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Research Article

Behavioral Predictors of Colonization with *Lactobacillus crispatus* or *Lactobacillus jensenii* after Treatment for Bacterial Vaginosis: A Cohort Study

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Objective: Evaluate predictors of vaginal colonization with lactobacilli after treatment for bacterial vaginosis (BV). *Methods*. Vaginal fluid specimens from women with BV underwent qPCR for *Lactobacillus crispatus*, *L. jensenii*, and *L. iners* pre- and posttreatment. *Results*. Few women with BV were colonized with *L. crispatus* (4/44, 9%) or *L. jensenii* (1/44, 2%), though all had *L. iners.* One month posttreatment 12/44 (27%) had *L. crispatus*, 12/44 (27%) *L. jensenii*, and 43/44 (98%) *L. iners.* Presence of *L. jensenii* posttreatment was associated with cure (Risk Ratio (RR) 1.67; 95% CI 1.09–2.56); *L. crispatus* showed a similar trend (RR 1.41; 95% CI 0.89–2.24, $P = 0.14$). Receptive oral sex was associated with 2.2-$\log_{10}$ lower concentration of *L. crispatus* (95% CI −4.38, −.02), and digital-vaginal sex with 2.6-$\log_{10}$ lower concentration (95% CI −4.87, −.33). *Conclusion*. One month after BV treatment, few women established colonization with *L. crispatus* or *L. jensenii*. Few behaviors were associated with colonization.

1. Introduction

Bacterial vaginosis (BV) is the most common cause of vaginal discharge in reproductive age women [1], is present in approximately 29% of women in the United States [2], and is characterized by vaginal colonization with anaerobic bacterial species along with loss of lactobacilli. The clinical sequelae of BV are significant—a nearly two-fold-increased risk of HIV-1 acquisition [3, 4], preterm delivery [5, 6], and pelvic inflammatory disease (PID) [7]—and affect millions of women worldwide each year, making BV a significant health problem. Treatment with antibiotics has a cure rate of 50–80% [8, 9] but recurrence within 1 to 3 months is common (30–52%) [10–12].

Hydrogen peroxide (H_2O_2-) producing species of vaginal lactobacilli are associated with decreased rates of BV [13, 14], and better reproductive health outcomes [15, 16] compared to non-H_2O_2-producing species. *Lactobacillus crispatus* is the most common vaginal H_2O_2-producing *Lactobacillus* species [17, 18]. *L. jensenii* is another frequently isolated H_2O_2-producing species [18, 19]. Some hypothesize that the recurrence rate of BV is high because these protective lactobacilli do not recolonize the vagina after antibiotic treatment aimed at eradicating BV-associated anaerobes, and so leave an ecologic void that is quickly refilled by opportunistic organisms. In one study, only 40% of women were recolonized with any H_2O_2-producing species of lactobacilli 30 days after oral metronidazole treatment and 57% were recolonized 30 days after vaginal clindamycin [20]. Most women are colonized with a single dominant species of *Lactobacillus* [21], but it is unclear if this is because there is competition between species for the vaginal niche. A majority of women studied in the US are colonized with *Lactobacillus iners* [22, 23], a fastidious species that does not commonly produce H_2O_2

and that has been associated with increased risk of abnormal vaginal microbiota in pregnant women [24]. Little is known about the effect of *L. iners* on a woman's ability to colonize with beneficial H_2O_2-producing species.

Presence of H_2O_2-producing lactobacilli [14, 25, 26], specifically *L. crispatus* [24], has been associated with decreased risk of abnormal vaginal microbiota and BV; thus, recolonization with these species after treatment for BV is likely an important marker of vaginal health. We undertook this nested cohort study to evaluate the effect of sexual behavior on vaginal recolonization with two hydrogen peroxide producing *Lactobacillus* species, *L. crispatus* and *L. jensenii,* one month after treatment for BV.

2. Methods

2.1. Study Population and Design. We conducted an analysis of women diagnosed with and treated for BV while enrolled in an observational cohort study in Seattle, WA. As previously described, participants were recruited through advertisements, media, and community referral, and had to be ≥16 years old and report having had sex with at least one woman in the previous year, a group with relatively high BV prevalence [27]. Study visits were scheduled every three months for a year, with additional visits for vaginal symptoms and/or 4 weeks after treatment for BV. At each visit, participants completed a computer-assisted self-interview (CASI) that collected information about demographics, sexual practices, medical, and reproductive history. The study was approved by the University of Washington Institutional Review Board and all participants provided informed consent at enrollment. Participants underwent pelvic examination with collection of vaginal swabs for saline microscopy, Gram stain, and bacterial culture. A separate foam swab was collected by rolling along the vaginal wall and was then frozen at −80°C for use in molecular assays. Women diagnosed with BV by Amsel's clinical criteria [28] were treated with vaginal metronidazole gel, 37.5 mg nightly for 5 nights, and assessed at a followup visit after 4 weeks. Vaginal fluid Gram stains were scored using the criteria outlined by Nugent et al. [29], however, treatment success was defined solely as absence of BV by Amsel's criteria.

We included all participants who were diagnosed with BV during the study and whose followup visits occurred 3 to 8 weeks after treatment. Only the first BV-positive visit was included for participants who were diagnosed with BV more than once. The study was conducted between October 2003 and December 2006, but between 3/2/2004 and 12/8/2005, only women with vaginal pH > 4.5 at the followup visit had samples taken follow-up (due to limitations in study funding). Because of this differential assessment and the resulting potential bias, all women whose followup visits fell within this time period were excluded. Participants whose samples did not have enough material to complete all PCR assays were also excluded.

2.2. Molecular Assays. Frozen vaginal swabs from the BV-positive visit and a followup visit within 3–8 weeks were processed as previously described [30]. All extracted DNA was tested in a quantitative PCR assay using primers targeting the human 18S rRNA gene to validate that successful DNA extraction occurred. An internal amplification control PCR using exogenous DNA from a jellyfish gene was used to test for presence of PCR inhibitors [31].

Vaginal fluid samples were then subjected to taxon-directed 16S rRNA gene quantitative PCR assays for the detection and quantification of *L. crispatus, L. jensenii*, and *L. iners* [30, 32]. Each assay has previously been validated and proven sensitive (to a level of 1–10 DNA copies/reaction) and specific (does not detect other bacteria at a concentration of 10^6 copies/reaction). The assays use a TaqMan format, and are run on an ABI 7500 Thermocycler (Applied Biosystems, Foster City, CA) or Eppendorf Mastercycler ep Realplex thermal cycler (Eppendorf, Westbury, NY).

2.3. Statistical Analysis. The primary outcome of interest was presence or absence of *L. crispatus* or *L. jensenii* after treatment for BV. In secondary analyses, we assessed the relationship between sexual behaviors and quantities of bacteria, expressed as 16S rDNA gene copies/vaginal swab and log transformed. Univariate log binomial regression was used to assess the relationship between presence or absence of either *L. crispatus* or *L. jensenii* and (a) different behaviors, and (b) presence and quantity of *L. iners*. Univariate linear regression was used to assess the relationship between sexual behaviors and quantity of *L. crispatus* or *L. jensenii* in the subset of women who were colonized. Given the relatively small number of women, we did not perform multivariate analyses.

3. Results

A total of 336 women were enrolled in the observational cohort. Of these, 136 (40%) were diagnosed with BV during the study: 96 at enrollment, and 40 at a routine study visit or a nonscheduled visit with symptomatic BV. Eleven women never returned for followup, 58 women had followup visits that fell during the period of exclusion, and 23 did not have adequate sample remaining for all of the assays and were excluded, leaving 44 women available for this analysis.

3.1. Baseline Characteristics. The 44 women had a mean age of 25 ± 3 years and were primarily white (35/44; 80%). Half of the visits occurred during the proliferative phase of the menstrual cycle, and half in the luteal phase. The majority of women had only female partners (31/44; 70%), while smaller percentages had male only (4/44; 9%), partners of both genders (4/44; 9%) or no sexual partner in the last 3 months (5/44; 11%). All women had BV by Amsel's criteria, and 98% (43/44) also had BV by Nugent's score, which was significantly different than women excluded from this substudy, of whom only 85% (78/92) had BV by Nugent's score ($P = .02$). This was the only characteristic that differed between women in the substudy and those that were excluded. Women in the substudy were as likely to complete antibiotic treatment for BV as women in the larger cohort (89% versus 90%; $P = .95$).

At diagnosis, 29/44 (66%) women reported having had receptive oral-vaginal contact in the previous 90 days. Slightly more reported digital-vaginal sex (82%), while fewer

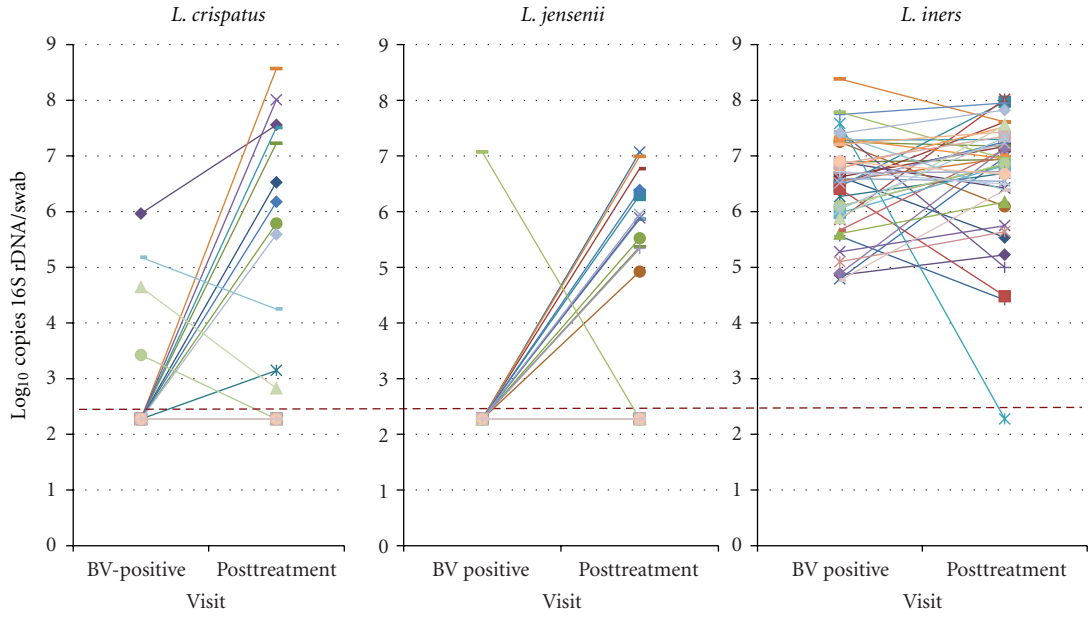

Figure 1: Change in concentrations of three different species of lactobacilli, as measured by species-specific quantitative PCR, 4 weeks after treatment for bacterial vaginosis with vaginal metronidazole. Each line represents an individual patient and her concentration of each bacterium before and after treatment. The dotted line represents the lower limit of detection of the qPCR assay.

reported toy-vaginal sex (36%) during that same time. Only 8 (18%) women reported sexual contact with a male partner in the 3 months prior to BV diagnosis, 7 of whom reported having penile-vaginal sex during that time. All 44 women were colonized with *L. iners* at BV diagnosis, while few were colonized with *L. crispatus* (4/44, 9%) *or L. jensenii* (1/44, 2%).

3.2. Posttreatment Characteristics. Nearly all women (43/44; 98%) were colonized with *L. iners* after treatment. Only 12/44 (27%) were colonized with *L. crispatus* and 12/44 (27%) with *L. jensenii.* Of those, six women were colonized with both species, and six each with only one of the two species. Posttreatment, 18 women (41%) still met Amsel's criteria for BV and were considered to have failed treatment, all of whom also had a Nugent score ≥7. Among these women, only 3 (16.7%) were colonized with *L. crispatus* and 2 (11.1%) with *L. jensenii*. Of the 26 women who achieved cure, a slightly higher percentage (but still a minority) were colonized with *L. crispatus* (9/26; 35%) and *L. jensenii* (10/26; 38%). Presence of *L. crispatus* at diagnosis or followup trended towards association with cure (Risk Ratio 1.41; 95% CI .89, 2.24; $P = .14$), but this was not statistically significant. Women colonized with *L. jensenii* after treatment had significantly higher rates of treatment success (RR 1.67; 95% CI 1.09, 2.56; $P = .02$).

Of the four women colonized with *L. crispatus* at BV diagnosis, one achieved cure and had higher concentrations after treatment, while 3 failed treatment, and had lower ($n = 2$) or undetectable ($n = 1$) concentrations. The one woman colonized with *L. jensenii* at BV diagnosis no longer had detectable colonization after treatment, and also failed treatment (Figure 1). Among colonized women, mean Log_{10} concentration of *L. crispatus* after treatment was 6.1 ± 1.9 gene copies/mL and for *L. jensenii* was 6.0 ± .7 gene copies/mL. All 44 women were colonized with high quantities of *L. iners* at the BV diagnosis visit (mean Log_{10} copies 6.5 ± .9), and the quantity did not change significantly at the followup visit (mean Log_{10} copies 6.7 ± 1.1; $P = .40$).

Between the visit at which BV was diagnosed and treatment provided, and subsequent followup (median 33 days, IQR 28–37), 21/44 women (48%) reported oral-vaginal sex, 26 (59%) digital-vaginal sex, 8 (18%) penile-vaginal sex, and 9 (20%) toy-vaginal sex. Among all 44 women, no interim sexual behaviors were associated with presence or absence of either *L. jensenii* or *L. crispatus* at the followup visit or with treatment failure (Table 1). Among women who were colonized with *L. jensenii* or *L. crispatus* at the followup visit, we examined whether behaviors reported in the interim period between treatment and followup at 32 days were associated with quantity of bacteria detected at the followup visit (Table 2). In the subset of 12 women establishing colonization by followup, report of digital-vaginal sex was significantly associated with 2.6-Log_{10} lower concentrations of *L. crispatus* (95% CI −4.87, −.33). Report of receptive oral sex was associated with 2.2-Log_{10} lower concentrations of *L. crispatus* (95% CI −4.38, −.02). No behaviors were associated with quantity of *L. jensenii* detected at that visit.

4. Discussion

In this cohort of women reporting sex with women, rates of vaginal colonization with two species of commensal H_2O_2-producing lactobacilli four weeks after treatment

Table 1: Univariate association between reported sexual behaviors during treatment and followup and presence of *L. crispatus* or *L. jensenii* at the posttreatment visit.

		Presence of *L. crispatus* ($n = 44$)	Presence of *L. jensenii* ($n = 44$)	BV diagnosis by Amsel's at followup ($n = 18$)
Sexual behavior during followup period	*N*	Prevalence ratio	Prevalence ratio	Prevalence ratio
Number of partners	12	Reference	Reference	Reference
0				
1	26	1.62 (.39, 6.65)	1.12 (.34, 3.46)	1.1 (.50, 2.44)
2+	6	3.0 (.67, 13.4)	1.33 (.30, 5.96)	.4 (.06, 2.70)
Oral vaginal sex	21	2.19 (.77, 6.22)	1.53 (.57, 4.1)	1.10 (.54, 2.23)
Digital-vaginal sex	26	2.08 (.65, 6.63)	2.08 (.65, 6.63)	.87 (.43, 1.76)
Toy-vaginal sex	9	1.30 (.44, 3.82)	.78 (.21, 2.94)	1.11 (.48, 2.56)
Penile-vaginal sex	8	.9 (.24, 3.34)	.9 (.24, 3.34)	.9 (.34, 2.38)
Use of vaginal lubricant	11	2.42 (.66, 8.93)	.30 (.04, 2.21)	.73 (.30, 1.78)

Table 2: Association between reported sexual behaviors during treatment and followup and quantity of *L. crispatus* or *L. jensenii* in women who were colonized at the posttreatment visit.

Sexual behavior during followup period	*L. crispatus*		*L. jensenii*	
	N	Log_{10} difference in 16S rRNA copies/mL	*N*	Log_{10} difference in 16S rRNA gene copies/mL
Number of partners				
0	2	Reference	3	Reference
1	7	−1.66 (−4.87, 1.55)	7	−.03 (−1.21, 1.16)
2+	3	−3.13 (−6.52, .25)	2	−.16 (−1.73, 1.40)
Oral vaginal sex	8	**−2.20 (−4.38, −.02)**	7	−.11 (−1.05, .83)
Digital-vaginal sex	9	**−2.60 (−4.87, −.33)**	9	−.06 (−1.13, 1.02)
Toy-vaginal sex	3	−1.65 (−4.32, 1.03)	2	−.15 (−1.39, 1.09)
Penile-vaginal sex	2	.69 (−2.67, 4.04)	2	−.14 (−1.39, 1.10)
Use of vaginal lubricant	4*	−2.92 (−6.09, .24)	1*	−.53 (−2.07, 1.0)

*Missing data for 5 women.

for bacterial vaginosis were low. Though colonization was infrequent, women who were able to establish colonization achieved high concentrations of each of these bacteria.

For clinicians and affected women, the high rate of BV recurrence after antibiotic treatment is exceedingly frustrating [10–12]. Several groups have evaluated whether adding probiotic compounds containing lactobacilli to treatment improves outcomes, but results have been mixed [33–36]. In a study of healthy women treated with vaginal probiotic capsules containing *L. crispatus*, participants who reported penile-vaginal sex between treatment and followup were less likely to establish colonization with the probiotic strain [37]. We hypothesized that sexual activity in the month after treatment may inhibit vaginal colonization with beneficial lactobacilli, possibly through reinoculation with BV-associated bacteria from vulvar or rectal reservoirs, which might increase risk for BV recurrence.

In the parent study of nearly 350 women from which this nested case control study was derived, we demonstrated that women cured of BV had higher rates of colonization by *L. crispatus* after treatment (42%) than women with persistent BV (26%; $P = .0003$); data on *L. jensenii* were not available [30]. A different study obtained vaginal swabs for culture and found that by 4 weeks after treatment with vaginal metronidazole 59% of women were colonized with hydrogen peroxide producing lactobacilli [20]. Other studies used Nugent score to characterize shifts of the vaginal bacteria, and reported that as many as 66% of treated women had at least some lactobacilli at 21–30 days after treatment [38], though H_2O_2 production was not measured. Our group previously measured posttreatment quantity of *L. crispatus* in a cohort of pregnant women using PCR and found that only 9/53 (17%) of women had detectable levels 4–6 weeks after treatment [39].

Few studies have evaluated behavioral predictors of colonization with lactobacilli. In women with BV, those who report more sexual partners are less likely to be colonized with H_2O_2-producing lactobacilli [40]. In our cohort, women colonized with *L. crispatus* who reported digital-vaginal and/or oral-vaginal sex had lower quantities of this bacterium. Although it did not reach statistical significance, we saw a paradoxical opposite trend in the risk related to these behaviors for vaginal colonization with *L. crispatus* or *L. jensenii*, suggesting that women with more partners, or

reporting more frequent oral-vaginal or digital-vaginal sex, were more likely to be colonized. One possible explanation is that women colonized by *L. crispatus* and *L. jensenii* more likely achieved cure of BV, thus reducing the likelihood of vaginal symptoms that might deter them from engaging in sex. This observation highlights the difficulty in studying the complex relationships between sexual behaviors and the dynamic nature of vaginal microbiology—temporal associations are difficult to ascertain unless both outcomes are measured frequently (ideally, daily).

The main limitation of this study is the small sample size, which reduced our power to detect potential associations between behaviors and colonization with specific lactobacilli. A significant number of participants with BV did not have a posttreatment sample, which limited our ability to examine the entire study group. Participants selected for this substudy were similar to the larger cohort except for having higher Nugent scores at diagnosis, which may partially explain their high rate of treatment failure. This cohort is composed primarily of women who have sex exclusively with women, and our results may differ from those obtained in a cohort of primarily heterosexual women. However, this allowed us to study the effect of several different types of sexual behavior on the vaginal microbiota. The population had well-characterized information about sexual activity during the treatment period, and a very high rate of followup (92%). Our quantitative PCR analysis allowed detection of small quantities of bacteria and analysis of changes in quantity of bacteria after treatment with respect to sexual behaviors.

5. Conclusions

Vaginal colonization with H_2O_2-producing lactobacilli 4 weeks after treatment for BV was uncommon, suggesting that there is a window of vulnerability during which women may be more susceptible to reinfection or recurrence. While no sexual behaviors were found to impact presence of colonization, quantity of *L. crispatus* was decreased in women reporting digital-vaginal and oral-vaginal contact. Quantity of *L. jensenii* was not affected by any reported sexual behaviors. This suggests that some species of commensal lactobacilli may be more sensitive to the effect of sexual activity on the vaginal environment.

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