Published in final edited form as:

Sex Transm Dis. 2011 December ; 38(12): 1137–1144. doi:10.1097/OLQ.0b013e31822e6121.

Effect of sexual activity on vaginal colonization with hydrogen-peroxide producing Lactobacilli and *Gardnerella vaginalis*

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Abstract

Background—Bacterial vaginosis is associated with sexual activity, but mechanisms for this association are unclear.

Study Design—Cross-sectional analysis of data from women reporting sex with women who provided information on sexual behaviors as part of a study of vaginal bacteria. Vaginal bacteria were detected by semi-quantitative culture. Binomial regression with log-link evaluated associations between detection of bacteria and sexual behaviors reported to occur prior to enrollment. Linear regression evaluated associations between these behaviors and quantity of bacteria.

Results—Of 320 women, 216 (68%) were colonized with H₂O₂-producing lactobacilli and 142 (44%) with *G. vaginalis*. Colonization with *G. vaginalis* was associated with >20 digital-vaginal sex acts (RR 2.01; 1.22, 3.29) or >10 toy-vaginal acts in the past 3 months (RR 1.76; 1.32, 2.36). Quantity of H₂O₂-producing lactobacilli was 1.3 log lower in colonized women reporting >10 acts of insertive vaginal sex toy use in the past 3 months (95%CI −2.04, −.56), 1.19 log lower with toy-vaginal sex in past 7 days (−1.75, −.62), and 0.78 log lower in women sharing toys with a partner (−1.25, −.31).

Conclusions—Vaginal insertive use and sharing of sex toys was associated with decreased quantities of H₂O₂-producing lactobacilli and higher risk of colonization with *G. vaginalis*.

Keywords

bacterial vaginosis; lactobacilli; *Gardnerella vaginalis*

Introduction

Bacterial vaginosis (BV) is characterized by vaginal discharge, loss of normal vaginal lactobacilli and increased vaginal concentrations of anaerobic bacterial species, including *Gardnerella vaginalis*. Women with BV are at increased risk for preterm delivery[1], pelvic inflammatory disease[2], and HIV-1 acquisition[3], making it a significant public health concern. BV is associated with having a new sexual partner, female sexual partners, multiple sexual partners, and with not using condoms[4, 5] leading many investigators to argue that it is a sexually transmitted infection [6]. Previously, the evidence presented against sexual

transmission of BV included studies reporting BV in sexually naïve women[7], but recent work suggests that those studies did not consider sexual activities other than intercourse, including oral or digital sexual activity,[8] and that BV is rare in women who report no sexual contact of any kind. Whether BV is associated with sex because of transfer of a BV-associated bacterial species, such as *G. vaginalis*, or because sexual activity has a detrimental effect on protective vaginal lactobacillus colonization is unknown.

While several bacterial species have been associated with BV[9], some investigators argue that *G. vaginalis* is a key species in the development of BV[10], and others that a deficiency in healthy *Lactobacillus* species is the cause. Sexual and vaginal hygiene practices have been associated with differences in *Lactobacillus* colonization and vaginal microbial composition [11, 12]. In one small study, vaginal colonization with *G. vaginalis* was associated with oral-vaginal contact as well as non-penetrative digital vaginal contact [13], although this has not been consistently demonstrated [14]. One trial of vaginal capsules containing a *Lactobacillus crispatus* strain in healthy women showed that more frequent sexual activity during treatment was associated with lower rates of colonization with the probiotic strain[15]. Few studies have conducted a comprehensive evaluation of the range of sexual behaviors in relation to the vaginal microbiota.

We hypothesize that sexual activity disrupts the vaginal ecosystem and increases the risk of bacterial vaginosis, either by decreasing colonization with protective H₂O₂-producing lactobacilli or by increasing the presence of BV-associated species like *Gardnerella vaginalis*. We conducted an analysis of the association between sexual behaviors and vaginal colonization with H₂O₂-producing lactobacilli or *G. vaginalis* among women who reported sex with women in the past year, many of whom also reported recent sex with men. This population offers a unique opportunity to study a wide range of sexual behaviors in addition to penile-vaginal intercourse.

Material & Methods

Study population and design

Data were obtained from the enrollment visit for women entering an observational cohort study in Seattle, WA who were ≥16 years old and reported having sex with at least one woman in the previous year [16]. Women recruited through advertisements, media and community referral between October 2003 and December 2006 completed a computer-assisted self-interview (CASI) that collected information about demographics, medical and reproductive history and detailed information about type, frequency and timing of sexual behaviors. The study was approved by the University of Washington Institutional Review Board and all participants signed informed consent at enrollment. Participants underwent a standardized pelvic exam with collection of vaginal swabs. Bacterial vaginosis was diagnosed using Amsel's clinical criteria[17]. All participants with vaginal bacterial culture results available at the enrollment visit were included in this cross-sectional analysis.

Vaginal samples

Dacron swabs were used to test vaginal pH, and create slides for wet mount and Gram-stain for evaluation of the Nugent score [18]. Swabs collected for bacterial culture were placed in a Port-a-Cul anaerobic transport device (Becton Dickinson, Cockeysville, MD) and delivered to the lab for quantitative culture within 24 hours of collection[19]. Microaerophilic microorganisms were identified after 48–72 hours of incubation at 37°C in 5–10% CO₂. Plates for anaerobes were incubated within an anaerobic chamber (Bactron IV, Sheldon Manufacturing, Cornelius, OR) at 37°C for 5–7 uninterrupted days. All isolates were identified by standard biochemical methods[20].

Statistical analysis

We performed two analyses examining the association between sexual behavior and (a) presence and (b) quantity of H₂O₂-producing *Lactobacillus* species and *G. vaginalis*. Among all participants, a log binomial regression model was developed with presence of the bacteria of interest as the outcome. Among the subset of participants with bacteria detected, linear regression models were developed with log₁₀ quantity of either H₂O₂-producing lactobacilli or *G. vaginalis* as the outcome. For categorical characteristics with more than one category, the lowest value was chosen as the reference group and a Wald test used to test whether any categories were significantly different from the others. The frequency of a behavior (i.e., number of acts) and time since engaging in a behavior were analyzed as categorical variables. Separate multivariable models were developed for each behavior that was significantly associated with the bacteria of interest at $p < 0.05$ in univariable analyses. In addition to adjusting for age and race, behavioral characteristics were assessed and retained in the multivariable model if p remained < 0.05 or if their inclusion changed the point estimate by more than 10%. Stata v10.0 was used for statistical analyses (StataCorp, College Station, TX).

Results

Baseline characteristics

Of 335 women enrolled in the parent study, 320 had baseline vaginal cultures completed and were eligible for inclusion. These women were primarily (76%) white and ranged in age from 15 to 35 (mean 25 ± 3.5 years). At enrollment very few had sexually transmitted infections such as *Chlamydia trachomatis* ($< 1\%$) or trichomoniasis (0%), but 94 (29%) had bacterial vaginosis by Amsel's clinical criteria. A slightly higher number had BV by Nugent score ($n = 104$; 33%), while a small number ($n = 23$; 7%) had an intermediate Nugent score. Over two-thirds (216/320, 68%) of the women were colonized with H₂O₂-producing *Lactobacillus* species, with a mean quantity of $1.3 \times 10^8 \pm 3.6 \times 10^8$ colony forming units (CFU). One hundred forty-two (44%) were colonized with *G. vaginalis*, with a mean colony count of $5.74 \times 10^8 \pm 1.4 \times 10^9$ CFU. Of the 193 women with normal Nugent scores 13% (26/193) had *G. vaginalis* and 89% (172/193) H₂O₂-producing lactobacilli detected by culture. Of the 23 women with intermediate scores 65% ($n = 15$) were positive for *G. vaginalis* and 74% ($n = 17$) for H₂O₂-producing lactobacilli. Among women with Nugent score of 7–10, 101/104 (97%) were culture positive for *G. vaginalis* and 27/104 (26%) for H₂O₂-producing lactobacilli.

Most women reported sex with only female partners in the last 90 days ($n = 218$; 68%), while 48 (15%) reported sex with both women and men, and 27 (8%) sex with only men. The majority had one sexual partner ($n = 193$; 60%), a small number were abstinent ($n = 27$; 8%), and 100 (31%) reported 2 or more partners in the past 90 days. The most frequently reported receptive sexual behaviors in the prior 90 days were digital vaginal sex ($n = 276$; 87%) and oral-vaginal sex ($n = 252$; 80%), while a smaller number reported toy-vaginal sex ($n = 133$; 42%). The most infrequent behaviors were penile-vaginal sex ($n = 66$; 21%), receptive oral-anal sex ($n = 61$; 19%), and penetrative anal sex ($n = 75$; 24%). Many sexual behaviors were reported to be concurrent. For example, women reporting toy-vaginal sexual contact ($n = 133$) were also very likely to report digital-vaginal contact (99%; 132/133) and oral-vaginal contact (94%; 125/133) but not penile-vaginal contact (9%; 12/133), oral-anal (30%; 40/133) or penetrative anal sex (38%; 51/133).

Association of sexual behavior with presence of *Lactobacillus* and *Gardnerella vaginalis* colonization

In univariable analysis of all 320 women, none of the measured sexual behaviors (Table 1) were associated with the presence (versus absence) of H₂O₂-producing lactobacilli. A higher likelihood of colonization with *G. vaginalis* was observed in women reporting the highest number of penetrative digital-vaginal sex acts (Risk Ratio 2.01; 1.22, 3.29) or the most penetrative toy-vaginal acts in the past three months (RR 1.76; 1.32, 2.36), compared to women not reporting those behaviors. In multivariable analysis, after adjusting for number of partners, report of penile-vaginal and toy-vaginal sex, risk of colonization with *G. vaginalis* increased with the number of acts of both digital-vaginal and toy-vaginal sex. Women reporting >20 acts of digital-vaginal sex were 30% (ARR 1.3; 0.9–1.7) more likely to be colonized. Women reporting >10 acts of toy-vaginal sex were 70% (ARR 1.7; 1.3–2.3) more likely to be colonized after adjusting for number of partners and report of digital-vaginal sex. Inclusion of race and age in the model did not significantly alter these results.

Association of sexual behavior with quantity of bacteria in colonized women

Among the 142 women colonized with *G. vaginalis* no behaviors were significantly associated with quantity of the bacterium (Table 2). In the 216 women with H₂O₂-producing lactobacilli detected, concentrations of bacteria were lower in women reporting the most penetrative vaginal sex with a toy in the past 3 months (1.3 log lower in women reporting >10 acts; 95% CI –2.04, –.56), with toy use in the past 1–6 days (1.19 log lower; 95% CI –1.75, –.62) and with vaginal sharing of toys with a female partner (.78 log lower in women who shared toys vaginally; 95% CI –1.25, –.31) compared with women not reporting that behavior (Table 2). Women reporting more than 5 penile-vaginal sex acts in the past three months had higher quantities of H₂O₂-producing lactobacilli. Among 67 women who reported any penile-vaginal sex in the past 90 days, 15 (22%) reported always using condoms with all partners, 29 (43%) used condoms sometimes and 20 (30%) never used condoms. Women reporting penile-vaginal sex in the past three months reported high rates of receptive oral sex (61/67; 91%) and digital-vaginal sex (64/67; 96%) but low rates of toy-vaginal sex (12/67; 18%).

In multivariable analysis, controlling for number of partners in the past three months and report of penile-vaginal sex, quantities of H₂O₂-producing lactobacilli decreased as the number of toy-vaginal sex acts in the past 90 days increased (lower by 1.25 log for >10 acts, 95% CI –2.0, –.5). Similarly, more recent use of a vaginal insertive toy was related to decreased amounts of H₂O₂-producing lactobacilli: quantities were 1.2 log lower in women who had engaged in toy-vaginal sex in the past one to six days (95% CI –1.7, –.5), compared to women who had not. Sharing of sex toys was associated with 0.7 log lower quantity of lactobacilli (95% CI –1.2, –.3), compared to not using toys. In contrast, after adjusting for number of partners, report of digital-vaginal and toy-vaginal sex, reporting >5 acts of penile-vaginal sex in the past 90 days was associated with a 0.7 log increase in the quantity of lactobacilli (95% CI .1, 1.3).

Discussion

In our study of women who reported recent sex with women, receptive vaginal sex with a toy was associated with a decreased quantity of protective H₂O₂-producing lactobacilli and a higher likelihood of colonization with *G. vaginalis*. We focused on these bacterial species because vaginal detection of *G. vaginalis* and absence of H₂O₂-producing lactobacilli among women without BV have been independently associated with an increased risk of subsequent acquisition of BV [21]. We also found that report of more frequent receptive digital-vaginal sex increased the likelihood of vaginal colonization with *G. vaginalis*, but

that oral-vaginal and anal sexual behaviors were not significantly associated with colonization or quantity of either *G. vaginalis* or H₂O₂-producing lactobacilli. In contrast, women reporting a greater number of penile-vaginal sex acts had higher quantities of H₂O₂-producing lactobacilli compared to women reporting no penile-vaginal sex. Taken together, these findings suggest that some forms of sexual activity may predispose women to develop BV, though whether this is due to a reduction in protective factors or the introduction of a specific pathogen is unclear.

Studies of primarily heterosexual women show an increased risk of BV in women who have unprotected [5, 22, 23] or more frequent penile-vaginal sex [24, 25]. In both heterosexual and lesbian women, receptive vaginal sex with a toy has been associated with higher risk of BV [8, 26]. Reporting anal sex or vaginal sex after receptive anal sex was also a risk for BV in some studies [22], though not in all [24, 25]. Receptive oral sex has been associated with BV in some studies [11], though not others [22, 24]. An analysis of this cohort found that sharing sex toys with a female partner, having a female partner with BV or using vaginal lubricant were all associated with presence of BV. [27] Few of these studies describe the effect of sexual behavior on presence or quantity of individual BV-associated bacteria, and no causal pathway for the association between sex and BV has been identified.

When first identified, *G. vaginalis* was thought to be the causative agent in BV; however, the bacterium is also present in a majority of women without BV. [28] Although this suggests that *G. vaginalis* alone is not causative, several investigators have suggested that its presence is critical for the development of BV. [10, 29] Our study adds to the few that have specifically assessed the effect of sexual activity on vaginal colonization with *G. vaginalis*. In a small study of women who reported never having had penile-vaginal intercourse, oral-vaginal sexual contact and digital-vulvar contact were associated with higher rates of colonization with *G. vaginalis* [13]. We also found that digital-vaginal contact increased the likelihood of colonization. Recently, a study comparing male partners of women with and without BV found no difference in presence of *G. vaginalis* in penile shaft swabs between the two groups [30]. In our cohort, whose participants reported a low frequency of recent penile-vaginal intercourse (22%), this behavior was not associated with vaginal colonization by *G. vaginalis*. Among colonized women we did not find any behaviors associated with quantity of *G. vaginalis*, suggesting that sexual activity may facilitate acquisition of the bacterium but not its subsequent proliferation. That women with the highest number of digital-vaginal or toy-vaginal sex acts had the highest risk of colonization could potentially be due to more contact with the peri-anal skin and rectal bacteria.

Vaginal colonization with H₂O₂-producing lactobacilli is a marker of vaginal health, but data are mixed regarding specific factors that increase the likelihood of colonization with these bacteria. In a study of vaginal probiotic capsules containing *L. crispatus*, women who had more penile-vaginal intercourse were less likely to establish colonization with the probiotic lactobacilli [15]. However, in studies of both adolescents and adults, the number of partners and frequency of vaginal intercourse was not associated with vaginal colonization by H₂O₂-producing lactobacilli [14, 31, 32]. In the present study, no behavioral factor was significantly associated with the presence of H₂O₂-producing lactobacilli, suggesting that there may be genetic or environmental factors that may prevail in establishing and/or supporting colonization. However, among women colonized with H₂O₂-producing lactobacilli, higher quantities were observed in those reporting the highest rates of penile-vaginal sex in the past 3 months (more than 5 acts), which was surprising. This may not reflect what happens in a primarily heterosexual population; the women who did not report penile-vaginal sex in this cohort were more likely to report toy-vaginal or digital-vaginal behavior, behaviors that may strongly contribute to the development of BV. These findings

confirm the need to carefully assess different sexual behaviors, and their concurrence, in studies that attempt to relate sexual practices to detection of vaginal bacteria.

In the participants in this study, use of sex toys was consistently associated with differences in the vaginal ecosystem. Women who reported the greatest number of acts with sex toys were more likely to be colonized with *G. vaginalis*. Use of a sex toy in the past week, reporting more than 10 acts of toy-vaginal sex or sharing of sex toys with a partner in the past 90 days was associated with decreased quantity of H₂O₂-producing lactobacilli. This suggests that use of toys may result in the transmission of some factor that changes the balance of the vaginal bacteria. Alternatively, toy use may be associated with some unmeasured confounder associated with loss of protective H₂O₂-producing lactobacilli and colonization with *G. vaginalis*, both of which favor the development of BV. This could be due to vaginal lubricant use, which has been associated with prevalent BV [27], sharing of vaginal secretions between partners, or a higher likelihood of anal contact when using a toy. In our cohort, use of vaginal lubricant was strongly correlated with use of vaginal toys, as well as anal behaviors and could not be analyzed separately.

Our analysis has several limitations. First, our results may not be able to be generalized to a heterosexual population; however, women who have sex with other women are at high risk of BV and offer a unique opportunity to study a range of sexual behaviors. Moreover, a substantial minority of our participants (21%) reported vaginal-penile sex in the three months prior to assessment. Additionally, our analysis was an exploratory analysis and will need to be confirmed in separate cohorts of women. Finally, we only queried participants about the number of sex acts that occurred during the preceding 3-month period; more recent behaviors (for example, the past week) may be more relevant to current vaginal colonization status. Many of the sexual behaviors examined were highly correlated, making inference of causality difficult; similar analyses should be conducted in heterosexual populations where some of these behaviors are less correlated. Nevertheless, our cohort offers a well-characterized assessment of frequency and timing of a wide variety of sexual activities collected by CASI, which allowed a detailed assessment of the relationship between sexual behaviors and vaginal colonization with individual bacterial species. This method has been shown to yield significantly higher rates of disclosure for same-sex behavior and undesirable social behaviors when compared directly with self-administered questionnaires [33–35].

In summary, our data indicate that recent penetrative vaginal sex with a toy was associated with decreased quantities of H₂O₂-producing lactobacilli in women who were colonized with those bacteria. Penetrative sexual behavior with fingers or a toy was associated with a higher likelihood of colonization with *G. vaginalis*. These data add to the increasingly persuasive evidence base suggesting that BV is a sexually facilitated, if not sexually transmitted, condition. Until a causal pathway involving sexual behavior is identified, women should be counseled that all types of penetrative sexual activity may affect the vaginal ecosystem in a way that could increase the risk of BV.

Acknowledgments

Financial support: National Institute of Allergy and Infectious Diseases grant RO1 AI052228 (JMM). Dr. Mitchell is supported by the Women's Reproductive Health Research Award, and the UW ITHS Tuition Support Degree Program Award.

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Table 1

Univariate analyses among 320 women assessing the relationship of sexual behavior in the past 90 days and presence of either hydrogen peroxide-producing lactobacilli or *Gardnerella vaginalis* by culture.

Baseline characteristic	Hydrogen peroxide producing lactobacilli			<i>Gardnerella vaginalis</i>		
	N = 320*	Risk Ratio	Wald test p-value	N = 320*	Risk Ratio	Wald test p-value
Age						
< 25	155	Ref		155	Ref	.43
≥ 25	164	1.02 (.87, 1.18)	.82	164	.91 (.71, 1.16)	
Race						
White	243	Ref		243		.30
Non-white	77	.90 (.74, 1.09)	.29	77	1.15 (.88, 1.51)	
Menstrual phase						
Proliferative	155	Ref		155	Ref	.19
Luteal	147	1.11 (.94, 1.30)	.21	147	.84 (.65, 1.09)	
Behavior in the past 3 months						
Number of sexual partners						
0	27	Ref		27	Ref	
1	193	1.09 (.81, 1.48)		193	1.31 (.75, 2.28)	.40
2	59	1.13 (.81, 1.58)	.58	59	1.58 (.88, 2.83)	
≥3	41	.93 (.63, 1.37)		41	1.32 (.70, 2.49)	
Lubricant use						
No	78	Ref		78	Ref	.18
Yes	156	1.06 (0.87, 1.30)	.57	156	1.26 (.90, 1.76)	
Number of acts of oral sex						
No oral sex	66	Ref		66	Ref	
1–10	141	1.05 (0.85, 1.29)	.98	149	1.07 (.76, 1.5)	.16
11–20	34	1.04 (0.78, 1.39)		35	.98 (.59, 1.61)	
>20	44	1.05 (0.80, 1.37)		44	1.44 (.99, 2.11)	

Hydrogen peroxide producing lactobacilli				<i>Gardnerella vaginalis</i>			
	N = 320*	Risk Ratio	Wald test p-value	N = 320*	Risk Ratio	Wald test p-value	
Days since last oral sex							
No oral sex	66	Ref		66	Ref		
1–6	135	1.10 (.89, 1.35)	.52	135	1.12 (.80, 1.58)	.47	
7–30	77	.96 (.745, 1.23)		77	1.11 (.76, 1.62)		
30–90	25	1.04 (.76, 1.44)		25	.68 (.34, 1.37)		
Number of acts digital-vaginal sex							
No digital-vaginal sex	42	Ref		42	Ref		
1–10	92	1.19 (.91, 1.56)	.37	92	1.47 (.89, 2.44)	.002	
11–20	50	1.13 (.84, 1.52)		50	.97 (.52, 1.80)		
>20	58	1.00 (.73, 1.37)		58	2.01 (1.22, 3.29)		
Days since last digital-vaginal sex							
No digital-vaginal sex	42	Ref		42	Ref		
1–6	184	1.14 (.88, 1.47)	.54	184	1.49 (.93, 2.41)	.36	
7–30	63	1.00 (.74, 1.36)		63	1.54 (.91, 2.59)		
30–90	23	1.12 (.78, 1.61)		23	1.26 (.64, 2.50)		
Number of acts penile-vaginal sex							
No penile-vaginal sex	248	Ref		248	Ref		
1–5	22	1.02 (.76, 1.37)	.83	22	1.32 (.91, 1.92)	.17	
>5	42	1.07 (.86, 1.32)		42	.80 (.52, 1.22)		
Days since last penile-vaginal sex							
No penile-vaginal sex	253	Ref		253	Ref		
1–6	32	1.04 (.81, 1.33)	.45	32	1.04 (.70, 1.54)	.37	
7–30	24	1.19 (.95, 1.49)		24	.65 (.34, 1.23)		
30–90	10	.90 (.54, 1.51)		10	1.33 (.79, 2.25)		
Number of acts toy-vaginal sex							
No toy-vaginal sex	185	Ref		185	Ref		
1–10	83	.88 (.73, 1.08)	.47	90	1.04 (.77, 1.41)	<.001	
>10	28	.93 (.69, 1.24)		28	1.76 (1.32, 2.36)		

Hydrogen peroxide producing lactobacilli						Gardnerella vaginalis	
	N = 320*	Risk Ratio	Wald test p-value	N = 320*	Risk Ratio	Wald test p-value	
Days since last toy-vaginal sex							
No toy-vaginal sex	185	Ref		185	Ref		
1–6	47	1.01 (.82, 1.25)		47	1.21 (.86, 1.70)		
7–30	60	.89 (.71, 1.11)	.65	60	1.19 (.87, 1.63)	.40	
30–90	20	.87 (.60, 1.26)		20	1.36 (.88, 2.09)		
Sharing of toys with partner							
No use of toys	185	Ref		185	Ref		
Toys, no vaginal sharing	32	.90 (.68, 1.20)	.48	34	1.23 (.84, 1.80)	.11	
Toys, vaginal sharing	76	.89 (.73, 1.09)		76	1.33 (1.01, 1.75)		
Number of acts oral-anal sex							
None	257	Ref		257	Ref		
1–5	38	1.11 (.90, 1.37)	.45	38	.95 (.64, 1.41)	.95	
> 5	5	1.21 (.77, 1.89)		5	.90 (.31, 2.66)		
Had oral-anal sex in past week							
No	56	Ref		56	Ref	.81	
Yes	14	1.08 (.74, 1.58)	.69	14	.92 (.47, 1.80)		
Number of acts penetrative anal sex (finger, dildo, penis)							
None	257	Ref		257	Ref		
1–5	27	1.05 (.81, 1.35)	.94	27	1.10 (.73, 1.67)	.90	
> 5	16	1.02 (.73, 1.44)		16	1.00 (.57, 1.78)		
Had penetrative anal sex in past week							
No	66	Ref		66	Ref	.97	
Yes	20	.84 (.57, 1.24)	.39	20	.99 (.57, 1.72)		

* Not all responses have complete data for all 320 participants

Table 2

Univariate analyses assessing the relationship of sexual behavior and quantity of bacteria (by semi-quantitative culture) in women with vaginal colonization with either hydrogen peroxide producing lactobacilli or *Gardnerella vaginalis*.

	Hydrogen peroxide-producing lactobacilli			Gardnerella vaginalis		
	N = 216*	Difference in log ₁₀ bacterial quantity (cfu)	Wald test p value	N = 142*	Difference in log ₁₀ bacterial quantity (cfu)	Wald test p value
Baseline characteristics						
Age						
< 25	104	Ref		72	Ref	.70
≥ 25	112	-.03 (-.44, .38)	.88	69	-.09 (-.56, .38)	
Race						
White	168	Ref		104		.77
Non-white	48	.07 (-.42, .56)	.78	38	.08 (-.45, .61)	
Menstrual phase						
Proliferative	98	Ref		75	Ref	.77
Luteal	103	0.40 (-.03, .82)	.07	60	.07 (-.40, .54)	
Behavior in past 3 months						
Number of sexual partners						
0	17	Ref		9	Ref	
1	133	-.004 (-.78, .77)		84	.54 (-.44, 1.51)	.53
2	42	-.02 (-.88, 0.85)	.98	31	.24 (-.82, 1.29)	
≥3	24	.14 (-.82, 1.10)		18	.63 (-.51, 1.77)	
Lubricant use						
No	49	Ref		29	Ref	.12
Yes	104	-.43 (-0.98, 0.11)	.12	73	.47 (-.13, 1.07)	
Number of acts oral sex						
No oral sex	43	Ref		27	Ref	
1-10	96	.03 (-0.52, 0.59)		65	.28 (-.33, .90)	.27
11-20	23	.06 (-.73, .84)	.51	14	.48 (-.40, 1.36)	
>20	30	-.43 (-1.15, .29)		26	.72 (-.02, 1.45)	

		Hydrogen peroxide-producing lactobacilli			Gardnerella vaginalis		
		N = 216*	Difference in log ₁₀ bacterial quantity (cfu)	Wald test p value	N = 142*	Difference in log ₁₀ bacterial quantity (cfu)	Wald test p value
Days since last oral sex							
No oral sex		43	Ref		27	Ref	
1-6		97	-.28 (-.83, .27)		62	.47 (-.18, 1.12)	
7-30		48	.28 (-.35, .90)	.15	35	.32 (-.41, 1.04)	.48
30-90		17	.29 (-.57, 1.14)		7	.69 (-.50, 1.89)	
Number of acts digital-vaginal sex							
No digital-vaginal sex		28	Ref		13	Ref	
1-10		68	-.39 (-1.10, .32)		42	.31 (-.49, 1.1)	
11-20		35	-.17 (-.97, .63)	.16	15	.13 (-.82, 1.08)	.18
>20		36	-.84 (-1.64, -.05)		36	.75 (-.06, 1.56)	
Days since last digital-vaginal sex							
No digital-vaginal sex		26	Ref		13	Ref	
1-6		130	-.48 (-1.12, .17)		85	.42 (-.41, 1.25)	
7-30		39	-.05 (-.81, .70)	.29	30	.30 (-.63, 1.22)	.57
30-90		16	-.32 (-1.27, .62)		9	.82 (-.39, 2.02)	
Number of acts penile-vaginal sex							
No penile-vaginal sex		166	Ref		111	Ref	
1-5		15	.10 (-.70, .91)		13	-.04 (-.83, .76)	.75
>5		30	.77 (.18, 1.35)	.04	15	-.28 (-1.03, .46)	
Days since last penile-vaginal sex							
No penile-vaginal sex		168	Ref		114	Ref	
1-6		22	.08 (-.59, .76)	.22	15	-.20 (-.98, .57)	.94
7-30		19	.53 (-.19, 1.26)		7	.04 (-1.05, 1.14)	
30-90		6	1.01 (-.23, 2.25)		6	-.21 (-1.38, .97)	
Number of acts toy-vaginal sex							
No toy-vaginal sex		128	Ref		75	Ref	
1-10		51	-.33 (-.81, .16)	.002	38	.11 (-.46, .67)	.10
>10		18	-1.3 (-2.04, -.56)		20	.76 (.07, 1.45)	

	Hydrogen peroxide-producing lactobacilli			Gardnerella vaginalis		
	N = 216*	Difference in log ₁₀ bacterial quantity (cfu)	Wald test p value	N = 142*	Difference in log ₁₀ bacterial quantity (cfu)	Wald test p value
Days since last toy-vaginal sex						
No toy-vaginal sex	128	Ref		75	Ref	
1-6	33	-1.19 (-1.75, -.62)		23	.72 (.07, 1.38)	
7-30	37	-.17 (-.71, .36)	.001	29	.23 (-.37, .83)	.19
30-90	12	-.30 (-1.17, .57)		11	.28 (-.60, 1.17)	
Sharing of toys with partner						
No toys	128	Ref		75	Ref	
Toys, no vaginal sharing	20	-.13 (-.79, .53)	.005	17	.64 (-.11, 1.40)	.24
Toys, vaginal sharing	47	-.78 (-1.25, -.31)		41	.18 (-.37, .72)	
Number of acts oral-anal sex						
None	170	Ref		114	Ref	
1-5	28	-.21 (-.81, .38)	.77	16	.20 (-.55, .96)	.67
> 5	4	-.13 (-1.62, 1.35)		2	.75 (-1.26, 2.76)	
Had oral-anal sex in past week						
No	37	Ref	.35	26	Ref	.75
Yes	10	-.53 (-1.66, .61)		6	-.1 (-.73, .53)	
Number of acts penetrative anal sex (finger, dildo, penis)						
None	173	Ref		112	Ref	
1-5	19	-.01 (-.74, .71)	.95	13	-.43 (-1.24, .39)	.27
> 5	11	-.16 (-1.10, .78)		7	.64 (-.44, 1.73)	
Had penetrative anal sex in past week						
No	47	Ref	.27	30	Ref	.88
Yes	12	-.56 (-1.56, .44)		9	-.05 (-.78, .67)	

* Not all responses have complete data for all participants