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Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women

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Abstract

Urogenital infections afflict an estimated one billion people each year. The size of this problem and the increased prevalence of multi-drug resistant pathogens make it imperative that alternative remedies be found. A randomized, placebo-controlled trial of 64 healthy women given daily oral capsules of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 for 60 days showed no adverse effects. Microscopy analysis showed restoration from asymptomatic bacterial vaginosis microflora to a normal lactobacilli colonized microflora in 37% women during lactobacilli treatment compared to 13% on placebo ($P = 0.02$). Lactobacilli were detected in more women in the lactobacilli-treated group than in the placebo group at 28 day ($P = 0.08$) and 60 day ($P = 0.05$) test points. Culture findings confirmed a significant increase in vaginal lactobacilli at day 28 and 60, a significant depletion in yeast at day 28 and a significant reduction in coliforms at day 28, 60 and 90 for lactobacilli-treated subjects versus controls. The combination of probiotic *L. rhamnosus* GR-1 and *L. fermentum* RC-14 is not only safe for daily use in healthy women, but it can reduce colonization of the vagina by potential pathogenic bacteria and yeast.

Keywords: Placebo-controlled; Woman; Probiotic; *Lactobacillus*

1. Introduction

The microorganisms that colonize the vaginal vault play a major role in the maintenance of health of a woman. When this flora is dominated by lactobacilli or a commensal flora, the person is regarded as being healthy in terms of the urogenital tract, unless other specific disease traits are evident. When the vault is colonized primarily or solely by pathogenic bacteria, such as *Escherichia coli* or *Gardnerella vaginalis*, the patient is generally regarded as having an abnormal flora, and in the latter case she would be diagnosed as having asymptomatic bacterial vaginosis (BV). Women with an abnormal flora are likely at higher risk of developing symptomatic infections in the vagina and/or bladder, as well as having an increased risk of sexually transmitted diseases and preterm labor [1–3]. Antimicrobial therapy has been reasonably effective at curing bacterial infections of the bladder and vagina, but mounting drug resistance and failure of antibiotics to change host receptivity to pathogen recurrences, plus a negative impact on patient quality of life, make it imperative that alternative therapeutics be found [4–7].

Probiotics are regarded as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" [8]. A recent Food and Agriculture Organization of the United Nations and the World Health Organization Working Group has developed guidelines for

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what constitutes a true 'probiotic', and very few so-called health products currently meet these criteria because they have no published clinical studies showing a benefit of their strains on the host [9]. Probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 have been shown in open studies to colonize the vagina following oral intake [10,11]. The present randomized, placebo-controlled study was designed to determine if ingestion of these strains was safe in healthy women, and to test whether it induced any changes in pathogen load in the vagina.

2. Materials and methods

2.1. Bacterial strains

L. rhamnosus GR-1 and *L. fermentum* RC-14 were selected because they originated from the female urogenital tract, colonized the vagina and could inhibit the growth and adhesion of urogenital pathogens [12]. They were grown in bulk fermentation vats under Good Manufacturing Practice by Chr Hansen, Denmark, then freeze-dried and placed in gelatin capsules with a viability that remained at $>10^9$ per strain per vial for the duration of the trial.

2.2. Subjects

Sixty four women, mean age 35 years (range 19–46) were recruited from the London, Ontario community. They had no history of urogenital infection in the previous 12 months, no urogenital abnormalities, were not on any medications and all reported to a physician in full health. They were randomly allotted to receive either a freeze-dried capsule containing the *L. rhamnosus* GR-1 and *L. fermentum* RC-14 or calcium carbonate placebo by mouth once daily for 60 days. Each subject read, understood and signed a Consent Form approved by the Human Ethics Review Board of the University of Western Ontario. The subjects and investigators were blinded to the therapy. The subjects began the study immediately following menses. No vaginal swabs were collected during the menstrual period, and therefore all swabs represent early or late period timepoints. This was done to avoid the influence of mid-cycle estrogen peaks which can increase pathogen levels [13], and because local physicians who have followed women with recurrent urogenital infections perceived that such infections often tended to occur just prior to menses.

Two vaginal swabs were collected at days 0 (before treatment), 7, 28, 60 and 90: one was sent blinded (in Port-A-Cul transport tubes, Becton Dickinson Microbiology Systems) to an independent laboratory where it was cultured for total lactobacilli, yeast and coliforms using standard diagnostic media and biochemical tests; and the other was rolled onto a glass slide, Gram stained and given a Nugent score (indicative of normal, intermediate or BV)[14] by a technician blinded to all patient data. This scoring system examines vaginal cells at random and applies a 0–3 score for normal (dominated by Gram-positive bacilli resembling lactobacilli), 4–6 for intermediate (lactobacilli present along with Gram-negative or Gram-variable rods), and 7–10 for BV (no lactobacilli seen and cells colonized by Gram-negative rods).

The research nurse, supervised by a physician, followed every patient throughout the study. This entailed regular contact to obtain samples and monitor any perceived adverse events. Upon completion of the study, each subject filled out a questionnaire to determine whether any adverse events or perceived improvement in well-being occurred, including vaginal well-being (lack of irritation, pain, odors, discharge, changes on mucus or other noticeable changes; or improved sensation in the vagina) during the treatment period.

2.3. Statistical analysis

The Nugent data was analyzed by Fisher's exact test and the culture data was assessed using analysis of variance and a one-sided Wilcoxon test.

3. Results

The patients did not report any side effects associated with probiotic therapy. Two subjects taking the placebo developed a yeast infection during the second month of the study. Although all subjects reported vaginal well-being at time of recruitment, 16/64 (25%) had asymptomatic BV on day 0, as measured by Nugent scoring. Of those patients who did not have BV at baseline, 6 of 25 (24%) receiving placebo developed BV at day 35 and 4 (16%) at day 56 compared to 0 of 23 (0%) in the lactobacilli-treated group ($P<0.05$). Nugent scores showed lactobacilli present in 94% of women in the lactobacilli treated group at day 35 and 97% at day 56 which represented significantly more than in the controls ($P=0.08$ at day 35; $P=0.05$ at day 56). Significantly more subjects developed a microflora with lactobacilli present in the lactobacilli-treated group compared to placebo ($P<0.01$), and more placebo-treated subjects had a microflora that had fewer or no lactobacilli compared to the sample taken at entry ($P<0.01$)(Table 1).

The culture findings showed that lactobacilli oral therapy led to a significant (log 10) increase in vaginal lactobacilli within 4 weeks ($P=0.01$), plus a 0.8 log 10 decrease in yeasts ($P=0.01$) and coliforms ($P=0.001$) compared to the placebo (Table 2). This distribution of flora continued through day 60 with more lactobacilli and less coliforms and yeast in the treatment group compared to placebo ($P<0.1$). At day 90, 30 days after cessation of probiotics,

Table 1
Nugent Gram stain scores of vaginal cells from healthy women (32 per group) given lactobacilli or placebo for 60 days then followed until day 90

Nugent scoring	Placebo	Lactobacilli treated
Normal every sample to day 90	6 (19%)	9 (28%)
Normal worse during treatment	13 (41%)	4 (13%)*
BV better during treatment	10 (31%)	19 (59%)*
No change for 90 days	3 (9%)	0
Normal at 90 days follow-up	9/32 (28%)	13/31 (42%)

*Significant $P < 0.01$. Samples were collected on day 0 (before treatment) then day 7, 28, 60 and 90. Nugent scoring was normal (dominated by Gram-positive bacilli resembling lactobacilli), intermediate (lactobacilli present along with Gram-negative or Gram-variable rods), or BV (no lactobacilli present, and cells colonized by Gram-negative or Gram-variable rods).

there was still fewer coliforms in the lactobacilli-treated group compared to placebo ($P < 0.01$).

A patient questionnaire showed that 30% women taking lactobacilli reported a perceived improvement in vaginal health compared to 12% given placebo ($P = 0.17$). There was no statistical difference between the groups with respect to limiting intimate contact, vaginal itchiness or odor, or desire to have sex, although two subjects on placebo treatment felt worse than before in terms of vaginal discomfort and symptoms of low-grade vaginal infection.

4. Discussion

The study demonstrated that probiotic *L. rhamnosus* GR-1 and *L. fermentum* RC-14 can be taken orally on a daily basis for 2 months without any side effects. The therapy resulted in a significant improvement in the vaginal flora in terms of increased lactobacilli presence and decreased yeast and coliforms. The outcome was not designed to be mechanism-based, but the results indicate that intestinal passage of these probiotic strains led to a beneficial impact on the vaginal microflora. This may have occurred due to the strains themselves ascending to the vagina from the rectal area [10], or altering the ability of the pathogens to transfer to this niche. It is feasible that the therapy caused an alteration in the mucosal immunity of the host (via the gut and/or vagina) and that this played a part in reducing pathogen counts. Previous studies have shown that whilst oral therapy with these strains does not appear to mount a peripheral immune response [15], it can induce an intestinal antibody and phagocytic response that appears to benefit the host in terms of reduced impact of pathogens [16].

Studies have shown that the vaginal microflora is constantly in a state of flux and at many time points during the menstrual cycle it can be dominated by BV pathogens in the absence of lactobacilli without symptomatic infection developing [2,17]. A similar result was obtained here. While this so-called abnormal microflora obviously does not always convert to a symptomatic infection, it increases the risk of that happening by several fold [18,19]. Thus, the log higher levels of coliforms and yeast indicate that even women who appear healthy (as expressed personally and by reference from their physician), are indeed at some risk of urogenital infection each month. This reflects the large numbers of women who suffer from urogenital infections on an annual basis. Two such infections did arise in the placebo group, but none in the lactobacilli group up to 1 month following cessation of therapy, suggesting perhaps a carry-over effect of treatment that was statistically significant in terms of reduced coliforms.

The vaginal yeast counts were shown to change by log 10 within a given month, indicating that healthy women can be at risk of vaginitis even when they feel perfectly normal. The two lactobacilli strains used here can inhibit the growth of *Candida* in vitro [16], so the reduced total vaginal yeast counts could be due in part to this inhibition or to fewer yeast emerging from the rectum and ascending into the vagina. Alternatively, the lactobacilli displaced the yeast in the vagina and created an environment less suitable for continued yeast colonization. The mechanism of displacement could involve competition for binding sites to mannose used by both species [20,21] or inhibition of spread by deposition of biosurfactant proteins produced by *Lactobacillus* RC-14 [22]. In previous studies, the use of vaginally applied lactobacilli showed a correlation with reduced yeast vaginitis in women at high risk [23].

In post-menopausal women, vaginal estriol therapy depletes *E. coli* colonization and increases lactobacilli with

Table 2
Summary of culture results pooled from all 64 subjects

Organisms tested	Mean log difference from day 0 values				
	Subjects	Day 7	Day 28	Day 60	Day 90
Lactobacillus counts	Lactobacilli	0.2	0.9*	0.5*	0
Lactobacillus counts	Placebo	−0.3	−0.4	0.1	−0.4
Yeast counts	Lactobacilli	0	0.2*	1.1	0.5
Yeast counts	Placebo	0.4	1.0	1.7	0.9
Coliform counts	Lactobacilli	−0.2	0.1*	−0.1*	0.3*
Coliform counts	Placebo	0	0.9	0.5	1.1

The standard deviations are within 20%. Significance was $P < 0.05$ as denoted by *. Negative values indicate a reduced number of organisms compared to baseline.

the result that the incidence of recurrent UTI drops significantly [24]. This illustrates an association, if not a direct correlation, between lactobacilli and vaginal health. Strains *L. rhamnosus* GR-1 and *L. fermentum* RC-14 do not induce side effects, unlike estrogen replacement therapy, and would be worthy of study in a post-menopausal group. It should be noted that not all lactobacilli or probiotic strains are effective in the vagina. A commercial probiotic strain, *L. rhamnosus* GG, is apparently not well suited to colonizing the vagina nor is *L. acidophilus*, perhaps explaining why both these strains failed to prevent recurrence of UTI [25,26]. Further studies are required to understand this difference in functionality between strains.

In summary, *L. fermentum* RC-14 and *L. rhamnosus* GR-1 represent the first probiotic combination taken orally to reduce vaginal colonization by pathogenic bacteria and yeast. Daily intake of scientifically selected probiotics could provide a natural, safe and effective means to stabilize the fluctuating vaginal flora and thereby lower the risk of infections in healthy women as well as those prone to urogenital disease.

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