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Probiotic *Lactobacillus* dose required to restore and maintain a normal vaginal flora

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Abstract

Forty-two healthy women were randomized to receive one of three encapsulated *Lactobacillus rhamnosus* GR-1 plus *Lactobacillus fermentum* RC-14 probiotic dosage regimens or *L. rhamnosus* GG by mouth each day for 28 days. However, the vaginal flora, assessed by Nugent scoring, was only normal in 40% of the cases, and 14 patients had asymptomatic bacterial vaginosis. Treatment with *L. rhamnosus* GR-1/*L. fermentum* RC-14 once and twice daily correlated with a healthy vaginal flora in up to 90% of patients, and 7/11 patients with bacterial vaginosis converted to normal or intermediate scores within 1 month. Ingestion of *L. rhamnosus* GG failed to have an effect. This study confirms the potential efficacy of orally administered lactobacilli as a non-chemotherapeutic means to restore and maintain a normal urogenital flora, and shows that over 10^8 viable organisms per day is the required dose.

Keywords: Bacterial vaginosis; Lactobacillus; Vaginal flora; Probiotics

1. Introduction

Recent studies have emphasized the importance of a healthy, lactobacilli dominated flora not only to prevent sexually transmitted diseases [1] and preterm labour [2], but also to maintain the quality of life of women [3]. In an attempt to develop a non-chemotherapeutic means to restore and maintain a healthy urogenital tract, probiotic therapy using lactobacilli has been considered, and there is evidence to indicate that certain strains can be effective when inserted directly into the vagina or when ascending from the rectum after oral ingestion [4–6]. Two strains, *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14, appear to be particularly adept at the latter [6]. Repeated intake of probiotics could be important not only in women prone to urogenital infections, but also for healthy women as their vaginal flora is often depleted of lactobacilli thereby increasing the risk of infection [7,8].

The present study was designed to determine the dose required for probiotic lactobacilli to impact the vaginal flora, and to compare a commercial product with strains GR-1 and RC-14. A Gram stain system, proven to be effective in assessing the normality or 'health' of the vaginal flora without the need for culture [9], was used. *L. rhamnosus* GG was selected as a control arm of the study, because its ingestion fails to affect the vaginal flora [10].

2. Methods

2.1. Lactobacillus strains

Strains *L. rhamnosus* GR-1 and *L. fermentum* RC-14 were grown in MRS broth (Sigma, Detroit, MI, USA), tested for purity, and freeze dried in equal amounts into gelatin capsules in dosage forms of 8×10^8 and 6×10^9 viable organisms. Commercially available capsules containing 10^{10} viable *L. rhamnosus* GG were purchased. All capsules were placed in vials and stored in a household refrigerator until use. Viable counts were carried out at regular intervals to determine shelf life, and no significant loss of viability was found during the duration of the study.

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Table 1
Nugent scoring outcomes of patients in Group 1 given once daily 8×10^8 *L. rhamnosus* GR-1 and *L. fermentum* RC-14

Patient No.	History	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
104	YV	BV	BV	BV	BV	BV	BV	N/A
110	YV/UTI	Normal	Normal	Normal	Normal	Normal	Normal	Normal
111	YV	Int.	Int.	Int.	Int.	Normal	Normal	Int.
113	UTI	Int.	Normal	Int.	Int.	Int.	BV	Int.
116	UTI	Normal	Normal	Normal	Normal	Normal	Normal	Normal
117	UTI/YV/BV	Normal	Normal	Int.	Normal	Normal	Normal	Normal
118	YV	BV	Normal	Normal	Normal	Normal	Normal	Normal
120	YV	BV	Int.	Int.	Int.	Int.	Int.	BV
125	YV/UTI	Normal	Normal	Normal	Normal	Normal	Normal	Normal
133	None	BV	BV	BV	BV	BV	BV	BV

None, no past history of urogenital infection; BV, asymptomatic bacterial vaginosis and scores within the BV Nugent range of 7–10; YV, yeast vaginitis; UTI, urinary tract infection; Normal, scores within the normal Nugent range of 0–3; Int., scores within the intermediate Nugent range of 4–6; N/A, not available.

2.2. Subjects and randomization

The subjects, primarily Caucasian, were recruited through family practices in London, ON, Canada. Each subject voluntarily signed an informed consent using a format approved by the Ethics Review Board of the University of Western Ontario. The inclusion criteria were for healthy women, 17 years and over (actual range was 17–50 with mean of 31 ± 8), who were currently free from urogenital infections (urinary tract infection (UTI), bacterial vaginosis (BV), or yeast vaginitis) and were not on long term, low dose antibiotics for UTI. The exclusion criteria were patients with abnormal renal function (serum creatinine >110 μmol l^{-1}, upper limit 90 μmol l^{-1}) or pyelonephritis, women who were pregnant, women who were lactose intolerant or receiving prednisone, immunosuppressive drugs, antimicrobial therapy or were using nonoxynol-9 as a spermicide agent. No subject received antibiotics during the study.

The capsules were dispensed in a randomized manner into vials containing one capsule per day of 10^{10} of GG (Group 4), 8×10^8 (Group 1) and 6×10^9 (Group 3) of GR-1/RC-14, and two per day of the $8\times10^8=1.6\times10^9$ (Group 2) GR-1/RC-14 dose. Treatment was given for 28 days. The technician evaluating the Nugent scores was blinded as to the treatments given, as were the researchers and patients, except for those subjects given twice daily therapy.

2.3. Sample processing

Deep vaginal swabs were collected within 2 days prior to study, then on days 7, 14, 21, 28, 35, and 41. The swabs were placed onto glass slides and examined using the Nugent scale (0–3 normal; 4–6 intermediate; 7–10 BV) [9].

3. Results

Compliance was excellent and all 42 subjects completed the study, although four did not provide a final vaginal swab. None of the patients reported symptomatic yeast vaginitis, UTI or BV or adverse side effects during the 6 week test period. While all the subjects reported feeling normal with respect to the urogenital tract upon entry into the study, only 17/42 (40%) actually showed a normal

Table 2
Nugent scoring outcomes of patients in Group 2 given 8×10^8 *L. rhamnosus* GR-1 and *L. fermentum* RC-14 twice daily (that is 1.6×10^9 daily dose)

Patient No.	History	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
101	YV	Normal	Normal	Normal	Normal	Normal	Normal	Normal
102	YV	Int.	Int.	Normal	Normal	Normal	Int.	Normal
103	UTI	Int.	Normal	Normal	Int.	Normal	Normal	Normal
105	YV/UTI	BV	BV	Int.	Int.	Int.	Normal	Normal
106	YV/UTI	BV	BV	Int.	Normal	Normal	Normal	Normal
107	YV/BV	Normal	Normal	Int.	N/A	Int.	Int.	Int.
108	UTI	BV	BV	Int.	N/A	Int.	N/A	N/A
109	YV/UTI	Normal	Normal	Normal	Normal	Normal	Normal	Normal
121	YV	Normal	Normal	Normal	Normal	Normal	Normal	Normal
139	YV	Int.	Int.	Int.	Normal	Normal	Normal	Normal
143	BV/YV	Normal	Normal	Int.	Normal	Normal	Normal	N/A
145	YV	Normal	Normal	Normal	Normal	Normal	Normal	Normal

None, no past history of urogenital infection; BV, asymptomatic bacterial vaginosis and scores within the BV Nugent range of 7–10; YV, yeast vaginitis; UTI, urinary tract infection; Normal, scores within the normal Nugent range of 0–3; Int., scores within the intermediate Nugent range of 4–6; N/A, not available.

Table 3
Nugent scoring outcomes of patients in Group 3 given once daily 6×10^9 *L. rhamnosus* GR-1 and *L. fermentum* RC-14

Patient No.	History	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
114	UTI/YV	Normal	Normal	Normal	Normal	Normal	Normal	Normal
119	UTI/YV	Int.	Int.	Int.	Int.	Normal	Int.	Int.
123	YV	Normal	Normal	Normal	Normal	Normal	N/A	Normal
124	YV	BV	BV	Int.	Int.	BV	BV	BV
126	None	Int.	BV	Int.	Int.	Int.	Int.	Int.
127	YV	Int.	Int.	Int.	Int.	Int.	Int.	Int.
128	UTI/BV/YV	BV	BV	N/A	Int.	Normal	Int.	Int.
129	UTI/BV/YV	Normal	Normal	Normal	Normal	Normal	Normal	Int.
130	UTI	BV	BV	BV	Int.	Int.	Int.	Normal
131	UV	Int.	Int.	Normal	Int.	Int.	Int.	Normal
132	None	BV	BV	BV	BV	BV	BV	BV

None, no past history of urogenital infection; BV, asymptomatic bacterial vaginosis and scores within the BV Nugent range of 7–10; YV, yeast vaginitis; UTI, urinary tract infection; Normal, scores within the normal Nugent range of 0–3; Int., scores within the intermediate Nugent range of 4–6; N/A, not available.

Nugent score. Indeed, asymptomatic BV was diagnosed by Nugent scoring in 4/10 women in Group 1, 3/12 in Group 2, 4/11 in Group 3 and 3/9 in the GG control Group 4 (Tables 1–4). BV scores reverted to normal or intermediate at day 28 in 7/11 (64%) subjects given one of three forms of GR-1/RC-14 therapy. At 2 week follow-up, 2/9 (22.2%) patients in the GG control Group 4 had a BV score, compared to 4/29 (13.8%) in the GR-1/RC-14 treated group ($P=0.613$ Fisher's Exact test).

As shown in Table 4 and summarized in Table 5, the control GG group showed no improvement in the number of subjects with normal vaginal flora at the end of 28 days of treatment. On the contrary, treatment with the GR-1/RC-14 combination twice daily (still almost one log less than one dose of the GG control) resulted in 50% more normal scores than before treatment started. Within 2 weeks of completion of treatment, the vaginal flora remained normal (comparing day 0 and day 28) in 90% of women given the twice daily GR-1/RC-14 lactobacilli, and this was significantly better than the GG control ($P=0.017$).

Of the subjects who had a history of yeast vaginitis during the previous 5 years, 7/13 (54%) who did not have a normal flora at entry, developed a normal flora within 28 days of treatment with GR-1/RC-14, while one 1/4 (25%) of the GG controls converted to normal. Of the five subjects who had a history of BV and presented with a BV Nugent score, one (No. 128) converted to a normal flora with GR-1/RC-14 treatment, while none of the GG controls converted. Of subjects with a past history of UTI, treatment with one of the three GR-1/RC-14 doses was better at converting an abnormal flora on day 0 to a normal flora within 28 days ($P=0.043$).

The conversion to a normal flora within 28 days was significant for women in Group 2 ($P=0.017$), with a tendency towards significance for Group 3 ($P=0.147$), and lack of significance for Group 1 ($P=0.611$ on day 28; $P=0.994$ on day 42) compared to control Group 4. The dosage forms higher than 8×10^8 appeared to be necessary for clinical effect.

4. Discussion

The pool of subjects was quite typical of healthy women in a fairly well educated, middle class community. However, the nature of the study attracted more women with a history of previous urogenital infection than in a random

Table 4
Nugent scoring outcomes of patients in Group 4 given once daily 10^{10} *L. rhamnosus* GG control

Patient #	History	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
134	UTI/YV	Normal	Int.	Int.	Normal	Int.	Int.	Int.
135	YV	BV	BV	N/A	N/A	Int.	Int.	Int.
136	BV/YV	Normal	Normal	Normal	Normal	Normal	Normal	Normal
137	UTI/YV	Int.	Int.	Int.	Normal	Int.	Int.	BV
138	YV	BV	BV	BV	Normal	Normal	Normal	Normal
140	UTI/YV	Int.	Int.	Int.	Int.	Int.	Int.	Int.
141	None	BV	BV	BV	N/A	N/A	N/A	BV
142	YV/BV	Normal	Int.	Normal	Normal	Normal	Normal	Normal
144	YV	Normal	Normal	Normal	Int.	Int.	Int.	Int.

None, no past history of urogenital infection; BV, asymptomatic bacterial vaginosis and scores within the BV Nugent range of 7–10; YV, yeast vaginitis; UTI, urinary tract infection; Normal, scores within the normal Nugent range of 0–3; Int., scores within the intermediate Nugent range of 4–6; N/A, not available.

Table 5
Summary of women with a healthy Nugent score for their vaginal flora before treatment and at days 28 and 42 following treatment with *L. rhamnosus* GR-1 and *L. fermentum* RC-14 (Groups 1–3) or *L. rhamnosus* GG

	Percentage of women with healthy[a] vaginal flora			
	Group 1 (8×10^8)	Group 2 (1.6×10^9)	Group 3 (6×10^9)	Group 4 (10^{10} GG)
Before treatment	40	50	27	44
At end of treatment	60	82	45	38
Two weeks after treatment	56	90	30	33

[a]As defined by Nugent scoring.

population, and these women likely represent consumers wishing to utilize functional foods as a means of maintaining health [11]. The women were not at high risk of sexually transmitted diseases, unlike some populations studied elsewhere [8], such as those with BV and at greater risk of HIV-1 through sexual intercourse [12]. Their overall history of bladder and vaginal infection was not unexpected, given the high incidence of these diseases amongst women. The low incidence of a history of BV was also not surprising, given its poor diagnosis in community clinics. The prevalence of normal flora at 40% at the start of the study was lower than the 76.5% reported by Schwebke et al. [8], perhaps illustrating the innate abnormality of women with a history of urogenital infections and antibiotic treatment.

The finding that the flora was normal in the majority of subjects after treatment with lactobacilli strains GR-1 and RC-14, and not the control patients taking *L. rhamnosus* GG, suggested strongly that the two strain combination therapy had an impact on the flora. The ingestion of the GR-1/RC-14 probiotic therapy resulted in the vaginal flora being restored to normal within 28 days in 82% in one group of patients. This is much higher than normal Nugent values reported in a general healthy population of women [8]. Whether or not a longer duration of treatment would mean that the flora could be maintained as normal in all subjects remains to be determined. In subjects who started with a normal flora, 12/13 (92%) retained normality at day 28 and one scored intermediate following GR-1/RC-14 therapy. This is almost double the 48% of women found elsewhere to maintain a normal flora over a menstrual cycle as determined by Nugent scoring [7]. This suggests that daily probiotic use could be considered as a means to maintain a healthy urogenital flora.

The question of how long on therapy did it take to increase the number of normal Nugent scores was not an aim of the study, and while there is an increase in Groups 1 and 2 on day 7 and day 21, no statement of significance can be made.

The dosage forms did not reveal significant differences, and it appears that the range of doses selected for study was too narrow.

There was no significant difference between Group 1 and the control at day 28 or day 42, suggesting that a daily dose greater than 8×10^8 is required for an effect. The twice daily dose of GR-1/RC-14 contained considerably fewer organisms than those in the GG control product. The results for *L. rhamnosus* GG are consistent with its inability to colonize the vagina or protect the host from recurrence of UTI [10].

The prevalence of asymptomatic bacterial vaginosis was 26% (11/42) at study commencement as assessed by Nugent evaluation. This is similar to the 20% figure found in a larger study of 635 women [13]. The conversion of BV scores to normal with GR-1/RC-14 therapy (64%) is higher than the spontaneous conversion rate reported at 12% in a previous study [14], implying potential clinical significance with probiotic therapy.

For women prone to recurrence of UTI, GR-1/RC-14 and not GG therapy corresponded with maintenance of a lactobacilli dominated vaginal flora. This reaffirms an earlier finding that prophylactic antibiotics could one day be replaced by oral probiotics as a means to prevent UTI [5].

The study was not designed to determine if the ingested strains were the ones that colonized the vagina. Rather, even if the effect was a result of the therapy creating an environment better able to support indigenous lactobacilli growth [5], the clinical outcome is still important.

In summary, the findings indicate that a daily oral dose of 10^8 viable probiotic lactobacilli can restore and maintain the urogenital health of women.

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