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Effectiveness of *Lactobacillus*-containing vaginal tablets in the treatment of symptomatic bacterial vaginosis

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Abstract

The purpose of this study was to determine the effectiveness of *Lactobacillus*-containing vaginal tablets in the treatment of bacterial vaginosis (BV) and in the restoration of a healthy vaginal flora. Thirty-nine women with BV were enrolled in a double-blind, placebo-controlled clinical trial. Patients received either one *Lactobacillus*-containing tablet or placebo daily for 7 days. Clinical criteria, vaginal Gram stain scores and symptoms were compared with those at the initial visit and those at completion of therapy and 2 weeks later. After completion of therapy, all of the patients in the *Lactobacillus*-treated group (n = 18) were free of BV, showing a normal (83%) or intermediate (17%) vaginal flora, as compared with only two patients free of BV with intermediate flora (12%) from among the 16 placebo-treated women ($p < 0.001$). Two weeks after completion of therapy, treatment was successful (score <7) in 61% of *Lactobacillus*-treated patients as compared with 19% of those in the placebo group ($p < 0.05$). In the treatment group, the total number of symptomatic patients and the intensity of their symptoms, in particular vaginal malodour, were significantly reduced at both follow-up visits. The data indicate that intravaginal administration of exogenous selected strains of lactobacilli can restore a normal vaginal microbiota and be used in treating bacterial vaginosis.

Keywords: Bacterial vaginosis, lactobacilli, probiotics, vaginal microbiota, vaginal polyamines
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Introduction

Bacterial vaginosis (BV) is the most common vaginal disorder among women of reproductive age, with a prevalence of 10–29% in the entire female population [1,2]. BV is not caused by one specific pathogenic microorganism, but rather by an imbalance of vaginal microbial flora, as well as, possibly, other, still unknown, cofactors. In the presence of BV, the lactobacilli, which represent the predominant microorganisms in the healthy human vagina, are reduced, absent or lacking specific antimicrobial properties (i.e. production of H_2O_2). These are replaced by *Gardnerella vaginalis* and other anaerobic organisms, e.g. *Atopobium vaginae*, *Bacteroides*, *Mobiluncus*, *Prevotella*, *Peptostreptococcus* spp., *Ureaplasma urealyticum* and *Mycoplasma hominis*, most of which are normally found in small amounts in the vagina [3]. Lactobacilli, particularly those producing H_2O_2, play a pivotal role in controlling the microenvironment of the vagina and in inhibiting the overgrowth of potentially pathogenic organisms [4]. Possible mechanisms of this protection include inactivation of pathogens by different *Lactobacillus* products (lactic acid, H_2O_2 and bacteriocins), competition for epithelial cell attachment sites and stimulation of the local immune system [5–7]. Women with BV typically complain of vaginal discomfort and clinical symptoms such as homogeneous malodorous vaginal discharge, which is more noticeable after unprotected intercourse, although a substantial fraction of women are asymptomatic [8]. The 'fishy' odour, characteristic of vaginal discharges in BV-affected women, is linked to the high levels of polyamines produced by the abnormally growing anaerobic microorganisms [9,10]. Amines do indeed volatilize in an alkaline environment, giving rise to the malodour [11,12].

Alterations in the vaginal microbiology, such as those occurring in BV, have been associated with ascending infections and obstetrical complications [13], as well as with

urinary tract infections [14,15]. A significant association between the depletion of vaginal lactobacilli and an increased risk of prevalent and incident human papillomavirus infection has been recently reported [16]. Increasing data also indicate that BV facilitates the acquisition of sexually transmitted diseases such as human immunodeficiency virus (HIV), herpes simplex virus type 2, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection [17–20]. Moreover genital tract shedding of herpes simplex virus type 2 and cytomegalovirus is significantly higher in women affected by BV than in BV-free women, and female genital tract HIV load correlates inversely with *Lactobacillus* count [19,21,22].

Therapy of BV involves oral or local administration of metronidazole or intravaginal clindamycin, and varies in efficacy (48–85% [13] for absence of infection 4 or more weeks after treatment).

There are several unpleasant side effects and disadvantages associated with these therapies, including superinfections with pathogenic microorganisms, susceptibility of lactobacilli to clindamycin and high relapse rates [13,23,24]. Moreover, vaginal pathogens, particularly *G. vaginalis* and anaerobic bacteria, are showing increasing drug resistance [25,26].

The use of lactobacilli to re-establish a physiological microbial flora of the female urogenital tract dates back to the early 1900s (reviewed by Sieber and Dietz [27]). Renewed attention has recently been focused on approaches involving alternative 'natural' treatments that could be effective in the microbiological and clinical resolution of the condition without side effects. As recently reviewed by Falagas *et al.* [28], several attempts have been made in recent years to treat BV with *Lactobacillus*-containing products, with conflicting results [29–33].

Vaginal tablets containing a combination of three strains of lactobacilli (*Lactobacillus brevis* (CD2), *Lactobacillus salivarius* (FV2) and *Lactobacillus plantarum* (FV9), characterized and selected for the prophylaxis and treatment of vaginal infections, have been recently developed [34,35]. *L. salivarius* and *L. plantarum* strains produce anti-infective agents, including hydrogen peroxide, and are able to co-aggregate efficiently with vaginal pathogens [35]. Co-aggregation produces a microenvironment around the pathogen where the concentration of inhibitors is increased. *L. plantarum* and *L. brevis* strains are able to adhere at high levels to human epithelial cells, displacing vaginal pathogens [35]. All the strains were able to temporarily colonize the human vagina after 5 days of treatment [36]. The present randomized, placebo-controlled trial was designed to test the effectiveness of these vaginal tablets for the treatment of BV and their ability to restore physiological conditions in the vaginal environment by re-establishing a normal, healthy flora.

Materials and Methods

Patients

Women with a known history of recurrent bacterial vaginosis were screened for the study. Bacterial vaginosis was defined according to Amsel *et al.* [37] by the presence of at least three of the following criteria: (i) thin, homogeneous vaginal discharge; (ii) vaginal pH higher than 4.5; (iii) 'fishy' odour of vaginal fluid after addition of 10% KOH (whiff test); and (iv) presence of clue cells on microscopic evaluation of saline wet preparations. The patients were selected by gynaecologists of the local gynaecological services from among women attending the day clinic for recurrent bacterial vaginosis between December 2002 and May 2004. Eligible subjects were premenopausal women older than 18 years who fulfilled three or more of the Amsel criteria for clinical diagnosis of BV and who complained of vaginal symptoms and signs such as discharge and/or malodour. Exclusion criteria were pregnancy, diabetes, the use of antibiotics or vaginal antimicrobials in the previous 14 days, *Trichomonas vaginalis* infection, yeast infection or cultures positive for *N. gonorrhoeae*.

The study was conducted in accordance with the Declaration of Helsinki and current standards of good clinical practice, and was approved by a review committee responsible for ensuring the rights and safety of the research subjects. Written informed consent was obtained from the patients.

Study medication

The test preparation consisted of vaginal tablets (Florisia; VSL Pharmaceuticals, Inc., Towson, MD, USA,) containing at least 10^9 viable lactobacilli (*L. brevis* (CD2), *L. salivarius* subsp. *salicinius* (FV2), and *L. plantarum* (FV9)). The number of viable *Lactobacillus* cells was verified every 6 months after preparation of vaginal tablets as previously described [34]. The placebo preparation did not contain lactobacilli, but contained the same excipients as the test preparation, as described by Maggi *et al.* [34], for fast-release formulation of vaginal tablets. Treatment consisted of one *Lactobacillus* tablet daily at bedtime for 7 days. Therapy was initiated after the enrolment visit or immediately after the end of menstruation if menses were expected within a 7-day period. Patients were asked to avoid sexual intercourse and vaginal douching during treatment.

Study design

This was a randomized, double-blind, placebo-controlled study. Patients were assigned to therapy with active or placebo preparation according to a computer-generated randomization scheme.

None of the staff or patients had access to the randomization codes during the study. The medications were dispensed by the investigator at the initial visit; compliance was assessed by counting returned tablets and questioning the patients.

Evaluation and scheduling

Demographics and medical history concerning previous contraception, infectious disease history, sexual activity and history and last menstrual period were assessed at baseline. Pelvic examination, assessment of clinical signs and symptoms of vaginosis (vaginal discharge, unpleasant odour and subjective vulvar discomfort, graded according to a score: 0, absence; +1, low; +2, intermediate; +3, high) and microbiological/biochemical sampling were performed at baseline, and after 1 and 3 weeks from the beginning of therapy.

Patients were assessed for BV according to the Amsel criteria [37] and the Gram stain score of vaginal smears according to Nugent *et al.* [38] The categories used to quantify bacterial morphotypes in vaginal smears were 1+ (<1 cell per field), 2+ (1–4 cells per field), 3+ (5–30 cells per field) and 4+ (>30 cells per field). Two definitions of resolution were considered: a Gram stain score of <7 (i.e. not BV) and a Gram stain score of <4 (i.e. reversion to 'normal flora') with negativization of Amsel criteria.

At each follow-up visit, patients were requested to report any unexpected symptom during the study period. Adverse events were recorded in the case report form.

Specimen collection

Specimens were obtained from the lateral vaginal wall and the posterior vaginal fornix with a cotton-tipped swab, and then rolled over a glass slide for Gram stain analysis. The pH of the vaginal contents was measured with indicator strips (range 0–6, Merck). A second swab loaded with vaginal discharge was placed in a sterile tube containing 0.5 mL of physiological solution for amine test and identification of clue cells. Specimens for culture were placed in Amies transport medium (Tansystem Venturi, Copan) and delivered to the laboratory. Vaginal rinsing for polyamine analysis was performed with 2 mL of 0.9% NaCl by flushing and re-aspirating the fluid three times on vaginal walls.

Microbiological analysis

For the culture, vaginal swabs were inoculated on *G. vaginalis* selective medium agar plates (Oxoid, SpA, Milan, Italy) and HHD agar plates (Biolife, Italiana Srl, Milan, Italy) for lactobacilli isolation. The plates were incubated under micro-aerophilic conditions for 48 h at 37°C. The cultures on *G. vaginalis* selective medium were checked for colony morphology, and Gram stain and catalase test were performed for presumptive identification of *G. vaginalis*. The strains were identified at species level with the API 20 Strep system (bioMérieux Italy) and by testing susceptibility to metronidazole and sulphonamide. Colonies grown on HHD agar were analysed for morphology, for homofermentative and heterofermentative characteristics and for cell morphology after Gram stain. Final confirmation was based on the carbohydrate fermentation profile determined with API 50 CHL test strips (bioMérieux Italy).

Polyamine analysis

The vaginal fluid lavages were prepared according to Fu *et al.* [39]. Two millilitres of vaginal washings was lyophilized and then redissolved in 300 μL of 0.05 M phosphate buffer (pH 7.0). To remove proteins, 150 μL of trichloroacetic acid (30%) was added. The supernatant was neutralized with saturated sodium carbonate and derivatized with dansyl chloride. For derivatization, the reactant solution consisted of 200 μL of dansyl chloride dissolved in acetone (10 mg/mL), 400 μL of neutralized sample supernatant, 50 μL of 140 μM hexamethylendiamine and 50 μL of saturated sodium carbonate. The mixture was incubated in a water bath for 10 min at 70°C. One hundred microlitres of this solution was analysed by HPLC. HPLC analysis was performed with a Waters-Millipore apparatus (Milford, MA, USA). Samples were applied to a reverse-phase column (Symmetry C18, 5 μm, 4.6 $\times$ 250 mm). The chromatography was carried out in gradient conditions at 50°C in a thermostatic apparatus, using a one-step-linear gradient from 80% to 100% of methanol in 20 min, with a flow rate of 1 mL/min. Detection was carried out using a fluorescence detector (λ_{ex} = 370 nm, λ_{em} = 506 nm). Peak quantitation was performed by automatic peak area integration using dedicated software (Millennium 32, Waters). Results were expressed as nanomoles per millilitre of vaginal washing.

Statistical analysis

The statistical analysis was performed using SPSS 13.0 software. The Mann–Whitney *U*-test was used to compare differences between the two treatment groups. A p-value of less than 0.05 was considered to be statistically significant.

Results

Study population

Forty-nine patients were screened, 39 of whom were eligible and participated in this trial. Twenty patients were randomly assigned to receive the test preparation and 19 to receive

TABLE 1. Characteristics of evaluable patients at enrolment by treatment group

	Lactobacilli (*n* = 18)	Placebo (*n* = 16)	p-value
Age (years, mean ± SD)	33 ± 9.9	35 ± 9.2	0.54
Nugent Gram stain score			0.62
Score 4–6	2 (11%)	1 (6%)	
Score 7–10	16 (89%)	15 (94%)	
Mean Gram stain score ± SD	7.4 ± 1.3	7.7 ± 0.9	0.37
Amsel criteria			
pH >4.5	16 (89%)	15 (94%)	0.61
Positive amine odour	17 (94%)	14 (87%)	0.47
Clue cells present	18 (100%)	15 (94%)	0.28
Thin homogeneous discharge	18 (100%)	16 (100%)	NA
Mean no. of positive Amsel criteria	3.83	3.75	0.46
Positive culture for *Gardnerella vaginalis*	14 (78%)	14 (87%)	0.46
Lactobacilli/field			0.72
5+ (score 0–1)	2 (11%)	1 (6%)	
>0–4 (score 2–3)	6 (33%)	4 (25%)	
0 (score 4)	10 (56%)	11 (69%)	

SD, standard deviation; NA, not applicable.

TABLE 2. Response to therapy by treatment group

	Lactobacilli (*n* = 18)		Placebo (*n* = 16)	
	No.	%	No.	%
First follow-up				
Normalization of Amsel criteria	15	83	1	6
Nugent Gram stain score[a]				
Normal (0–3)	15	83	0	0
Intermediate (4–6)	3	17	2	12
Bacterial vaginosis (7–10)	0	0	14	88
Mean Gram stain score ± SD	2 ± 1.9		7.6 ± 1.1	
Lactobacilli/field[a]				
5+ (score 0–1)	12	67	0	0
>0–4 (score 2–3)	6	33	4	25
0 (score 4)	0	0	12	75
Positive culture for *Gardnerella vaginalis*	2	11	13	81
Second follow-up				
Normalization of Amsel criteria	12	67	2	12
Nugent Gram stain score[b]				
Normal (0–3)	9	50	1	6
Intermediate (4–6)	2	11	2	12
Bacterial vaginosis (7–10)	7	39	13	81
Mean Gram stain score ± SD	4.3 ± 3.3		7.4 ± 1.5	
Lactobacilli/field[c]				
5+ (score 0–1)	8	44	1	6
>0–4 (score 2–3)	6	33	2	12
0 (score 4)	4	22	13	81
Positive culture for *G. vaginalis*	7	39	13	81

[a] <0.001 for comparison between *Lactobacillus*-treated and placebo-treated groups.
[b]p 0.017 and [c]p 0.02 for comparison between *Lactobacillus*-treated and placebo-treated groups.

placebo. Ten patients were excluded; four of them refused to give consent and six did not meet the inclusion criteria. Five patients dropped out of the study (one patient required antibiotic therapy and four did not return for the follow-up visits), leaving 34 evaluable patients (active treatment, *n* = 18; placebo, *n* = 16). The baseline characteristics of women randomly assigned to the test preparation or placebo were demographically similar (Table 1). Gram stain scores of vaginal smears did not significantly differ by treatment group. All of the women with BV diagnosed according to the clinical criteria of Amsel had vaginal Gram stains that were either intermediate (9%) or consistent with bacterial vaginosis (91%). At the time of inclusion, the majority of patients (31/34) showed an absence of or very low amounts of lactobacilli in the vaginal fluid, as assessed by culture and Gram-stained smear evaluation. At that time, only three women with intermediate flora had more than five lactobacilli per high-power field in vaginal samples. Vaginal fluids from these patients had a pH value of 4.5. All but two patients (one in each group) had the maximum score for *Gardnerella* morphotypes, with seven women also showing presence of *Mobiluncus*.

Seven-day assessment

At the first follow-up visit (Table 2), all of the patients in the *Lactobacillus*-treated group were free of BV, showing a normal (83%) or intermediate (17%) vaginal flora. All of the women had lactobacilli in vaginal samples, as assessed by culture and Gram-stained smear evaluation. Of the 16 women presenting with an absence of or a very low amount of lactobacilli at inclusion, 12 (75%) showed a very good colonization after probiotic administration (11 patients, 4+; one patient, 3+), two showed intermediate colonization (2+), and two remained unchanged (fewer than one lactobacillus per high-power field). Interestingly, in the two women with an initially high *Lactobacillus* count (intermediate flora), the amount of lactobacilli decreased to lower values (2+). The score of *Gardnerella* morphotypes also decreased from the maximum (4+) to intermediate and low values in these patients. Thus, one of these patients returned to normal and one retained the same Gram stain score. In the actively treated group all of the women showed a strong reduction in the number of *Gardnerella* morphotypes that were absent, or present in very low amounts, in ten women (56%), whereas seven patients (39%) had intermediate colonization (2+) (data not shown). None of the patients in the placebo group recovered, although one woman with initial BV shifted to intermediate flora. The patient with intermediate flora at the initial visit retained the same Gram stain score. No variation in the number of lactobacilli was observed among controls in comparison to that at enrolment; lactobacilli were absent in 12 subjects (75%) or present at very low concentrations. *G. vaginalis* was isolated from the vaginal fluid of two and 13 women from the *Lactobacillus*-treated group and the placebo-treated group, respectively.

Twenty-one-day assessment

By the second follow-up visit, Nugent Gram stain of vaginal smears was significantly different between the *Lactobacillus*-treated group and the placebo-treated group (p 0.017).

Treatment was successful (score <7 and normalization of Amsel criteria) in 11 of the *Lactobacillus*-treated patients (61%) as compared with three (19%) of the placebo group. Nine patients (50%) among the actively treated women had normal vaginal smear results (score <4) in comparison to one (6%) in the control group. In the treatment group, both women with a high number of lactobacilli at entry (intermediate flora) showed a return to normal flora. The Gram stain *Lactobacillus* score was significantly higher in patients treated with lactobacilli in comparison with the placebo group (p 0.02). Microbiological analysis demonstrated that 14/18 (78%) women treated with *Lactobacillus*-containing vaginal tablets harboured lactobacilli in vaginal fluid. In eight of these patients, lactobacilli were present at very high levels, whereas six had lower amounts of lactobacilli. *Gardnerella* and *Bacteroides* spp. morphotypes were absent, or present in small amounts, in women recolonized with lactobacilli, but were found at high levels in non-recolonized patients.

The tablets caused no detectable side effects, and good compliance was observed in all the patients.

Effect of treatment on symptoms

The symptoms of patients before and after treatment are reported in Table 3. A significant reduction in the number of patients with symptoms and clinical signs at both follow-up visits, in comparison to the number with symptoms and clinical signs at the enrolment visit, was observed in the treatment group (p <0.05). Moreover, all of the still-symptomatic women, upon further examination, showed a reduction in the score of symptoms. Increased vaginal discharge and subjective vulvar discomfort disappeared at the first follow-up visit in 56% (10/18) and 71% (10/14), respectively, of the women who received *Lactobacillus*-containing tablets, in comparison with none and 25% (3/12), respectively, of the placebo recipients. Similar findings were observed at the second follow-up visit. At the control endpoint, malodorous vaginal discharge was significantly reduced in most of the actively treated patients. Sixteen of 17 patients reported improvement by one to three gradations. The median odour score of all patients was 2.5 at the inclusion visit and 0 at the two follow-up visits. Overall, 71% (12/17) of patients, including three women still affected by BV, showed complete resolution of vaginal malodour, in comparison with 12% (2/16) of the placebo group. The median odour score of symptomatic patients decreased from 3 to 1.5 and 3 to 1, respectively, at the two follow-up visits.

Vaginal secretions of a subset of patients (five in the active treatment group and three in the placebo group) were analysed for the presence of and the amount of polyamines, e.g. putrescine and cadaverine, before and after treatment. The mean concentrations of putrescine and cadaverine in the secretions from three actively treated women who recovered shifted from 149 ± 28 and 321 ± 70 nmol/mL to 6 ± 3.6 and 1.1 ± 1 nmol/mL, respectively, and 5.7 ± 1.9 and 2.3 ± 2 nmol/mL, respectively, at the two follow-up visits. A representative HPLC profile of polyamines in vaginal samples from a patient treated with the probiotic preparation is shown in Fig. 1. Putrescine and cadaverine, which were found in high concentrations at inclusion (Nugent score 7), were drastically reduced at the two follow-up visits (Nugent score 0 and 3, respectively). The amine concentration in five women with persistent BV (two in the treatment group and three in the placebo group) did not show significant changes.

TABLE 3. Patients with clinical symptoms and signs before and after treatment

	Treatment					
	Lactobacillus tablets (*n* = 18)			Placebo (*n* = 16)		
Symptom	Day 0	Day 7[a]	Day 21[b]	Day 0	Day 7	Day 21
Increased discharge	18 (2)	8 (1.5)	10 (1)	16 (2.5)	16 (2.5)	15 (2.5)
Vulvar discomfort	14 (2)	4 (1.5)	2 (1.5)	12 (2)	9 (2)	8 (2)
Malodour	17 (3)	6 (1.5)	5 (1)	16 (2.5)	14 (2)	14 (2.5)

[a]p <0.022 and [b]p <0.011 for comparison of each symptom at each time-point between *Lactobacillus*-treated and placebo-treated groups.
Median score of clinical symptoms and signs of symptomatic patients is reported in parenthesis. Range is from 0 (none) to 3 (high).

Discussion

The current study demonstrates the effectiveness of vaginal tablets containing selected strains of lactobacilli in the resolution of BV. At the first follow-up, all patients were free of BV, presenting with a normal (83%) or intermediate (17%) vaginal flora. Treatment with the probiotic preparation was 61% effective in eliminating BV and 50% effective in restoring 'normal vaginal flora' as determined by Gram stain at the final follow-up. The statistical analysis demonstrates a significant difference between the actively treated and the placebo groups; however, as five participants dropped out of the study, the effect of attrition bias should not be excluded. Different variables, e.g. contraceptive methods or initiation of therapy in a different phase of the menstrual cycle, could have influenced the effectiveness of the probiotic treatment, but the limited number of enrolled patients did not permit subgroup analysis.

It must be emphasized that the therapy, although not typically pharmacological but entirely probiotic, achieved a cure

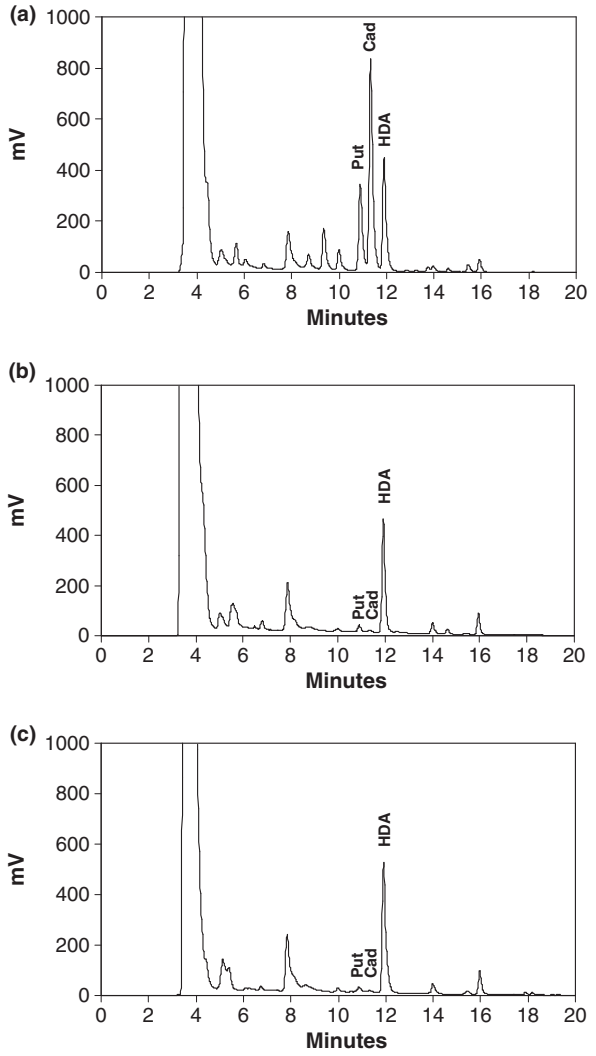

FIG. 1. HPLC analysis of polyamines in vaginal samples of a patient treated with the probiotic preparation. (a) Baseline (Nugent score 7). (b) First follow-up (Nugent score 0). (c) Second follow-up (Nugent score 3). HDA, hexamethylendiamine; Put, putrescine; Cad, cadaverine.

rate in the lower range of typical pharmacological therapies, which have cure rates from 60% to 85% 10–21 days after the completion of treatment [13]. BV resolution has been observed 2 weeks after treatment in 75% of non-pregnant women treated with clindamycin vaginal cream [40]. In asymptomatic pregnant women, Klebanoff *et al.* [41] found two 2-g doses of metronidazole to be 60% effective in reducing Nugent scores to <4 at 2–3.9 weeks after the start of therapy.

It is important to note that the probiotic treatment was very well tolerated and no side effects have been reported. The absence of side effects is a great advantage of probiotic treatment in comparison to antibiotic treatment. In fact, a longer treatment regimen or repeated treatments could be suggested for those women with continuing or recurring clinical symptoms.

We observed spontaneous resolution of BV (score <7) in 19% of placebo-treated women and spontaneous reversion to 'normal vaginal flora' in 6% (score <4). Schwebke [42] reported that, after administration of placebo vaginal gel to 30 asymptomatic non-pregnant women, 22% of the patients had Gram stain scores <7 and 4% Gram stain scores <4 2 weeks after enrolment. Klebanoff *et al.* [41] observed Gram stain scores <7 in 13% and <4 in 5% of placebo-treated women at 2–3.9 weeks after the start of therapy; both of these results are similar to ours.

Different species of lactobacilli have been evaluated for the treatment of BV. *Lactobacillus acidophilus* was used intravaginally in a double-blind, placebo-controlled clinical trial. Hallén *et al.* [30] demonstrated that immediately after completion of a 6-day treatment, 57% of 28 patients were free of BV, as compared to none of the 29 women in a placebo group. Resolution of BV was maintained in 21% of *Lactobacillus*-treated patients 20–40 days after the start of treatment. The only property of the strain used in this study that may be relevant for the treatment of BV was the production of H_2O_2. Using well-characterized and well-selected strains, Reid *et al.* [32] recently demonstrated that oral intake of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 resulted in a significant increase in vaginal lactobacilli and a significant depletion in yeasts and coliforms for *Lactobacillus*-treated healthy women, as compared with controls, during a 60-day treatment period. Two weeks after the end of a 1-month course of oral treatment with different amounts of the same strains, restoration from bacterial vaginosis microflora to a normal *Lactobacillus*-colonized microflora was observed in four of nine (44%) asymptomatic women [31]. Intravaginal administration of these strains showed cure of BV in significantly more probiotic-treated subjects than metronidazole-treated subjects [33]. Ten and 25 days after the end of a 5-day treatment with lactobacilli or metronidazole vaginal gel, resolution of BV (Nugent score <7) was achieved in 17/20 (85%) and 15/17 (88%), respectively, probiotic-treated women, in comparison to 9/20 (45%) 9/18 (50%), respectively, of metronidazole-treated women.

The strains of lactobacilli present in the vaginal tablets used in this study were carefully selected for properties relating to mucosal colonization, i.e. their ability to adhere at high levels to human epithelial cells [35] and to colonize the human vagina [36]. The results of this study demonstrate that 78% of women with an absence of, or very low amounts of, lactobacilli at inclusion in the study had lactobacilli in vaginal fluid 3 weeks after the start of probiotic treatment.

The observation that two women with intermediate vaginal flora and a high level of lactobacilli at inclusion in the study recovered after treatment with lactobacilli is intriguing. Although this aspect was not analysed in detail, it may be suggested that endogenous lactobacilli are not able to counteract BV-related microorganisms, whereas the strains present in the vaginal tablets are.

An additional advantage observed with the *Lactobacillus* preparation used in this study is the reduction of malodorous vaginal discharge, which is the primary subjective symptom and complaint of women affected with BV. Vaginal malodour disappeared in most of the actively treated patients, including non-BV-free women and, if persistent, it was of lower intensity. This is confirmed by the significant reduction in vaginal concentrations of polyamines observed in the vaginal fluid of women cured of BV after administration of *Lactobacillus*-containing vaginal tablets. Indeed, amines produced by the overgrowing anaerobic microorganisms are responsible for the vaginal malodour observed in BV-affected women [11,12]. The effect on vaginal malodour could be ascribed to the CD2 strain of *L. brevis* present in the vaginal tablets. This strain produces high levels of the enzyme arginine deiminase, which is able to downregulate polyamine synthesis [43]. As the primary objective of BV treatment in non-pregnant women is to alleviate symptoms, particularly the 'fishy' odour that is characteristic of vaginal discharges, the probiotic preparation used in this trial could represent an important step towards improving the efficacy of bacteriotherapy for BV.

In conclusion, it is feasible to repopulate the vagina of women having recurrent BV with the use of exogenous lactobacilli. Carefully selected strains of lactobacilli can restore a normal vaginal flora and eliminate bacterial vaginosis. The possibility of restoring a healthy vaginal microbiota is of great importance, not only for the therapy of BV, but also as a potential intervention to reduce the risk of acquiring HIV-1 infection and other sexually transmitted diseases [17].

Finally, vaginal probiotic administration could be suggested in cases of recurrent bacterial vaginosis, to avoid repeated use of antibiotics and as prophylaxis for dismicrobism with depletion of lactobacilli in the female genital tract following systemic antibiotic administration.

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Transparency Declaration

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References

1. Cauci S, Driussi S, De Santo D *et al.* Prevalence of bacterial vaginosis and vaginal flora changes in peri- and postmenopausal women. *J Clin Invest* 2002; 40: 2147–2152.
2. Allsworth JE, Peipert JF. Prevalence of bacterial vaginosis: 2001–2004 National Health and Nutrition Examination Survey data. *Obstet Gynecol* 2007; 109: 114–120.
3. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. *N Engl J Med* 2005; 353: 1899–1911.
4. Hawes SE, Hillier SL, Benedetti J *et al.* Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. *J Infect Dis* 1996; 174: 1058–1063.
5. Redondo-Lopez V, Cook RL, Sobel JD. Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. *Rev Infect Dis* 1990; 12: 856–872.
6. Aroutcheva A, Gariti D, Simon M *et al.* Defense factors of vaginal lactobacilli. *Am J Obstet Gynecol* 2001; 185: 375–379.
7. Valore EV, Park CH, Igreti SL, Ganz T. Antimicrobial components of vaginal fluid. *Am J Obstet Gynecol* 2002; 187: 561–568.
8. Klebanoff MA, Schwebke JR, Zhang J, Nansel TR, Yu KF, Andrews WW. Vulvovaginal symptoms in women with bacterial vaginosis. *Obstet Gynecol* 2004; 104: 267–272.
9. Cook RL, Redondo-Lopez V, Schmitt C, Meriwether C, Sobel JD. Clinical, microbiological, and biochemical factors in recurrent bacterial vaginosis. *J Clin Microbiol* 1992; 30: 870–877.
10. Wolrath H, Forsum U, Larsson PG, Borén H. Analysis of bacterial vaginosis-related amines in vaginal fluid by gas chromatography and mass spectrometry. *J Clin Microbiol* 2001; 39: 4026–4031.
11. Sobel JD. Vaginitis. *N Engl J Med* 1997; 337: 1896–1903.
12. Brand JM, Galask RP. Trimethylamine: the substance mainly responsible for the fishy odor often associated with bacterial vaginosis. *Obstet Gynecol* 1986; 63: 682–685.
13. Koumans EH, Markowitz LE, Hogan V. Indications for therapy and treatment recommendations for bacterial vaginosis in nonpregnant and pregnant women: a synthesis of data. *Clin Infect Dis* 2002; 35 (suppl 2): S152–S172.
14. Harmanli OH, Cheng GY, Nyirjesy P, Chatwani A, Gaughan JP. Urinary tract infections in women with bacterial vaginosis. *Obstet Gynecol* 2000; 95: 710–712.
15. Hillebrand L, Harmanli OH, Whiteman V, Khandelwal M. Urinary tract infections in pregnant women with bacterial vaginosis. *Am J Obstet Gynecol* 2002; 186: 916–917.
16. Watts DH, Fazarri M, Minkoff H *et al.* Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1-infected and high-risk HIV-1-uninfected women. *J Infect Dis* 2005; 191: 1129–1139.
17. Martin HL, Richardson BA, Nyange PM *et al.* Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J Infect Dis* 1999; 180: 1863–1868.
18. Cherpes TL, Meyn LA, Krohn MA, Lurie JG, Hillier SL. Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. *Clin Infect Dis* 2003; 37: 319–325.

19. Ross SA, Novak Z, Ashrith G *et al.* Association between genital tract cytomegalovirus infection and bacterial vaginosis. *J Infect Dis* 2005; 192: 1727–1730.
20. Wiesenfeld H, Hillier S, Krohn MA, Landers D, Sweet R. Bacterial vaginosis is a strong predictor of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection. *Clin Infect Dis* 2003; 36: 663–668.
21. Cherpes TL, Melan MA, Kant JA, Cosentino LA, Meyn LA, Hillier SL. Genital tract shedding of Herpes simplex virus type 2 in women: effects of hormonal contraception, bacterial vaginosis and vaginal group B *Streptococcus* colonization. *Clin Infect Dis* 2005; 40: 1422–1428.
22. Sha BE, Zariffard MR, Wang QJ *et al.* Female genital-tract HIV load correlates inversely with *Lactobacillus* species but positively with bacterial vaginosis and *Mycoplasma hominis*. *J Infect Dis* 2005; 191: 25–32.
23. Sobel JD, Ferris D, Schwebke J *et al.* Suppressive antibacterial therapy with 0.75% metronidazole vaginal gel to prevent recurrent bacterial vaginosis. *Am J Obstet Gynecol* 2006; 194: 1283–1289.
24. Bayer AS, Chow AW, Concepcion N, Guze LB. Susceptibility of 40 lactobacilli to six antimicrobial agents with broad gram-positive anaerobic spectra. *Antimicrob Agents Chemother* 1978; 14: 720–722.
25. McLean NW, McGroarty JA. Growth inhibition of metronidazole-susceptible and metronidazole-resistant strains of *Gardnerella vaginalis* by lactobacilli in vitro. *Appl Environ Microbiol* 1996; 62: 1089–1092.
26. Beigi RH, Austin MN, Meyn LA, Krohn MA, Hillier SL. Antimicrobial resistance associated with the treatment of bacterial vaginosis. *Am J Obstet Gynecol* 2004; 191: 1124–1129.
27. Sieber R, Dietz UT. *Lactobacillus acidophilus* and yogurt in the prevention and therapy of bacterial vaginosis. *Int Dairy J* 1998; 8: 599–607.
28. Falagas ME, Betsi GI, Athanasiou S. Probiotics for the treatment of women with bacterial vaginosis. *Clin Microbiol Infect* 2007; 13: 657–664.
29. Fredricsson B, Englund K, Weintraub L, Olund A, Nord CE. Bacterial vaginosis is not a simple ecological disorder. *Gynecol Obstet Invest* 1989; 28: 156–160.
30. Hallén A, Jarstrand C, Påhlson C. Treatment of bacterial vaginosis with lactobacilli. *Sex Transm Dis* 1992; 19: 146–148.
31. Reid G, Beuerman D, Heinemann C, Bruce EW. Probiotic *Lactobacillus* dose required to restore and maintain a normal vaginal flora. *FEMS Immunol Med Microbiol* 2001; 32: 37–41.
32. Reid G, Charbonneau D, Erb J *et al.* Oral use of *L. rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. *FEMS Immunol Med Microbiol* 2003; 35: 131–134.
33. Anukam KC, Osazuwa E, Osemene GI, Ehigiagbe F, Bruce AW, Reid G. Clinical study comparing probiotic Lactobacillus GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. *Microbes Infect* 2006; 8: 2772–2776.
34. Maggi L, Mastromarino P, Macchia S *et al.* Technological and biological evaluation of tablets containing different strains of lactobacilli for vaginal administration. *Eur J Pharm Biopharm* 2000; 50: 389–395.
35. Mastromarino P, Brigidi P, Macchia S *et al.* Characterization and selection of vaginal *Lactobacillus* strains for the preparation of vaginal tablets. *J Appl Microbiol* 2002; 93: 884–893.
36. Massi M, Vitali B, Federici F, Matteuzzi D, Brigidi P. Identification method based on PCR combined with automated ribotyping for tracking probiotic *Lactobacillus* strains colonizing the human gut and vagina. *J Appl Microbiol* 2004; 96: 777–786.
37. Amsel R, Totten PA, Spiegel CA, Chen KCS, Eschenbach D, Holmes KK. Non specific vaginitis: diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983; 74: 14–22.
38. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. *J Clin Microbiol* 1991; 29: 297–301.
39. Fu S, Zou X, Wang X, Liu X. Determination of polyamines in human prostate by high performance liquid chromatography with fluorescence detection. *J Chromatogr B* 1998; 709: 297–300.
40. Sobel J, Peipert JF, McGregor JA *et al.* Efficacy of clindamycin vaginal ovule (3-day treatment) vs. clindamycin vaginal cream (7-day treatment) in bacterial vaginosis. *Infect Dis Obstet Gynecol* 2001; 9: 9–15.
41. Klebanoff MA, Hauth JC, MacPherson CA *et al.* Time course of the regression of asymptomatic bacterial vaginosis in pregnancy with and without treatment. *Am J Obstet Gynecol* 2004; 190: 363–370.
42. Schwebke JR. Asymptomatic bacterial vaginosis: response to therapy. *Am J Obstet Gynecol* 2000; 183: 1434–1439.
43. Di Marzio L, Russo FP, D'Alò S *et al.* Apoptotic effects of selected strains of lactic acid bacteria on human T leukemia cell line are associated with bacterial arginine deiminase and/or sphingomyelinase activities. *Nutr Cancer* 2001; 40: 185–196.