

Randomized, Placebo-Controlled Phase 2 Trial of a *Lactobacillus crispatus* Probiotic Given Intravaginally for Prevention of Recurrent Urinary Tract Infection

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Background. Urinary tract infections (UTIs) are common among women and frequently recur. Depletion of vaginal lactobacilli is associated with UTI risk, which suggests that repletion may be beneficial. We conducted a double-blind placebo-controlled trial of a *Lactobacillus crispatus* intravaginal suppository probiotic (Lactin-V; Osel) for prevention of recurrent UTI in premenopausal women.

Methods. One hundred young women with a history of recurrent UTI received antimicrobials for acute UTI and then were randomized to receive either Lactin-V or placebo daily for 5 d, then once weekly for 10 weeks. Participants were followed up at 1 week and 10 weeks after intervention and for UTIs; urine samples for culture and vaginal swabs for real-time quantitative 16S ribosomal RNA gene polymerase chain reaction for *L. crispatus* were collected.

Results. Recurrent UTI occurred in 7/48 15% of women receiving Lactin-V compared with 13/48 27% of women receiving placebo (relative risk [RR], .5; 95% confidence interval, .2–1.2). High-level vaginal colonization with *L. crispatus* ($\geq 10^6$ 16S RNA gene copies per swab) throughout follow-up was associated with a significant reduction in recurrent UTI only for Lactin-V (RR for Lactin-V, .07; RR for placebo, 1.1; $P < .01$).

Conclusions. Lactin-V after treatment for cystitis is associated with a reduction in recurrent UTI. Larger efficacy trials of this novel preventive method for recurrent UTI are warranted.

Clinical Trials Registration. NCT00305227.

Urinary tract infection (UTI) is an exceedingly common outpatient problem among young healthy women and results in considerable morbidity and health care cost

[1–3]. A recent population survey of women in the United States found that the lifetime risk of UTI was >60% and that the estimated societal cost of UTI exceeds \$25 billion over 20 years [1]. Approximately 30% of women develop frequent recurrent episodes, which often necessitate repeated antimicrobial treatment courses [4–7]. Increasing resistance to commonly used antimicrobials such as trimethoprim-sulfamethoxazole and fluoroquinolones, even among uropathogens causing community-acquired UTIs, is making treatment and prevention of these infections more problematic [8–10]. Thus, novel, safe, and effective nonantimicrobial prevention and treatment strategies are needed.

One nonantimicrobial adjunct treatment approach for which there is strong mechanistic evidence is use of a hydrogen peroxide-producing ($H_2O_2^+$) lactobacillus

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probiotic to restore the normal vaginal microbiota in women who are prone to UTI [11–13]. We and others have demonstrated that women with recurrent UTI (rUTI) often have specific alterations in their vaginal microbiota, especially at the time of UTI, namely, increased rates of colonization with *Escherichia coli* and depletion of the normally predominant $H_2O_2^+$ lactobacilli [14, 15]. If repletion of the normal $H_2O_2^+$ lactobacilli were effective in reducing *E. coli* colonization and UTI, this strategy would reduce the costs and morbidity associated with UTIs, including time lost from work, health care costs, antimicrobial use, and the ultimate development of antimicrobial resistance [16]. Although there is strong in vitro evidence to support this approach, there have been few clinical studies. Randomized, double-blind, placebo-controlled studies of adequate sample size with a physiologically relevant probiotic strain are needed to ascertain whether this approach is of value.

Lactin-V (Osel) contains a carefully selected $H_2O_2^+$ *Lactobacillus crispatus* strain CTV-05 isolated from a healthy woman's vagina and was developed as a vaginal probiotic for use in pathophysiological states characterized by detrimental alterations in vaginal flora, such as bacterial vaginosis (BV) [17, 18], rUTI, and potentially others. We recently published the results of a randomized, double-blind, phase 1 trial of the safety and tolerance of Lactin-V in women with rUTI, showing that *L. crispatus* CTV-05 can be given as a vaginal suppository with minimal adverse effects to healthy women with a history of rUTI [19].

Subsequently, we conducted a randomized, double-blind, placebo-controlled phase 2 trial of Lactin-V among women with rUTI. Major goals of the study were to assess the ability of the probiotic to reduce the incidence of rUTI, to evaluate whether the probiotic achieved vaginal colonization, to assess effects on the vaginal microbiota of women after treatment for UTI, and to confirm the safety of the probiotic. We found that Lactin-V reduced the risk of rUTI approximately as effectively as antimicrobial prophylaxis, achieved high-level vaginal colonization in most women, and was well tolerated. Lactin-V is a promising candidate for prevention of rUTI.

METHODS

Participants

We recruited premenopausal women aged 18–40 years with current, symptomatic, uncomplicated cystitis from the student health center at the University of Washington (Seattle, WA) from February 2006 through February 2009. Cystitis at enrollment and during follow-up was defined as 1 or more typical UTI symptoms (dysuria, frequency, or urgency) and pyuria (cell count, ≥ 8 white blood cells per high-power field on urinalysis) and a positive voided midstream urine culture, defined as $\geq 10^2$ colony-forming units (CFUs)/mL of 1 or more uropathogens

(*E. coli* or other Enterobacteriaceae, *Enterococcus* species, or group B streptococci) or $\geq 10^5$ CFUs/mL of *Lactobacillus* species present as a single infecting organism. Eligible participants had a history of at least 1 prior symptomatic UTI treated within the past 12 months prior to the current UTI. Eligibility requirements also included the following: a normal Papanicolaou test result documented in the past year or at the screening clinic visit; not being pregnant and having regular menstrual cycles or amenorrhea for at least 6 months secondary to use of a hormonal contraceptive or hysterectomy; agreement not to use other intravaginal products, self-medication for UTI, or antimicrobial prophylaxis while using the product; agreement not to use tampons for 24 h after insertion of the product; agreement to use birth control and, if using condoms, to use non-spermicidal condoms provided by the study site personnel; and capability of understanding English and providing informed consent.

Exclusion criteria included the following: current complicated cystitis or uncomplicated pyelonephritis; a history of urologic abnormality or renal calculi; recent sexually transmitted infection (STI) or BV or a history of recurrent BV; risk factors for STI and human immunodeficiency virus (HIV) infection; pregnancy or within 2 months of pregnancy; lactation; menopause; diabetes, HIV infection, or other immunocompromised state; drug or alcohol abuse; abnormal pelvic examination results; and persistent symptoms and/or pyuria after treatment of the enrollment acute UTI.

Randomization

The study participants were randomly assigned to Lactin-V or placebo by use of a computer-generated randomized number system in blocked assignments to achieve equal sample sizes in both groups. The assigned intervention substance (Lactin-V or placebo) was packaged in identically appearing packets according to assignment and sequential study number. The list was not available for viewing by clinicians who saw the study participants or by laboratory personnel who handled their specimens.

Study Design

The participants were treated for acute UTI at visit 1 with standard therapy. At visit 2, scheduled for 7–10 d after UTI treatment, participants were randomized to receive Lactin-V (gelatin capsules with no applicator; dose, 10^8 CFUs/mL) or placebo vaginal suppositories for self-administration once daily for 5 d. Participants then self-administered vaginal suppositories once weekly for 10 weeks. Participants were seen in scheduled follow-up visits at 1 week (visit 3) and 10 weeks (visit 4) after beginning Lactin-V or placebo and for symptomatic UTIs. At routine follow-up or symptomatic visits, participants underwent a structured interview, including a review of adverse events, and a structured physical examination, which included the

following: assessment of the appearance of external genitalia; speculum examination of the vagina and cervix; bimanual examination; vaginal wet mount and further assessment of vaginal discharge (pH test, sniff test, and microscopic examination for clue cells, yeast, and trichomonads); urine dipstick for leukocyte esterase, nitrite, and blood; and enumeration of urine white blood cells by use of a hemocytometer. Specimens were collected for urinalysis, urine, and vaginal culture. Vaginal swab specimens for real-time 16S ribosomal RNA (rRNA) gene quantitative polymerase chain reaction (qPCR) for *L. crispatus* to assess vaginal colonization were collected as described elsewhere [20], by use of polyurethane foam swabs (Epicenter Biotechnologies) brushed against the lateral vaginal wall, re-sheathed, and frozen immediately at -20°C and then held at -80°C until analysis.

If participants developed acute cystitis symptoms during the course of the study, they were instructed to contact the clinic or, if the clinic was closed, to contact the covering physicians. If the episode occurred while the clinic was open, participants came into the clinic, were evaluated for clinical parameters, and provided specimens for testing as described above. If the episode occurred while the clinic was closed, participants were instructed to collect a specimen for urine culture, refrigerate it, and bring it to clinic as soon as the clinic was open. Participants who developed symptomatic UTI during study follow-up were treated and continued on the study.

Laboratory Procedures

Laboratory procedures were performed on clinical specimens with the use of standard methods, as described elsewhere [19], and included urine dipstick testing, urinalysis, and vaginal and urine cultures for facultative bacteria and *Lactobacillus* species. We quantified *L. crispatus* in vaginal swabs collected and stored at each visit, extracting DNA and performing real-time 16S rRNA gene qPCR with an assay specific for *L. crispatus*, as described elsewhere [21].

L. Crispatus Colonization Levels and Patterns

On the basis of qPCR results, *L. crispatus* colonization was analyzed by comparing the level of *L. crispatus* colonization at the end of the study in the 2 groups of participants, as well as the patterns of colonization over the course of the study in each participant. High-level *L. crispatus* colonization was defined as having $\geq 10^6$ 16S RNA gene copies of *L. crispatus* per swab, whereas low-level colonization was defined as having $< 10^6$ 16S RNA gene copies of *L. crispatus* per swab. A high-level colonization pattern was defined within each participant as demonstrating high-level colonization of *L. crispatus* at the first follow-up visit (visit 3) and maintaining this level throughout follow-up (visit 4 and any symptomatic visits during follow-up). A low-level colonization pattern was defined as all other patterns not meeting these criteria.

Sample Size, Objectives, and Institutional Review Board Approval

The target sample size and final enrollment was 100 participants, 50 in each group, to provide information on the biological activity of the Lactin-V intervention and its effect on the incidence of rUTI in high-risk women, and to provide information on possible adverse events. The primary objective was to evaluate Lactin-V in healthy premenopausal women with rUTI for the ability of the probiotic to reduce the incidence of cystitis and produce high-level vaginal colonization with *L. crispatus* at the end of 10 weeks. Secondary objectives were to evaluate patterns of vaginal colonization with *L. crispatus* in the vaginal microbiota and to confirm the safety of the probiotic. The study was approved by the institutional review board of the University of Washington and registered as a clinical trial (Clinicaltrials.gov ID no. NCT00305227).

Data Analysis

All women enrolled and randomized were described using means, medians, and frequency counts. The effect of the intervention was described using relative risks (RRs) and 95% confidence intervals (CIs). Per-protocol analyses were performed and produced similar results.

RESULTS

Enrollment and Demographics

The total enrollment was 100 participants; 50 received Lactin-V and 50 received placebo (Figure 1). The median age of participants was 21 years in both groups. The median number of lifetime UTIs was 4.5. Three-quarters of the women in both groups had never been married, and 99% were sexually active with an equal median rate of sexual activity in the month

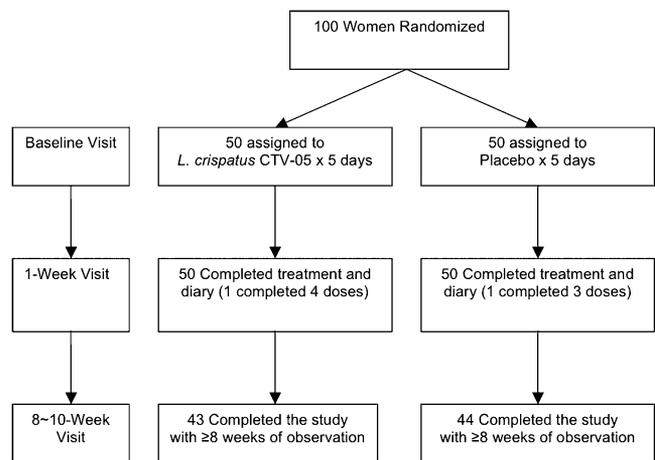


Figure 1. Randomization and follow-up of participants. *L. crispatus* CTV-05, *Lactobacillus crispatus* strain CTV-05.

Table 1. Baseline Characteristics of Study Participants

Characteristics	Participants receiving Lactin-V (n = 50)	Participants receiving placebo (n = 50)	All participants (n = 100)
Age in years, median (range)	21 (18–31)	21 (18–36)	21 (18–36)
Never married	39 (78)	37 (74)	76 (76)
Race or ethnicity			
White	33 (66)	42 (84)	75 (75)
Asian	8 (16)	3 (6)	11 (11)
African American	0 (0)	1 (2)	1 (1)
Native American	0 (0)	1 (2)	1 (1)
Other	9 (18)	3 (6)	12 (12)
Hispanic	4 (8)	3 (6)	7 (7)
No. of UTIs in lifetime, median (range)	4 (1–45)	5 (1–30)	4.5 (1–45)
1–2	18 (36)	12 (24)	30 (30)
≥3	32 (64)	38 (76)	70 (70)
Sexually active in the past month	50 (100)	49 (98)	99 (99)
Episodes of sexual intercourse in the past month, median	10	10	10
Any spermicide use ^a in the past month	5 (10)	2 (4)	7 (7)
Abnormal Papanicolaou test result	2 (4)	3 (6)	5 (5)

NOTE. Data are no. (%) of participants, unless otherwise indicated. UTI, urinary tract infection.

^a Diaphragm or spermicidal condom use.

prior to enrollment; <10% of women had used a spermicide-containing birth control method in the past month (Table 1).

Clinical Outcomes and Probiotic Effect on Vaginal Microbiota

Seven (15%) of the women receiving Lactin-V had at least 1 UTI compared with 13 (27%) of the women receiving placebo (RR, .5; 95% CI, .2–1.2) (Table 2). The prevalence of *E. coli* rUTIs was similar in the 2 groups (prevalence in Lactin-V group, 75%; prevalence in placebo group, 69%). On the basis of qPCR analysis, most women receiving Lactin-V achieved high-level ≥ vaginal colonization with *L. crispatus* (≥10⁶ 16S RNA gene copies per swab). At the final visit at 10 weeks (visit 4), the prevalence of high-level *L. crispatus* colonization among Lactin-V recipients was 39 (93%) of 42 participants, compared with 30 (68%) of 44 placebo recipients (*P* = .004).

Women who received Lactin-V and achieved a high-level *L. crispatus* vaginal colonization pattern throughout the course of the study had a significant reduction in the risk of rUTI, whereas when this high-level colonization pattern occurred in women who received placebo, it was not protective (RR for Lactin-V, .07; RR for placebo, 1.1; *P* < .01) (Table 2). There was no significant difference in causative organisms for rUTI events in the 2 arms of the study.

Safety and Tolerability

We confirmed the findings in the phase 1 study regarding safety and tolerance of Lactin-V [19]. Adverse effects were reported by 56% of participants who received Lactin-V and by 50% of participants who received placebo; the most common adverse

effects included vaginal discharge or itching or moderate abdominal discomfort. One participant in the placebo group discontinued treatment because of adverse effects. The incidence of BV or candidal vaginitis was low in both treatment groups (0%–5%), and unlike the findings in the phase 1 study [19], there was no significant difference in rates of pyuria between the 2 groups (rate at visit 3 among women in the Lactin-V group, 13%; rate at visit 3 among women in the placebo group, 22%; rate at visit 4 among women in the Lactin-V group, 32%; rate at visit 4 among women in the placebo group, 33%). No episodes of pyelonephritis were reported in either group.

Table 2. Urinary Tract Infection Rates by Intervention and *Lactobacillus crispatus* Colonization Pattern

Intervention	No. (%) of participants developing recurrent UTI	Relative risk (95% CI)
Lactin-V (n = 48)	7 (15)	.5 (.2–1.2)
Placebo (n = 48)	13 (27)	...
Intervention, <i>L. crispatus</i> colonization pattern		
Lactin-V, High level (n = 41)	2 (5)	.07 (.02–.3)
Lactin-V, Low level (n = 7)	5 (71)	...
Placebo, High level (n = 32)		
Placebo, Low level (n = 16)	4 (25)	...

NOTE. CI, confidence interval; Lactin-V, *L. crispatus* intravaginal suppository probiotic (Osel); UTI, urinary tract infection

DISCUSSION

The results of this randomized, double-blind, phase 2 trial suggest that Lactin-V, an intravaginal probiotic composed of *L. crispatus* CTV-05, may reduce the rate of rUTI in UTI-prone women by about one-half. Thus, among the 50 women who received Lactin-V, the rate of culture-confirmed UTI was 15%, as compared with 27% among women who received placebo (RR, .5; 95% CI, .2–1.2). Moreover, women in the Lactin-V group who achieved a high-level *L. crispatus* vaginal colonization pattern had a significant reduction in rUTI compared with those who did not ($P < .01$). Women receiving placebo had no such reduction, regardless of their pattern of colonization. The safety and tolerability of Lactin-V, previously demonstrated in a phase 1 study [19], was confirmed in this study.

Lactin-V readily achieved high-level and sustained vaginal colonization, as measured by *L. crispatus* qPCR. Nearly every woman who received Lactin-V had *L. crispatus* colonization at follow-up as shown with qPCR. In contrast, among the 14 study participants who had no *L. crispatus* identified using the highly sensitive qPCR method, 12 had received the placebo. Both Lactin-V recipients in whom *L. crispatus* colonization failed to establish during follow-up had rUTI.

We and others have shown that women with rUTI typically have depletion of the normally predominant $H_2O_2^+$ lactobacilli at the time of UTI [14, 15]; however, previous studies have used standardized methods of quantifying lactobacilli cultivatable from vaginal samples on a scale of 1–4+ [22] or by using repetitive element sequence-based PCR repPCR, which also requires cultivatable lactobacilli [19]. This study is novel in its application of a quantitative molecular method to assess vaginal microbiota following UTI, and thus to more precisely define sentinel changes in vaginal microbiota following standard treatment for UTI, either with or without a specific intervention designed to repopulate the vaginal bacterial biota, namely, the Lactin-V probiotic.

We assessed *L. crispatus* colonization as a sentinel measure of the vaginal microbiota because surveys of healthy, premenopausal women worldwide show that this bacterium is very common, although colonization with other lactobacilli, including *L. iners*, *L. jensenii*, or *L. gasseri*, is also found [23–27]. Thus, using a qPCR method to assess *L. crispatus* colonization in women in both arms of the study allowed us to distinguish the natural recovery of the vaginal microbiota after UTI, as may have potentially occurred in the participants receiving placebo, from specific effects attributable to the intervention with the Lactin-V probiotic.

This analysis showed a most striking finding, namely, that although women who received placebo often had high concentrations of vaginal *L. crispatus* during follow-up, this colonization did not protect them from rUTI (RR, 1.1; 95% CI,

.4–3.1), whereas those women who received Lactin-V and achieved high colonization were protected from rUTI (RR, .07; 95% CI, .02–.3; $P < .01$). Thus, since women in both arms of the study had reduction in lactobacillus colonization after UTI (not shown), receiving Lactin-V after treatment for acute UTI confers a significant advantage over repopulation of the vaginal microbiota with endogenous *L. crispatus*. We hypothesize that this advantage reflects unique properties for protection against UTI associated with repopulation by the probiotic lactobacillus isolate. This is supported by in vitro studies showing that the probiotic strain adheres to vaginal epithelial cells (VECs) in high numbers, especially cells from women with rUTI [28]. In addition, *L. crispatus* CTV-05 competitively inhibits uropathogenic *E. coli* adherence to VECs and inhibits growth of uropathogenic *E. coli* in vitro (Stapleton, unpublished data, 2010 unpublished data). Ongoing studies in our group are directed at understanding the mechanisms of protection in vivo and optimizing this prophylactic regimen.

In a recent review of randomized controlled trials (RCTs) of lactobacillus probiotics for BV or UTI, only 4 RCTs for prevention of UTI were identified, and only 1 of these 4 demonstrated a significant reduction in rates of recurrence [29]. Most studies did not assess vaginal colonization with lactobacilli after treatment, including the 1 positive study of probiotic prevention of UTI [29]. In our study, we found that a quantitative assessment of the vaginal microbiota was useful in determining the efficacy of this intervention to prevent rUTI.

We did not directly compare this intervention with antimicrobial prophylaxis for rUTI, but a comparison of our results with historic controls is instructive. Sen [30] reviewed multiple modalities for prevention of rUTI in 2008, noting that RCTs demonstrate efficacy of both continuous and postcoital antimicrobial prophylaxis regimens. There were more RCTs of continuous prophylaxis regimens, and the administration schemas of these regimens are more similar to our study design. Sen [30] reported that in a meta-analysis of 10 RCTs of continuous antimicrobial prophylaxis over 2–6 months of follow-up, rates of rUTI were reduced to 12% (24 of 195 participants) compared with 65% (116 of 177 participants) among placebo recipients, with a RR of rUTI that is very comparable to our findings (RR, .21; 95% CI, .13–.33).

In summary, Lactin-V treatment in women with rUTI resulted in robust and prolonged colonization with *L. crispatus*, with a trend of reducing the incidence of rUTI by ~50%. The protective effects of Lactin-V were even greater in those women who achieved the most robust colonization with *L. crispatus* and reflect an apparent treatment advantage for Lactin-V over natural recovery of the vaginal microbiota after an episode of rUTI. This antimicrobial-sparing, well-tolerated intervention compares favorably with historical data regarding antimicrobial prophylaxis, the current standard of care for the prevention of

rUTI. Our results strongly support the need for a larger, randomized, double-blind, placebo-controlled trial to confirm the efficacy of Lactin-V for prevention of rUTI in the general population of at-risk women. Studying this intervention in a population-based cohort may also supply useful information on target subsets of women who would most benefit from this type of intervention.

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Potential conflicts of interest. All authors: no conflicts.

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