal soaps, salt solution, lemon juice, or vinegar). Almost all users (96%) reported drying the vagina by inserting cloth, paper, or cotton wool after finger-cleansing and after sex, and 49% of them reported inserting traditional substances (derived from herbs, trees, stones, and soil) a few times per month to dry or tighten the vagina. By definition, none of the nonusers finger-cleanse with liquids other than plain water, wiped, or inserted traditional substances. However, 91% of the nonusers finger-cleanse with plain water once or twice per day, and only 9% did not finger-cleanse at all. The women started having sex at a mean age of 18 years and started intravaginal practices at a mean age of 21 years. All but 3 women who reported intravaginal practices at baseline, including finger-cleansing with plain water, had engaged in these practices for ≥1 year, and 84% for ≥2 years. There were no crossovers between study groups during follow-up.

Prevalence of vaginal flora characteristics at baseline and 6-month follow-up. Table 1 shows that lactobacilli were present in the vaginal flora of 31% of the users and 46% of the nonusers at baseline (P = .054). The prevalence of lactobacilli was 40% and 45%, respectively, at 6-month follow-up (P = .601). Clue cells and group B streptococci were common, and they were found more frequently in users than nonusers at baseline (35% and 21% for clue cells; P = .061; 26% and 15% for group B streptococci; P = .106). Yeasts were frequently found in both groups at all visits (table 1). Only 2 women (both users) tested positive for G. vaginalis.

Our study confirmed that vaginal flora characteristics are of a transient nature. Twenty-eight of the 94 women who had no vaginal lactobacilli at baseline had normal flora at 6-month follow-up. In contrast, 22 of the 51 women who had normal flora at baseline had no vaginal lactobacilli at 6-month follow-up (McNemar’s OR, 1.27; 95% CI, 0.70–2.33; P = .396). When the women were stratified by study group, the McNemar’s OR was 1.45 (95% CI, 0.63–3.47; P = .336) for users and 1.09 (95% CI, 0.44–2.73; P = .835) for nonusers. Similar statistically non-significant results were found for yeasts and group B streptococci. Clue cells, however, showed a different pattern. Only 4 users who were clue-cell negative at baseline were positive at 6-month follow-up, whereas 21 users were clue-cell positive at baseline but negative at 6-month follow-up (McNemar’s OR,
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Table 1. Prevalence of vaginal flora, human immunodeficiency virus, and sexually transmitted diseases (STDs) among the study group of Zimbabwean women at baseline (n = 169) and at 6-month follow-up (n = 135).

<table>
<thead>
<tr>
<th>Vaginal flora (Gram’s stain)</th>
<th>Baseline</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Users (n = 99)</td>
<td>Nonusers (n = 70)</td>
<td>P*</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>30.9</td>
<td>45.7</td>
</tr>
<tr>
<td>Clue cells</td>
<td>35.1</td>
<td>21.4</td>
</tr>
<tr>
<td>Yeasts</td>
<td>38.1</td>
<td>35.7</td>
</tr>
<tr>
<td>GBS</td>
<td>26.3</td>
<td>14.7</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>2.3</td>
<td>0</td>
</tr>
</tbody>
</table>

HIV and other STDs

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Users (n = 99)</td>
<td>Nonusers (n = 70)</td>
<td>P*</td>
</tr>
<tr>
<td>HIV</td>
<td>35.1</td>
<td>30</td>
</tr>
<tr>
<td>Confirmed STDb</td>
<td>12.4</td>
<td>7.1</td>
</tr>
<tr>
<td>Possible STDb</td>
<td>22.7</td>
<td>17.1</td>
</tr>
</tbody>
</table>

NOTE. Data are % of patients. GBS, group B streptococci.

a Determined by 2-sided Fisher’s exact test.
b Defined as a positive laboratory test for gonorrhea, Chlamydia, trichomoniasis, or syphilis.

* Includes abnormal vaginal or cervical discharge indicative of STD, genital warts, genital herpes, cervical friability/cervicitis, and clinical diagnosis of pelvic inflammatory disease.

0.19; 95% CI, 0.05–0.57; P < .001). The McNemar’s OR was 0.70 (95% CI, 0.23–2.04; P = .467) for nonusers.

Prevalence and incidence of HIV and other STDs. Table 1 shows that there were no statistically significant differences in prevalence of HIV, laboratory-confirmed STDs (gonorrhea, chlamydia, trichomoniasis, or syphilis), or findings suggestive of STDs (abnormal discharge, cervicitis, pelvic inflammatory disease, genital warts, herpes, or ulcers) between the 2 study groups at baseline and 6-month follow-up. At each study visit, ~1/3 of the participants (28%, 25%, and 33% respectively) received treatment for an RTI, and a total of 55% of the participants received antibiotic or antifungal treatment at least once during the study.

The baseline HIV prevalence was 35% in the user group and 30% in the nonuser group, with no new HIV infections during follow-up. Users were not statistically significantly more likely to develop a laboratory-confirmed STD during follow-up than were the nonusers (relative risk, 2.15; 95% CI, 0.64–9.28; P = .188). Incidence rates for individual STDs in the entire cohort were 1.5 for gonorrhea, 7.4 for chlamydia, 7.4 for trichomoniasis, and 7.4 for syphilis per 100 person-years of follow-up.

Predictors for the presence of lactobacilli in the vaginal flora. The baseline multivariate model in table 2 shows that users were statistically significantly less likely to have lactobacilli in their vaginal flora than nonusers (OR, 0.44; 95% CI, 0.22–0.89; P = .022) after controlling for several potential confounding variables (see table 2, footnotes). No statistical interactions were found. Use of a hormonal method of contraception (low-dose combined oral contraceptive pills, Progesterin-only pills, Depo Provera injections, or Norplant insertions, as compared with no modern method of contraception or inconsistent condom use) was associated with having lactobacilli in the vaginal flora (OR, 2.38; 95% CI, 0.93–6.08; P = .070), but this did not reach statistical significance. Women who were HIV-infected were much less likely (OR, 0.24; 95% CI, 0.11–0.55; P = .001) to have lactobacilli in their vaginal flora.

In contrast, no association between intravaginal practices and the presence of lactobacilli at 6-month follow-up was found in the transition model (OR, 0.79; 95% CI, 0.38–1.68; P = .554). The presence of lactobacilli at the 6-month follow-up visit was strongly associated with the presence of lactobacilli at baseline (OR, 2.38; 95% CI, 1.10–5.14; P = .027). Other variables associated with the presence of lactobacilli at 6-month follow-up were being HIV positive (OR, 0.39; 95% CI, 0.17–0.89; P = .025) and frequency of sex in the preceding 4 weeks (OR, 1.06; 95% CI, 0.99–1.13; P = .094), but the latter did not reach statistical significance. A significant interaction between the presence of lactobacilli at baseline and condom use was found (P = .019), but it did not change the overall conclusions of the model in table 2.

To determine which self-reported gynecological complaints, physical examination findings, and laboratory results were associated with the presence of lactobacilli (in addition to HIV serostatus), all such findings were added to the baseline and transition models one at a time (see Subjects and Methods). Only findings that were associated with the presence of lactobacilli at P<.150 are shown in table 2. Women with normal vaginal flora at baseline were less likely to have dysuria (OR, 0.26; 95% CI, 0.08–0.79; P = .018), a friable cervix during the physical examination (OR, 0.17; 95% CI, 0.04–0.69; P = .013), dysplasia on Pap smear (OR, 0.07; 95% CI, 0.01–0.44; P = .005), and clue cells on Gram’s stain (OR, 0.11; 95% CI, 0.02–0.57; P = .009). They were, however, more likely to have abnormal vaginal discharge at the 6-month pelvic examination (OR, 3.40; 95% CI, 1.38–8.35; P = .008).

Predictors for the presence of clue cells in the vaginal flora.
Table 2. Odds ratios (ORs) for the presence of lactobacilli in sequentially defined multivariate models.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Baseline model&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Transition model&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 162)</td>
<td>(n = 132)</td>
</tr>
<tr>
<td>Intravaginal practices</td>
<td>0.44 (0.22–0.89)</td>
<td>0.79 (0.38–1.68)</td>
</tr>
<tr>
<td>Frequency of sex in the 4 w before BL</td>
<td>2.38 (1.10–5.14)</td>
<td>1.06 (0.99–1.13)</td>
</tr>
<tr>
<td>Use of hormonal contraceptives at BL&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.38 (0.93–6.08)</td>
<td>0.07</td>
</tr>
<tr>
<td>Breast-feeding during study period</td>
<td>1.84 (0.75–4.52)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Clinical findings and lab results<sup>g</sup>

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain during sex</td>
<td>0.46 (0.17–1.21)</td>
</tr>
<tr>
<td>Dysuria&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26 (0.08–0.79)</td>
</tr>
<tr>
<td>Friable cervix on PE</td>
<td>0.17 (0.04–0.69)</td>
</tr>
<tr>
<td>Abnormal discharge on PE</td>
<td>3.4 (1.38–8.35)</td>
</tr>
<tr>
<td>Mucosal dryness on PE</td>
<td>0.64 (0.37–1.11)</td>
</tr>
<tr>
<td>Dysplasia on Pap smear</td>
<td>0.07 (0.01–0.44)</td>
</tr>
<tr>
<td>Yeast on Gram’s stain</td>
<td>2.13 (0.90–5.05)</td>
</tr>
<tr>
<td>Chac cells on Gram’s stain</td>
<td>0.11 (0.02–0.57)</td>
</tr>
</tbody>
</table>

NOTE. BL, baseline; CI, confidence interval; HIV, human immunodeficiency virus; PE, pelvic examination.
<sup>a</sup> Defined as lactobacilli present at baseline. The following variables were considered in the baseline model but did not remain: age, socioeconomic status, number of children, current condom use, use of antibiotics in the 2 weeks before the baseline visit, any other treatment received, and the presence of a sexually transmitted disease (STD) at baseline (as determined either by a positive laboratory result or by syndromic assessment).
<sup>b</sup> Defined as lactobacilli present at 6 months. The following variables were considered in the transition model but did not remain: age, socioeconomic status, number of children, current condom use at 6-month follow-up, breast-feeding at any time during the study, use of antibiotics in the 2 weeks before the baseline and 6-month visits, any other treatment received, and the presence of an STD at 6-month follow-up (as determined either by a positive laboratory result or by syndromic assessment).
<sup>c</sup> Determined by backward stepwise multiple logistic regression with 0.200 as the significance level for removal from the model and 0.150 as the significance level for addition to the model.
<sup>d</sup> An interaction was found between condom use and the presence of lactobacilli at baseline (P < 0.019). Among condom users, but not among nonusers, the presence of lactobacilli at baseline and at 6-month follow-up was strongly correlated. There was no association between use of intravaginal practices and the presence of lactobacilli at 6-month follow-up regardless of condom use.
<sup>e</sup> Hormonal contraception included low-dose combined oral contraceptives, Progestin-only pills, Depo Provera injections, and Norplant insertions. The comparison group included women who were not using any modern method of contraception or were inconsistent condom users.
<sup>f</sup> There were no new HIV infections during follow-up.
<sup>g</sup> Variables were added to the baseline and transition models one at a time because they were strongly correlated with each other.
<sup>h</sup> Self-reported at any time during the year before enrollment.

Table 3 shows that users were more likely than nonusers to have clue cells in their vaginal flora at baseline (OR, 2.08; 95% CI, 1.43–3.02; P = 0.049) after controlling for various potential confounding variables (see table 3, footnotes). This association did not persist in the transition model (OR, 0.38; 95% CI, 0.21–0.63; P = 0.303), partially because of the strong association between the presence of clue cells at baseline and 6-month follow-up (OR, 2.91; 95% CI, 1.05–8.07; P = 0.040). A few statistical interactions were found (see table 3, footnotes), but they did not change the overall conclusions of the transition model.

A variety of self-reported complaints and clinical and laboratory findings were associated with the presence of clue cells in the baseline and transition models. Only findings that were associated with the presence of clue cells at P < 0.150 are shown in table 3. Women who had clue cells on the baseline Gram’s stain were less likely to have lactobacilli (OR, 0.30; 95% CI, 0.13–0.68; P = 0.004) on the same Gram’s stain. Other variables associated with the presence of clue cells at the 6-month follow-up visit were having had genital warts in the year before enrollment (OR, 6.42; 95% CI, 1.09–37.77; P = 0.040) and having swollen inguinal lymph nodes (OR, 4.96; 95% CI, 1.33–18.47; P = 0.017) and abnormal vaginal discharge (OR, 3.24; 95% CI, 0.89–11.81; P = 0.087) at the 6-month pelvic examination. The latter did not reach statistical significance.

Vaginal flora characteristics and incidence of STDs. To investigate whether disturbances in vaginal flora were associated with increased incidence of STDs, we compared the incidence of STDs during the 6-month follow-up period in women with and without certain vaginal flora characteristics at baseline. We could not compare the incidence of HIV in the various groups, because there were no new HIV infections during follow-up. Table 4 shows that there was no difference in STD incidence in women with and without lactobacilli, yeasts, or group B streptococci at baseline. Women who tested positive for clue cells at baseline, however, were 1.94 times more likely to develop an STD during follow-up than women who tested negative (95% CI, 1.15–3.28; P = 0.02). The same trends were found when the data were stratified by HIV serostatus.

We also determined whether women who were persistently lactobacillus negative at both visits (baseline and 6-month fol-
low-up) as compared with those transiently positive or persistently positive had a higher incidence of STDs. We did not find a difference between the proportions of women acquiring an STD in each of these 3 groups (29.5% for women who were persistently negative, 21.8% for women who were transiently positive, and 29% for women who were persistently positive). Similarly, we did not find any differences for yeast and group B streptococci. However, we did find a statistically significant trend for clue cells: 40% of the women who had clue cells at both study visits acquired an STD during follow-up, as compared with 36.7% of the women who had clue cells at one study visit, and 19.3% of the women who had no clue cells at both visits (Cuzick nonparametric test for trend, \( P = .020 \)).

**Discussion**

This study confirms that vaginal flora disturbances are indeed common in Zimbabwean women. Less than 46% of study participants throughout the study had lactobacilli present in their vaginal flora, and the prevalences of clue cells, yeasts, group B streptococci, and clinical signs and symptoms of RTIs were high.

Users of intravaginal practices were more likely than non-users to have disturbances of the vaginal flora, as characterized by the absence of lactobacilli and the presence of clue cells, in baseline univariate and multivariate analyses. This was not confirmed at 6-month follow-up, possibly because more than half of the participants received treatment for an RTI while in the study (even though this variable was added to all multivariate models), or because vaginal flora characteristics are of a transient nature. This transient nature also complicates the interpretation of the observed associations between intravaginal practices and disturbances of the vaginal flora at baseline. Although it seems likely that certain intravaginal practices disrupt growth and metabolic conditions for lactobacillus species in the vagina, it is also possible that the displacement of lactobacilli by anaerobes or other pathogens causes discomfort for the woman, prompting her to finger-cleanse or wipe.

Several self-reported complaints, clinical findings, and laboratory results were associated with the absence of lactobacilli
and the presence of clue cells in multivariate models, suggesting that these vaginal flora characteristics are associated with RTIs. The presence of lactobacilli and clue cells were negatively associated: women with lactobacilli were much less likely to have clue cells and vice versa, confirming that lactobacilli are often replaced by clue cells and other microorganisms. Estrogen levels may also play a role in the regulation of vaginal flora. In this study, only one of the associations between hormonal contraception, breast-feeding, and vaginal flora characteristics was statistically significant, but this was likely due to a lack of statistical power. Further study in this area is needed.

Although we controlled for a variety of potential confounders in all multivariate analyses presented in this paper (such as age, socioeconomic status, number of children, frequency of sex in the 4 weeks before each examination, current condom use, use of antibiotics in the 2 weeks before examination, any other treatment received, and the presence of HIV and other STDs), we could not control for the characteristics of the male partner. However, we are not aware of any sexual or hygienic practices that are common among Zimbabwean but not American or European men that could explain the stark differences in vaginal flora between Zimbabwean and American or European women. An exception is the high prevalence of STDs and HIV in Zimbabwe as compared with American or European men. We did, however, control all analyses for the presence of STDs and HIV in the female participants, which is a marker for the presence of STDs and HIV in their male partners. A longitudinal couples study may be well suited to further study the temporal relationship between intravaginal practices and vaginal flora disturbances and the effect of male partner characteristics on this relationship.

It should be noted that the nonuser group consisted mainly of women who finger-cleansed with plain water only, despite intensive efforts to also recruit women who did not engage in intravaginal practices at all. Finger-cleansing with plain water could have an effect on the vaginal flora, thereby weakening our ability to determine the effects of other intravaginal practices on the vaginal flora. Furthermore, the effect of finger-cleansing with plain water itself could not be determined. Our only alternative, however, would have been to actively discourage finger-cleansing with plain water in the nonuser group.

The next question we would like to explore is whether women with vaginal flora disturbances were at higher risk for HIV and other STDs. This study was not designed to examine the association between vaginal flora disturbances and HIV incidence, but we were able to explore associations with HIV prevalence at baseline and STD incidence. We found that women without lactobacilli were much more likely to be HIV positive in the baseline and transition multivariate models and that women with clue cells at baseline were more likely to acquire an STD during follow-up. Because we could not examine HIV incidence, we cannot distinguish between the following 2 scenarios: (1) HIV infection alters the vaginal flora; or (2) the altered vaginal flora (and inflammation that may be the result of that) facilitates HIV transmission. There are, however, a number of observations that suggest the latter. First, the relative absence of lactobacilli in women in Zimbabwe has been documented since the early 1980s, before HIV infection became prevalent [2]. The HIV epidemic appears to have had little impact on the prevalence of lactobacilli, with only 40% of healthy pregnant women having lactobacilli in 1982 compared with 31%-46% in this study. Second, bacterial vaginosis and nonulcerative STDs have been shown to facilitate HIV transmission in a few prospective cohort studies [4, 27], and in this study, the presence of clue cells was associated with a higher incidence of STDs. Finally, it is biologically plausible, and it has been shown in vitro, that HPO-producing lactobacilli inhibit the survival of HIV in the genital tract [8]. Nonetheless, large cohort studies that have adequate statistical power to address the relationships between intravaginal practices, vaginal flora disturbances, and HIV acquisition are urgently needed.

In this study, we were not able to use clinical diagnostic criteria for bacterial vaginosis (the presence of abnormal vaginal discharge, vaginal pH > 4.5, and a positive whiff test) in our analyses. Many women, particularly users, finger-cleansed and wiped their vaginas before coming to the clinic, thereby making the measurement of these criteria unreliable. However, several studies have shown a high correlation between the clinical diagnosis of bacterial vaginosis and the presence of lactobacilli and clue cells on Gram’s stain [28, 29]. We were also not able to establish the presence of HPO-producing lactobacilli by culture. Although the detection of lactobacilli by Gram’s stain alone is more reliable than culture, it is nonspecific [30]. The role of HPO-producing lactobacilli in the various relationships described in this paper should be investigated further. Finally, we could not determine the effect of vaginal flora disturbances on the acquisition of each different type of STD because our data lacked statistical power. This should also be addressed in future studies.

Intravaginal practices are widespread in sub-Saharan Africa, and many cases of STDs and HIV may therefore be attributable to them. Further studies are urgently needed to gain a better

| Table 4. Six-month cumulative incidence of sexually transmitted diseases in women who did and did not have vaginal flora characteristic(s) (VFC) at baseline (BL). |
|-----------------------------------------------|-----------------|---------------------------------|
| VFC at BL No VFC at BL RR (95% CI) | p* |
|-----------------------------------------------|-----------------|---------------------------------|
| Lactobacillus | 23.6 | 28.3 | 0.84 (0.47-1.49) | .569 |
| Clue cells | 40 | 20.6 | 1.94 (1.15-3.28) | .025 |
| Yeasts | 29.3 | 24.7 | 1.19 (0.67-2.03) | .570 |
| GBS | 22.2 | 30.2 | 0.74 (0.34-1.58) | .482 |

NOTE. Sexually transmitted diseases were defined as a positive laboratory test for gonorrhea, chlamydia, trichomoniasis, syphilis, or a syndromically diagnosed sexually transmitted disease (eg, genital warts, genital herpes, and clinical diagnosis of pelvic inflammatory disease). GBS, group B streptococci.

* Determined by Gram’s stain.

1 Determined by 2-sided Fisher’s exact test.
understanding of the relationships explored in this paper. If intravaginal practices cause or perpetuate vaginal flora disturbances, and if these disturbances facilitate the acquisition of STDs and HIV, many cases of HIV and other STDs could potentially be prevented by interventions such as the discouragement of intravaginal practices and the inclusion of bacterial vaginosis in the syndromic treatment procedure of abnormal vaginal discharge. Because the prevalence of bacterial vaginosis is extremely high in sub-Saharan Africa and its clinical management is often disappointing [31], it may also be worthwhile to study artificial recolonization of lactobacilli in the vaginal tract.

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