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Risk and protective factors associated with BV chronicity among women in Rakai, Uganda

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Abstract

Objectives—To assess risk and protective factors associated with bacterial vaginosis (BV) chronicity ascertained by Nugent score criteria.

Methods—A longitudinal cohort study included 255 sexually experienced, postmenarcheal women who provided weekly self-collected vaginal swabs for up to 2 years. Vaginal swabs were scored using Nugent criteria and classified as normal (≤3), intermediate (4–6) and Nugent-BV (≥7). Detailed behavioural/health information were assessed every 6 months. A per-woman longitudinal summary measure of BV chronicity was defined as the percentage of each woman's weekly vaginal assessments scored as Nugent-BV over a 6-month interval. Risk and protective factors associated with BV chronicity were assessed using multiple linear regression with generalised estimating equations.

Results—Average BV chronicity was 39% across all follow-up periods. After adjustment, factors associated with BV chronicity included baseline Nugent-BV (β=35.3, 95% CI 28.6 to 42.0) compared with normal baseline Nugent scores and use of unprotected water for bathing (ie,

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Competing interests None declared.

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rainwater, pond, lake/stream) (β=12.0, 95% CI 3.4 to 20.5) compared with protected water sources (ie, well, tap, borehole). Women had fewer BV occurrences if they were currently pregnant (β= −6.6, 95% CI −12.1 to 1.1), reported consistent condom use (β=−7.7, 95% CI −14.2 to 1.3) or their partner was circumcised (β=−5.8, 95% CI −11.3 to 0.3).

Conclusions—Factors associated with higher and lower values of BV chronicity were multifactorial. Notably, higher values of BV chronicity were associated with potentially contaminated bathing water. Future studies should examine the role of waterborne microbial agents in the pathogenesis of BV.

INTRODUCTION

Bacterial vaginosis (BV) is characterised by relatively low levels of *Lactobacillus* species and an abundance of diverse anaerobic bacteria.[1] BV prevalence varies widely with the highest prevalence found in Sub-Saharan Africa.[2] Symptomatic BV is the most common vaginal complaint among women of reproductive age and has been shown to adversely impact sexual relationships and self-esteem.[3] Moreover, women with symptomatic and asymptomatic BV are at increased risk of adverse pregnancy and reproductive health outcomes, including preterm birth, endometritis and STIs.[4] The causal mechanisms remain under investigation, although lactic acid, low pH and immune responses are believed to be the main properties underlying protection against BV.[5] While current BV treatments can produce remission, rates of recurrence are substantial.[6] Thus, identifying potential risk and protective factors may elucidate the aetiology of this complex condition and identify prevention strategies.

Fluctuations in vaginal bacterial communities associated with BV may be due to a complex interplay between the host, bacteria and environment.[7] Risk factors for BV include menses, vaginal hygiene practices (eg, douching), risky sexual behaviours and partner characteristics (eg, uncircumcised partner), while protective factors include hormonal contraception.[8,9] Extravaginal reservoirs of BV-associated organisms have also been shown to increase BV,[10] possibly through sexual or hygiene practices.

We previously reported on a 2-year prospective study among rural women in Rakai, Uganda that examined factors related to short-term (weekly) variability of Nugent scores.[11] Here, we seek to examine risk and protective factors associated with BV chronicity, or the number of weekly vaginal microbiota assessments with Nugent-BV[12] divided by the total number of assessments per woman over a 6-month interval. Given the dynamic nature of vaginal microbiota, this longitudinal summary measure offers another approach to measuring a woman's predominant BV state.

MATERIALS AND METHODS

Study design and procedures

A 2-year cohort study was conducted between 2001 and 2003 to assess changes in BV status among 312 consenting women aged 13–39 years, residing in rural Rakai District, Uganda. Households were randomly selected from 24 subcommunity clusters in close proximity to Rakai Health Sciences Program (RHSP) field offices. One index and seven contiguous

households were selected within each subcommunity (192 households identified). All eligible, consenting women in each household were enrolled. Adolescent girls (13–19 years) were oversampled. Participants were included regardless of HIV status, current/prior pregnancy or history of sexual experience. A detailed description of this cohort has been published previously.[11] This analysis is based on 255 sexually experienced, postmenarcheal women. BV prevalence in this study was comparable with the larger cohort of women in Rakai; however, some characteristics (eg, contraceptive use, partners in the past year) differed given the younger age range and selection of communities closer to trading centres.[13]

Women provided self-collected vaginal swabs at weekly intervals. Swabs were assessed using the Nugent quantitative morphological classification,[1] which provides a standardised and reliable method for population-based assessment of vaginal microbiota.[2] Nugent scores were classified as normal (0–3 points), intermediate (4–6 points) or bacterial vaginosis (7–10 points, Nugent-BV).[12] Two laboratory technicians performed primary readings and a third reader validated a random sample of primary readings. HIV status was assessed at baseline and every 6 months using two HIV enzyme immunosorbent assays (EIAs) (Vironostika HIV-1; Organon Teknicka, Charlotte, North Carolina, USA; Cambridge Biotech, Worcester, Massachusetts, USA) with Western blot (HIV-1 Western Blot; BioMerieuz-Vitek, St. Louis, Missouri, USA) for discordant EIA results. *Trichomonas vaginalis* was detected at baseline and every 6 months using the InPouch TV culture method (BioMed Diagnostics, San Jose, California, USA). Pregnancy was assessed at baseline via urinary hCG in all women who were not visibly pregnant. Thereafter, women whose last menstrual period occurred over 30 days ago, were not using Depo Provera, self-reported being pregnant but not yet visible, or had lactational amenorrhoea for 4 months or longer were tested monthly.

Interview data were collected at baseline and every 6 months to ascertain demographic characteristics, household environment, reproductive health history, sexual behaviours, partner characteristics (eg, circumcision status), vaginal and personal hygiene behaviours, general health status and self-reported treatment for vaginal symptoms. Information on sexual behaviours and partner changes over 30 days, changes in hygiene practices, vaginal symptoms, syndromic treatment and treatment provider, menstrual history and pregnancy histories was collected monthly. Information on sexual intercourse, menstrual history, vaginal symptoms and treatment in the past week was collected weekly.

Vaginal symptoms (genital discharge, dysuria and ulcers) were treated syndromically. For women self-reporting abnormal discharge, RHSP provided a single, directly observed 2 g dose of metronidazole and cotrimazole pessaries (1 bd for 5 days). Since persistent discharge could occur as a result of gonorrhoea or chlamydia, non-pregnant women with persistent discharge were treated with single-dose azithromycin (1000 mg) plus single-dose ciproflaxin 500 mg; pregnant women with persistent discharge received single-dose azithromycin and cefixime (400 mg). Given the limited response to treatment of asymptomatic Nugent-BV,[14,15] RHSP did not provide antibiotics if symptoms were not present. Women in the community may have also sought treatment from providers outside of RHSP.

Variable definitions

To characterise a woman's predominant BV state, each woman's 2-year follow-up period was divided into four 6-month intervals. A longitudinal summary measure of BV chronicity for each woman was calculated for each of the four 6 month intervals as follows: the total number of weekly vaginal microbiota assessments that were scored as Nugent-BV were divided by the total number of vaginal microbiota assessments for each woman, and multiplied by 100. BV chronicity was expressed as a percentage value for each woman and treated as a continuous variable for analysis. The 6-month period was chosen because it provided sufficient observation time to assess the frequency of Nugent-BV occurrences and could be linked to detailed behavioural assessment from the 6-monthly interviews.

Explanatory variables at baseline included age, religion, relative wealth status, BV at enrolment, source of bathing water and genital washing frequency. Responses for source of bathing water were grouped at the time of analysis as protected (protected well, tap or borehole), partially protected (unprotected well) and unprotected (rainwater, pond/lake/stream) and were based on the least protected source reported by the respondent out of three possible responses. These categories were determined in consultation with the RHSP field team and represent bathing water sources that had a fresh supply of water (eg, from an underground spring or well) and/or complete, partial or no protection from external environmental contaminates (see online supplementary table 1 for a detailed description). The relative household wealth index was based on the weighted sum of possession of modern objects (radio, bicycle, vehicle) and dwelling characteristics (materials used for roof, walls and floor, and availability of electricity and latrine), divided into tertiles.

Potential time-varying characteristics included HIV status and symptoms of AIDS, *T. vaginalis*, self-reported treatment for vaginal symptoms during weekly visits within each 6-month interval (none, one, two or more times), pregnancy and breastfeeding status, hormonal contraceptive use, items used to bathe the vagina, intravaginal practices (IVPs, defined as substances or objects inserted into the vagina and whether they were obtained from a health worker or not), lifetime number of sex partners, sexual activity in the past 6 months, condom use with the most recent partner and partner characteristics (ie, partner's other relationships, circumcision status). Two women who reported washing with salt or detergent were excluded from the analysis. For HIV status, if a woman reported symptoms of AIDS at one point in follow-up, she was categorised as such and remained in that category throughout follow-up. Antiretroviral therapy was not available in Rakai at the time of this study (prior to 2004). Pregnancy and breastfeeding status were based on whether they were pregnant, breastfeeding (but not pregnant) or neither.

Statistical methods

Descriptive characteristics at baseline and BV chronicity categories of none to 100 (divided into 11 groups) and self-reported treatment for vaginal symptoms across each 6-month visit were assessed. Mean differences in BV chronicity (continuous variable) and 95% CIs were estimated from a linear regression model using generalised estimating equations with an unstructured correlation matrix and robust standard errors. Covariates were retained in the model if they were associated with the outcome in unadjusted models (alpha-level=0.10) or

were potential confounders reported in other studies. A strong association between religion and partner's circumcision status precluded inclusion of both variables in the model. Therefore, only partner's circumcision status was selected for inclusion. IVP was not included in the final model because the main reason for vaginal insertion was having vaginal symptoms (ie, discharge, itching), thus a potential for reverse causality. Change in covariate associations were assessed in sensitivity analyses that excluded baseline BV status or adjusted for *T. vaginalis*.

We used history matrix visualisation to illustrate longitudinal BV patterns ordered from lowest to highest values of BV chronicity by bathing water source for participants with near complete data (≤1 missing) in the first 6 months (n=154). The upper quartile (ie, 70% or higher) of the distribution of BV chronicity values was compared by bathing water source using a non-parametric Wilcoxon-type test for trend. Statistical analyses and history transition matrices were performed in Stata V.14.2 (StataCorp, College Station, Texas, USA) and R V.2.8.1 statistical software, respectively.

RESULTS

Baseline characteristics of 255 women are shown in table 1. The majority of women in the sample were between the ages of 20 and 29 years, Christian and in the lowest wealth tertile. Forty-eight per cent of women had BV at enrolment.

During follow-up, 82.3% (n=210) women contributed observation time across all four 6-month intervals. Losses to follow-up were 6.3% (n=16) after 0–6 months, 6.3% (n=16) after 6–12 months and 4.3% (n=11) after 12-month to 18-month intervals. Two women (0.01%) did not contribute observation time after baseline. The percentage of women with no Nugent-BV per 6-month interval (ie, BV chronicity=0) increased over the course of the study (6 months, 12.3%; 24 months, 20.5%), whereas the percentage with the highest BV chronicity values (90.1%–100%) remained relatively constant (6 months, 12.7%; 24 months, 11.4%) (table 2). Self-reported treatment for vaginal symptoms decreased over the study period (6 months, 35.2%; 24 months, 26.7%).

After adjustment for all covariates retained in the model (table 3), BV chronicity among all referent groups was 20.8% (95% CI 7.9 to 33.6), or approximately 5 weeks per 6-month interval. There were significantly lower BV chronicity values in older women (β=−9.2, 95% CI −16.3 to −2.1 for ages 20–29, and β=−11.6, 95% CI −21.1 to −2.2 for ages 30–39), pregnant women (β=−6.6, 95% CI −12.1 to −1.1), women reporting consistent condom use (β=−7.7, 95% CI −14.2 to −1.3) and women reporting their partner was circumcised (β=−5.8, 95% CI −11.3 to −0.3) compared with each respective referent group. Factors associated with higher BV chronicity values included having Nugent score categories of intermediate or BV at baseline (β=8.2, 95% CI 0.4 to 15.9 and β=35.3, 95% CI 28.6 to 42.0, respectively) and unprotected water for bathing (β=12.0, 95% CI 3.4 to 20.5). Partitioning the unprotected water source category further into rain (β=13.6, 95% CI 4.2 to 23.1) or pond (β=12.6, 95% CI 0.3 to 24.9) showed similar associations (data not in table 3). Other factors retained in the model were not associated with BV chronicity (table 3).

In sensitivity analyses, we found minimal change in covariate associations, but partner circumcision status was no longer significant after including *T. vaginalis* (β=−3.5, 95% CI −8.7 to 1.8) or excluding baseline BV status from the model (β=−5.6, 95% CI −11.4 to 0.2).

Figure 1 shows a higher proportion of women with chronic BV (ie, upper quartile of BV chronicity values) for women reporting unprotected (40.6%) compared with protected (11.5%) or partially protected (18.6%) bathing water sources (p<0.001, test for trend).

DISCUSSION

BV was common in this population of rural Ugandan women with close to 50% of women having BV at enrolment and average BV chronicity values of 40% across all women in the study over time. Consistent with the recurrent or persistent nature of BV, women spent almost 36% more time with BV if they had BV at enrolment compared with a normal Nugent score. Factors associated with lower BV chronicity included older age, pregnancy, circumcision status and consistent condom use. Notably, unprotected sources of bathing water, were associated with higher BV chronicity.

While prior studies have examined hygiene factors related to BV in Sub-Saharan Africa,[16–18] few studies have investigated sources of bathing water. A study conducted in The Gambia reported no association between BV and water source (tap or protected well only) among an urban, clinic-based population.[16] In contrast, our study was conducted among women who resided mainly in rural areas, where microbial contamination of water sources is known to be higher than urban areas.[19] In Rakai, women bathe by the home using water collected in bins from sources identified in this study. Surface water (eg, ponds, streams) is susceptible to faecal contamination from animals.[20] Given that the rectum has been postulated as a reservoir for BV-associated bacteria,[21] it is conceivable that faecal contamination may increase exposure to potential microbial pathogens that influence BV development. Additionally, rainwater is collected in wide-mouthed bins that capture water falling from roofs. Studies document that wide-mouthed water storage vessels are more likely to be contaminated with total coliforms and *E. coli* compared with narrow-mouthed vessels.[2223]

There is also a potential for water collection bins to be cross-contaminated by multiple water sources. In our analysis, however, we account for the possibility that multiple sources of water were used and classified the groups according to the least protective source. Thus, women who reported protected bathing water sources would have only used this source, minimising the likelihood of cross-contamination. Alternatively, bathwater was highly correlated with drinking water. Thus, we cannot exclude the possibility that unsafe drinking water may increase the possibility of vaginal contamination through exposure to gut microbiota. We also cannot exclude the possibility that unmeasured confounders, including shared environmental factors and bathing practices, may explain this relationship. However, the relationship held after sensitivity analyses and controlling for several sociodemographic characteristics and hygiene practices.

Several factors were also associated with decreased BV chronicity including older age, pregnancy, consistent condom use and a circumcised male partner. Consistent with our findings, studies have reported a more *Lactobacillus*-dominant vaginal microbiota during pregnancy.[24] The association between higher oestrogen and *Lactobacillus*-dominant vaginal microbiota has been shown in other studies; however, the mechanism remains unclear.[25] The reduction or absence of menstruation during pregnancy may also reduce susceptibility to BV. In contrast to some studies,[9] we found no association between hormonal contraceptive use and persistent BV. A recent randomised controlled pilot study by Vodstrcil *et al* found no difference in the rate of BV recurrence between women randomised to combined oral contraception or non-hormonal contraception,[26] suggesting protective associations may be limited in the context of recurrence.

Consistent condom use and having a circumcised partner reduced BV chronicity. A randomised trial of male circumcision showed a significant reduction of BV in women whose partners were circumcised[27] and reductions in penile proinflammatory anaerobic bacteria after circumcision.[28] Exposure to the same partner before and after treatment for BV and inconsistent condom use have also been associated with higher recurrence of BV.[29]

We found no relationship between BV chronicity and the number of sex partners, male partners having multiple female sex partners or the woman having a new partner; however, sample size was limited for these risk factors. In addition, self-reported treatment for vaginal symptoms was not associated with BV chronicity. Weekly reports of self-reported treatment was examined in this analysis because it had been associated with short-term resolution of BV in this cohort[11] and because RHSP is not the sole provider of treatment in this district. However, we could not differentiate the type of treatment and this measure may not provide the level of specificity to assess treatment effects across longer intervals.

There are a number of strengths to this analysis. BV chronicity per woman at each 6-month interval may better characterise the predominant vaginal microbiota state and reduce misclassification due to transient BV episodes. In addition, we had extensive information on sexual and hygiene practices, behaviours and environment.

There are also limitations. Although the number of women (n=255) and observations (weekly visits up to 2 years) was relatively large, power may be limited to detect associations in smaller subgroups. In addition, Gram stain is based on morphological assessment. Higher resolution tools, such as 16S rRNA gene amplicon sequencing, may reveal other important findings in the vaginal microbiota with species-specific results, such as *Lactobacillus iners*. Furthermore, we could not validate bathing water sources with measures of microbial contamination (eg, faecal indicator bacteria, such as *E. coli* or thermotolerant coliforms) or delineate how bathing practices, such as sharing of water collection bins for vaginal washing, may have influenced the development of BV. However, we would anticipate that if the causal mechanism were due to sharing bins rather than bathing water source, exposure to BV-associated organisms would be found across all categories of bathing water source and attenuate associations. Thus, this practice may be less likely to explain the differences found in our study. Finally, we did not have complete

information on other STIs (eg, herpes simplex virus-2) that may affect the vaginal microenvironment and susceptibility to BV.[30]

Summary and implications

Identifying modifiable risk factors for BV is needed to improve strategies for prevention and care of this common gynaecological condition. Using frequent sampling in a cohort of rural Ugandan women, our findings were consistent with a multifactorial aetiology, including pregnancy-related changes and sexual transmission. Additionally, we found that unprotected bathing water sources were consistently associated with BV, suggesting a potential role of environmental factors. Future studies should examine the importance of waterborne microbial agents in the pathogenesis of BV.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key messages

- This study examined factors associated with bacterial vaginosis (BV) chronicity (ie, percentage of weekly vaginal microbiota assessments scored as Nugent-BV per woman over a 6-month interval), which may provide a better measure of a woman's predominant BV state.
- Most notably, use of unprotected bathing water sources (eg, pond, stream) increased BV frequency, suggesting environmental factors may contribute to the aetiology of BV.
- Consistent with other studies, pregnancy, consistent condom use and a circumcised partner were associated with reduced BV chronicity.

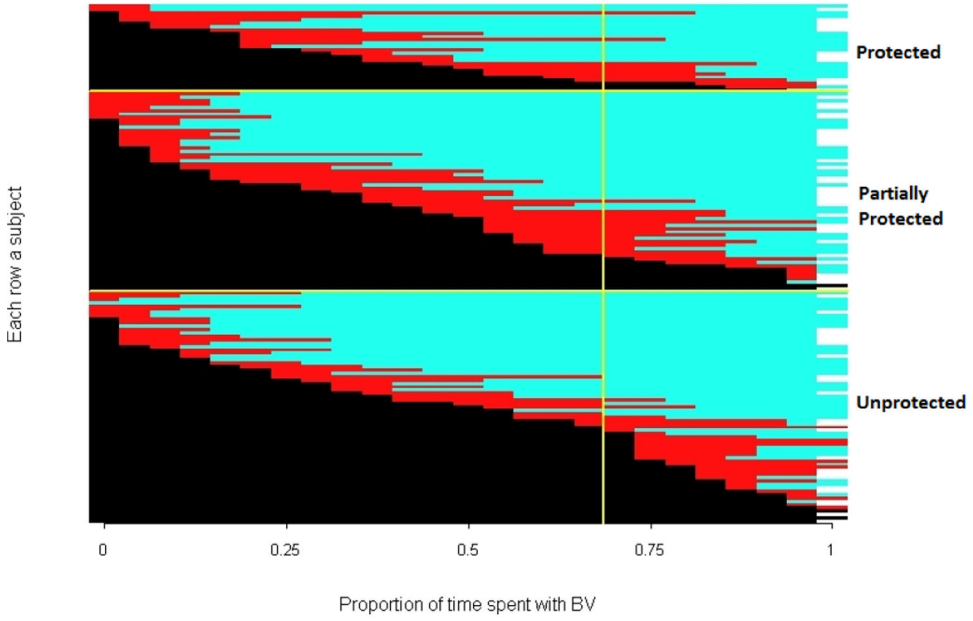

Figure 1.

Source of bathing water and the proportion of time spent with bacterial vaginosis (BV) within women ranked from lowest to highest in the first 6 months of the study. In the graph, light blue bars correspond to normal vaginal smears, red bars to intermediate Nugent score, black bars to BV and white bars to interval-censored visits. Horizontal yellow lines divide women based on source of bathing water and the vertical yellow line corresponds to the upper quartile of the distribution of the proportion of time spent with BV (ie, greater than 70% of weekly observation time with BV). Protected sources include a protected well, tap or borehole; partially protected sources include an unprotected well; and unprotected sources include rainwater, pond/lake/stream.

Table 1

Descriptive characteristics of ever sexually active, postmenarcheal women at enrolment, n=255

Characteristics	n (%)
Sociodemographic	
Age	
14–19	65 (25.5)
20–29	130 (51.0)
30–39	60 (23.5)
Religion	
Christian	205 (80.4)
Muslim	50 (19.6)
Wealth tertiles	
Low	105 (41.2)
Middle	76 (29.8)
High	74 (29.0)
General and reproductive health	
Nugent score at baseline	
Normal (0–3)	79 (31.1)
Intermediate (4–6)	54 (21.3)
BV (7–10)	121 (47.6)
HIV status	
Negative	220 (88.7)
Positive (without AIDS symptoms)	19 (7.7)
Positive (with AIDS symptoms)	9 (3.6)
Self-reported treatment for vaginal symptoms (past week)	
No	233 (91.7)
Yes	21 (8.3)
Currently pregnant	
No	219 (86.6)
Yes	34 (13.4)
Currently breast feeding	
No	157 (61.6)
Yes	98 (38.4)
Current hormonal contraceptive use	
None	200 (78.7)
Contraceptive pill/oral contraceptives	19 (7.5)
Injectable methods (Depo Provera)	35 (13.8)
Norplant	0 (0)
Vaginal hygiene practices	
Source of bathing water	
Protected	46 (18.0)
Partially protected	97 (38.0)

Characteristics	n (%)
Unprotected	112 (43.9)
Items used to bathe the vagina	
Soap and water	198 (78.0)
Water only	56 (22.1)
Genital washing frequency (times per day)	
1 to 3	208 (81.9)
4 or more	46 (18.1)
Intravaginal practices	
No substances or objects inserted into the vagina	178 (77.4)
Substances or objects inserted were obtained from health provider *	21 (9.1)
Other substances or objects †	31 (13.5)
Sexual behaviours and partner characteristics	
Condom use with most recent partner	
Never used	151 (59.2)
Inconsistent use	70 (27.5)
Consistent use	34 (13.3)
No of sex partners in the past year	
None	21 (8.2)
1	222 (87.1)
2 or 3	12 (4.7)
Partner has other sex partners	
No	47 (18.4)
Yes	104 (40.8)
Don’t know	104 (40.8)
Partner is circumcised	
No	171 (68.1)
Yes	80 (31.9)

*Foam/jelly/tablet/cream obtained from a health worker/pharmacist. The reasons cited for insertion were related to vaginal symptoms (66.7% cited discharge, 33.3% cited itching).

†Included substances not obtained from a health provider. The majority of respondents indicated “herbs/leaves/powder” (83.9%), medicated soaps (12.9%) or other (3.2%). The main reasons cited for insertion was “widening pelvic girdle during pregnancy” (38.17%), attributed mainly to herbs/leaves/powders, followed by vaginal symptoms or hygiene reasons (12.9%).

BV, bacterial vaginosis.

Table 2

BV chronicity deciles and self-reported treatment of vaginal symptoms across each 6-month visit

	6-month visits N (%)			
	6 months	**12 months**	**18 months**	**24 months**
BV chronicity categories *				
None	31 (12.3)	44 (18.6)	32 (14.5)	43 (20.5)
0.1%–10.0%	39 (15.4)	36 (15.2)	29 (13.1)	30 (14.3)
10.1%–20.0%	20 (7.9)	28 (11.8)	27 (12.2)	24 (11.4)
20.1%–30.0%	19 (7.5)	19 (8.0)	20 (9.1)	13 (6.2)
30.1%–40.0%	20 (7.9)	17 (7.2)	17 (7.7)	8 (3.8)
40.1%–50.0%	19 (7.5)	16 (6.8)	15 (6.8)	13 (6.2)
50.1%–60.0%	22 (8.7)	13 (5.5)	18 (8.1)	9 (4.3)
60.1%–70.0%	10 (4.0)	18 (7.6)	16 (7.2)	14 (6.7)
70.1%–80.0%	22 (8.7)	7 (3.0)	11 (5.0)	14 (6.7)
80.1%–90.0%	19 (7.5)	15 (6.3)	10 (4.5)	18 (8.6)
90.1%–100%	32 (12.7)	24 (10.1)	26 (11.8)	24 (11.4)
Self-reported treatment for vaginal symptoms †				
None	164 (64.8)	170 (71.7)	160 (72.4)	154 (73.3)
Once	49 (19.4)	39 (16.5)	33 (14.9)	27 (12.9)
2 or more times	40 (15.8)	28 (11.8)	28 (12.7)	29 (13.8)

*Calculated as the total number of weekly vaginal microbiota assessments that were scored as Nugent-BV divided by the total number of vaginal microbiota assessments, and multiplied by 100. This measure was then categorised into deciles of BV chronicity for each 6-month visit.

†Treatment reported in the past 6 months.

BV, bacterial vaginosis.

Table 3

Unadjusted and adjusted differences in the average percentage of BV fluctuations by sociodemographic, health, vaginal washing, sexual behaviour and partner characteristics

Characteristics	Number of observations	Mean BV chronicity[†] (95% CI)	Unadjusted β (95% CI)[‡]	Adjusted β (95% CI)[§]
Total	921	39.2 (37.0 to 41.4)	–	–
Average across all referent groups (intercept)	–	–	–	20.8 (7.9 to 33.6)
Sociodemographic				
Age				
14–19	225	42.9 (38.4 to 47.4)	ref	ref
20–29	475	39.0 (35.9 to 42.1)	−4.5 (−13.1 to 4.0)	−9.2 (−16.3 to −2.1) **
30–39	221	35.8 (31.4 to 40.1)	−7.2 (−17.2 to 2.7)	−11.6 (−21.1 to −2.2) **
Wealth tertiles				
Low	381	37.3 (33.9 to 40.7)	ref	ref
Middle	280	43.6 (39.5 to 47.7)	5.3 (−3.2 to 13.8)	5.3 (−1.6 to 12.1)
High	260	37.2 (33.1 to 41.3)	−0.5 (−9.2 to 8.1)	1.9 (−5.5 to 9.4)
General and reproductive health				
BV status at enrolment				
Normal	288	22.7 (19.3 to 26.0)	ref	ref
Intermediate	200	27.6 (23.6 to 31.6)	5.5 (−2.1 to 13.0)	8.2 (0.4 to 15.9) **
BV	429	55.7 (52.6 to 58.7)	35.7 (28.9 to 42.4) **	35.3 (28.6 to 42.0) **
HIV status				
Negative	808	38.5 (36.1 to 40.8)	ref	ref
Positive without AIDS symptoms	59	40.4 (32.0 to 48.8)	10.6 (−1.5 to 22.6) *	10.0 (−2.2 to 22.2)
Positive with AIDS symptoms	39	48.2 (35.8 to 60.7)	10.1 (−7.8 to 28.0)	4.7 (−9.9 to 19.2)
Self-reported treatment for vaginal symptoms (past 6 months)				
None	648	39.3 (36.7 to 42.0)	ref	ref
Once	148	38.9 (33.3 to 44.4)	0.9 (−3.1 to 4.8)	0.5 (−3.3 to 4.2)
2 or more times	125	38.7 (33.3 to 44.1)	−1.6 (−5.6 to 2.4)	−2.3 (−6.7 to 2.1)
Pregnancy and breastfeeding status (past 6 months)				
Neither	399	41.2 (37.8 to 44.7)	ref	ref

Characteristics	Number of observations	Mean BV chronicity[†] (95% CI)	Unadjusted β (95% CI)[‡]	Adjusted β (95% CI)[§]
Breast fed but not pregnant	309	38.3 (34.5 to 42.1)	−1.1 (−6.0 to 3.8)	−2.2 (−7.1 to 2.7)
Pregnant	213	36.6 (32.2 to 41.0)	−6.8 (−11.8 to −1.9) **	−6.6 (−12.1 to −1.1) **
Current hormonal contraceptive use				
None	609	37.3 (34.6 to 40.0)	ref	ref
Contraceptive pill/oral contraceptives	54	43.2 (34.5 to 51.9)	5.9 (−0.2 to 12.1) *	2.4 (−3.9 to 8.8)
Injectables (Depo Provera)	183	45.4 (40.4 to 50.3)	3.7 (−1.6 to 9.1)	1.3 (−4.3 to 7.0)
Norplant	29	23.9 (13.2 to 34.5)	−1.5 (−13.0 to 10.0)	2.5 (−8.3 to 13.2)
Vaginal washing practices				
Source of bathing water[¶]				
Protected	171	34.8 (30.2 to 39.4)	ref	ref
Partially protected	343	35.4 (31.9 to 38.8)	1.0 (−7.8 to 9.8)	4.4 (−3.9 to 12.7)
Unprotected	407	44.2 (40.7 to 47.7)	8.6 (−0.2 to 17.4) *	12.0 (3.4 to 20.5) **
Items used to bathe the vagina				
Soap and water	722	38.6 (36.2 to 41.1)	ref	ref
Water only	151	40.6 (34.9 to 46.2)	1.3 (−4.1 to 6.7)	2.1 (−3.2 to 7.5)
Genital washing frequency (times per day)				
1 to 3	752	38.7 (36.2 to 41.1)	ref	ref
4 or more	165	40.7 (35.3 to 46.1)	1.6 (−8.3 to 11.5)	0.78 (−7.8 to 9.4)
Sexual behaviours and partner characteristics				
Condom use or sexual activity (past 6 months)				
Never used condoms	566	40.4 (37.6 to 43.2)	ref	ref
Inconsistent use	153	39.5 (34.1 to 45.0)	4.6 (0.01 to 9.18) **	3.8 (−0.9 to 8.5)
Consistent use	65	31.7 (24.1 to 39.3)	−6.7 (−13.2 to −0.2) **	−7.7 (−14.2 to −1.3) **
No sex in the past 6 months	90	34.1 (26.8 to 41.5)	−4.5 (−11.1 to 2.1)	−3.1 (−8.9 to 2.7)
No of sex partners ever				
One	237	32.9 (28.8 to 37.1)	ref	ref
Two	234	41.5 (36.9 to 46.0)	3.4 (−3.5 to 10.2)	3.5 (−4.0 to 10.9)
Three or more	404	41.0 (37.7 to 44.3)	2.3 (−4.8 to 9.3)	4.1 (−2.9 to 11.1)
Partner has other sex partners				
No	138	34.3 (28.9 to 39.8)	ref	ref

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Characteristics	Number of observations	Mean BV chronicity[†] (95% CI)	Unadjusted β (95% CI)[‡]	Adjusted β (95% CI)[§]
Yes	392	43.1 (39.7 to 46.5)	2.6 (−3.0 to 8.3)	−0.7 (−6.8 to 5.5)
Don't know	345	36.0 (32.5 to 39.6)	−1.0 (−6.4 to 4.4)	−3.7 (−9.4 to 2.1)
Partner is circumcised				
No	590	40.6 (37.8 to 43.3)	ref	ref
Yes	282	35.2 (31.2 to 39.2)	−4.9 (−10.3 to 0.55) [*]	−5.8 (−11.3 to −0.3) [**]

[*] 0.05<p<0.1 and

[**] p< 0.05.

[†] Average across four 6-month intervals of BV chronicity. BV chronicity was calculated as the total number of weekly vaginal microbiota assessments that were scored as Nugent-BV divided by the total number of vaginal microbiota assessments, and multiplied by 100.

[‡] Uses total number of observations available for each variable.

[§] Number of observations=856; number of women=234; adjusts for all factors displayed in the table.

[¶] Based on the least protected source reported by respondent out of 3 possible responses: protected sources (protected well, tap or borehole), partially protected (unprotected well) and unprotected (rainwater, pond/lake/stream).

BV, bacterial vaginosis.