

## Correlation of Behaviors with Microbiological Changes in Vaginal Flora

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Bacterial vaginosis (BV) is characterized by dramatic changes in the vaginal ecosystem. Women without evidence of vaginal infection may exhibit transient changes in their flora. We prospectively followed up women by using diaries and self-obtained vaginal smears to correlate behaviors with changes in flora. The majority of women (38/51, 78%) had significant, although transient, changes. Behaviors associated with unstable flora were a history of BV, a greater number of partners, and more frequent episodes of receptive oral sex. Only the latter remained significantly associated in the multivariate analysis. Variables that were associated with day-to-day variability in the flora included use of vaginal medication, menses, greater number of partners, spermicide use, more frequent vaginal intercourse, and less frequent use of condoms. Only a minority of women (11/51, 22%) maintained a "normal" lactobacillus-predominant flora. Factors associated with instability of the flora are similar to those epidemiologically associated with BV.

Despite the fact that the etiology of bacterial vaginosis (BV) remains unknown, the dramatic microbiological changes that are associated with this syndrome are well understood. Significant shifts in the vaginal ecosystem occur in patients with BV, specifically a decrease in the number of hydrogen peroxide-producing lactobacilli and a large increase in the number of anaerobic and facultatively anaerobic bacteria [1, 2]. Although the healthy vagina is often characterized as having a predominance of lactobacilli, transient shifts in the vaginal flora (VF) have been shown to occur in many women without evidence of genital tract infection. These shifts have previously been shown to be associated with menses [3–8]; however, the contributions of behavioral factors to microbiological changes in the VF have been less well studied. We examined the day-to-day changes in VF in a group of women without evidence of genital tract infection, and we correlated those changes to specific reported behaviors.

### Materials and Methods

**Study population.** Premenopausal, nonpregnant women aged  $\geq 18$  years without clinical evidence of genital tract infection, including BV, were recruited for the study. Women attending the

Jefferson County Department of Health (JCDH) Sexually Transmitted Diseases Clinic for a routine evaluation were eligible; however, the majority of women were recruited via an advertisement in the university newspaper. Participants were screened for sexually transmitted diseases (STDs) at the enrollment visit, and if an STD was detected they were promptly treated and removed from the study.

**Clinical methods.** Women who consented to the study were interviewed concerning their sexual and behavioral history, including sexual activity, contraception, douching, tampon use, antibiotic use, and history of STD and vaginal infections. A pelvic examination was performed by using a nonlubricated speculum. Specimens obtained for immediate examination included a vaginal pH, "whiff" test, and wet-prep microscopy. If evidence of vaginal infection was found, the patient was excluded from the study. Additional vaginal specimens were obtained for Gram stain and for cultures of *Trichomonas vaginalis*. Endocervical specimens were obtained for culture for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Blood was obtained for syphilis serology. Subjects were instructed to keep a diary of events such as sexual activity, menses, antibiotic use, douching, and so forth. They were also instructed to perform daily self-obtained vaginal smears by inserting a sterile cotton swab into the vaginal vault and immediately rolling the specimen gently onto a clean glass slide. Subjects were seen after 3 weeks to review their diary and to collect slides obtained thus far. The final study visit took place  $\sim 6$  weeks after enrollment. At this visit, the history, examination, and diagnostic testing were repeated, and the final diary and slides were collected.

**Laboratory methods.** Cultures for *N. gonorrhoeae* were done by using modified Thayer–Martin media according to standard procedures [9]. Chlamydial infections were diagnosed by cell culture in microtiter plates [10]. Cultures for *T. vaginalis* were done by using the InPouch TV test (BioMed Diagnostics Inc., Santa Clara, CA) [11]. Pouches were incubated at 37°C and examined daily for up to 5 days. Vaginal smears were Gram stained and interpreted by using the Nugent method. This technique relies on the quantification of 3 different bacterial morphotypes: large gram-positive bacilli, small gram-variable coccobacilli, and curved rods. Nugent

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Informed consent was obtained from all patients enrolled in this study, which conformed to the guidelines for human experimentation of the US Department of Health and Human Services and the Institutional Review Board of the University of Alabama at Birmingham.

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scores of 0–3 represent normal VF, 4–6 represent intermediate flora, and 7–10 represent BV [12]. Gram stains were interpreted by individuals who were unaware of the subject’s diary entries or historical data. Clinical criteria were used for the bedside diagnosis of BV [13].

*Statistical analyses.* Descriptive statistics such as frequencies and proportions were calculated for categorical demographic, clinical, and behavioral variables, whereas means and standard deviations were calculated for continuous variables. The correlation between quantitative demographic variables, such as number of episodes of sexual intercourse, and the corresponding diary data was estimated by calculating the Spearman’s correlation coefficient. The  $\chi^2$  test was used to assess the association between demographic and diary data for dichotomous variables such as condom use (yes/no). Further, sensitivity and specificity estimates were calculated to compare diary information with baseline demographic information.

The proportion of normal VF scores was calculated for each woman on the basis of data from her self-obtained vaginal smears. The distribution of the percentage of slides with normal VF scores from the women indicated a median of 85%. Thus, from the distribution of these proportions, subjects were categorized as having <85% normal VF scores (“unstable”) versus  $\geq$ 85% normal VF scores (“stable”). The  $\chi^2$  test was used to univariately compare various demographic, clinical, and behavioral variables between these 2 groups of women. The Wilcoxon rank-sum test was used to compare continuous variables between the 2 groups. Multiple logistic regression was done to determine the significant predictors of having <85% normal VF scores. Demographic and diary data were included in the full logistic model, and a stepwise selection procedure was used to determine the significant variables in the final model.

To assess the relationships of reported behaviors to day-to-day changes in VF scores, each 2 subsequent scores over the entire observation period were compared for each woman. A change was defined as the current score being higher than the previous score; otherwise, no change was defined for a specific pair of scores. As a result, each woman had repeated measures of change or no change in VF scores over the entire observation period. Longitudinal data analysis was used to determine the significant predictors of change in scores. Specifically, a generalized linear model by using a binomial distribution with a logit link was used to model the effect of demographic and diary variables on the change in VF score. Demographic and diary variables were included in the full model. Because the predictor variables from the diary data were also measured repeatedly over the entire observation period, we lagged the effect of these variables on change in VF scores by 1 day. Model-building was done to determine the significant predictors of change in VF scores. Relative risks and 95% confidence intervals were calculated for each significant predictor of change in VF score [14].

**Results**

*Description of the study population.* Sixty women were enrolled in the study. A description of the study population is shown in table 1. There was a nearly even distribution of blacks

**Table 1.** Description of study population (n = 60).

Characteristic	No. (%)
Age (years)	
Mean	30
Median	28
Minimum	19
Maximum	56
19–24	17 (28.3)
25–29	17 (28.3)
30–34	12 (20.0)
$\geq$ 35	14 (20.0)
Race	
Black	31 (51.7)
White	28 (46.7)
Asian	1 (1.7)
Sexual history/behavior	
Type of contraception <sup>a</sup>	
Oral contraceptives	21 (35)
Diaphragm	1 (1.7)
Spermicide	3 (5.0)
Condoms	51 (85.0)
Depoprovera	2 (40.0)
Douching	22 (36.7)
Type of douche	
Betadine	2 (9.1)
Vinegar/water	19 (86.4)
Baking soda	1 (4.5)
Douching frequency (per month)	
0–1 times	19 (86.4)
2–4 times	3 (13.6)
Tampon use	38 (63.3)
Frequency of use	
Rare	3 (7.9)
$\leq$ 50% of time	8 (21.0)
>50% of time	12 (31.6)
100% of time	14 (36.8)
Unknown	1 (2.6)
History of bacterial vaginosis (BV)	
Yes	16 (26.7)
No	44 (73.3)
No. of previous BV episodes	
1	7 (41.1)
2	4 (23.5)
3	3 (17.6)
4	0
5	2 (11.7)
History of STD <sup>b</sup>	
Trichomoniasis	17 (28.3)
Gonorrhea	5 (8.3)
Chlamydia	10 (16.7)
Syphilis	1 (1.7)
Herpes	2 (3.3)
Any of these STDs	24 (40)
Lifetime sex partners, mean (median)	9.4 (7.0)
Partners during past year, mean (median)	1.2 (1.0)
Partners during past 30 days, mean (median)	0.65 (1.0)
Episodes of vaginal intercourse per month <sup>c</sup>	7.2 (6.0)
Episodes of receptive oral sex per month <sup>c</sup>	2.15 (0.5)

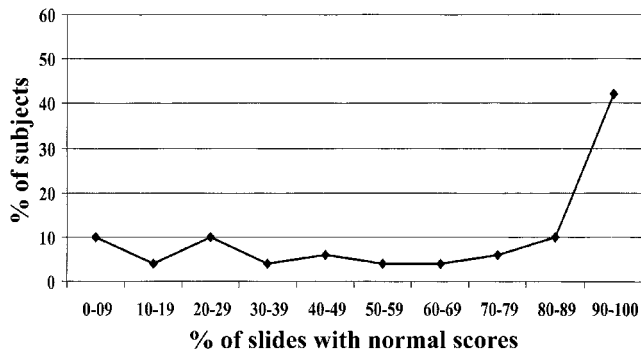
NOTE. Values are no. (%) unless otherwise indicated.

<sup>a</sup> Some women reported multiple types of contraception.

<sup>b</sup> Some women may have had >1 sexually transmitted disease (STD).

<sup>c</sup> Current level of activity.

and whites. The mean age of those enrolled was 30 years (median, 28; SD, 8.16; range, 19–56). Although 40% of the women admitted to a prior history of STD, only 27% reported a prior history of BV. The median number of lifetime sex partners was



**Figure 1.** Distribution of vaginal fluid Gram stains with normal Nugent scores among study participants.

7; however, the median number of sex partners in the past year was 1. None of the women were found to have an STD at the time of screening. Thus, these women represented a population of women who were currently at low risk for STD. One woman dropped out of the study immediately after enrollment as a result of psychological discomfort associated with self-collection of vaginal specimens. Of the remaining 59 women, 55 completed all study visits, and 51 submitted sufficient numbers of satisfactory self-collected slides (median number of slides per patient = 40, with a minimum of 18) to be included in all of the analyses.

**Microbiological and clinical changes.** Of the 51 fully assessable women, none of whom had BV by Amsel criteria at entry [14], 8 (15.7%) entered into the study with an intermediate VF score and 4 (7.8%) with a VF score of 7 or 8. The remaining 39 (76.5%) women had normal Gram stain scores on entry to the study. During the course of the study, various patterns of VF among individual women were observed. Eleven women (22%) maintained normal VF scores throughout. The remaining women had unstable patterns of flora, with 25 (49.0%) demonstrating fluctuations between normal and intermediate VF scores during the observation period and 13 (25.5%) fluctuating between normal scores and VF scores consistent with BV. Two women never had normal scores and instead varied between the intermediate and BV categories. The percentage of days that individuals had normal scores varied from 0% to 100%, with a median of 85% (figure 1). Having an abnormal VF score at the beginning of the study was highly predictive of having normal VF on <85% of the days ( $P < .001$ ). Of the 13 women who fluctuated between normal and BV scores, 6 had *Mobiluncus* morphotypes on Gram stain.

During the course of the study, 5 of the women who completed all follow-up visits complained of vaginal discharge at the third visit and developed the clinical criteria for the diagnosis of BV. All of these women had VF scores consistent with BV, and 3 of them had *Mobiluncus* on Gram stain. Thus, the incidence of symptomatic BV among this cohort during the observation period was 5 (9%) of 55 by clinical (Amsel) criteria.

One subject who had a normal VF score at entry and then fluctuated to intermediate levels was diagnosed with asymptomatic BV by clinical criteria at her final visit.

**Correlation of diary and interview data.** Behavioral data collected during the initial interview and from the diary entries were reviewed and correlated for all of the 57 women who submitted diaries during the course of the study. There was a positive correlation between the behaviors reported during the initial interviews and the diary entries (table 2). For example, women reporting more frequent episodes of oral sex and vaginal intercourse at intake into the study had a corresponding increase in the number of episodes reported in their diary ( $r = .62$ ,  $P < .001$  and  $r = .34$ ,  $P = .01$ , respectively). With the interview as the gold standard to which the validity of the diary entries could be compared, the sensitivity of the diary as an instrument for recording behaviors varied between 26% and 76%. The specificity was in the range of 87.5%–100%. These comparisons indicate that if a behavior was not reported in the interview, it was also not reported in the diary (specificity). The positive correlations between the interview and diary data permitted the use of both of these data sets (interview and diary variables) in the analytical models.

**Association of reported behaviors with stable versus unstable VF.** Univariate analyses of the association of reported behaviors with changes in VF were done for women having stable ( $\geq 85\%$  of days with normal VF [ $n = 26$ ]) versus unstable ( $< 85\%$  of days with normal flora [ $n = 25$ ]) patterns. A prior history of BV (44% vs. 12%;  $P = .01$ ), a greater mean number of lifetime sex partners (13.4 vs. 7.15;  $P = .01$ ), a greater mean number of sex partners in the past 12 months (1.56 vs. 0.88;  $P = .03$ ), and a greater number of current episodes of receptive oral sex (3.6 vs. 1.4;  $P = .003$ ) were all significantly associated with patterns of unstable flora (table 3). In the final logistic model of this analysis, number of episodes of receptive oral sex was the only behavior significantly associated with unstable flora ( $P < .001$ ). Age was not a correlate of VF patterns.

**Longitudinal data analysis model of behaviors and changing VF.** To further examine potential direct cause and effect re-

**Table 2.** Correlation of diary entries with interview responses.

Interview variable	Diary	Interview response <sup>a</sup>		P	Sensitivity <sup>b</sup> (%)	Specificity <sup>b</sup> (%)
		Yes	No			
Douching	Yes	5 (26)	0 (0)	.003	26	100
	No	14 (74)	38 (100)			
Tampons	Yes	28 (76)	0 (0)	.001	76	100
	No	9 (24)	20 (100)			
Condom use	Yes	14 (29)	1 (12)	.880	29	87.5
	No	35 (71)	7 (88)			

NOTE. The Spearman correlation coefficient, a measurement of the degree of association between diary and interview variables, is .34 for no. of episodes of vaginal intercourse ( $P = .01$ ) and .62 for no. of episodes of receptive oral sex ( $P < .001$ ).

<sup>a</sup> Responses are given as no. (%).

<sup>b</sup> Sensitivity and specificity of diary entries versus interview data as gold standard.

**Table 3.** Variables from interview date significantly associated with unstable vaginal flora.

Variable	Stable (n = 26)	Unstable (n = 25)	P
History of bacterial vaginosis, no. (%)			
Yes	3 (12)	11 (44)	.01
No	23 (88)	14 (56)	
Lifetime sex partners, mean ±SD	7.15 ± 1.5	13.4 ± 2.6	.01
Partners during last 12 months, mean ± SD	0.88 ± 0.14	1.5 ± .24	.03
Episodes of receptive oral sex per month, <sup>a</sup> mean ± SD	1.04 ± 0.40	3.6 ± 0.90	.003

<sup>a</sup> Remained significant in the logistic regression analysis.

relationships between behavior and changes in VF, longitudinal data analysis was done as previously described. Variables from both the diary and the interview were included. Two variables from the diary data and 4 variables from the interview data were significant predictors of day-to-day change (table 4). Use of vaginal medication and the occurrence of menses were the only 2 diary variables associated with changes in flora demonstrated between subsequent self-obtained vaginal specimens. Women who reported having menses the previous day were 2.15 times ( $P < .001$ ) more likely to demonstrate a change in VF score the following day compared with those women who did not report having menses. Women who reported using vaginal medication the previous day were 4.1 times ( $P < .001$ ) more likely to experience a change in VF score the following day. Interview variables associated with changing flora included a greater number of sex partners in the past 12 months, more frequent episodes of vaginal intercourse, less frequent use of condoms, and the use of spermicide for contraception. The latter women were 3 times ( $P < .001$ ) more likely to experience a change in VF scores. Of the 7 women who recorded use of a vaginal medication in their diary, 5 self-treated for presumed yeast infections. One woman self-treated with metronidazole gel for the symptom of vaginal discharge and the remaining woman noticed odor and self-treated with an unknown medication. Among the 3 women who recorded douching in their diaries, no immediate subsequent changes in VF were observed. Receptive oral sex was not associated with immediate changes in flora in this model.

**Discussion**

Microbiological changes in the VF of sexually active women are common. These changes may be thought of as a continuum, with the most extreme changes being associated with the clinical syndrome of BV. Epidemiologically, BV has been associated with sexual activity, including an increased number of sex partners, STDs, and douching [13, 15–17]. However, the actual cause of BV is unknown. Less dramatic changes in the VF have been reported previously; however, attempts to determine the causes of these fluctuations have been limited and have yielded little insight [7]. Information about the relationships of specific

behaviors to subsequent changes in VF could provide clues as to the pathogenesis of BV. In prior studies, by using sequential vaginal cultures, changes in VF were noted to occur in some women around the time of menses [3–8]. In our previous study of 10 asymptomatic volunteer women who collected self-obtained vaginal smears on a daily basis, only 2 women (20%) had normal VF (Nugent scores of 0–3) for the entire observation period. Among women with variable flora, changes occurred throughout the cycle but were most highly correlated with menses. Behavioral variables were not collected as part of that study [8]. Priestley et al. evaluated 26 health care workers over an 8-week period by using patient-collected vaginal smears and cultures. Subjects collected an average of 3.2 specimens per week and recorded behaviors in a diary. Thirty-five percent of these women had intermittent changes in their flora consistent with BV demonstrated by Gram stain. The investigators were unable to show any significant correlations between reported behaviors and alterations in flora except for the fact that those women with BV more frequently used scented soap for bathing [7].

In our present study, we again used daily self-obtained vaginal smears, a technique that we have previously shown to be valid when compared with clinician-obtained specimens [8]. In addition, we relied on diaries to capture information on behaviors that were practiced by the patients during the observation period. The majority of women were willing to obtain the vaginal smears. We were able to show positive correlations between behaviors reported by the subject at the enrollment interview and in diary entries. Positive correlations between diary and interview data have been previously shown in a study of physical activity [18]. However, overall, our subjects reported fewer episodes of some behaviors in their diary than they said was their practice during the interview. This variation in sensitivity could be explained by incomplete recording of events in the diary or by alteration in usual behaviors, such as douching, during the course of the study.

Among the 60 women studied, only 11 (22%) had vaginal smears with Nugent scores consistent with normal flora during the entire observation period, a percentage nearly identical to that which we found in our smaller study of 10 women [8]. Transient fluctuations in VF were common and often marked. The incidence of BV by clinical criteria over a 6-week obser-

**Table 4.** Variables significantly associated with day-to-day shifts in vaginal flora.

Variable	RR (95% CI)	P
Menses <sup>a</sup>	2.15 (1.51–3.06)	<.001
Vaginal medication use <sup>a</sup>	4.10 (2.3–7.25)	<.001
Spermicide use <sup>b</sup>	3.30 (2.2–4.8)	<.001
Lack of condom use <sup>b</sup>	1.65 (0.98–2.76)	.06
No. of partners during past 12 months <sup>b</sup>	1.60 (1.41–1.77)	<.001
Episodes of vaginal intercourse per month <sup>b</sup>	1.06 (1.0–1.10)	<.001

NOTE. RR, relative risk; CI, confidence interval.

<sup>a</sup> Variables from diary.

<sup>b</sup> Variables from interview.

vation period in this cohort was 9%, which was higher than anticipated. As in our previous study, changes in flora were often noted around the time of menses, suggesting the possibility of hormonal influences; however, there was no correlation between use of hormonal contraception and changing flora.

Behavioral variables that were associated with unstable flora (normal VF scores on <85% of days) were similar to those that have been shown by others to be associated with BV. These include a history of BV and a greater number of sex partners. In addition, we found an association between receptive oral sex and unstable VF. Interestingly, strong similarities exist between the anaerobic bacteria associated with gingivitis and those associated with BV. Our attempt to link changes in flora to a proximate behavior showed that use of vaginal medication and the occurrence of menses were the only 2 diary variables associated with adverse changes in flora the day after the behavior. However, women who reported in their enrollment interview a greater number of sex partners in the past 12 months, more frequent episodes of vaginal intercourse, less frequent use of condoms, and use of spermicide were more likely to exhibit day-to-day changes in their VF. The association of the first 3 of these variables with changes in VF lends support to the hypothesis that unprotected sexual activity is an important factor in the development of unstable flora, BV, or both.

A potential limitation of this study was the possibility of incomplete recording of behaviors in the diaries. If this occurred, it may have interfered with our ability to detect relationships between certain behaviors and changes in flora. An additional consideration is that we made the assumption that changes in flora linked to a specific behavior would be detectable the following day. It is possible that changes effected by particular behaviors may take longer to evolve.

In summary, maintenance of consistently normal VF occurs in only a minority of women. Fluctuations occur that may be marked. For the most part these changes are transient; however, the incidence of symptomatic BV in this cohort approached 10%. Behaviors that were associated with unstable flora are very similar to those that are associated with BV and STDs. Future studies with similar techniques in different populations of women may provide additional information regarding the influence of behavior on the composition of the VF and subsequent risk for the development of BV.

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