Characterization of Vaginal Flora and Bacterial Vaginosis in Women Who Have Sex with Women

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Bacterial vaginosis (BV) may be common among women who report having sex with women (WSW) and frequently occurs in both members of monogamous couples. The results of Gram staining of a vaginal smear were consistent with BV in 81 (25%) and intermediate in 37 (11%) of 326 WSW included in this study. Lactobacilli were detected in 64% of subjects, and 42% of subjects had H_2O_2-producing strains. BV was associated with a higher lifetime number of female sex partners, failure to always clean an insertive sex toy before use, and oral-anal sex with female partners. Neither recent douching nor sexual practices with male partners were associated with BV. Vaginal smears from each partner were concordant in 55 (95%) of 58 monogamous couples; BV was present in both partners in 16 couples (28%) ($P < .001$, compared with expected distribution). BV was common among subjects who did not douche, who did not have concurrent sex with male partners, or who did not have a new sex partner, which suggests that other risk factors for BV exist. These data support the hypothesis that sexual exchange of vaginal secretions is a possible mechanism for acquisition of BV.

Bacterial vaginosis (BV) is a condition characterized by overgrowth of commensal anaerobic flora relative to the H_2O_2-producing lactobacilli that predominate in the healthy vagina. BV confers an increased risk of preterm delivery, low birth weight, and postpartum and postabortal endometritis [1–5] and has been associated with pelvic inflammatory disease [6, 7] and, in prospective studies [8, 9], acquisition of human immunodeficiency virus (HIV). The presence of H_2O_2-producing strains of lactobacilli in the vagina is strongly associated with a decreased prevalence and rate of acquisition of gonorrhea [8, 10], and some anaerobes associated with BV augment expression of HIV in T cells in vitro [11].

Although recent douching for hygiene and having a new sex partner are associated with the acquisition of BV in heterosexual women [10, 12–14], the cause of BV is not understood. Avail-

able data, including data from studies that evaluated antibiotic treatment of male partners of women with BV, weigh against transmission of bacteria playing a role as a causative event in BV in heterosexual women [15–18]. However, prospective studies involving heterosexual women indicate that a strong association exists between report of a new sex partner and the development of BV [10, 12].

The prevalence of BV has been reported to be high (27%–52%) among women who report having sex with women (WSW) [19–23], compared with the prevalence among women who are attending prenatal (16%) or sexually transmitted disease (STD; 24%–37%) clinics [24–25]. A high degree of concordance found in one study for the presence or absence of BV among members of lesbian couples prompted the authors to define BV as an STD in WSW [19]. The plausibility of this hypothesis is supported by the successful attempt of Gardner and Dukes to transmit BV from one woman to another by the transfer of vaginal secretions in early studies of "*Hemophilus vaginalis* vaginitis" [26]. However, other investigators have questioned whether sexual transmission has a role in the high prevalence of BV among WSW [20].

The present study was undertaken to investigate the vaginal flora of WSW, with emphasis on the microbiological characteristics of BV and its association with previous or concurrent sex with male partners, douching history, and sexual behavior with current female partners.

Subjects and Methods

Subjects. The study population comprised self-selected female volunteers ≥16 years of age who reported having had sex with

another woman in the previous year. Beginning in February 1998, women were recruited through advertisements posted in community gathering places (restaurants, bookstores, clubs, and bars) and newspaper and magazine articles and by referral from community clinicians. Because self-identification as "lesbian" may not predict actual participation in same-sex sexual behavior or its frequency, we oriented recruitment materials to WSW rather than to "lesbians." Neither current STD symptomatology nor STD history was mentioned in recruitment materials. Women were enrolled for a single study visit. Once enrolled, they were invited to refer female sex partners for possible enrollment.

After participants phoned to make an appointment, an extensive standardized questionnaire inquiring about demographic characteristics and medical, reproductive, and sexual history was mailed to them, which they completed and brought to the appointment. The questionnaire was reviewed for completion with the subject. After the first 54 subjects were enrolled, the questionnaire was amended to include partner-specific information about recent sexual behavior. A standardized physical examination was performed by a study clinician (K.S.) that included visual inspection and collection of vaginal fluid for Gram staining, saline microscopic examination, pH measurement, and potassium hydroxide preparation. Vaginal swab specimens from the lateral vaginal wall were collected and placed immediately into Port-a-Cul anaerobic transport tubes (Becton Dickinson), which were held and transported at room temperature to the laboratory and set up for culture within 24 h of collection.

Microbiological testing. Vaginal swabs were used in cultures for aerobic and anaerobic organisms and genital mycoplasmas done by a quantitative method that has been described elsewhere [27]. Lactobacilli were identified to the genus level by Gram staining and examination of colony morphology. *Lactobacillus* isolates were tested for production of H_2O_2 in a qualitative assay on a tetramethylbenzidine agar plate. Organisms grown anaerobically for 2–3 days were exposed to ambient air. Colonies of H_2O_2-positive organisms form a blue pigment as horseradish peroxidase reacts with tetramethylbenzidine in the presence of H_2O_2 [27]. BV was defined by Gram staining, using the Nugent criteria [28]. All women with BV who were symptomatic (defined as the presence of increased or malodorous vaginal discharge) were offered treatment with a standard regimen of intravaginal metronidazole (0.75%, 5 g, 1 application nightly for 5 nights) [29]. Women who were asymptomatic were offered treatment but also were educated about the possibility that vaginal flora might return to normal without it [30]. Women who declined treatment were advised to return to us or to the primary care provider for evaluation if symptoms appeared.

Statistical analysis. Using SPSS software, the association between BV and subject characteristics was measured by χ^2 analysis, the Mann-Whitney U test (for nonparametric comparison), and logistic regression (for multivariate analysis). For multivariate analysis, "abnormal vaginal flora" was defined as a reading by the Nugent criteria of intermediate vaginal flora or BV. All tests for statistical significance were 2 sided; $P < .05$ was considered to be statistically significant. Interactions between terms entered into the multivariate analysis were assessed. For the analysis, a "couple" was defined as 2 women who had been monogamous partners for ≥ 3 months previous to study entry and whose study visits occurred within 3 months of each other. The multivariate analysis included

a variable to control for whether a woman's partner was also seen in the study.

Results

The majority (81%) of the 326 women enrolled in this study reported having had sex with a male partner at some point in the past, and 26% reported having had sex with a male partner in the previous year. Receptive digital-vaginal and oral-vaginal sex with female partners in the previous 6 months were reported by nearly all (99%) of the 273 women who supplied partner-specific information on recent sexual behavior (84% of the total study group). Forty-six (14%) of the 326 subjects stated that they used male condoms on insertive sex toys; of these, 64% used condoms 100% of the time. More than one-half of the women (53%) reported using a lubricant with their partners; of these, 73% used a nonspermicidal lubricant, 22% used another type of lubricant, and 5% did not know what type was used. Of 153 women who answered a question about cleaning of sex toys, 51 (33%) never or only sometimes cleaned a dildo or vibrator between uses with a female partner. Twenty-one percent of all subjects specifically acknowledged having had a previous episode of BV.

The microbiological characteristics of subjects' vaginal flora are shown in table 1. *Lactobacillus* was present at a concentration of $\geq 10^7$ cfu/g of vaginal fluid in 156 (48%) of all subjects; of 223 women who had any *Lactobacillus* species, 152 (68%) had H_2O_2-producing strains. After *Lactobacillus,* the most predominant facultative isolates found in women with normal vaginal flora were coagulase-negative staphylococci, *Enterococcus* species, and *Ureaplasma urealyticum;* few women with normal flora were colonized with *Gardnerella vaginalis* (7%). *Mycoplasma hominis* was present in 11% of 263 women tested for that organism. Anaerobic gram-positive cocci and *Prevotella* species were isolated in samples from more than one-half of all women. Of the 39 women who were colonized with group B streptococci (12% of all women), 4 women (10%) had never had sex with a male partner. The pattern of most common vaginal flora did not differ when data from women who had never been sexually active with a male partner were analyzed separately, nor was the pattern affected by time since last sex with a male partner (data not shown). However, mean concentrations of both *G. vaginalis* and *Escherichia coli* were lower among women who reported never having had sex with a male partner ($P = .05$).

When the Nugent criteria for Gram staining were used to classify test results [28], 208 (64%) of the 326 women had normal vaginal flora, 37 (11%) had intermediate vaginal flora, and 81 (25%) had flora consistent with BV. The organisms most frequently associated with the presence of BV were *G. vaginalis, U. urealyticum,* coagulase-negative staphylococci, anaerobic gram-positive cocci, black-pigmented anaerobic gram-negative rods, and *Prevotella* species (table 1). Fifty-eight percent of women with BV complained of either increased vaginal discharge or itching.

Table 1. Type, frequency, and concentration of microorganisms found in the vaginal flora of 326 women who reported having had sex with other women in the previous year, stratified by vaginal flora pattern (as assessed by Gram stain).

Organism	Normal vaginal flora ($n = 208$)		Intermediate vaginal flora ($n = 37$)		Bacterial vaginosis ($n = 81$)	
	No. (%) of subjects	Mean concentration, cfu/mL	No. (%) of subjects	Mean concentration, cfu/mL	No. (%) of subjects	Mean concentration, cfu/mL
Facultative isolates						
Lactobacillus species[a]	185 (89)	NA	26 (70)	NA	12 (15)	NA
H$_2$O$_2$ producing	133 (64)	2×10^8	14 (38)	1×10^8	5 (6)	4×10^5
Non–H$_2$O$_2$ producing	106 (51)	1×10^8	14 (38)	3×10^8	6 (7)	3×10^7
Gardnerella vaginalis[b]	14 (7)	1×10^7	15 (41)	3×10^8	74 (91)	9×10^8
Group B streptococci	24 (12)	9×10^7	6 (16)	1×10^7	9 (11)	1×10^8
Enterococcus species	105 (51)	2×10^8	14 (38)	5×10^8	12 (15)	4×10^7
Staphylococcus aureus	7 (3)	6×10^4	1 (3)	5×10^5	4 (5)	3×10^4
Coagulase-negative staphylococci	169 (81)	1×10^5	29 (78)	9×10^4	71 (88)	4×10^5
Streptococcus viridans species	0	NA	2 (5)	3×10^7	1 (1)	1×10^8
Escherichia coli	41 (20)	7×10^7	14 (38)	9×10^7	19 (23)	5×10^6
Other gram-negative rods	38 (18)	5×10^7	7 (19)	2×10^7	12 (15)	3×10^7
Candida albicans	31 (15)	1×10^6	4 (11)	3×10^6	5 (6)	3×10^5
Other yeasts	2 (1)	1×10^6	0	NA	0	NA
Ureaplasma urealyticum[c]	98 (58)	4×10^4	17 (63)	9×10^5	60 (88)	2×10^6
Mycoplasma hominis[d]	5 (3)	3×10^3	2 (7)	7×10^5	16 (24)	3×10^6
Anaerobic isolates						
Anaerobic gram-positive cocci	91 (44)	3×10^6	24 (65)	2×10^7	55 (68)	3×10^8
Prevotella species	74 (36)	3×10^4	25 (68)	5×10^6	77 (95)	8×10^7
Bacteroides ureolyticus	1 (0.5)	3×10^2	0	NA	1 (1)	3×10^4
Black-pigmented anaerobic gram-negative rods	26 (13)	7×10^3	12 (32)	3×10^6	54 (67)	4×10^6
B. fragilis	18 (9)	2×10^5	8 (22)	3×10^7	5 (6)	2×10^6
Fusobacterium species	0	NA	0	NA	1 (1)	2×10^6

NOTE.   NA, not applicable.
[a] Some women had both H$_2$O$_2$-producing and non–H$_2$O$_2$-producing lactobacilli.
[b] Media overgrown or unavailable for 8 subjects; $n = 318$.
[c] Media overgrown for 62 subjects; $n = 264$.
[d] Media overgrown for 63 subjects; $n = 263$.

Women with BV did not differ from those without BV with regard to age, race, whether they had ever had sex with a man, time since last sex with a man, recent (data not shown) or lifetime number of male sex partners, or new female sex partner (table 2). Only 15 women reported douching ≥ 1 time each month, and it had been several years since most of the remaining women douched. Thus, it was not possible to evaluate the temporal association between douching and BV. A history of receptive oral-anal sex was associated with the presence of BV ($P < .001$), as were younger age at first intercourse with a male partner ($P = .02$), history of pregnancy ($P = .03$), and higher lifetime number of female partners ($P = .005$). BV was also associated with report of never or only sometimes cleaning an insertive sex toy between uses on the subject and on a partner ($P = .01$) and with a shorter time since last genital-genital contact with a female partner ($P = .04$). When the presence of abnormal vaginal flora was analyzed as the primary outcome, ever having douched was also a significant risk factor ($P = .03$), as was lifetime number of male sex partners ($P = 0.05$). In a multivariate model that also included age, race, previous pregnancy, lifetime number of male and female sex partners, and whether a woman's sex partner was enrolled in the study, a higher lifetime number of female sex partners ($P = .03$), history of receptive oral-anal sex ($P = .004$), and report of not always cleaning an insertive sex toy between uses ($P = .02$) were significantly associated with a finding of BV (table 3). There were no significant first-level interaction effects between the variables. In separate models, neither the frequency (rather than simply the history) of sexual behaviors reported during the previous 6 months, the proximity of these behaviors to the time of BV diagnosis, nor the duration of partnerships was related to the presence of BV (data not shown).

Fifty-eight monogamous couples (116 women) participated in the study, and 55 couples (95%) were concordant for the presence or absence of BV. Both partners had BV in 16 couples, neither partner had BV in 39 couples, and one partner had BV in only 3 couples. This degree of concordance differed from the calculated expected distribution ($P < .001$). Among these monogamous couples, BV was present in 30 (94%) of 32 women whose partners had BV and in 7 (8%) of 84 women whose partners did not have BV ($P < .001$). Among all women with BV in these partnerships, 32 (91%) of 35 had partners with BV; among all women without BV, 3 (4%) had partners with BV. Fifty-six percent of couples in which lactobacilli were found in both partners were concordant

Table 2. Association between bacterial vaginosis (BV) and subjects' characteristics as reported on the study questionnaire.

Characteristic	Subjects with BV (n = 81)	Subjects without BV (n = 245)	Univariate OR (95% CI)	P
Age, mean years	31	30	—	.5
Race				
Nonwhite	14 (17)	29 (12)	1.6 (0.8–3.3)	.2
White	64 (79)	216 (88)		
Sex with male partners				
Ever had intercourse with male				.9
partner	66 (82)	196 (80)	1.1 (0.6–2.0)	
Age at first sex with male				.02
partner, mean years	18	16		
No. of months since last sex				.2
with male partner, mean	56	69		
No. of male sex partners in				.1
lifetime, median	9	6		
Sex with female partners				
Age at first sex with female				.7
partner, mean years	21	21	—	
Any sex with female partner in				.5
previous 6 months	65 (80)	214 (87)	0.9 (0.3–2.3)	
No. of female sex partners,				
median				
Lifetime	11	8		.005
Previous year	2	2		.2
Previous 6 months	1	1		.2
Previous 30 days	1	1		.9
No. of times participated in sexual				
behavior in the previous 6 months				
Receptive digital-vaginal	23	23		.7
Receptive oral	14	12		.8
Receptive digital-anal	1.1	1.5		.6
Genital-genital contact	7	7		.9
Days since last genital-genital contact	2	5		.04
Ever participated in sexual behavior				
Receptive digital-vaginal	81 (100)	243 (99)	—	1.0
Receptive oral-vaginal	80 (99)	236 (96)	2.7 (0.3–22)	.5
Receptive digital-anal	53 (65)	132 (54)	1.6 (1.0–2.7)	.07
Receptive oral-anal	39 (48)	62 (25)	2.7 (1.6–4.6)	<.001
Insertive sex toy not routinely				
cleaned between uses[a]	19 (53)	33 (28)	2.8 (1.3–6.1)	.01
Douching history				
Ever douched	35 (43)	83 (34)	1.5 (0.9–2.5)	.14
Months since last douche, mean	79	75		.8
Frequency of douching				
<10 Times in life	26 (32)	52 (21)		.1
<1 Time/month	6 (7)	15 (6)		
1–2 Times/month	2 (3)	10 (4)		
3–4 Times/month	0	3 (1)		
History of pregnancy	29 (36)	56 (23)	1.9 (1.1–3.2)	.03
Partner enrolled in study	34 (42)	88 (36)	1.2 (0.8–1.8)	.4

NOTE. Data are no. (%) of subjects, unless otherwise indicated. CI, confidence interval; OR, odds ratio.
[a] Data on use of insertive sex toys were not available for all subjects.

for type of lactobacillus (H_2O_2 producing or non–H_2O_2 producing). No members of couples without BV were colonized with *G. vaginalis,* but this organism was predominant in most couples (87%) who were concordant for BV. Couples concordant for BV also were concordant for the presence of *U. urealyticum* (92%), *M. hominis* (77%), *Prevotella* species (100%), and black-pigmented anaerobic gram-negative rods (88%). Neither sexual behaviors of couples in the previous 6 months nor duration of partnership differed according to the couples' BV status (data not shown).

Table 3. Multivariate analysis of subjects' risk for bacterial vaginosis.

Characteristic	Multivariate OR (95% CI)	P
Age	—	.7
Nonwhite race	1.4 (0.7–3.0)	.4
History of pregnancy	1.6 (0.8–3.1)	.2
Lifetime no. of female sex partners		
1–3	Reference	
4–6	2.0 (0.9–4.4)	.08
≥7	2.2 (1.1–4.5)	.03
Lifetime no. of male sex partners		
0	Reference	
1–3	0.8 (0.3–1.9)	.6
4–6	1.2 (0.5–2.9)	.7
≥7	1.1 (0.5–2.5)	.8
Receptive sexual behaviors (ever)		
Digital-anal	1.1 (0.6–2.0)	.7
Oral-anal	2.4 (1.3–4.4)	.004
Insertive sex toy cleaned between uses		
Always	Reference	
Never/sometimes	2.7 (1.2–6.1)	.02
Question not answered	1.8 (1.0–3.6)	.07

NOTE.   CI, confidence interval; OR, odds ratio.

Discussion

BV was common in our study population of women who reported having sex with women in the previous year and was characterized by vaginal bacteria similar to those associated with BV in heterosexual women. However, BV in our subjects was independently associated with report of not always cleaning an insertive sex toy between uses with a female partner, a higher lifetime number of female sex partners, and report of ever having participated in receptive oral-anal sex with a female partner. BV occurred independently of previous or current sex with male partners, new male or female sex partner, and douching history. In fact, recent douching was reported rarely.

In our subjects, the prevalence of vaginal colonization with lactobacilli was low, compared with that among heterosexual women studied in STD clinic settings and among pregnant women [24, 25]. Few subjects colonized with lactobacilli had H₂O₂-producing strains. Monogamous couples had a high concordance (95%) for the presence or absence of BV, and presence of BV in one woman was strongly associated with the presence of BV in her partner. Couples who were concordant for BV reported no sexual behaviors that distinguished them from couples without BV. Only one-half of couples were found to be concordant for type of lactobacilli (H_2O_2 producing or non–H_2O_2 producing); however, we used a qualitative method to detect H_2O_2. Use of enhanced H_2O_2 detection protocols might provide a more accurate assessment [31].

Our subjects had a low rate of recovery of *G. vaginalis* (32% overall and only 7% among women with normal vaginal flora). The reasons for this are unclear; colonization rates for *G. vaginalis* are generally 46%–65% among heterosexual women of reproductive age [25]. *Candida albicans* was present in 12% of

our subjects, compared with a reported general prevalence of 21%–31% [25]. Twelve percent of subjects were colonized with group B streptococci, a prevalence only slightly lower than that generally noted for heterosexual women (15%–21%) [25].

Our findings concur with those of the few studies that have specifically assessed BV prevalence among WSW. Berger et al. [19] found a BV prevalence of 29% among 103 women, as defined by the Amsel criteria, and noted a high prevalence of infection in both members among 22 couples. In another study that included 91 WSW seen in a London genitourinary medicine clinic, 52% had BV, and neither specific sexual behavior with female partners nor recent sex with male partners was reported more commonly among the women who had BV [20]. The authors of that study concluded that only the reported lifetime number of female sex partners and use of lubricants might be associated with BV. In a study that included 859 WSW attending an STD clinic in Seattle, BV was diagnosed in 26% by the Amsel criteria [32]. In another study, WSW who were attending an STD clinic in Sydney were more than twice as likely to have BV as were matched heterosexual control subjects [33].

The cause of the high prevalence of BV among WSW and in both members of monogamous couples is unclear. Our finding of a relationship between BV and reports of not always cleaning an insertive sex toy between uses with a partner, as well as of a shorter time since last genital-genital contact, suggest that exchange of vaginal secretions from one woman to another plays a role in acquisition of BV. The attempts of Gardner and Dukes [26] to demonstrate that pure *G. vaginalis* is the sole infectious cause of BV failed; they introduced the organism into the vagina in 13 healthy women, and only 1 woman developed BV. However, 11 (73%) of 15 women developed BV when they were inoculated with the vaginal secretions of women with BV. This suggests that a transmissible component other than *G. vaginalis* is present in vaginal fluid. Our results further support the hypothesis that an exchange of vaginal secretions between women is a possible mechanism of transmission.

Explanations for the potential relationship between BV and receptive oral-anal or digital-anal sex and history of pregnancy are less clear. Among our subjects, report of higher lifetime numbers of female sex partners was associated with an increased prevalence of BV, a finding that suggests a relationship similar to that seen for both male and female sex partners in other studies [10, 12, 20]. An association between frequency of receptive oral sex and BV [34] or unstable vaginal flora [35] has been observed by some investigators but not by others [10]. Because receptive oral sex was practiced frequently by almost all our subjects, its effect was difficult to examine. Furthermore, the low rate of recovery of lactobacilli in culture, compared with that seen on Gram stain (65%), in this study group contrasts with the higher rates of recovery found among heterosexual women studied in the same laboratory, which generally approach 92% (K.A., unpublished data). The possibility that some inhibitory factor in the vaginal fluid of WSW could affect growth of lactobacilli both in vitro

and in vivo should be examined. Further study may also eluci-date the possibility that a mechanism exists by which frequent receptive oral sex could affect vaginal colonization with and growth of *Lactobacillus*.

Our study has several limitations. First, subjects were volun-teers who may not be a representative sample of all WSW; thus, findings may not be generalizable to all WSW. In particular, 38% of subjects were members of monogamous partnerships. Second, most subjects were white. BV is strongly associated with nonwhite race, and assessments of a more racially diverse group of WSW should be performed. Third, small numbers in some subgroups did not allow meaningful comparisons, par-ticularly with regard to the contribution of douching behavior to BV, and detailed information about sexual behavior was not available for 30% of subjects who were members of a monog-amous partnership. Conversely, almost all subjects practiced re-ceptive digital-vaginal and receptive oral sex, which precluded meaningful analysis of the relationship of these behaviors to the presence of BV. Finally, bacterial overgrowth was present in some cultures for *M. hominis,* which prevented accurate identification of this organism.

Study of larger and more diverse populations of WSW should allow better characterization of factors associated with BV, espe-cially among couples and women of color. Whether the natural history of BV in WSW differs from that of BV in heterosexual women is not known, and the effect of reducing transmission of vaginal secretions or treating female sex partners of infected individuals has not been studied. Concurrent enrollment of a control group of heterosexual women would be useful for direct comparison of characteristics of lactobacilli, including species identification by DNA homology and genomic fingerprinting [36], and of epidemiologic features and sexual behaviors asso-ciated with BV. Finally, pelvic inflammatory disease has been reported to have occurred in a woman who had never been sexu-ally active with a male partner and in whom no bacterial STD was identified [37]; the frequency of pelvic inflammatory dis-ease in WSW, and whether BV may play a role, is not yet known.

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