MAJOR ARTICLE

Vaginal Colonization by Probiotic *Lactobacillus crispatus* CTV-05 Is Decreased by Sexual Activity and Endogenous Lactobacilli

May A. D. Antonio,[1] Leslie A. Meyn,[1] Pamela J. Murray,[1,3] Barbara Busse,[3] and Sharon L. Hillier[1,2]

[1]Magee–Womens Research Institute and Departments of [2]Obstetrics, Gynecology, and Reproductive Sciences and [3]Pediatrics, University of Pittsburgh, Pittsburgh, Pennsylvania

Background. Two potencies of gelatin capsules containing *Lactobacillus crispatus* CTV-05 were evaluated for safety and vaginal colonization in 90 young women.

Methods. Sexually active females aged 14–21 years were randomized to receive either 10^6- or 10^8-cfu CTV-05 capsules inserted intravaginally twice daily for 3 days. At enrollment and at 4 weekly follow-up visits, behavioral and demographic information and quantitative vaginal cultures were collected. *Lactobacillus* species were identified by DNA hybridization, and the CTV-05 strain was discerned using repetitive-sequence polymerase chain reaction DNA fingerprinting.

Results. Of the 90 participants, 87 returned for at least 2 follow-up visits. Of 40 participants who lacked *L. crispatus* colonization at enrollment, 36 (90%) were successfully colonized by CTV-05 at 1 or more follow-up visits, whereas only 24 (51%) of 47 participants colonized by *L. crispatus* at enrollment were positive for CTV-05 at follow-up ($P < .001$). Compared with sexually abstinent participants, females engaging in sexual intercourse with the use of condoms (odds ratio [OR], 6.3 [95% confidence interval {CI}, 1.3–29.4]; $P = .02$) or having unprotected sex (OR, 75.5 [95% CI, 6.9–820.6]; $P < .001$) during the first week were less likely to become colonized by CTV-05.

Conclusions. These data suggest that the factors that predict failure to become colonized by probiotic lactobacilli include exposure to semen, vaginal intercourse, and the presence of lactobacilli of the same species at enrollment.

Women who have hydrogen peroxide (H_2O_2)–producing lactobacilli in the vagina have a reduced incidence of bacterial vaginosis (BV) [1, 2], one of the most common vaginal syndromes among reproductive-age women [3]. BV is associated with increased acquisition of herpes simplex virus type 2 [4, 5], human papillomavirus [6], and HIV [7, 8] infection as well as pelvic inflammatory disease [9] and adverse pregnancy outcomes [10–13]. BV is microbiologically characterized by a reduction in or absence of lactobacilli and increases in anaerobic gram-negative rods, *Gardnerella vaginalis, Mobiluncus* species, other anaerobic gram-positive rods, and *Mycoplasma hominis* [3, 14]. Although the cure rate for BV with either metronidazole or clindamycin may initially be as high as 94% a week after treatment, studies consistently show recurrence, and the recurrence rate for BV may be >50% over a 12-month period [15]. Austin et al. [16] reported that H_2O_2-producing lactobacilli significantly increased after treatment, but only 48% of women were colonized by H_2O_2-producing lactobacilli 70–90 days after metronidazole treatment (M. N. Austin, personal communication). Because H_2O_2-producing lactobacilli decrease the risk of BV and many women lack H_2O_2-producing lactobacilli after BV treatment, several researchers have proposed that introducing exogenous probiotic strains of lactobacilli could help restore normal vaginal microflora [17–19].

Although strains of *Lactobacillus fermentum* and *Lactobacillus rhamnosus* have been extensively investigated as probiotics to help prevent urogenital infections [17, 18], researchers recommend using *Lactobacillus* species commonly recovered from the vagina [20–22]. Ge-

Received 18 August 2008; accepted 9 December 2008; electronically published 30 March 2009.

Potential conflicts of interest: none reported.

Presented in part: 33rd Annual Scientific Meeting and Symposium of the Infectious Diseases Society for Obstetrics and Gynecology, Monterey, California, 3 August 2006 (poster 8).

Financial support: National Institutes of Health (Public Health Service grant 1U01 AI 47785).

Reprints or correspondence: May Antonio, Magee–Womens Research Institute, 204 Craft Ave., A520, Pittsburgh, PA 15213 (beamerma@upmc.edu).

The Journal of Infectious Diseases 2009; 199:1506–13

0022-1899/2009/19910-0014$15.00
DOI: 10.1086/598686

nomic methods, including whole genomic characterization of vaginal microflora [14], have documented that *Lactobacillus crispatus* is a predominant *Lactobacillus* species that vaginally colonizes women without BV—including pregnant women [20, 21]—in the United States [23], Japan [24], and Europe [20, 21, 25, 26]. Of women vaginally colonized by *L. crispatus,* an H_2O_2-producing species, 43% are also colonized rectally [23]. Compared with vaginal colonization alone, both vaginal and rectal colonization by H_2O_2-producing lactobacilli reduced the risk of BV 4-fold in a cross-sectional study [23]. The population-based prevalence of *L. crispatus* may be due to its superior capacity to persist in the vagina [27]. One strain, *L. crispatus* CTV-05, had a high mean adherence to vaginal epithelial cells in vitro [28] and established vaginal colonization in 7 of 9 women [19].

The primary objective of the present double-blind, randomized single-center study was to evaluate the safety and colonization efficacy of 2 potencies of gelatin capsules containing *L. crispatus* CTV-05. The secondary objectives were to evaluate the effects of sexual activity and endogenous lactobacilli on vaginal colonization by probiotic lactobacilli.

METHODS

A double-blind, randomized study was conducted at the Adolescent Health Clinic at Children's Hospital of Pittsburgh and the Family Planning Clinic at Magee–Womens Hospital, Pittsburgh, Pennsylvania. Before enrollment, written informed consent was obtained from participants who were ⩾18 years old and from guardians of participants who were 14–17 years old. The participants were scheduled for follow-up visits at 7, 14, 21, and 28 days after enrollment, for a total of 5 visits. The study population comprised 90 sexually active, nonpregnant female subjects who were 14–21 years of age and free of genital infections. Subjects were excluded from enrollment if they were positive for BV, candidiasis, trichomoniasis, or cervicitis or if they had colposcopic abnormalities of the vulva, vagina, or cervix. In the conduct of clinical research, the human-experimentation guidelines of the US Department of Health and Human Services were followed.

The 90 participants were randomized with equal frequency to the 2 intervention groups, using a permuted block design with a block size of 4. One group received gelatin capsules containing 10^6 cfu of *L. crispatus* CTV-05 in a desiccated state, and the other group received identical capsules containing 10^8 cfu of *L. crispatus* CTV-05. All capsules were packaged identically and were indistinguishable in appearance. Both study staff and participants were masked as to the allocation group.

Participants were given detailed verbal instructions for digital insertion of the vaginal capsules and were directed to avoid using intravaginal products, including douches and medications, during the 48 h preceding a follow-up visit. During the enrollment visit, 1 vaginal capsule was inserted by the participant under the supervision of a study clinician. Participants were given 5 additional envelopes, each containing 1 vaginal gelatin capsule. They were instructed to insert 1 gelatin capsule on the evening of the enrollment visit, at least 6 h after the insertion of the first capsule, and to insert the remaining capsules twice a day for the next 2 days.

All visits included a speculum examination of the cervix and vaginal walls and an inspection of the external genitalia for erythema, edema, and lesions. Participants also underwent colposcopic examinations during enrollment and at the 7-day follow-up visit. The vulva, vagina, and cervix were evaluated for erythema, epithelial disruption, and sloughing. One nurse practitioner conducted all of the colposcopic examinations.

At each visit, participants were interviewed using a standardized questionnaire to obtain demographic, behavioral, and medical information, which included urethral, external genitalia, vaginal, and abdominal symptoms. The participants were asked to complete daily diaries for the 14 days after enrollment. The diaries involved circling "yes" or "no" for a list of symptoms and writing short answers about intercourse, medication, vaginal products, and general wellness.

At enrollment and at subsequent visits when clinically indicated, cervical specimens were obtained for *Neisseria gonorrhoeae* culture and *Chlamydia trachomatis* nucleic acid amplification testing. A vaginal swab sample was collected to inoculate modified Diamond's medium for *Trichomonas vaginalis*. A suspension made from a vaginal swab placed into sterile saline was examined under high power (×400) for the presence of clue cells, motile trichomonads, and yeast. Vaginal fluid was tested for amine odor and vaginal pH.

For all visits, a vaginal swab sample was rolled onto a slide that was Gram stained and scored for BV using the Nugent criteria [29]. Additionally, 2 Dacron swabs were used to obtain samplings from the lateral vaginal wall and placed into an anaerobic transport gel contained in a BBL Port-A-Cul tube (Becton Dickinson). The sample was delivered to the research laboratory within 12 h for quantitative culture set up for the detection of microorganisms, as described elsewhere [16].

To differentiate *L. crispatus* CTV-05 from other lactobacilli and other *L. crispatus* strains, repetitive-sequence polymerase chain reaction (rep-PCR) DNA fingerprinting was performed, as described elsewhere [19]. A total of 1670 *Lactobacillus* isolates from 436 visits were recovered by culture and subjected to rep-PCR. A mean of 3.8 *Lactobacillus* isolates were evaluated per visit.

Species-level identification of lactobacilli was performed using DNA hybridization, as described elsewhere [23]. If >2 isolates from the same participant had similar rep-PCR DNA fingerprints (i.e., were the same strains), only 1 was identified to the species level. Of the 246 lactobacilli isolated from the enrollment visits, 146 isolates were subjected to species-level identification.

Fisher's exact test was used to compare enrollment characteristics between the randomization arms, colonization efficacy according to the potency of CTV-05 capsules, and colonization efficacy according to the presence or absence of endogenous *Lactobacillus* species at enrollment. Colonization was defined as the presence of the CTV-05 strain at a follow-up visit. Changes in the prevalence of vaginal microorganisms within each potency group over the 5 visits were evaluated using Cochran's *Q* test for several related samples. Changes in the median log concentrations of vaginal microflora within each potency group over the 5 visits were evaluated using Friedman's test; participants who were negative for the presence of a specific microorganism were included. Median concentrations of H_2O_2-producing lactobacilli at the 7-day follow-up visit for participants who were never colonized with CTV-05 were compared with concentrations at the first-colonizing visit for participants who were colonized with CTV-05 by the Mann-Whitney *U* test. Logistic regression models were used to identify factors associated with noncolonization with *L. crispatus* CTV-05 at any follow-up visit. Models were developed using forward stepwise regression, and variables were retained if the Wald χ^2 test statistic yielded $P \leqslant .05$.

RESULTS

The demographic and behavioral characteristics of the 90 enrolled participants are shown in table 1. There were no differences in these characteristics between the 2 randomization groups. Although all participants reported previous sexual activity, only 58 (64%) reported vaginal intercourse in the month before enrollment. None of the participants reported having female sex partners.

Of the 90 enrolled participants, 3 did not return for any follow-up visits. The resulting modified intention-to-treat data set of 87 females includes 8 participants with 1 visit out of window and 1 participant who did not return for her 21- and 28-day follow-up visits. Of 450 potential study visits for the trial, 436 (97%) were completed.

No statistically significant difference was found between the 2 potency groups with respect to vaginal colonization by *L. crispatus* CTV-05 at each individual visit or overall colonization, suggesting that the 2 capsules were similarly efficacious at establishing colonization. Overall, CTV-05 was recovered at 1 follow-up visit or more for 60 (69%) of the 87 participants. Of the 86 participants who completed all visits, 51 (59%) were positive for CTV-05 colonization at the 28-day visit. Of these 51 participants, 38 sustained colonization of CTV-05 from the 7-day visit, 8 from the 14-day visit, and 2 from the 21-day visit. CTV-05 was first detected at the 28-day visit for 3 participants. For 8 of the 86 participants, CTV-05 was detectable at a single follow-up visit, but colonization was not sustained.

Because endogenous *Lactobacillus* species could competitively inhibit colonization by the probiotic *L. crispatus* CTV-05, the vaginal *Lactobacillus* species colonizing the participants at enrollment were evaluated as a predictor of CTV-05 colonization at follow-up. At enrollment, only 9 (10%) of the 87 subjects were lacking lactobacilli, 34 (39%) were colonized by 1 *Lactobacillus* species, and 44 (51%) were colonized by >1 *Lactobacillus* species. The most prevalent vaginal *Lactobacillus* species at enrollment was *L. crispatus,* which colonized more than half (54%) of the 87 participants, followed by *L. iners* (43%), *L. jensenii* (28%), and *L. gasseri* (20%) (table 2). Of 31 participants colonized by any *Lactobacillus* species other than *L. crispatus*, 28 (90%) were subsequently colonized by *L. crispatus* CTV-05, suggesting that other species do not antagonize subsequent colonization by *L. crispatus*. Of 9 participants initially lacking lactobacilli, 8 (89%) were successfully colonized by CTV-05, suggesting that there is not a host resistance to *L. crispatus* among women who lack this *Lactobacillus* species. By comparison, only 24 (51%) of 47 participants colonized by *L. crispatus* at enrollment were positive for *L. crispatus* CTV-05 at follow-up ($P < .001$), suggesting that endogenous *L. crispatus* competitively inhibit the probiotic strain of *L. crispatus* (table 2).

To determine whether CTV-05 colonization was sustained throughout the 5 weeks of follow-up, CTV-05 colonization frequency was compared among participants who were colonized by *L. crispatus* at enrollment and participants who lacked endogenous *L. crispatus* before capsule use (figure 1). CTV-05 was detected at the first follow-up visit in 83% of the participants who lacked *L. crispatus* colonization at enrollment, compared with only 28% of those who had *L. crispatus* colonization at enrollment. Colonization by CTV-05 was sustained at the 14-, 21-, and 28-day follow-up visits in a higher proportion of females initially lacking *L. crispatus* colonization, compared with the females colonized by endogenous *L. crispatus*. The modest increase in CTV-05 detection at the 21- and 28-day visits was due to the increased detection of CTV-05 in participants who had *L. crispatus* colonization at enrollment.

Multivariable analysis also showed that participants already vaginally colonized by *L. crispatus* before the introduction of probiotic *L. crispatus* CTV-05 were significantly less likely to be colonized by the exogenous strain (table 3). Additional factors associated with failure to be colonized by CTV-05 included sexual intercourse with the use of condoms for contraception or unprotected sexual intercourse during the week the vaginal capsules were used (table 3). Participants who remained sexually abstinent for the first 14 days after enrollment were the most likely to be colonized by CTV-05, compared with participants who had sexual intercourse with the use of condoms for contraception or unprotected sexual intercourse during the week of capsule use. No other factors were found to be associated with noncolonization, including race, age, potency, and vaginal pH at enrollment.

To evaluate whether colonization by the capsule strain resulted in higher-density *Lactobacillus* colonization, total coloni-

Table 1. Demographic and behavioral characteristics of the 90 randomized female participants, by *Lactobacillus crispatus* CTV-05 capsule potency.

Characteristic	Capsule potency		*P*[a]
	10^6 cfu	10^8 cfu	
Age			.959
14–17 years	24 (53)	22 (49)	
18–19 years	13 (29)	15 (33)	
20–21 years	8 (18)	8 (18)	
Race			.589
White	14 (31)	19 (42)	
Black	24 (53)	21 (47)	
Other	7 (16)	5 (11)	
Single marital status	44 (98)	40 (89)	.203
Education[b]			.112
≤8 years	7 (16)	4 (9)	
9–10 years	13 (30)	15 (33)	
11–12 years	17 (40)	11 (24)	
>12 years	6 (14)	15 (33)	
Employed part or full time	16 (36)	14 (31)	.823
Cigarette use in past 30 days	24 (53)	28 (62)	.522
Alcohol use in past 30 days	21 (47)	19 (42)	.832
Marijuana use in past 30 days	15 (33)	15 (33)	>.999
Ever douched	24 (53)	20 (44)	.527
Ever pregnant	15 (33)	12 (27)	.646
Frequency of sexual intercourse in past 30 days			.860
None	15 (33)	17 (38)	
1–4 times	20 (44)	17 (38)	
≥5 times	10 (22)	11 (24)	
Male sex partners in past 3 months			.887
0	9 (20)	7 (16)	
1	32 (71)	33 (73)	
≥2	4 (9)	5 (11)	
Lifetime no. of sex partners			.356
1	10 (22)	8 (18)	
2–3	11 (24)	15 (33)	
4–5	13 (29)	7 (16)	
≥6	11 (24)	15 (33)	

NOTE. Data are no. (%) of participants, unless otherwise indicated.

[a] Fisher's exact test.

[b] Education information was missing for 2 participants in the 10^6-cfu group.

zation density at enrollment was compared with that at the first visit when CTV-05 was detected. Of the 60 participants colonized by CTV-05 during the study, 42 were colonized at enrollment by H_2O_2-producing lactobacilli, at a median concentration of $10^{7.4}$ cfu/mL of vaginal fluid (range, $10^{3.1}$–$10^{8.8}$). By comparison, the median concentration of H_2O_2-producing lactobacilli at the first visit when CTV-05 was detected was $10^{7.5}$ cfu/mL of vaginal fluid (range, $10^{3.1}$–$10^{8.9}$). CTV-05 was not detected at any visit for 27 participants (31%). For this group, the median concentration of H_2O_2-producing lactobacilli was $10^{7.8}$ cfu/mL of vaginal fluid (range, $10^{3.0}$–$10^{8.5}$) at enrollment and $10^{7.5}$ cfu/mL of vaginal fluid (range, $10^{5.1}$–$10^{8.6}$) at the first follow-up visit among the participants colonized by these microorganisms. Thus, there was no significant difference in the median concentration of H_2O_2-producing lactobacilli between participants colonized with CTV-05 at any visit and participants who were never colonized ($P = .508$).

Another measure of safety was the analysis of vaginal microflora before and after capsule use. Across the follow-up period, there were no significant changes in prevalence or median log concentration for anaerobic gram-negative rods, *Prevotella bivia,* black-pigmented anaerobic gram-negative rods, *G. vaginalis,* anaerobic gram-positive cocci, group B *Streptococcus, Escherichia coli,* and any yeast. There was, however, a significant

Table 2. *Lactobacillus* species colonizing the vagina at enrollment and subsequent *Lactobacillus crispatus* CTV-05 colonization at any follow-up visit.

Colonizing species at enrollment (no. of participants colonized[a])	*L. crispatus* CTV-05 follow-up status,[b] no. (%)		
	Positive (n = 60)	Negative (n = 27)	P[c]
L. crispatus (n = 47)	24 (40)	23 (85)	<.001
L. jensenii (n = 24)	16 (27)	8 (30)	.799
L. gasseri (n = 17)	13 (22)	4 (15)	.567
L. iners (n = 37)	26 (43)	11 (41)	>.999
None (n = 9)	8 (13)	1 (4)	.263

[a] At enrollment, 45 (52%) of the participants were colonized by >1 *Lactobacillus* species.
[b] Positive indicates that colonization by *L. crispatus* CTV-05 was seen at any follow-up visit, and negative indicates that colonization was not seen at any visit.
[c] Fisher's exact test.

increase in the prevalence of H_2O_2-producing lactobacilli in both potency groups. The proportion of females with colonization by H_2O_2-producing lactobacilli increased from 69% at enrollment to 93% at the 7-day follow-up visit in the group receiving 10^6-cfu capsules. At enrollment, a higher proportion (83%) of participants in the 10^8-cfu potency group had H_2O_2-producing lactobacilli colonization, and this proportion increased to 100% by the 7-day follow-up visit. The frequency of colonization by H_2O_2-producing lactobacilli was higher at the 28-day follow-up visit than at the enrollment visit for both potency groups.

The colposcopic examinations at enrollment and the 7-day follow-up visit, the weekly pelvic examinations, and the symptom reviews from diaries and interviews from a total of 436 patient visits were important for assessing physiologic safety and revealed 47 adverse events. None of the adverse events were severe enough to prompt a participant to contact a study clinician before a scheduled research visit. The 47 adverse events recorded occurred in 32 (37%) of 87 participants. Of the 47 adverse events, 22 were coded by the study clinician as being unrelated to capsule use. For the remaining 25 events coded as being possibly related or having an unknown relationship to *L. crispatus* capsule use, 5 had no physical findings or symptoms; 17 were of mild severity, including pruritis (n = 3), BV (n = 2), urinary symptoms (n = 2), yeast vaginitis (n = 3), menstrual pain (n = 1), retained condom (n = 1), and other varied but minor events (n = 5); and 3 were moderately severe and required antifungal therapy for yeast vaginitis. None of the adverse events were severe enough that the participant had to be removed from the study.

Vaginal discharge was the only symptom whose frequency increased significantly, to a maximum of 74% during the second

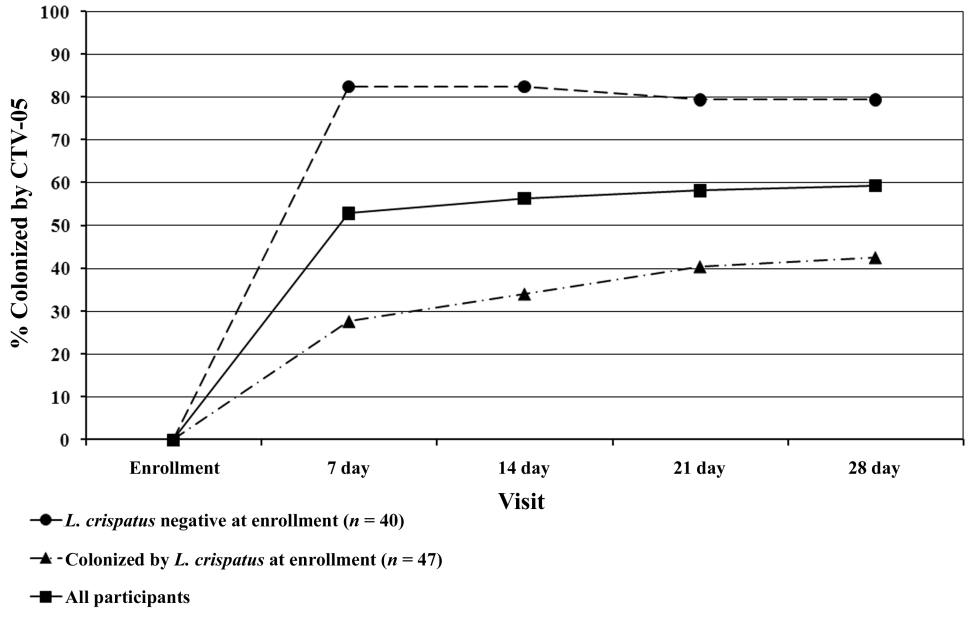

Figure 1. Frequency of colonization by probiotic *Lactobacillus crispatus* CTV-05, by the *L. crispatus* status of participants at enrollment.

Table 3. Adjusted risks of noncolonization with *Lactobacillus crispatus* CTV-05 at any follow-up visit for the 87 participants who returned for follow-up (logistic regression model).

Characteristic	Adjusted OR (95% CI)[a]	*P*[b]
L. crispatus colonization at enrollment	34.61 (4.18–286.65)	.001
Sexual activity reported at follow-up visits		
Abstinent between enrollment and 14-day visit	1.00 (referent)	. . .
Abstinent between enrollment and 7-day visit only	5.46 (0.89–33.56)	.067
Protected sexual intercourse between enrollment and 7-day visit	6.29 (1.34–29.44)	.020
Unprotected sexual intercourse between enrollment and 7-day visit	75.46 (6.94–820.59)	<.001

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

[a] Adjusted for all factors shown.

[b] Wald χ^2 test.

day of capsule use. The frequency of discharge returned to levels reported at enrollment by day 5, and discharge was reported in only 18% of the subjects by the end of the study. The temporal association between capsule use and the symptoms of discharge suggested that discharge resulted from the dissolution of the capsule, not a physiologic source.

DISCUSSION

L. crispatus CTV-05 established vaginal colonization at 1 follow-up visit or more in 69% of young females overall and in 90% of females not already colonized by *L. crispatus*. Although this suggests that the CTV-05 strain of *L. crispatus* adheres to the vaginal epithelium of women and can establish colonization, there is also some evidence of competition for adherence sites with endogenous lactobacilli, particularly other *L. crispatus* strains. Of the females who were not colonized by CTV-05 at any visit, 85% were already colonized by an endogenous *L. crispatus* strain. Vaginal colonization by endogenous *L. crispatus* appears to preclude the successful colonization by an exogenous *L. crispatus* strain.

Unprotected vaginal intercourse during the days surrounding capsule use also substantially decreased the likelihood of successful colonization. The high pH of seminal fluid may affect the adherence or survivability of CTV-05. Alternatively, a component of seminal fluid may block the adherence of CTV-05 to vaginal epithelial cells. It is also possible that CTV-05 is adherent to and is transferred by sperm [30]. Sexual intercourse with the use of condoms also affects colonization, although less significantly than unprotected sex. In one study, the levels of vaginal lactobacilli already colonizing the vagina 1–2 days before sexual intercourse with or without a condom were not significantly affected 8–12 h after intercourse [31]. Therefore, every effort should be made to instruct women to remain abstinent during actual capsule use and for at least 11 days after the first capsule, which should allow sufficient time for CTV-05 to adhere to vaginal epithelial cells. The lack of colonization among women having unprotected sex may be a serious limitation of the probiotic approach.

Ninety percent of the enrolled young women were vaginally colonized by lactobacilli before using the *L. crispatus* CTV-05 capsules, and 76% were vaginally colonized by H_2O_2-producing strains. This was not surprising given that all females with BV and other genital tract infections were excluded. *L. iners,* a non–H_2O_2-producing species, had an unexpectedly higher prevalence and frequency of cocolonization with other *Lactobacillus* species than reported elsewhere for cultivated lactobacilli from the vagina [23].

The high prevalence of endogenous lactobacilli before capsule use minimized the "net increase" in *Lactobacillus* colonization that was theoretically possible in this trial. One possible safety concern regarded the potential for *Lactobacillus* "overgrowth" with multiple doses of exogenous lactobacilli. Twice-daily insertion of the vaginal capsules for 3 days did not amplify the vaginal *Lactobacillus* population, which remained relatively constant, suggesting that the exogenous strain of *L. crispatus* adjusted to normal physiologic levels (10^7–10^8 cfu/mL of vaginal fluid) and remained at these levels throughout the follow-up period. The failure to successfully colonize women who already have optimal levels of *L. crispatus* should not be seen as a shortcoming of this product, but rather as a self-regulatory mechanism within the vaginal ecosystem.

A 3-day twice-daily dose of 10^6 or 10^8 cfu of *L. crispatus* CTV-05 is sufficient to establish lactobacilli colonization in women lacking *Lactobacillus*. Other studies using DNA fingerprinting methods found that a 3-day daily vaginal capsule dose of 10^9 cfu (total) of *L. rhamnosus* GR-1 and *L. fermentum* RC-14 or 10^{10} cfu of *L. rhamnosus* GG resulted in low frequencies of colonization by the microorganisms by day 21 [18, 32]. In contrast, CTV-05 was detected in more than half (58%) of the participants in this study at the 21-day visit, suggesting that a *Lactobacillus* species that is predominantly found in the vagina may be a more successful vaginal probiotic than *L. rhamnosus* and *L. fermentum,* which are not prevalent in the vagina.

A limitation of this study is the reliance on visual detection of the probiotic strain by culture. The colony morphology of the

probiotic strain and that of endogenous *L. crispatus* may be very similar and difficult to discern. During the early follow-up visits, CTV-05 may be present in very low quantities and masked by other bacteria. Aside from fingerprinting strain-typing methods, there are no direct detection genetic methods to identify the CTV-05 strain. A second limitation was the reliance on self-report to assess participants' adherence to product use. Finally, results may not be generalizable to adult women or women with vaginal infections, because only females 14–21 years old without genital infections were included in the study.

The purpose of the present study was to better characterize the safety of *L. crispatus* CTV-05 capsules inserted intravaginally. The only event recorded in the diaries that was temporally associated with use of the vaginal *Lactobacillus* capsule was vaginal discharge, which was also frequently reported in a study of CTV-05 for women with recurrent urinary tract infection [33]. The sensation of discharge was most likely attributable to the melting of the capsule, given that gelatin melts at body temperature in the presence of genital fluid. The dissolution of the capsule may result in increased discharge, but no significant adverse effects were attributable to capsule use.

L. crispatus CTV-05 effectively colonized the vagina of 90% of women lacking endogenous *L. crispatus* but successfully colonized only half of women already colonized by *L. crispatus* at enrollment. Lengthening the 3-day dosing period evaluated in this study may increase colonization by the probiotic strain. Additional studies of the product will be needed to assess whether vaginal capsules containing *L. crispatus* CTV-05 will be a useful adjunct to antibiotic treatment for the prevention of recurrent BV.

References

1. Cherpes TL, Hillier SL, Meyn LA, Busch JL, Krohn MA. A delicate balance: risk factors for acquisition of bacterial vaginosis include sexual activity, absence of hydrogen peroxide-producing lactobacilli, black race, and positive herpes simplex virus type 2 serology. Sex Transm Dis **2008**; 35:78–83.
2. Hawes SE, Hillier SL, Benedetti J, et al. Hydrogen peroxide–producing lactobacilli and acquisition of vaginal infections. J Infect Dis **1996**; 174: 1058–63.
3. Hillier S, Marrazzo J, Holmes KK. Bacterial vaginosis. In: Holmes KK, Sparling PF, Stamm WE, et al., eds. Sexually transmitted diseases. 4th ed. New York: McGraw-Hill, **2008**:737–68.
4. Cherpes TL, Meyn LA, Krohn MA, Lurie JG, Hillier SL. Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. Clin Infect Dis **2003**; 37:319–25.
5. Gottlieb SL, Douglas JM Jr, Foster M, et al. Incidence of herpes simplex virus type 2 infection in 5 sexually transmitted disease (STD) clinics and the effect of HIV/STD risk-reduction counseling. J Infect Dis **2004**; 190: 1059–67.
6. Watts DH, Fazzari M, Minkoff H. Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1–infected and high-risk HIV-1–uninfected women. J Infect Dis **2005**; 191:1129–39.
7. Taha TE, Hoover DR, Dallabetta GA, et al. Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. AIDS **1998**; 12:1699–706.
8. Myer L, Denny L, Telerant R, Souza M, Wright TC Jr, Kuhn L. Bacterial vaginosis and susceptibility to HIV infection in South African women: a nested case-control study. J Infect Dis **2005**; 192:1372–80.
9. Haggerty CL, Hillier SL, Bass DC, Ness RB. Bacterial vaginosis and anaerobic bacteria are associated with endometritis. Clin Infect Dis **2004**; 39:990–5.
10. Gravett MG, Nelson HP, DeRouen T, Critchlow C, Eschenbach DA, Holmes KK. Independent associations of bacterial vaginosis and *Chlamydia trachomatis* infection with adverse pregnancy outcome. JAMA **1986**; 256:1899–903.
11. Hay PE, Lamont RF, Taylor-Robinson D, et al. Abnormal bacterial colonization of the genital tract and subsequent preterm delivery and late miscarriage. BMJ **1994**; 308:295–8.
12. Hillier SL, Nugent RP, Eschenbach DA, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group. N Engl J Med **1995**; 333:1737–42.
13. Lahli-Camp JM, Rai R, Ison C, et al. Association of bacterial vaginosis with history of second trimester miscarriage. Human Reprod **1996**; 11: 1575–8.
14. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. N Engl J Med **2005**; 353:1899–911.
15. Bradshaw CS, Morton AN, Hocking J, et al. High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. J Infect Dis **2006**; 193: 1478–86.
16. Austin MN, Beigi RH, Meyn LA, Hillier SL. Microbiologic response to treatment of bacterial vaginosis with topical clindamycin or metronidazole. J Clin Microbiol **2005**; 43:4492–7.
17. Reid G, Charbonneau D, Erb J, et al. Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. FEMS Immunol Med Microbiol **2003**; 35:131–4.
18. Gardiner GE, Heinemann C, Bruce AW, Beuerman D, Reid G. Persistence of *Lactobacillus fermentum* RC-14 and *L. rhamnosus* GR-1 but not *L. rhamnosus* GG in the human vagina as demonstrated by randomly amplified polymorphic DNA. Clin Diagn Lab Immunol **2002**; 9:92–6.
19. Antonio MAD, Hillier SL. DNA fingerprinting of *Lactobacillus crispatus* strain CTV-05 by repetitive sequence-based PCR analysis in a pilot study of vaginal colonization. J Clin Microbiol **2003**; 41:1881–7.
20. Kiss H, Kögler B, Petricevic L, et al. Vaginal *Lactobacillus* microbiota of healthy women in the late first trimester of pregnancy. BJOG **2007**; 114: 1402–7.
21. Wilks M, Wiggins R, Whiley A, et al. Identification and H_2O_2 production of vaginal lactobacilli from pregnant women at high risk of preterm birth and relation with outcome. J Clin Microbiol **2004**; 42:713–7.
22. McGroarty JA. Probiotic use of lactobacilli in the human female urogenital tract. FEMS Immunol Med Microbiol **1993**; 6:251–64.
23. Antonio MAD, Rabe LK, Hillier SL. Colonization of the rectum by *Lactobacillus* species and decreased risk of bacterial vaginosis. J Infect Dis **2005**; 192:394–8.
24. Song Y, Kato N, Matsumiya Y, Liu C, Kato H, Watanabe K. Identification of and hydrogen peroxide production by fecal and vaginal lactobacilli isolated from Japanese women and newborn infants. J Clin Microbiol **1999**; 37:3062.
25. Giorgi A, Torriani S, Dellaglio F, Bo G, Stola E, Bernuzzi L. Identification of vaginal lactobacilli from asymptomatic women. Microbiologica **1987**; 10:377–84.
26. Vásquez A, Jakobsson T, Ahrné S, Forsum U, Molin G. Vaginal lactobacillus flora of healthy Swedish women. J Clin Microbiol **2002**; 40: 2746–9.
27. Vallor AC, Antonio MAD, Hawes SE, Hillier SL. Factors associated with acquisition of, or persistent colonization by, vaginal lactobacilli: role of hydrogen peroxide production. J Infect Dis **2001**; 184:1431–6.

28. Kwok L, Stapleton AE, Stamm WE, Hillier SL, Wobbe CL, Gupta K. Adherence of *Lactobacillus crispatus* to vaginal epithelial cells from women with or without a history of recurrent urinary tract infection. J Urol **2006**; 176:2050–4.
29. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. J Clin Microbiol **1991**; 29:297–301.
30. Toth A, O'Leary WM, Ledger W. Evidence of microbial transfer by spermatozoa. Obstet Gynecol **1982**; 59:556–9.
31. Eschenbach DA, Patton DL, Hooton TM, Meier AS, et al. Effects of vaginal intercourse with and without a condom on vaginal flora and vaginal epithelium. J Infect Dis **2001**; 183:913–8.
32. Burton JP, Cadieux PA, Reid G. Improved understanding of the bacterial vaginal microbiota of women before and after probiotic instillation. Appl Environ Microbiol **2003**; 69:97–101.
33. Czaja CA, Stapleton AE, Yarova-Yarovaya Y, Stamm WE. Phase I trial of a *Lactobacillus crispatus* vaginal suppository for prevention of recurrent urinary tract infection in women. Infect Dis Obstet Gynecol **2007**; 2007:35387.