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Prospective study of correlates of vaginal *Lactobacillus* colonization among high-risk HIV-1 seronegative women

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Abstract

Objective—Vaginal colonization with *Lactobacillus* species is characteristic of normal vaginal ecology. The absence of vaginal lactobacilli, particularly hydrogen peroxide (H_2O_2)-producing isolates, has been associated with symptomatic bacterial vaginosis (BV) and increased risk for HIV-1 acquisition. Identification of factors associated with vaginal *Lactobacillus* colonization may suggest interventions to improve vaginal health.

Methods—We conducted a prospective cohort study of correlates of vaginal *Lactobacillus* colonization among Kenyan HIV-1 seronegative female sex workers. At monthly follow-up visits, vaginal *Lactobacillus* cultures were obtained. Generalized estimating equations were used to examine demographic, behavioral, and medical correlates of *Lactobacillus* isolation, including isolation of H_2O_2-producing strains.

Results—*Lactobacillus* cultures were obtained from 1020 women who completed a total of 8896 follow-up visits. Vaginal washing, typically with water alone or with soap and water, was associated with an approximately 40% decreased likelihood of *Lactobacillus* isolation, including isolation of H_2O_2-producing strains. Recent antibiotic use, excluding metronidazole and treatments for vaginal candidiasis, reduced *Lactobacillus* isolation by ~30%. H_2O_2-producing lactobacilli were significantly less common among women with *Trichomonas vaginalis* infection and those who were seropositive for herpes simplex virus type 2. In contrast, H_2O_2-producing lactobacilli were significantly more common among women with concurrent vaginal candidiasis.

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Authorship Contributions

Study concept: JMB, WMH, RSM
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Conclusions—Modifiable biologic and behavioral factors are associated with *Lactobacillus* colonization in African women. Our results suggest intervention strategies to improve vaginal health in women at high risk for HIV-1.

Keywords

vaginal infection; Lactobacillus; vaginal washing; female sex workers; Kenya

Key Messages

- In this prospective longitudinal cohort study among Kenyan women, modifiable biologic and behavioral factors were associated with *Lactobacillus* colonization.
- Vaginal washing, with water alone or with soap and water, was associated with an approximately 40% decreased likelihood of *Lactobacillus* isolation.
- Recent antibiotic use reduced *Lactobacillus* isolation by ~30%.
- H_2O_2-producing lactobacilli were less common among women with concurrent *Trichomonas vaginalis* infection and among those who were seropositive for herpes simplex virus type 2.

Introduction

The normal vaginal microflora in women of reproductive age is composed predominantly of *Lactobacillus* species [1]. These Gram positive rods create an inhospitable environment for infectious pathogens by producing antimicrobial substances including hydrogen peroxide (H_2O_2) and lactic acid, which maintains a low vaginal pH [2].

There is increasing recognition that vaginal lactobacilli may mediate HIV-1 risk. *In vitro*, H_2O_2-producing lactobacilli are virucidal to HIV-1 [3]. One longitudinal study found a 2-fold increased HIV-1 risk among women without vaginal lactobacilli [4]. Moreover, in several prospective studies, bacterial vaginosis (BV), a vaginal infection characterized by decreased *Lactobacillus* colonization, has been associated with elevated HIV-1 risk [5–7]. Epidemiologic studies also found an association between a lack of lactobacilli and the presence of sexually transmitted infections (STIs), such as *Neisseria gonorrhoeae* [4] and *Trichomonas vaginalis* [8], which are risk factors for HIV-1 acquisition and could further amplify HIV-1 risk.

Understanding factors that influence vaginal *Lactobacillus* colonization may offer insight into novel vaginal health strategies to prevent HIV-1 transmission to women. We used data from a prospective observational cohort study to examine correlates of vaginal *Lactobacillus* isolation among African women at high risk for HIV-1.

Methods

Study population

In 1993, a prospective open cohort study was initiated of risk factors for HIV-1 and STI acquisition among women attending a municipal sex worker clinic in Mombasa, Kenya [9]. At monthly follow-up, data on recent sexual behavior and contraceptive use were recorded, and genital and blood samples were collected. STIs were treated according to Kenyan national guidelines, either syndromically at the time of examination or on the basis of laboratory testing at a scheduled results visit after one week. At each visit, risk reduction counseling and free condoms were provided, as well as primary care medical services. The study protocol was approved by the Kenyatta National Hospital/University of Nairobi Ethical Review Committee

and the Human Subjects Review Committee of the University of Washington. Participants provided informed consent.

Laboratory methods

HIV-1 serology was by ELISA (Detect HIV, Biochem ImmunoSystems, confirmed by Recombigen, Cambridge Biotech). Serological testing for herpes simplex virus type 2 (HSV-2) was performed on archived samples using a type-specific HSV-2 gG based ELISA (HerpeSelect 2, Focus Diagnostics), as described previously [10].

Endocervical secretions were cultured on modified Thayer-Martin media for *N. gonorrhoeae* and tested for *Chlamydia trachomatis* antigen by EIA (MicroTrak II, Syva). Testing for *C. trachomatis* was discontinued in June 1999 because of low incidence in the cohort (which may have related to low sensitivity of the EIA-based testing). Cervicitis was defined by a mean of >30 polymorphonuclear cells in three oil-immersion fields of Gram-stained cervical secretions.

Samples of vaginal secretions were examined microscopically using normal saline and 10% potassium hydroxide (KOH) for the presence of motile *T. vaginalis* and yeast. We defined vaginal candidiasis (VC) as the presence of yeast buds and/or pseudohyphae. BV was diagnosed by Nugent's criteria as a score of ≥7 on microscopic evaluation of Gram stained vaginal fluid [11].

Beginning in 1995, *Lactobacillus* species were isolated by culture of vaginal secretions collected at each monthly visit. Samples were directly inoculated onto Rogosa agar using Dacron swabs. Plates were incubated in anaerobic jars at 37°C for 72 h. Colonies were identified as *Lactobacillus* species on the basis of morphology and Gram stain appearance. Three *Lactobacillus* colonies from each plate were selected and stored in skim milk at −20°C for batch testing for H_2O_2 production, which was done by subculturing isolates on tetramethylbenzidine (TMB) agar containing horseradish peroxidase. After 72h of anaerobic incubation, subculture plates were exposed to air and H_2O_2-producing isolates were identified by visualization of blue coloration resulting from oxidation of TMB.

Data analysis

Data were analyzed using SPSS version 15.0 (SPSS) and S-PLUS 2000 (MathSoft). All visits between May 1995, when *Lactobacillus* cultures were begun, and May 2003, when a randomized trial of periodic presumptive treatment of vaginal infections was initiated in the cohort [12], were selected. Visits that took place as part of two studies of the vaginal microbicide nonoxynol-9 were excluded from the analysis [13,14]. Women who acquired HIV-1 during follow-up (n=120) were censored at their last HIV-1 seronegative visit.

To account for multiple measurements from individual women during prospective follow-up, we used generalized estimating equations with a logit link, exchangeable correlation structure, and robust variance estimates to examine correlates of *Lactobacillus* isolation. All models adjusted for the total number of visits contributed by each woman. Two outcomes were defined: (1) the presence of any lactobacilli and (2) the presence of H_2O_2-producing lactobacilli. Univariate analyses were performed for a number of characteristics including demographic factors, contraceptive use, sexual behavior, concurrently diagnosed genital tract infections, and recent antibiotic use. Multivariate models were then constructed using those variables demonstrating statistically significant associations (at $p \leq 0.05$) in univariate analysis.

Demographic and behavioral correlates included age, education, parity, tobacco use, alcohol use, and intravaginal washing practices. Of these, age was assessed as a time-dependent variable, while the remainder were from study enrollment data.

Contraceptive use was assessed in a time-dependent fashion. A 70-day window of persistent effect was estimated for women who changed their contraceptive method during follow-up, as we have done previously [10]. Sexual behavior correlates included number of sex partners and the occurrence of any unprotected sex, assessed as time-dependent variables measured during the week prior to each clinic visit. Sex during menses and whether lubrication was used for sex, both measured only at enrollment, were also assessed. The relationship between *Lactobacillus* isolation and the presence of concurrent genital tract infections (measured as time-dependent variables) was assessed.

Recent antibiotic use (as a time-dependent factor) was classified into 3 categories: (1) metronidazole, (2) treatment of VC (usually intravaginal clotrimazole or nystatin pessaries), and (3) any other antibiotics. Agents used for treatment of tuberculosis or helminths were excluded. Antibiotics were considered to have an effect for 60 days after administration, as we have done previously [15].

Results

Between May 1995 and May 2003, 1020 HIV-1 seronegative women contributed 8896 follow-up visits at which vaginal *Lactobacillus* cultures were performed. The median number of *Lactobacillus* cultures per woman was 5 [interquartile range (IQR) 2–11, range 1–84], and the median time between visits was 36 days (IQR 28-67). Most women worked as barmaids (Table 1) and supplemented their income with sex work.

*Lactobacillu*s cultures were collected at enrollment for 775 women (i.e., those who enrolled after May 1995). Of these, 169 (22%) had any lactobacilli and 75 (10%) had H_2O_2-producing lactobacilli. During follow-up, *Lactobacillus* species were isolated at 1694 (19.0%) follow-up visits, of which 731 (8.2% of total follow-up visits) had H_2O_2-producing lactobacilli isolated. Five hundred thirty women (52.0%) had lactobacilli detected at least once during follow-up, and 309 (30.3%) had H_2O_2-producing lactobacilli detected at least once.

Recent antibiotic exposure was present at 559 (6.3%), 214 (2.4%), and 639 (7.2%) follow-up visits for metronidazole, agents for treatment of VC, and other antibiotics, respectively.

Correlates of detection of Lactobacillus *species.* In univariate analysis, older age, alcohol use, vaginal cleansing, current bacterial vaginosis, HSV-2 seropositivity, recent use of metronidazole, and recent use of other antibiotics (except those for treatment of VC) were associated with decreased *Lactobacillus* isolation (Table 2). Tobacco use and a higher number of sexual partners were associated with increased detection.

In the multivariate analyses, two important factors were significantly associated with decreased *Lactobacillus* isolation: 1) vaginal washing, which decreased detection by approximately 40% both among women who used water alone and among those who used soap and 2) recent use of antibiotics other than metronidazole or preparations to treat VC, which decreased detection by approximately 30%. There was a step-wise decrease in the likelihood of *Lactobacillus* isolation with older age. A higher number of sexual partners (>1 per week) was associated with a 30% greater likelihood of *Lactobacillus* detection.

Correlates of detection of H_2O_2-producing Lactobacillus *species.* A number of factors associated with overall vaginal *Lactobacillus* colonization were also associated with vaginal H_2O_2-producing-*Lactobacillus* colonization. In univariate analysis, older age, parity, alcohol use, vaginal cleansing, current BV, *T. vaginalis* infection, HSV-2 seropositivity, and recent use of other antibiotics were associated with decreased *Lactobacillus* isolation (Table 3). Tobacco use, cervicitis, VC, and recent treatment for VC were associated with increased detection.

In multivariate modeling, vaginal washing and recent use of antibiotics other than metronidazole or preparations to treat VC were associated with significantly decreased detection of H_2O_2-producing lactobacilli, with magnitudes of effect similar to those seen in multivariate analysis for overall *Lactobacillus* isolation. Women older than 40 had an approximately 50% decreased likelihood of isolation of H_2O_2-producing lactobacilli. Alcohol use was not consistently associated with H_2O_2-producing *Lactobacillus* colonization, although intake of 1–7 drinks per week was associated with an approximately 40% decrease in detection. HSV-2 seropositivity and *T. vaginalis* infection were associated with decreased detection of H_2O_2-producing lactobacilli, whereas the identification of these *Lactobacillus* strains was increased when concurrent cervicitis and VC were detected.

Bacterial vaginosis and vaginal Lactobacillus. In both multivariate models, bacterial vaginosis was associated with an ~40% reduced likelihood of *Lactobacillus* detection. Because decreased *Lactobacillus* colonization may be reflective of bacterial vaginosis pathogenesis, we performed additional multivariate models, excluding the bacterial vaginosis variable – results for other variables in those models were essentially unchanged (results not shown).

Discussion

In this large, prospective study of HIV-1-uninfected Kenyan women, we found that biologic and behavioral factors were associated with isolation of vaginal *Lactobacillus* species, a key component of healthy vaginal ecology. The identification of modifiable biologic factors that influence vaginal *Lactobacillus* colonization may have important implications for the design of vaginal health strategies to prevent HIV-1 acquisition in women.

Intravaginal practices such as douching or wiping inside the vagina with water or antiseptic preparations have been associated with increased risk for HIV-1 acquisition [6]. We previously reported elevated HIV-1 risk among women who performed vaginal washing: adjusted hazard ratio (aHR) 2.64 (95% CI 1.00–6.97) for those who used water and aHR 3.84 (95% CI 1.51–9.77) for those who used soap, compared with those who did not vaginally cleanse [16]. An unadjusted preliminary analysis demonstrated decreased *Lactobacillus* colonization associated with vaginal washing [17]. Another report from our cohort found that *Lactobacillus* colonization was associated with decreased HIV-1 incidence [4]. The results of the present study bridge and reinforce these earlier findings, suggesting that decreased *Lactobacillus* colonization as a result of vaginal washing might be an important factor mediating the relationship between intravaginal practices and HIV-1 risk in this population. One limitation of our study was that vaginal washing was measured at enrollment and practices may have changed during follow-up; however, our previous report, associating vaginal washing with HIV-1 risk, used this same measure [16]. Our findings parallel the results of studies in U.S. women, which have also found douching to be associated with BV and decreased vaginal colonization with H_2O_2-producing lactobacilli [18,19].

Antibiotic use has been reported as a risk factor for loss of H_2O_2-producing lactobacilli [20], and our results confirm this association. We also found decreased *Lactobacillus* isolation after metronidazole use and increased H_2O_2-producing *Lactobacillus* isolation after antifungal treatment, although these did not achieve statistical significance in multivariate analysis. These latter associations may reflect confounding by indication, as metronidazole was very frequently used to treat BV and *T. vaginalis* infections in the cohort, while the antifungals were used to treat VC. These vaginal conditions were associated with decreased (BV and *T. vaginalis*) and increased (VC) identification of *Lactobacillus*, and perturbations of the vaginal flora may persist or recur after treatment.

The VC results are consistent with a recent analysis of women from this cohort who participated in a clinical trial of oral periodic presumptive treatment of vaginal infections between 2003 and 2006 [12]. Among women in the control arm, concurrent colonization with H_2O_2-producing *Lactobacillus* was associated with increased risk of symptomatic VC (adjusted OR 3.31, 95% CI 1.09–10.08), while prior metronidazole treatment was associated with a trend towards increased risk of symptomatic VC (adjusted OR 6.41, 95% CI 0.68–60.25) [21]. Given that BV, trichomoniasis, and VC have all been associated with increased HIV-1 risk [22], new approaches to treating vaginal pathogens need to investigate methods to support *Lactobacillus* colonization without trading one vaginal infection for another.

HSV-2 seropositivity was associated with an ~30% decrease in isolation of H_2O_2-producing lactobacilli in this cohort, consistent with other studies [23,24]. Subclinical HSV-2 reactivation is frequent in seropositive persons (~20% of days) and leads to long-lasting immunologic changes to the genital mucosa [25]. HSV-2 seropositivity has been associated with a >3-fold increased risk of HIV-1 acquisition [26], including in this cohort [10]. While the HSV-2/HIV-1 association has generally been hypothesized to be mediated through changes to mucosal integrity and mucosal immune cell populations, it is interesting to consider that an HSV-2 effect might be mediated in part through changes in vaginal ecology.

Older age was associated with a step-wise decrease in *Lactobacillus* isolation, potentially reflecting lower estrogen levels among older women. However, hormonal contraceptive use was not associated with *Lactobacillus* isolation. We and others have previously shown that hormonal contraception decreases BV risk [23,27]. Taken together these data suggest that hormonal contraception may reduce the level of disturbances of the vaginal flora, without promoting *Lactobacillus* colonization.

We were surprised to find that a higher number of sex partners was associated with detection of *Lactobacillus* (though not with H_2O_2-producing lactobacilli). Previous studies have found lactobacilli to be less common and BV more common among women with a higher number of sexual partners [19,23]. The reason for the association observed in our study is not clear, but could relate to incompletely controlled differences in sexual risk (e.g., reduced partner number when women had symptomatic vaginal discharge).

There are several strengths to this study. The large number of women and total number of visits makes this the largest study of correlates of vaginal *Lactobacillus* colonization to date. Longitudinal and frequent follow-up permitted careful analysis of time-dependent factors like age, antibiotic use, contraception, and sexual behavior.

We found a low colonization rate of vaginal *Lactobacillus*, especially H_2O_2-producing lactobacilli. The low isolation rates may be attributed to our population (Kenya vs. US, where most studies of vaginal flora have been conducted) or isolation methods (culture versus PCR [28]). Some *Lactobacillus* species, like *L. iners* (which is common among African women [28]), do not grow on Rogosa agar, and our colonization rates may be underestimates. The high prevalence of vaginal washing and high incidence of BV in our study population also likely contributed to low *Lactobacillus* isolation rates. Finally, the results of this study may not be generalizable to all populations – they may be most directly relevant to women at high risk for HIV-1; HIV-1 incidence in this cohort was >8% per year during the period included in this analysis [10].

Our findings suggest that modifiable biologic and behavioral correlates – intravaginal practices, antimicrobial use, and genital tract infections – influence vaginal colonization with *Lactobacillus* among African women at high risk for HIV-1. Increasing attention to vaginal health interventions as an HIV-1 prevention strategy [12] requires continued study of risk factors for vaginal pathogens and correlates of healthy vaginal flora. New interventions aimed

at improving vaginal *Lactobacillus* colonization have the potential to alter HIV-1 susceptibility.

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Table 1

Enrollment characteristics of study population (N = 1020)

	Median (IQR) or N (%)
Demographic	
Age, years	26 (22–31)
Education, years	8 (6–10)
Parity	2 (1–3)
Bar worker	723 (71%)
Substance use	
Tobacco use	199 (20%)
Alcohol use	796 (78%)
Vaginal cleansing	
None	65 (6%)
Water only	234 (23%)
Soap or other substances[a]	721 (71%)
Sexual behavior	
Sex partners, per week	1 (1–2)
100% condom use	639 (63%)
Lubrication used for sex	230 (23%)
Sex during menses	116 (11%)
Contraceptive use	
None	636 (62%)
Oral contraceptive pills	141 (14%)
Depot medroxyprogesterone acetate	206 (20%)
Intrauterine device	20 (2%)
Norplant	17 (2%)
Genital tract infections	
Neisseria gonorrhoeae	39 (4%)
Chlamydia trachomatis[b]	21 (3%)
Cervicitis	129 (13%)
Bacterial vaginosis	345 (34%)
Trichomonas vaginalis	54 (5%)
Vaginal candidiasis	137 (14%)
HSV-2 seropositive	798 (78%)
Genital ulcer disease (on examination)	14 (1%)

IQR, interquartile range; HSV-2, herpes simplex virus type 2

[a] Other substances include detergents or antiseptics, used by <5% of participants

[b] *C. trachomatis* testing done for 720 women (those enrolled through June 1999)

Table 2

Univariate and multivariate correlates of overall *Lactobacillus* isolation among HIV-1 seronegative women.

		UNIVARIATE[a]		MULTIVARIATE[a]	
	% of visits[b]	OR	95%CI	OR	95%CI
Demographic					
Age, years					
<25	20.0	reference		reference	
25–29	24.2	0.85	0.69–1.05	0.89	0.72–1.11
30–34	24.7	0.78	0.61–1.01	0.86	0.65–1.13
35–39	17.0	0.64	0.48–0.86	0.70	0.52–0.94
≥40	14.2	0.49	0.34–0.72	0.55	0.38–0.81
Education ≤8 years	64.0	0.93	0.78–1.11		
Parity					
0	16.3	reference			
1	29.8	0.81	0.63–1.04		
≥2	53.9	0.89	0.71–1.13		
Substance use					
Tobacco, cigarettes per day					
0	77.6	reference		reference	
1–9	13.9	1.27	1.02–1.60	1.15	0.91–1.46
≥10	8.5	1.35	1.01–1.80	1.16	0.87–1.56
Alcohol, drinks per week					
0	21.6	reference		reference	
1–7	39.2	0.79	0.63–1.00	0.85	0.66–1.09
>7	39.2	1.09	0.87–1.36	1.13	0.88–1.44
Vaginal cleansing					
None	11.5	reference		reference	
Water	25.9	0.72	0.54–0.97	0.64	0.47–0.87
Soap/other	62.6	0.66	0.52–0.85	0.56	0.43–0.74
Sexual behavior					
Number of sex partners, past week					
0	40.2	reference			

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		UNIVARIATE[a]		MULTIVARIATE[a]	
	% of visits[b]	**OR**	**95%CI**	**OR**	**95%CI**
1	45.0	1.10	0.97–1.25	1.10	0.97–1.25
>1	14.8	1.36	1.14–1.61	1.31	1.11–1.56
Unprotected sex, past week	20.9	0.99	0.85–1.15		
Lubrication used for sex	20.0	1.05	0.84–1.30		
Sex during menses	10.1	1.10	0.85–1.42		
Contraception					
None	64.9	reference			
Oral contraceptive pill	13.1	1.20	0.96–1.50		
Depot medroxyprogesterone acetate	19.5	0.95	0.78–1.17		
Intrauterine device	1.1	1.17	0.69–1.99		
Norplant	1.4	1.13	0.58–2.20		
Concurrent genital tract infections					
Neisseria gonorrhoeae	2.2	1.16	0.81–1.67		
Chlamydia trachomatis[c]	1.6	0.96	0.66–1.40		
Cervicitis	5.7	1.24	0.98–1.59		
Bacterial vaginosis	34.8	0.66	0.58–0.76	0.65	0.56–0.75
Trichomonas vaginalis	5.0	0.87	0.67–1.13		
Vaginal candidiasis	11.3	1.14	1.00–1.32		
HSV-2 seropositive	85.6	0.75	0.60–0.94	0.86	0.68–1.09
Genital ulcer disease (on examination)	1.1	0.79	0.48–1.31		
Antibiotic use within the past 60 days					
Metronidazole	6.3	0.75	0.58–0.98	0.78	0.60–1.03
Treatment for vaginal candidiasis	2.4	1.15	0.82–1.62		
Any other antibiotic	7.2	0.70	0.56–0.86	0.71	0.57–0.89

OR, odds ratio; CI, confidence interval; HSV-2, herpes simplex virus type 2

[a] Generalized estimating equations, adjusted for number of visits per woman.

[b] The percentage of visits (of total N=8896) considered “exposed” is presented for each covariate.

[c] *C. trachomatis* testing done for 4045 of 8896 (46%) follow-up visits (through June 1999).

Table 3

Univariate and multivariate correlates of H_2O_2-producing *Lactobacillus* isolation among HIV-1 seronegative women.

		UNIVARIATE[a]		MULTIVARIATE[a]	
	% of visits[b]	OR	95%CI	OR	95%CI
Demographic					
Age, years					
<25	20.0	reference		reference	
25–29	24.2	0.98	0.75–1.29	1.04	0.79–1.37
30–34	24.7	0.70	0.51–0.96	0.78	0.54–1.14
35–39	17.0	0.67	0.45–1.01	0.74	0.48–1.12
≥40	14.2	0.43	0.24–0.77	0.46	0.27–0.79
Education 8 years	64.0	0.88	0.68–1.13		
Parity					
0	16.3	reference			
1	29.8	0.66	0.46–0.94	0.83	0.58–1.18
≥2	53.9	0.77	0.55–1.08	1.20	0.85–1.70
Substance use					
Tobacco, cigarettes per day					
0	77.6	reference		reference	
1–9	13.9	1.39	1.02–1.88	1.31	0.96–1.79
≥ 10	8.5	1.21	0.82–1.79	1.21	0.83–1.76
Alcohol, drinks per week					
0	21.6	reference		reference	
1–7	39.2	0.54	0.39–0.75	0.61	0.43–0.87
>7	39.2	0.73	0.53–0.99	0.83	0.60–1.14
Vaginal cleansing					
None	11.5	reference		reference	
Water	25.9	0.72	0.48–1.11	0.64	0.41–1.01
Soap/other	62.6	0.65	0.46–0.91	0.58	0.40–0.85
Sexual behavior					
Number of sex partners, past week					
0	40.2	reference			

		UNIVARIATE[a]		MULTIVARIATE[a]	
	% of visits[b]	**OR**	**95%CI**	**OR**	**95%CI**
1	45.0	1.03	0.85–1.26		
>1	14.8	1.14	0.90–1.45		
Unprotected sex, past week	20.9	0.84	0.66–1.07		
Lubrication used for sex	20.0	1.04	0.75–1.43		
Sex during menses	10.1	0.69	0.44–1.09		
Contraception					
None	64.9	reference			
Oral contraceptive pill	13.1	1.10	0.81–1.50		
Depot medroxyprogesterone acetate	19.5	1.17	0.90–1.51		
Intrauterine device	1.1	0.86	0.35–2.13		
Norplant	1.4	1.06	0.46–2.48		
Concurrent genital tract infections					
Neisseria gonorrhoeae	2.2	1.15	0.68–1.93		
Chlamydia trachomatis[c]	1.6	0.75	0.31–1.86		
Cervicitis	5.7	1.43	1.03–2.00	1.39	1.00–1.90
Bacterial vaginosis	34.8	0.60	0.49–0.73	0.62	0.51–0.76
Trichomonas vaginalis	5.0	0.60	0.30–0.94	0.62	0.39–1.00
Vaginal candidiasis	11.3	1.34	1.11–1.61	1.31	1.08–1.59
HSV-2 seropositive	85.6	0.58	0.43–0.77	0.69	0.51–0.93
Genital ulcer disease (on examination)	1.1	0.77	0.39–1.51		
Antibiotic use within the past 60 days					
Metronidazole	6.3	0.91	0.66–1.27		
Treatment for vaginal candidiasis	2.4	1.55	0.99–2.42	1.47	0.94–2.31
Any other antibiotic	7.2	0.70	0.51–0.96	0.68	0.49–0.94

OR, odds ratio; CI, confidence interval; HSV-2, herpes simplex virus type 2

[a] Generalized estimating equations, adjusted for number of visits per woman.

[b] The percentage of visits (of total N=8896) considered “exposed” is presented for each covariate.

[c] *C. trachomatis* testing done for 4045 of 8896 (46%) follow-up visits (through June 1999).