

## Activity of a *Lactobacillus acidophilus*–Based Douche for the Treatment of Bacterial Vaginosis

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### ABSTRACT

**Objectives:** Bacterial vaginosis is characterized by alteration of the normal vaginal microflora, in which a mixed anaerobic bacterial flora becomes prevalent over the population of lacobacilli. Because administration of probiotics might be of some utility in restoring a normal flora, the present study aimed to evaluate the effect of a *Lactobacillus acidophilus*-strain-based douche on the vaginal environment in bacterial vaginosis.

**Patients and methods:** In an open-label pilot evaluation, 40 women with bacterial vaginosis as defined by Amsel's criteria were treated for 6 days with a douche containing *L. acidophilus*. Vaginal smears were collected from the patients and analyzed according to Nugent's criteria at the time of diagnosis, after 6 days of treatment, and again at 20 days after the last treatment. At the same times, determination of vaginal pH and a Whiff test were performed.

**Results:** The Nugent score decreased significantly from bacterial vaginosis or an intermediate flora toward a normal flora during treatment, and remained low during the follow-up period for almost all of the patients, indicating bacterial vaginosis in 52.5% and in 7.5% of the patients before treatment and at follow-up, respectively. After treatment, significant decreases in vaginal pH were observed, to less than pH 4.5 in 34/40 women, and the odor test became negative in all of the patients.

**Conclusions:** In this preliminary study, treatment of bacterial vaginosis with a vaginal douche containing a strain of *L. acidophilus* contributed to the restoration of a normal vaginal environment.

### INTRODUCTION

Bacterial vaginosis is considered one of the most common causes of vaginal inflammation among both pregnant and nonpregnant women, with a prevalence ranging between 4.9% and 36% in developed countries, but reaching a point prevalence of more than 50% in rural Uganda.<sup>1,2</sup> The vagina and its unique microflora form a well-balanced ecosystem, with the vaginal environment controlling the types of microbial organisms present and the microflora in turn controlling the vaginal environment.<sup>3</sup> This equilibrium is in a dynamic state that is determined by several factors

such as age, menarche, time in the menstrual cycle, pregnancy, infections, and sexual behavior.<sup>4–6</sup>

More important than symptoms *per se* are complications associated with bacterial vaginosis, which appears to be related to an increased risk of susceptibility to sexually transmitted diseases including human immunodeficiency virus (HIV) infection, and to an adverse outcome of pregnancy.<sup>7</sup>

The etiology of bacterial vaginosis continues to be debated, but it is generally acknowledged that vaginal lactobacilli play an essential role in maintaining an environment that limits the growth of pathogenic microorganisms in the vagina.<sup>8,9</sup> Bacterial vaginosis is characterized by alteration

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of the normal vaginal microflora, in which lactobacilli, the predominant species in the vagina in its healthy state, are supplanted by a mixed anaerobic bacterial flora.

Standard treatments of bacterial vaginosis with antibiotics are unpleasant, may induce bacterial resistance with repeated use, and are associated with a high relapse rate beyond 1 month after the completion of treatment.<sup>10</sup> If a decrease in the population of lactobacilli appears to be the first event leading to bacterial vaginosis, and relapses are often associated with failure to restore a healthy, lactobacillus-dominated vaginal flora, then the administration of lactobacilli might contribute to the treatment of bacterial vaginosis.<sup>11</sup> Because different species and strains of lactobacilli differ in their activity, it is necessary to evaluate their efficacy in restoring a normal flora. The present study was done to evaluate the effect of an *L. acidophilus*-strain-based douche on the vaginal environment in bacterial vaginosis.

## METHODS

### Patients and sample collection

One hundred and forty-six women in the pre- or postmenopausal state, reporting vaginal itching and attending gynecologic outpatient clinics in Milan, Italy, were asked to be included in this open-label pilot study. Eligible patients were nonpregnant women at least 18 years old with a clinical diagnosis of bacterial vaginosis, which was defined as meeting three of Amsel's criteria ( $\geq 20\%$  clue cells; an off-white [milky or gray], thin, homogenous vaginal discharge; a vaginal pH  $> 4.5$ ; and a fishy, amine-like odor upon the addition of 10% KOH to vaginal fluid [Whiff test]).<sup>12</sup> Patients were excluded if they were pregnant or nursing, had sexually transmitted or vulvovaginal infections other than bacterial vaginosis, had vulvovaginal or cervical abnormalities or disorders, were actively menstruating, or had received antifungal or antimicrobial treatment within 7 days of the study. Patients with a history of chlamydial or yeast infection previously diagnosed in other laboratories were also excluded. Before enrollment, each subject read, understood and signed a consent form.

TABLE 1. NUGENT SCORES BEFORE AND AFTER TREATMENT AND IN THE FOLLOW-UP

Visit	No. patients with Nugent score of		
	0-3 <sup>a</sup>	4-6	7-9
T <sub>0</sub>	3	16	21
T <sub>1</sub>	19	18	3
T <sub>2</sub>	31	6	3

T<sub>0</sub>, before treatment; T<sub>1</sub>, at the end of treatment; T<sub>2</sub>, during follow-up.

<sup>a</sup>Score: 0-3, normal; 4-6, intermediate; 7-10, bacterial vaginosis.

TABLE 2. NUGENT SCORE FOR LACTOBACILLI ONLY

Visit	No. patients with Nugent score of				
	0	1	2	3	4
T <sub>0</sub>	5	6	10	7	12
T <sub>1</sub>	15	9	11	1	4
T <sub>2</sub>	24	7	2	2	5

T<sub>0</sub>, before treatment; T<sub>1</sub>, at the end of treatment; T<sub>2</sub>, during follow-up

Point score: 4 = absence of lactobacilli, 3 =  $< 1$  lactobacillus/1000 $\times$ ; 2 = 1-5 lactobacilli/1000 $\times$ ; 1 = 6-30 lactobacilli/1000 $\times$ ; 0 =  $> 30$  lactobacilli/1000 $\times$ .

Vaginal swabs were collected at the time of diagnosis, after 6-7 days of treatment, and 20-23 days after the last treatment. Swabs were smeared on a glass slide and air-dried. The slides were Gram stained and examined at the end of the study by 2 of the study investigators using the Nugent scoring system. The 2 observers had previously examined 200 Gram-stained slides of vaginal smears in conjunction with one another, and showed negligible interobserver variation.

### Treatment

Each patient received a kit containing vaginal douches for 6 days (1 douche/day). Each douche contained an *L. acidophilus* strain, chosen because of its human origin and probiotic properties.\* The *L. acidophilus* was used in a lyophilized form with 10% hydroxypropyl guar gum plus 9.5% NaCl, with this preparation suspended immediately before use in 100 mL of tap water to obtain a final bacterial concentration of  $1 \times 10^9$  CFU/mL.

The treatment kit included a questionnaire to determine whether any adverse events or perceived improvement in well-being (lack of irritation, pain, discharges) occurred during the treatment and in the follow-up period.

### Statistical analysis

Differences in Nugent score, vaginal pH values and Whiff test during time of observation were analyzed by the Snedecor test. *p*-Values below 0.05 were considered statistically significant.

## RESULTS

One hundred and forty-six (146) outpatients with vaginal discharge as the major clinical complaint were screened for the study. Of these, 40 patients, with a mean age of 51 years (range: 45-58 years) fulfilled the inclusion criteria and com-

\*Each solution was prepared in our laboratory (data on file).

TABLE 3. VALUES OF pH BEFORE AND AFTER TREATMENT AND AT FOLLOW-UP

Visit	No. patients with vaginal pH of			
	<4	4-4.5	5	≥ 5.5
T <sub>0</sub>	0	11	17	12
T <sub>1</sub>	2	31	7	0
T <sub>2</sub>	6	28	6	0

T<sub>0</sub>, before treatment; T<sub>1</sub>, at the end of treatment; T<sub>2</sub>, during follow-up.

pleted the study. The main causes of exclusion were failure to meet the inclusion criteria (58 patients), noncompliance with the dosing regimen (25 patients), and one or both follow-up visits made outside the designated time periods (23 patients).

Nugent scores before and after treatment and at follow-up are reported in Table 1. At enrollment, Nugent's test was suggestive for bacterial vaginosis in 21/40 women, was intermediate for 16, and was normal for 3. At the end of the 6 days of treatment, 20 patients showed a normal vaginal microflora and 16 an intermediate microflora, and 4 patients showed a score suggestive of bacterial vaginosis. At the follow-up visit, the Nugent score was 3 or lower in 30 patients, indicating a normal flora, ranged from 4-6 in 7 patients and more than 7 in 3 patients. Differences between in Nugent score at different times of observation were statistically significant ( $p < 0.0001$ ).

Although no molecular analysis was performed, and it is therefore impossible to evaluate whether restoration of a normal vaginal microflora was due to the lactobacillus administered during treatment or to the growth of previously colonizing lactobacilli, Table 2 shows the Nugent score for lactobacilli only. At the end of the treatment period, the score for lactobacilli improved in 30 patients, was unchanged in 3, and decreased in the other 7. At 20 days after last treatment (follow-up visit), 29 patients still showed an improved score for lactobacilli, suggesting that recolonization was stable for the period under evaluation. Of the remaining patients, 6 showed a decrement in lactobacilli as compared with the first visit, while in 5, the score was the same as the first visit.

Vaginal pH decreased significantly at the end of treatment in 31 patients and remained below pH 4.5 in 34 patients at the follow-up visit (Table 3).

The Whiff test was initially positive for all patients and became negative in 30/40 and in 40/40 at the end of the treatment period and at the follow-up visit, respectively ( $p < 0.0001$  versus the pretreatment pH) (Table 4).

## DISCUSSION

Our results demonstrated that the use of a vaginal douche for 6 days can contribute to improvement of symptoms in bacterial vaginosis. Studies of reduction of the risk of infection by

lactobacilli are often discordant. Reid et al.<sup>13</sup> reported a cure rate of 37% in a placebo-controlled study after oral ingestion of *L. rhamnosus* and *L. fermentum* for 60 days. By contrast, studies involving vaginal suppositories containing *L. acidophilus* showed a beneficial effect in about 57% of cases only for a limited period, and tampons impregnated of *L. fermentum*, *L. rhamnosus*, and *L. gasseri* used for 5 days after 3 days of clindamycin suppository therapy failed to evidence any improvement in comparison with a placebo.<sup>13,14</sup> Different properties of the lactobacilli used in these studies may be responsible for the discrepancies observed, since not all lactobacillus species share the same probiotic effects, and even within the same species differences are found among different strains. In addition, it is also possible that the route and duration of administration may influence the activity of lactobacilli.<sup>10</sup>

In the present study, an increase in vaginal lactobacilli was observed after 6 days of treatment and at the follow-up visit 20 days later. However, this preliminary study did not include identification of microbial vaginal flora, making it impossible to determine whether the observed normalization of the vaginal environment was owing to the exogenous lactobacilli administered during treatment, which were able to colonize the vaginal mucosa for more than 15 days, or to the endogenous population of lactobacilli, the growth of which was favored by the treatment. Effects of treatment on the vaginal microflora were evaluated through the Nugent score, which conversely was not considered among inclusion criteria for the study. These criteria included the fulfillment of Amsel's criteria, which were also used to define clinical cure.

It is possible that the Nugent score in the present study was altered by the presence of exogenous lactobacilli as a result of the treatment with lactobacilli, and did not reflect true re-establishment of the original vaginal microflora. However, in most cases, normalization of the Nugent score toward a normal vaginal flora was confirmed by Amsel's criteria, particularly the Whiff test and pH, which returned to normal values after 6 days of treatment.

In conclusion, the results obtained in the present study, although preliminary, indicate that a selected strain of *L. acidophilus* contained in a vaginal douche contributes to recovery of a normal vaginal environment from bacterial vaginosis. Randomized, prospective, long-term trials with

TABLE 4. RESULTS OF WHIFF TEST BEFORE AND AFTER TREATMENT AND AT FOLLOW-UP

Visit	No. patients and Whiff test results	
	Positive	Negative
T <sub>0</sub>	40	0
T <sub>1</sub>	12	30
T <sub>2</sub>	0	40

T<sub>0</sub>, before treatment; T<sub>1</sub>, at the end of treatment; T<sub>2</sub>, during follow-up.

adequate sample sizes are needed to compare the efficacy of vaginal administration of a lactobacillus strain in treating bacterial vaginosis versus standard therapy.

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