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A Prospective Study of Risk Factors for Bacterial Vaginosis in HIV-1-Seronegative African Women

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Abstract

Background—Bacterial vaginosis (BV) is common and has been associated with increased HIV-1 susceptibility. The objective of this study was to identify risk factors for BV in African women at high risk for acquiring HIV-1.

Methods—We conducted a prospective study among 151 HIV-1-seronegative Kenyan female sex workers. Non-pregnant women were eligible if they did not have symptoms of abnormal vaginal itching or discharge at the time of enrollment. At monthly follow-up, a vaginal examination and laboratory testing for genital tract infections were performed. Multivariate Andersen-Gill proportional hazards analysis was used to identify correlates of BV.

Results—Participants completed a median of 378 (interquartile range 350–412) days of follow-up. Compared to women reporting no vaginal washing, those who reported vaginal washing 1–14 (adjusted hazard ratio [aHR] 1.29, 95% confidence interval [CI] 0.88–1.89), 15–28 (aHR 1.60, 95% CI 0.98–2.61), and >28 times/week (aHR 2.39, 95% CI 1.35–4.23) were at increased risk of BV. Higher BV incidence was also associated with the use of cloth for intravaginal cleansing (aHR 1.48, 95% CI 1.06–2.08) and with recent unprotected intercourse (aHR 1.75, 95% CI 1.47–2.08). Women using depot medroxyprogesterone acetate contraception were at lower risk for BV (aHR 0.59, 95% CI 0.48–0.73).

Conclusions—Vaginal washing and unprotected intercourse were associated with increased risk of BV. These findings could help to inform the development of novel vaginal health approaches for HIV-1 risk reduction in women.

Keywords

Bacterial vaginosis; vaginal washing; intravaginal practices; women; Africa

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INTRODUCTION

Bacterial vaginosis (BV) has been associated with increased risk for HIV-1 acquisition,[1–4] genital tract infections,[5] pelvic inflammatory disease,[6] and preterm delivery of a low-birth-weight infant.[7] Prospective studies have begun to shed light on the natural history of BV, and on the importance of specific demographic, behavioral, and biological risk factors for this condition. African ancestry,[8] lower educational level,[8] higher number of sexual partners,[8, 9] and oral-vaginal contact have all been associated with BV.[10] In contrast, condom use and hormonal contraception may be protective.[11, 12] Nonetheless, important questions remain about the role of some risk factors for BV. For example, intravaginal practices are common among African women and may increase the risk of BV, providing a potential mechanism through which intravaginal practices might increase HIV-1 susceptibility.[13–15] In addition, while it is generally accepted that hydrogen-peroxide producing *Lactobacillus* colonization is associated with lower risk of BV,[2, 16] the extent to which other BV risk factors act through effects on vaginal lactobacilli is not known.

We recently completed a randomized trial of monthly periodic presumptive treatment as an intervention to reduce vaginal infections in Kenyan women at risk for HIV-1.[17] A secondary aim was to utilize the prospectively collected data from the trial to identify modifiable risk factors for BV in this population.

MATERIALS AND METHODS

Population and Procedures

Detailed methods for the randomized trial are presented elsewhere.[17] Because the orally administered study drugs reduced the incidence of BV in the trial population, the present analysis focuses on women who were randomized to receive the matching oral placebo (hard gel capsules filled with lactose monohydrate). Briefly, non-pregnant, 18–45 year old, HIV-1-seronegative sex workers were eligible to enroll. The majority of women recruited to the cohort were bar workers who supported their regular income with occasional transactional sex. The protocol for the trial required that women did not have symptoms of abnormal vaginal itching or discharge at the time of enrollment. Subsequent visits were included in this analysis regardless of whether symptoms were present.

At baseline and at monthly follow-up visits, women completed an interview covering sexual risk behavior and personal hygiene practices during the past 7 days. In order to distinguish between external vulvar versus internal vaginal washing, we used a two-part question that was pilot tested prior to the study. Women were first asked whether they cleaned inside the vagina. Those who responded ‘yes’ were asked to clarify how far inside the vagina they cleaned, differentiating between ‘less than a finger tip length’ versus ‘more than a finger tip length’ beyond the vaginal introitus. We defined ‘finger tip length’ as the length of the distal phalanx of the second finger (approximately 2 centimeters). Women were classified as performing intravaginal washing if they reported vaginal cleaning practices that extended more than a finger tip length beyond the introitus.

At each visit, women had a pelvic speculum examination with collection of specimens for laboratory diagnosis of genital tract infections. Vaginal pH was measured using a test strip (ColorpHast 4.0–7.0, EM Reagents), and vaginal secretions were tested for the release of an amine odor after addition of a drop of 10% potassium hydroxide. Blood was collected for HIV-1 serological testing and a urine pregnancy test was performed. Women who reported abnormal vaginal itching or discharge were treated syndromically with metronidazole 2 grams as a single dose plus clotrimazole 200 mg vaginal suppositories nightly for three nights. Participants were invited to return for their laboratory results one week after each

examination. If additional treatment for sexually transmitted infections was indicated on the basis of the laboratory results, medications were provided according to WHO and Kenya Ministry of Health Guidelines.[18] Asymptomatic BV and vaginal candidiasis were not treated, as there is currently no indication for treatment of these conditions in non-pregnant women without symptoms. Women in the trial were asked to return for 12 monthly follow-up visits. Participation was discontinued if they acquired HIV-1 or became pregnant. Individual risk reduction education including instructions on condom use and a free supply of condoms were provided at each visit. Participants were counseled that vaginal washing may increase the risk for HIV-1, and were encouraged to minimize or eliminate these practices. This study was approved by the ethical review committees at Kenyatta National Hospital and the University of Washington. All participants provided written informed consent.

Laboratory Methods

Serological screening for HIV-1 was performed using an enzyme linked immunosorbent assay (ELISA; Detect-HIV; BioChem ImmunoSystems). Positive samples were confirmed using a second ELISA (Recombigen; Cambridge Biotech or Vironostika; Biomeriux).[19] Urine pregnancy testing was performed using a rapid β-hCG test (Plasmatec Laboratory Products).

Gram stained slides of vaginal secretions were evaluated according to standardized microscopic scoring criteria.[20] A vaginal saline wet mount was examined microscopically for the presence of budding yeast or hyphae, clue cells, and *Trichomonas vaginalis.* A drop of 10% potassium hydroxide was added, and the slide was examined a second time for the presence of yeast. Gram stained slides of endocervical secretions were examined microscopically. The number of polymorphonuclear leukocytes (PMN) in three non-adjacent high-power fields was counted, and the average cervical PMN count was calculated.

Lactobacillus culture was performed on Rogosa agar (Difco TM, Becton Dickinson), and production of H_2O_2 was assessed by sub-culture on tetramethylbenzadine agar with horseradish peroxidase.[21] Culture for *T. vaginalis* was performed in Diamond's modified media and culture for *Neisseria gonorrhoeae* was performed on modified Thayer-Martin media.

Statistical Methods

Analyses were performed using SPSS (version 15; SPSS) and S-Plus 2000 (Mathsoft). All women randomized to the placebo arm of the trial were considered eligible for inclusion in this analysis. Univariate and multivariate analyses of risk factors for incident BV were performed using Andersen-Gill proportional hazards models with robust variance estimates. This method of modeling allows for multiple events within a single individual over time. Variables associated with BV on univariate analysis ($P \leq 0.10$) were included in the multivariate models. Linear regression was used to assess the dose-response relationship between the frequency of vaginal washing and BV.

As in prior analyses,[11] we assumed an effect window of 85 days to capture the effect of hormonal contraceptive use on incident BV among women who changed or discontinued contraceptive methods (70 days of persistent effect of hormonal contraception after discontinuation + 15 days from BV acquisition to detection at a clinic visit, assuming acquisition at the midpoint between monthly visits). The window of effect for *Lactobacillus* on subsequent BV was set at 60 days, which allowed us to capture the presence of lactobacilli during 1–2 preceding visits.

The primary endpoint for this analysis was BV by microscopic criteria.[20] In addition, we analyzed risk factors for BV diagnosed on the basis of clinical criteria, defined as the identification of at least three out of four clinical signs: abnormal vaginal discharge, pH >4.5, clue cells present on vaginal saline wet preparation, and the release of an amine odor on addition of a drop of 10% potassium hydroxide to vaginal fluid.[22] Vaginal candidiasis was defined as the identification of budding yeast or hyphae on the vaginal saline wet mount or potassium hydroxide preparation. Women were considered to have *T. vaginalis* infection if motile trichomonads were observed on the vaginal wet mount or in culture.

RESULTS

Between May 2003 and November 2005, 378 women were screened for eligibility, of whom 310 were enrolled. Of the 68 women who did not enroll, 65 declined or did not return for a scheduled enrollment visit, one planned to leave the area, one reported intolerance to metronidazole, and one was pregnant. One hundred and fifty five women were randomized to the placebo arm, and 152 of these women returned for at least one follow-up visit. One woman was excluded from these analyses because she seroconverted to HIV-1 at her first follow-up visit, and therefore did not contribute HIV-1-seronegative follow-up time. The remaining 151 women are the focus of the longitudinal analyses presented here. The median duration of follow-up was 378 (interquartile range [IQR] 350–412) days per participant and the median interval between visits was 30 (IQR 28–35) days. Overall, the women contributed a total of 153 person-years of follow-up over 1,570 visits.

Baseline characteristics of the 151 sex workers included in these analyses are presented in Table 1. The median age of participants was 32 (IQR 27–39) years. They reported a relatively low number of sexual partners (median 1, IQR 0–1), and a low frequency of intercourse (median 1, IQR 0–2) during the past week. One-hundred and thirty (86%) women reported vaginal washing with a median frequency of 14 (14–21) times per week. The most common combination of practices, reported by 86 (57%) women, was use of a finger to wash inside the vagina with soap or antiseptic both during bathing and after sex. Although the women denied symptoms of abnormal vaginal itching or discharge at enrollment, BV was present by Gram stain criteria in 56 (37%) and by clinical criteria in 27 (18%) participants.

Risk Factors for Bacterial Vaginosis

Bacterial vaginosis was identified by Gram stain criteria at 553 follow-up visits (3.61 visits with BV per woman-year). Risk factors for BV by microscopic criteria are presented in Table 2. Several modifiable risk factors were associated with BV in univariate analyses. To ascertain the independent effects of each of these risk factors, an initial multivariate model was constructed including all covariates that were associated with BV in univariate analyses ($P \leq 0.10$), except *Lactobacillus*, since changes in vaginal *Lactobacillus* colonization could represent an intermediate step in the causal linkage between BV and other risk factors. In this model, more frequent vaginal washing, use of cloth to clean inside the vagina, unprotected intercourse, and *T. vaginalis* infection were independently associated with increased risk of BV. In contrast, the risk of BV was significantly lower in women using depot medroxyprogesterone acetate (DMPA) contraception and in those with concurrent vaginal candidiasis. A second multivariate model was constructed including vaginal *Lactobacillus* status. The absence of *Lactobacillus* remained a significant predictor of BV. However, addition of this variable did not substantially change the hazard ratios for any of the other covariates, suggesting that vaginal washing, use of cloth inside the vagina, unprotected intercourse, DMPA, and other vaginal infections may influence the risk of BV

through mechanisms that are at least partly independent of their effects on vaginal lactobacilli.

Vaginal washing practices may vary in relation to vaginal symptoms like odor or discharge. For this reason, we repeated the analyses in Table 2 using the vaginal washing practices reported at study enrollment, as these practices would be unrelated to subsequent symptoms. Baseline vaginal washing frequency was correlated with the average frequency of vaginal washing during follow-up ($R=0.52$, $p<0.001$). Women who reported the highest frequency of vaginal washing at baseline remained at a significantly higher risk of BV by Gram stain criteria during subsequent follow-up. Compared to women reporting no vaginal washing at baseline, the adjusted hazard ratios for BV in those who reported at enrollment that they performed vaginal washing 1–14 times per week, 15–28 times per week, and >28 times per week were 1.06 (95% confidence interval [CI] 0.83–1.35), 0.80 (95% CI 0.63–1.02), and 1.55 (95% CI 1.16–2.06) respectively. Similar results were observed upon further adjustment for vaginal *Lactobacillus* status (data not shown).

Bacterial vaginosis was identified by clinical criteria at 300 visits (1.96 visits with BV per woman-year). Because there was a lower rate of BV by clinical criteria compared to Gram stain criteria, analyses of risk factors for BV by clinical criteria had lower statistical power. In general, risk factors for BV measured by clinical criteria were similar to risk factors identified when BV was diagnosed microscopically (Table 3). However, the association between vaginal washing and BV by clinical criteria was not statistically significant in multivariate analysis.

DISCUSSION

This prospective study identified several modifiable risk factors associated with BV in this cohort of Kenyan sex workers. Vaginal washing and unprotected intercourse were both associated with increased risk of BV. In contrast, use of the injectable progesterone contraceptive DMPA was associated with a lower rate of BV.

Accumulating evidence from prospective studies of women in a variety of settings, and using very different intravaginal practices, suggests that women's risk for BV may be increased by common vaginal cleansing practices. Our data demonstrating an increased risk of BV in women who perform vaginal washing using fingers or cloth are consistent with a recently published study among US women, which demonstrated that vaginal douching (using a stream of liquid) increased the risk of BV in those with intermediate vaginal flora by Gram stain.[23] The dose-response relationship observed in our study provides additional epidemiological evidence in favor of a causal association. We also found a significant association between BV and the use of cloth to clean inside the vagina. These results, which are similar to our findings in an earlier cross-sectional study,[24] have implications for planning interventions aimed at modifying intravaginal practices. In particular, substantial reductions in BV might be achieved by reducing the frequency of vaginal washing or by modifying specific practices, even if complete cessation of vaginal washing proves difficult.

There is accumulating evidence that sexual risk behavior may influence the risk of BV, but different studies have provided somewhat conflicting results about the importance of specific risk factors. For example, in a multi-center study of US women, monogamy was associated with a significantly lower risk of BV.[8] This finding contrasts with the results from an Australian cohort in which having a regular sexual partner was associated with increased risk, while having a new partner was associated with lower risk.[25] The present study highlights the importance of unprotected intercourse as a risk factor for BV regardless of the number or type (new versus regular) of sexual partners. This contrasts with the

epidemiology of traditional sexually transmitted pathogens like gonorrhea and *Chlamydia*, for which new sex partners play a particularly important role in transmission.[26]

The finding that DMPA may lower the risk of BV is consistent with prior studies demonstrating similar associations.[11, 27] Changes in vaginal mucosal immune cell populations have been demonstrated in women using DMPA, likely reflecting altered local immune function.[28] This could in turn influence the risk for BV. Injectable progesterone contraception may also modify the risk of BV by reducing menstrual frequency and volume.[29]

Vaginal *Lactobacillus* colonization is an important mediator of the risk of vaginal infections.[2, 16] However, the present data suggest that the relationship between vaginal infections and specific risk factors such as intravaginal practices, sexual risk behavior, and hormonal contraceptive use, may not be explained entirely on the basis of their impact on lactobacilli. An important caveat to these results is the fact that some *Lactobacillus* species such as *L. iners* may be common among African women,[30] and can be difficult to culture successfully. Studies using cultivation-independent techniques to identify and quantify vaginal bacterial populations could provide important insights into the pathogenesis and natural history of disturbances of the vaginal flora.[31]

We used microscopic scoring criteria for the diagnosis of BV in the primary analyses presented here.[20] The risk factors for BV by clinical criteria were generally similar,[22] although there were fewer episodes using this definition, resulting in lower statistical power. Microscopic criteria were selected for defining the primary BV endpoint because 86% of these women reported vaginal washing, raising concern about whether the clinical criteria would produce reliable results. Specifically, we felt that vaginal washing might influence the examiners' ability to identify abnormal vaginal discharge, which is a component of the clinical definition of BV.

The findings from this prospective study differ in a few important respects from those of our earlier cross-sectional analysis of baseline data in this patient population.[24] In the cross-sectional study, BV was associated with use of petroleum jelly for vaginal lubrication, use of saliva for vaginal lubrication, and less frequent bathing. These associations were attenuated (lubrication with petroleum jelly) or eliminated (lubrication with saliva, less frequent bathing) in the longitudinal analysis. There are a number of possible explanations for the difference in these results. First, the cross-sectional analysis included only asymptomatic enrollment visits, whereas the longitudinal study included follow-up visits regardless of whether symptoms were present. Second, time-varying adjustment for potential confounding factors in the longitudinal analysis may have provided greater control of confounding. Finally, the associations may have occurred by chance in the cross-sectional study (Type-1 error), or the longitudinal study may have failed to show associations that truly exist (Type-2 error). Further research in other populations is needed to address these questions.

There were limitations to this study. Accurate assessment of sexual risk can be difficult. We have previously demonstrated that self-reported sexual behaviors in this population are associated with increased risk for sexually transmitted diseases.[32] Nonetheless, there remains a potential for residual confounding. It is also important to consider the fact that all participants in this study reported transactional sex. Their sexual practices, vaginal washing practices, and risk for BV may differ from women who do not engage in sex work. To date, there are limited data comparing intravaginal practices across populations.[13] Finally, all participants in this study were asymptomatic at enrollment. However, women were not excluded if they had a prior history of symptomatic vaginal conditions, and were retained after enrollment regardless of whether they became symptomatic. While the recruitment of

asymptomatic women differs from many studies of BV, we feel that this population may be particularly important, since women frequently do not recognize symptoms of BV. Indeed, the high frequency of asymptomatic BV was highlighted by the 37% baseline prevalence in our study.

The importance of understanding risk factors for BV has become increasingly evident in light of numerous studies linking this condition to HIV-1 acquisition.[1–4] The results presented here identify several modifiable risk factors for BV, which could help to inform the development of interventions to improve vaginal health. Ultimately, interventions aimed at reducing or eliminating vaginal washing could provide a simple, inexpensive, female-controlled strategy for reducing women's risk of acquiring HIV-1 infection.

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Table 1

Baseline Characteristics of Female Sex Workers in Enrolled Mombasa, Kenya between May 2003 and November 2005 (N=151)

Variable	Value
Age, median (IQR), years	32 (27–39)
Duration of sex work, median (IQR), years	4.3 (1.6–8.1)
Education, median (IQR), years	8 (7–11)
Bar worker[a]	116 (76.8%)
Tobacco use	18 (11.9%)
Alcohol use	115 (76.2%)
Vaginal washing in past week	130 (86.1%)
Frequency, median (IQR), per week[b]	14 (14–21)
Vaginal washing substance[b]	
Water only	43 (33.1%)
Soap or antiseptic[c]	87 (66.9%)
Vaginal washing method[b]	
Finger	127 (97.7%)
Cloth	3 (2.3%)
Timing of vaginal washing[d]	
Before sex	6 (4.7%)
After sex	128 (100%)
During bathing	127 (99.2%)
After urinating	35 (27.3%)
Other	1 (<1%)
Bathing frequency, median (IQR), per week	14 (14–14)
Vaginal lubricant for sex	
Vaseline	10 (6.7%)
Saliva	6 (4.0%)
Sex partners, median (IQR), in past week	1 (0–1)
Sex frequency, median (IQR), in past week	1 (0–2)
Any unprotected intercourse in past week	32 (21.2%)
New sex partner in past month	22 (14.7%)
Any history of vaginal intercourse	152 (100%)
Any history of anal intercourse	0 (0%)
Any history of oral-vaginal contact	5 (3.3%)
Contraception	
None or tubal ligation	93 (61.6%)
OCP	10 (6.6%)
DMPA	43 (28.5%)
Norplant	3 (2.0%)
IUD	2 (1.3%)

Variable	Value
Bacterial vaginosis (Gram stain criteria)	56 (37.1%)
Bacterial vaginosis (clinical criteria)	27 (17.9%)
Lactobacillus (any)	13 (8.6%)
Lactobacillus (H_2O_2 producing)	3 (2.0%)
Vaginal candidiasis	15 (9.9%)
Trichomonas vaginalis	2 (1.3%)
Neisseria gonorrhoeae	0 (0%)
Cervicitis[e]	0 (0%)

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

[a] Bar work has been associated with an increased risk of sexually transmitted infections and HIV acquisition in the Mombasa Cohort [1].

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

[c] Use of antiseptic for vaginal washing was reported by 3 women.

[d] N = 128. Data were missing for 2 of the 130 women who reported vaginal washing.

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

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Table 2

Risk Factors for Bacterial Vaginosis among Female Sex Workers in Mombasa, Kenya (Gram Stain Criteria)

	Univariate		Multivariate without *Lactobacillus*		Multivariate including *Lactobacillus*	
Characteristic	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**
Age[a]	0.80 (0.73–0.89)	<0.001	0.78 (0.70–0.86)	<0.001	0.78 (0.70–0.86)	<0.001
Years of sex work[a]	0.93 (0.81–1.06)	0.3				
Education <8 years	1.06 (0.91–1.24)	0.5				
Bar worker[b]	1.02 (0.85–1.23)	0.8				
Tobacco use	1.11 (0.89–1.37)	0.4				
Alcohol use	1.15 (0.95–1.39)	0.2				
Vaginal washing frequency						
None	1.0		1.0		1.0	
1–14 times per week	1.29 (0.96–1.72)	0.09	1.29 (0.88–1.89)	0.2	1.31 (0.89–1.91)	0.2
15–28 times per week	1.47 (1.08–1.99)	0.01	1.60 (0.89–2.61)	0.06	1.65 (1.01–2.70)	0.05
>28 times per week	1.94 (1.36–2.76)	<0.001	2.39 (1.35–4.23)	0.003	2.41 (1.36–4.27)	0.003
Test for trend	--	0.02	---	0.04	--	0.03
Vaginal washing substance						
None	1.0		1.0		1.0	
Water	1.18 (1.01–1.38)	0.04	0.95 (0.74–1.22)	0.7	0.93 (0.73–1.20)	0.6
Soap or antiseptic[c]	1.17 (1.00–1.38)	0.06	0.89 (0.69–1.15)	0.4	0.87 (0.68–1.12)	0.3
Cloth to clean inside vagina	1.58 (1.17–2.14)	0.003	1.48 (1.06–2.08)	0.02	1.50 (1.07–2.12)	0.02
Bathes <14 times per week	0.73 (0.52–1.04)	0.08	0.84 (0.59–1.21)	0.4	0.81 (0.57–1.17)	0.3
Vaginal lubricant for sex						
None	**1.0**		**1.0**		**1.0**	
Vaseline	1.39 (1.07–1.79)	0.01	1.22 (0.95–1.55)	0.1	1.26 (0.99–1.60)	0.06
Saliva	1.04 (0.49–2.24)	0.9	0.95 (0.50–1.80)	0.9	0.94 (0.49–1.80)	0.9
Sex partners in last week						
None	1.0		1.0		1.0	
One	1.38 (1.16–1.95)	<0.001	0.95 (0.77–1.16)	0.6	0.94 (0.77–1.16)	0.6
More than one	1.55 (1.24–1.95)	<0.001	0.97 (0.74–1.26)	0.8	0.94 (0.73–1.23)	0.7
Unprotected sex in last week	1.66 (1.43–1.92)	<0.001	1.75 (1.47–2.08)	<0.001	1.72 (1.44–2.06)	<0.001

	Univariate		Multivariate without *Lactobacillus*		Multivariate including *Lactobacillus*	
Characteristic	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**
New partner in past month	1.23 (1.01–1.51)	0.04	1.18 (0.95–1.47)	0.1	1.19 (0.96–1.48)	0.1
Contraception						
None or tubal ligation	1.0		1.0			
OCP	1.34 (1.06–1.68)	0.01	1.20 (0.96–1.50)	0.1	1.18 (0.94–1.48)	0.2
DMPA	0.59 (0.48–0.73)	<0.001	0.59 (0.48–0.73)	<0.001	0.60 (0.48–0.74)	<0.001
Norplant	0.91 (0.54–1.55)	0.7	0.89 (0.60–1.30)	0.5	0.97 (0.66–1.42)	0.9
IUD	1.28 (0.57–2.84)	0.5	1.59 (0.74–3.40)	0.2	1.80 (0.90–3.62))	0.1
Neisseria gonorrhoeae	0.39 (0.06–2.43)	0.3				
Cervicitis[d]	1.25 (0.74–2.11)	0.4				
Vaginal candidiasis	0.40 (0.27–0.59)	<0.001	0.41 (0.28–0.60)	<0.001	0.42 (0.29–0.62)	<0.001
Trichomonas vaginalis	1.73 (1.13–2.66)	0.01	1.83 (1.17–2.84)	0.008	1.78 (1.14–2.76)	0.01
Lactobacillus[e]						
None	1.0		---		1.0	
H_2O_2-negative only	0.65 (0.47–0.90)	0.01	---		0.70 (0.52–0.94)	0.02
H_2O_2-positive	0.58 (0.38–0.89)	0.01	---		0.64 (0.42–0.99)	0.04

NOTE: Hazard ratios are based on Andersen-Gill proportional hazards analysis. aHR, adjusted hazard ratio; CI, confidence interval; DMPA, depot medroxyprogesterone acetate; H_2O_2, hydrogen peroxide; HR, hazard ratio; IUD, intrauterine device; OCP, oral contraceptive pill.

[a] Per ten years.

[b] Bar work has been associated with an increased risk of sexually transmitted infections and HIV acquisition in the Mombasa Cohort [1].

[c] Use of antiseptics was reported at <2% of visits.

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

[e] *Lactobacillus* species identified on vaginal culture in the prior 60 days.

Table 3

Risk Factors for Bacterial Vaginosis among Female Sex Workers in Mombasa, Kenya (Clinical Criteria)

	Univariate		Multivariate without *Lactobacillus*		Multivariate including *Lactobacillus*	
Characteristic	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**
Age[a]	0.83 (0.72–0.97)	0.02	0.80 (0.69–0.92)	0.002	0.80 (0.69–0.92)	0.002
Years of sex work[a]	0.99 (0.83–1.20)	1.0				
Education <8 years	1.26 (1.00–1.58)	0.05	1.18 (0.93–1.49)	0.2	1.17 (0.93–1.48)	0.2
Bar worker[b]	1.09 (0.84–1.43)	0.5				
Tobacco use	1.17 (0.85–1.61)	0.3				
Alcohol use	1.12 (0.86–1.46)	0.4				
Vaginal washing frequency						
None	1.0		1.0		1.0	
1–14 times per week	2.00 (1.22–2.31)	0.006	1.38 (0.74–2.56)	0.3	1.38 (0.74–2.57)	0.3
15–28 times per week	2.04 (1.21–3.42)	0.007	1.17 (0.53–2.59)	0.7	1.21 (0.55–2.66)	0.6
>28 times per week	3.24 (1.80–5.83)	<0.001	1.96 (0.80–4.80)	0.1	1.95 (0.79–4.80)	0.2
Test for trend	---	0.05	---	0.2	---	0.2
Vaginal washing substance						
None	1.0		1.0		1.0	
Water	1.38 (1.10–1.73)	0.006	1.27 (0.89–1.81)	0.2	1.25 (0.88–1.80)	0.2
Soap or antiseptic[c]	1.41 (1.12–1.79)	0.004	1.25 (0.84–1.86)	0.3	1.22 (0.82–1.81)	0.3
Cloth to clean inside vagina	0.82 (1.41–1.62)	0.6				
Bathes <14 times per week	0.83 (0.52–1.34)	0.5				
Vaginal lubricant for sex						
None	**1.0**					
Vaseline	1.10 (0.71–1.70)	0.7				
Saliva	0.63 (0.19–2.05)	0.4				
Sex partners in last week						
None	1.0		1.0		1.0	
One	1.83 (1.41–2.38)	<0.001	1.29 (0.95–1.74)	0.1	1.28 (0.94–1.74)	0.1
More than one	1.83 (1.30–2.60)	<0.001	1.33 (0.92–1.93)	0.1	1.31 (0.90–1.90)	0.2
Unprotected sex in last week	1.93 (1.56–2.39)	<0.001	1.67 (1.30–2.14)	<0.001	1.65 (1.29–2.12)	<0.001

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	Univariate		Multivariate without *Lactobacillus*		Multivariate including *Lactobacillus*	
Characteristic	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**
New partner in past month	1.13 (0.84–1.15)	0.4				
Contraception						
None or tubal ligation	1.0		1.0		1.0	
OCP	1.54 (1.10–2.14)	0.01	1.36 (0.97–1.91)	0.07	1.35 (0.96–1.89)	0.09
DMPA	0.58 (0.42–0.78)	<0.001	0.58 (0.42–0.80)	<0.001	0.59 (0.42–0.81)	0.001
Norplant	1.19 (0.65–2.18)	0.6	1.19 (0.71–2.00)	0.5	1.29 (0.77–2.17)	0.3
IUD[d]	NC		NC		NC	
Neisseria gonorrhoeae[d]	NC					
Cervicitis[e]	0.72 (0.26–2.01)					
Vaginal candidiasis	0.50 (0.31–0.81)	0.005	0.52 (0.33–0.82)	0.005	0.53 (0.34–0.85)	0.008
Trichomonas vaginalis	1.90 (1.04–3.48)	0.04	2.04 (1.09–3.79)	0.02	2.00 (1.07–3.73)	0.03
Lactobacillus[f]						
None	1.0		---		1.0	
H_2O_2-negative only	0.63 (0.41–0.98)	0.04	---		0.76 (0.50–1.16)	0.2
H_2O_2-positive	0.46 (0.24–0.90)	0.02	---		0.57 (0.30–1.11)	0.1

NOTE: Hazard ratios are based on Andersen-Gill proportional hazards analysis. aHR, adjusted hazard ratio; CI, confidence interval; DMPA, depot medroxyprogesterone acetate; H_2O_2, hydrogen peroxide; HR, hazard ratio; IUD, intrauterine device; OCP, oral contraceptive pill; NC, no convergence.

[a] Per ten years.

[b] Bar work has been associated with an increased risk of sexually transmitted infections and HIV acquisition in the Mombasa Cohort [1].

[c] Use of antiseptics was reported at <2% of visits.

[d] There was no convergence of the model for this covariate, so it was excluded from the reported model.

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

[f] *Lactobacillus* species identified on vaginal culture in the prior 60 days.