



Vaginal Colonization by Orally Administered *Lactobacillus rhamnosus* GG

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Abstract

Background: The lack of lactobacilli in the vagina of postmenopausal women due to estrogen deficiency plays an important role in the development of bacteriuria. In the last few years the use of lactobacilli for the prevention of genitourinary infections has been explored using different probiotic strains.

Objectives: To evaluate the vaginal colonization by *Lactobacillus rhamnosus* GG in postmenopausal healthy women following oral administration of the bacteria in a yogurt base for 1 month, as a first step in evaluating the potential probiotic role of LGG in the prevention of recurrent urinary tract infections.

Methods: One or two doses per day of yogurt containing 10⁹ colony-forming units of LGG were administered orally to 42 postmenopausal healthy women for 1 month. Vaginal and rectal swabs were cultured at the beginning and end of the study.

Results: At the end of the study the vaginas of only four women (9.5%) were colonized with LGG, at a very low number of bacteria, despite the fact that the gastrointestinal tracts of 33 women (78.6%) were colonized. There were no significant differences between one or two doses daily.

Conclusions: LGG should not be considered as a probiotic agent in urinary infections since it does not attach well to the vaginal epithelium.

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Urinary tract infection remains one of the most common bacterial infections in women. In postmenopausal women, the incidence of bacteriuria is 10–20% [1,2]. Among other causes, the lack of lactobacilli in the vagina of postmenopausal women due to estrogen deficiency plays an important role in the development of bacteriuria. Exogenous estrogen replacement, administered orally or vaginally, stimulates the proliferation of lactobacilli in the vaginal epithelium, reduces pH, and prevents the colonization of Enterobacteriaceae, eventually decreasing the incidence of urinary tract infection [3].

Studies have shown potential therapeutic benefits from the use of probiotics for the treatment and prevention of infection, especially gastroenteritis [4–6]. In recent years the use of lactobacilli for the prevention of genitourinary infections has been explored using different probiotic bacteria [7–9]. However, despite an impressive list of healthy effects attributed to probiotic agents, scientific methods for the selection and evaluation of bacterial strains having a potential probiotic effect in the prevention of UTI are limited.

Lactobacillus rhamnosus GG is the probiotic agent used by one of the major dairy product brands marketed in Israel. As a first step in the evaluation of this strain for the prevention of recurrent urinary tract infection in the postmenopausal woman, the present study evaluated the vaginal colonization by LGG when it was orally administered in a yogurt base.

Materials and Methods

This pilot study was conducted from July to September 2002. Postmenopausal healthy women who were not on hormonal replacement therapy were invited to participate. After signing a written informed consent, the enrolled women were randomly divided into two groups. Women in Group A received 100 ml of yogurt containing 10⁹ colony-forming units of *Lactobacillus rhamnosus* GG (Valio, Finland; and Tnuva, Israel) once daily for 1 month. Women in Group B received the same product and for the same period, but on a twice daily basis (i.e., 200 ml per day).

Two swabs, one rectal and one vaginal, were taken at two different visits: immediately before the first yogurt intake on visit 1, and within the last 2 days of the 1 month period on visit 2. The swabs were immediately sent to the microbiology laboratory for culturing, where they were plated on Rogosa Agar (Hy Laboratories, Rehovot, Israel) and streaked using a bacteriologic loop in a three-quadrant pattern. Following 72 hours incubation at 37°C in a 5% CO₂ enriched atmosphere, cultures were first evaluated for lactobacilli growth according to the morphologic appearance of colonies and Gram-stain. Suspected isolates were eventually identified to the species level using API-50 CHL (BioMerieux, Marcy l'Etoile, France). The phenotypic profile of *Lactobacillus rhamnosus* GG in this commercial kit was supplied by Valio (Finland) and corroborated in our facility using lactobacilli isolated directly from

LGG = *Lactobacillus rhamnosus* GG

the yogurt ingested by the study participants. The quantification of lactobacilli growth was interpreted according to the following criteria:

- Grade 1: Poor growth, colonies only in the first streaked quadrant
- Grade 2: Moderate growth, colonies in the second streaked quadrant
- Grade 3: Exuberant growth, colonies in the third streaked quadrant.

Results

Altogether, 46 postmenopausal women were enrolled and 42 completed the study, 21 in each group. Four women (two in each group) were discharged because of violation of protocol. The mean age was similar in both groups: 63.1 ± 8.3 years (range 49–75) in group A, and 62.0 ± 9.2 (range 49–79) in group B. No LGG growth was observed in any rectal or vaginal swab taken at visit 1. In other words, all women were free of LGG at the beginning of the study.

Table 1 presents the growth of LGG in rectal and vaginal swabs taken at visit 2. As shown in the table, women in both groups were similarly colonized by LGG in the rectum (81.0% in group A and 76.2% in group B) at the end of the study. The differences observed between the groups in terms of colony counts were randomly distributed, with no preference for any group. Vaginal colonization at the end of the study was observed in three women of group A and only one woman of group B. Colonization occurred in very low numbers (Grade 1) in three of these four women, and only one was colonized in grade 3.

Discussion

It is noteworthy that different species of lactobacilli have different probiotic effects and that the results obtained with one strain cannot be assumed for other strains [10]. It is expected that lactobacilli that are able to colonize the vaginal and urethral mucosa, which inhibit pathogen binding and subsequent growth, and are at the same time resistant to spermicidal killing, will show potential efficacy as probiotic factors for the prevention of urinary tract infection [11].

In a previous study performed in our institution, Shalev et al. [12] demonstrated the lower frequency of candidal vaginitis and/or bacterial vaginosis events in women following ingestion of yoghurt containing *Lactobacillus acidophilus*, compared to women who received pasteurized yoghurt without viable lactobacilli.

Lactobacilli that showed efficacy in reducing the frequency of bacteriuria events proved to be hydrogen peroxidase producers and harbored 29 kDa biosurfactant protein. The latter binds lactobacilli to collagen on vaginal epithelial cells and inhibits significant pathogen numbers from binding to surfaces [13]. LGG, the strain tested in the present study, produces hydrogen peroxidase but lacks the 29 kDa biosurfactant protein.

Osset and colleagues [14] studied the mechanisms whereby 15 strains of *Lactobacillus* block the adherence of uropathogens to vaginal epithelial cells and inhibit their growth. Among others,

Table 1. Growth of *L. rhamnosus*-GG in swabs taken at visit 2

	LGG growth							
	Rectal swab, No. (%)				Vaginal swab, No. (%)			
	Grade 1	Grade 2	Grade 3	Total	Grade 1	Grade 2	Grade 3	Total
Group A	11 (52.4)	3 (14.3)	3 (14.3)	17 (81.0)	2 (9.5)	0	1 (4.8)	3 (14.3)
Group B	6 (28.6)	9 (42.9)	1 (4.8)	16 (76.2)	1 (4.8)	0	0	1 (4.8)

Lactobacillus crispatus showed the greatest capacity to block uropathogen adherence [14]. The authors concluded that considerable variation exists among *Lactobacillus* strains regarding their adherence to uroepithelium, blockage of uropathogen attachment, and inhibition of uropathogen growth.

In our study, LGG did not colonize the vagina as the result of oral consumption of up to 2×10^9 CFU/day. Although the gastrointestinal tract of 33 women (78.6%) was colonized at the end of the study, the vaginal mucosa was colonized only in 4 women (9.5%), 3 of them with a very low bacterial load. Since attachment to the vaginal epithelium is one of the first and most important requisites to be met by a potential probiotic agent, it can be concluded that the possible role of LGG in the reduction of recurrent urinary tract infection in postmenopausal women is minimal if not impossible. Other strains exerting a potentially probiotic effect on the genitourinary tract have recently been explored. Gardiner et al. [15] researched lactobacilli, focusing in *L. rhamnosus* Gr-1 and *L. fermentum*, with very promising results in terms of vaginal colonization.

In this era of increasing bacterial resistance to antimicrobial agents, the development of alternative approaches is of major importance. Since infection site-oriented probiotic therapy is one of the most encouraging therapeutic alternatives for the treatment of infections in the post-antibiotic era, it is essential that the search be continued for the most appropriate probiotic strains to be used in prevention and treatment of urinary tract infection.

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CFU = colony-forming units

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