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Probiotics for the treatment of women with bacterial vaginosis

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ABSTRACT

This review considers whether probiotics are effective agents for the treatment and/or prevention of bacterial vaginosis (BV). There seems to be an association between the absence of, or low concentrations of, vaginal lactobacilli and the development of BV. Many studies have suggested that the presence of H_2O_2-producing vaginal lactobacilli may protect against BV, although some studies do not support this hypothesis. In-vitro studies have suggested that certain specific strains of lactobacilli are able to inhibit the adherence of *Gardnerella vaginalis* to the vaginal epithelium and/or produce H_2O_2, lactic acid and/or bacteriocins, which inhibit the growth of bacteria causing BV. Clinical trials showed that intra-vaginal administration of *Lactobacillus acidophilus* for 6–12 days, or oral administration of *L. acidophilus* or *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 for 2 months, resulted in the cure of BV (defined as a 0–1 positive score according to Amsel's criteria), and/or reduced the recurrences of BV, and/or caused an increase in vaginal lactobacilli and restoration of a normal vaginal microbiota, significantly more frequently than did a placebo, acetic acid or no treatment. However, several trials have found no significant difference in the cure rate of BV and in the number of vaginal lactobacilli after intra-vaginal instillation of lactobacilli when compared with the effect of a placebo or oestrogen. Thus, although the available results concerning the effectiveness of the administration of lactobacilli for the treatment of BV are mostly positive, it cannot yet be concluded definitively that probiotics are useful for this purpose.

Keywords Bacterial vaginosis, *Gardnerella vaginalis*, lactobacilli, probiotics, review, treatment, vaginosis

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INTRODUCTION

Bacterial vaginosis (BV) is a common vaginal infection causing significant gynaecological and obstetric morbidity. Apart from causing irritating symptoms, mainly a malodorous vaginal discharge, BV has been associated with pelvic inflammatory disease [1], infections following gynaecological surgery [2], and pre-term birth [3]. It has also been suggested that the presence of BV increases the risk for human immunodeficiency virus infection [4,5]. Treatment with metronidazole or clindamycin, administered orally or intra-vaginally, has been followed by frequent recurrences of BV [6]. Because of its high morbidity and frequent recurrence following treatment, alternative therapeutic agents need to be sought for the treatment of BV.

Probiotics are defined as 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host' [7]. Lactobacilli are the commonest organisms used as probiotics. Since the vaginal microbiota of women with BV has been found to contain a reduced number of lactobacilli in comparison with healthy women, lactobacilli administered orally or intra-vaginally have been tested for their effectiveness in colonising the vagina and curing women with BV, or at least preventing its recurrence.

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CHANGES IN VAGINAL MICROBIOTA DURING BV

The normal vaginal microbiota is normally dominated by lactobacilli, especially *Lactobacillus crispatus* [8–11], *Lactobacillus jensenii* [8,11], *Lactobacillus iners* [9,11,12] and *Lactobacillus gasseri* [8,10,11]. Compared with that of normal women, the vaginal microbiota of women with BV consists more commonly, and in higher numbers, of *Gardnerella vaginalis*, *Mycoplasma hominis*, *Prevotella*, *Peptostreptococcus*, *Mobiluncus* and *Bacteroides* spp., while lactobacilli are found less frequently and in lower numbers. Nugent *et al*. [13] suggested that BV should be diagnosed when vaginal smears examined following Gram's stain are given a score of ≥7, after adding the points (1–4+) allocated to each of the following morphotypes: lactobacilli (large Gram-positive rods), *G. vaginalis* (small Gram-variable rods), *Bacteroides* spp. (small Gram-negative rods) and *Mobiluncus* spp. (curved Gram-variable rods). The higher the number of the specific morphotypes per oil immersion field, the higher the number of points for each morphotype, except for lactobacilli, the absence of which obtained the highest score.

There is strong evidence that the absence of vaginal lactobacilli is associated with the development of BV. Alvarez-Olmos *et al*. [14] found a negative association between BV and the presence of lactobacilli in the vaginas of females aged 14–18 years (p 0.034). Similarly, vaginal lactobacilli were isolated from 73.7% of 825 women without BV, and from 29.8% of 131 women with BV (p <0.001) [15], from 74.3% of 2729 women without BV, and from 38.4% of 445 women with BV (OR 0.7, 95% CI 0.6–0.8), in a study of pregnant women aged >18 years [16], and from 96% of 28 women without BV, and from 52% of 67 women with BV (p <0.001) [17].

In addition, apart from the association between an absence of vaginal lactobacilli and BV, a possible association between low concentrations of vaginal lactobacilli and development of BV has been revealed by some studies. Thus, Zariffard *et al*. [18] analysed the cervicovaginal lavage samples of 21 human immunodeficiency virus-positive women by real-time PCR, and found that the median number of lactobacilli was significantly lower and the median number of *G. vaginalis* organisms was significantly higher in five women with BV, compared with 16 women without BV (8.5×10^6 vs. 1.1×10^9, p 0.013, and 1.3×10^{10} vs. 5.4×10^7, p 0.004, respectively). Mikamo *et al*. [19] also found that the mean count of *Lactobacillus* spp. was significantly lower in 129 *G. vaginalis*-positive women than in 110 *G. vaginalis*-negative women (7.02 vs. 8.66 $\log_{10}$ CFU/g, p <0.0001).

It has been suggested that H_2O_2-producing vaginal lactobacilli may prevent infection of the vaginal epithelium by bacteria that cause BV. Eschenbach *et al*. [17] isolated facultative H_2O_2-producing vaginal lactobacilli from 27 (96%) of 28 women without BV, and from four (6%) of 67 women with BV (p <0.001). In a study of pregnant women by Hillier *et al*. [20], H_2O_2-producing lactobacilli were isolated from 5% of women with BV (based on the Nugent score) and from 61% of those with a normal microbiota (p <0.001). A second study of pregnant women by Hillier *et al*. [21] showed that BV (based on Amsel's criteria) was significantly less common among women with H_2O_2-producing vaginal lactobacilli (10/127, 8%) than among women with non-H_2O_2-producing or no lactobacilli (29/86, 34%, and 37/62, 60%, respectively; p <0.001 for both comparisons). Similarly, Hawes *et al*. [22] found that BV developed in ten (25%) of 40 women with non-H_2O_2-producing vaginal lactobacilli, compared with only three (3%) of 118 women with H_2O_2-producing lactobacilli (p 0.02). Furthermore, a study by Antonio *et al*. [8] found that women with vaginal *L. crispatus* or *L. jensenii* (>94% of which were found to produce H_2O_2) were significantly less likely to have BV than were women colonised by other lactobacilli (only 22% of which produced H_2O_2) or women with no vaginal lactobacilli.

In contrast, some studies do not support the protective role of H_2O_2-producing lactobacilli against BV. In the study by Alvarez-Olmos *et al*. [14] among women with vaginal lactobacilli, H_2O_2-producing lactobacilli were isolated from 85% of 18 women with BV, and from 15% of 43 women without BV [14]. Moreover, Rosenstein *et al*. [23] isolated H_2O_2-producing lactobacilli from 11 (91.7%) of 12 pregnant women whose microbiota was indicative of BV following Gram's stain.

IN-VITRO EVIDENCE FOR THE EFFECT OF VAGINAL LACTOBACILLI ON BACTERIA CAUSING BV

Some in-vitro studies have shown that specific strains of lactobacilli are able to coaggregate with

G. vaginalis and block the adherence and/or displace previously adherent strains of *G. vaginalis* from vaginal epithelial cells. Boris *et al*. [24] showed that *Lactobacillus acidophilus*, *L. gasseri* and *L. jensenii*, isolated from the vaginal samples of healthy pre-menopausal women, coaggregated *in vitro* with *G. vaginalis*. *L. acidophilus* was also found to decrease the adherence and to displace previously adherent strains of *G. vaginalis* on vaginal epithelial cells. Mastromarino *et al*. [25] found that human isolates of *Lactobacillus salivarius* FV2 and *L. gasseri* 335 coaggregated *in vitro* with *G. vaginalis*, and that vaginal tablets containing a combination of these organisms with *Lactobacillus brevis* CD2 (which is strongly adherent to epithelial cells) reduced the adhesion by 57.7% and displaced 60.8% of *G. vaginalis* cells attached previously.

Some *Lactobacillus* strains have also been found to have an inhibitory effect on the in-vitro growth of pathogens that cause BV, which may be caused, in part, by the production of H_2O_2. Mastromarino *et al*. [25] found that *L. salivarius* FV2 and *L. gasseri* 335, isolated from the human vagina, produced large amounts of H_2O_2 and inhibited the growth of *G. vaginalis*. McLean and Rosenstein [26] showed that *L. acidophilus* 48101, isolated from the vagina of healthy women, produced large amounts of H_2O_2 and inhibited (although by less than the mean + SD) the growth of *Bacteroides* spp., *Prevotella bivia* and *G. vaginalis* isolated from vaginal swabs of women with BV.

Production of lactic acid by lactobacilli, which is mainly responsible for the low vaginal pH, contributes, probably even more than production of H_2O_2, to the inhibition of growth of *G. vaginalis*. An in-vitro study by McLean and McGroarty [27] showed that the bacteriostatic effect of *L. acidophilus* on *G. vaginalis* NCTC 11292 was reduced by 60% after increasing the culture pH by the addition of NaOH, and by 30% after denaturing H_2O_2 with catalase. Thus, production of lactic acid, which was mainly responsible for the low pH, and, to a lesser degree, production of H_2O_2 by *L. acidophilus*, affected the growth inhibition of *G. vaginalis* significantly. Klebanoff *et al*. [28] found that H_2O_2-producing lactobacilli at high concentrations inhibited the growth of *G. vaginalis* and *Bacteroides bivius*. Catalase inhibited the toxicity of H_2O_2-producing lactobacilli, but not that of non-H_2O_2-producing lactobacilli. Lower concentrations of H_2O_2-producing lactobacilli were toxic for *G. vaginalis*, but only when combined with myeloperoxidase and chloride, which have both been found in cervical mucus. The highest toxicity of this combination was obtained at pH 5–6. A pH of ≤4.5 had, by itself, an inhibitory effect on the growth of *G. vaginalis*, which was increased when the above combination was added.

Finally, production of bacteriocins by some *Lactobacillus* strains has also been found to play a role in the inhibition of growth of *G. vaginalis*, at least *in vitro*. Aroutcheva *et al*. [29] tested 22 *Lactobacillus* strains and found that 80% produced a bacteriocin that inhibited the growth of *G. vaginalis*. Simoes *et al*. [30] showed that the growth of 28 (78%) of 36 clinical isolates of *G. vaginalis* was inhibited by a bacteriocin-producing *L. acidophilus* strain, and characterised these *G. vaginalis* isolates as bacteriocin-susceptible.

CLINICAL TRIALS

Several clinical trials have been performed to investigate whether specific strains of lactobacilli, administered either orally or intra-vaginally, are able to colonise the vaginas of women with symptomatic or asymptomatic BV, to reduce the colonisation of pathogens, and to improve symptoms and/or signs of BV when they are present. Table 1 summarises the main characteristics and outcomes of randomised controlled trials (RCTs) that have been conducted with the aforementioned purpose.

Some RCTs have suggested that intra-vaginal administration of *L. acidophilus* for 6–7 days cures women with BV significantly more frequently than administration of a placebo, administration of acetic acid, or no treatment. Cure of BV was defined by the absence or presence of only one of Amsel's criteria, which are vaginal fluid with a pH >4.5, a thin homogeneous greyish-white adherent discharge, a fishy odour on addition of potassium hydroxide 10% w/v to the discharge (a positive amine test or sniff/whiff test), and clue cells on a saline wet mount [31]. Hallen *et al*. [32] found that significantly more women with BV were cured 7–10 days after the start of treatment with *L. acidophilus* when compared with those treated with a placebo. Restoration of a normal vaginal microbiota was established in significantly more (57%, 16/28) women with BV who were treated with *L. acidophilus* than in those receiving a placebo (none of 29). Another RCT involving pregnant

Table 1. Summary of randomised clinical trials of the use of probiotics in patients with bacterial vaginosis (BV)

Study	Number of women studied	Study population	Treatment		Outcome
			Lactobacilli	Control	
Fredricsson *et al.* [39]	61	Women with BV (≥3/4 Amsel's criteria[a] positive)	Group 1 (n = 14): 5 mL of fermented milk product with *Lactobacillus acidophilus* (5×10^8 to 2×10^9 CFU/mL) intra-vaginally twice-daily for 1 week	Group 2 (n = 17): acetic acid jelly intra-vaginally 5 mL 0.92% v/v twice-daily for 1 week Group 3 (n = 16): oestrogen intra-vaginally 5 mL cream twice-daily for 1 week Group 4 (n = 14): tablets metronidazole 500 mg intra-vaginally twice-daily for 1 week	Cure (0–1 Amsel's criteria) 4 weeks after start of treatment: Group 1: 1/14 (7.1%) Group 2: 3/17 (17.6%) Group 3: 1/16 (6.3%) Group 4: 13/14 (92.9%) No. of aerobic isolates: significant reduction 4 weeks after start of treatment in groups 2, 3 and 4 No. of anaerobic isolates: significant reduction 4 weeks after start of treatment only in group 4 No. of aerobic species: significant reduction 4 weeks after start of treatment in all groups No. of anaerobic species: significant reduction 4 weeks after start of treatment except for group 2
Hallen *et al.* [32]	57	Women with BV (≥3/4 Amsel's criteria positive)	Capsules of (H_2O_2-producing) *L. acidophilus* (10^8–10^9 CFU/capsule) intra-vaginally twice-daily for 6 days (n = 28)	n = 29 (placebo)	Presence of BV 7–10 days after start of treatment: 12/28 (43%) lactobacilli-treated vs. 29/29 (100%) placebo-treated, p <0.005 No significant difference between lactobacilli- and placebo-treated regarding the detection of *Mobiluncus* and *G. vaginalis* in the vaginal samples 7–10 days after start of treatment Detection of *Bacteroides* in vaginal samples: before treatment: 26/28 (93%) lactobacilli-treated vs. 28/29 (97%) placebo-treated, p >0.05; 7–10 days after start of treatment: 10/26 (38.4%) lactobacilli-treated vs. 27/28 (96.4%) placebo-treated, p <0.005 Detection of vaginal lactobacilli: before treatment: 12/28 (43%) lactobacilli-treated vs. 9/29 (31%) placebo-treated, p >0.05; 7–10 days after start of treatment: 18/28 (64%) lactobacilli-treated vs. 11/29 (38%) placebo-treated, p <0.05
Neri *et al.* [33]	84	Women aged 25–31 years with BV (≥3/4 Amsel's criteria positive, first trimester of pregnancy	Group 1 (n = 32): intra-vaginally 10–15 mL of yoghurt with *L. acidophilus* (>10^8/mL) twice-daily for 7 days and repetition after 1 week	Group 2 (n = 32): intra-vaginally tampons with 10–15 mL of 5% acetic acid and repetition after 1 week Group 3 (n = 20): no treatment	Subjective clinical improvement on second day after start of treatment: group 1: 32/32 (100%), group 2: 20/32 (62.5%), group 3: 0/20 (0%), p <0.0005 Cure of BV (0–1 Amsel's criteria) 1 month after end of second treatment: group 1: 28/32 (87.5%), group 2: 12/32 (37.5%), group 3: 3/20 (15%); group 1 vs. group 2: p 0.04; group 1 vs. group 3: p <0.0005; group 2 vs. group 3: p >0.05 Cure of BV 2 months after end of second treatment: group 1: 28/32 (87.5%), group 2: 12/32 (37.5%), group 3: 1/20 (5%), p <0.05; group 1 vs. group 2: p 0.04; group 1 vs. group 3: p <0.0005; group 2 vs. group 3: p <0.005
Parent *et al.* [34]	32	Pre-menopausal women with BV (≥2 Amsel's criteria positive, 6–30 vaginal lactobacilli per field of view (1000× magnification), eight pregnant	1–2 vaginal tablets/day with 50 mg of H_2O_2-producing *L. acidophilus* (≥10^7 CFU/tablet) and oestriol (0.03 mg/tablet) (Gynoflor) for 6 days (n = 17	n = 15 (placebo)	Cure of BV (positive: ≤1/4 Amsel's criteria): 2 weeks after start of treatment: 10/13 (76.9%) lactobacilli-treated vs. 3/12 (25%) placebo, p <0.05; 4 weeks after start of treatment: 7/8 (87.5%) lactobacilli-treated vs. 2/9 (22.2%) placebo, p <0.05 >30 vaginal lactobacilli per field of view (1000× magnification): 2 weeks after start of treatment: 7/11 (63.6%) lactobacilli-treated vs. 1/10 (10%) placebo, p <0.05; 4 weeks after start of treatment: 7/8 (87.5%) lactobacilli-treated vs. 1/7 (14.3%) placebo, p <0.01
Shalev *et al.* [41]	46	Women with recurrent vaginitis (≥4 episodes during the year prior to the study, 20 women with BV and eight women with VVC and BV at the start of the study)	Group 1 (n = 23): 150 mL/day yoghurt with *L. acidophilus* for 2 months + no yoghurt for the next 2 months + 150 mL/day pasteurised yoghurt for the last 2 months Group 2 (n = 23): 150 mL/day pasteurised yoghurt for 2 months + no yoghurt for the next 2 months + 150 mL/day yoghurt with *L. acidophilus* for the last 2 months		Women with positive *L. acidophilus* cultures[b]: before treatment: 20% (group 1) vs. 31% (group 2); after 1 month: 71% (group 1) vs. 27% (group 2), p <0.05; after 2 months: 92% (group 1) vs. 27% (group 2), p <0.05 Women with BV (3/4 Amsel's criteria-positive: pH >4.5, positive amine test, clue cells)[b]: before treatment: 56% (group 1) vs. 68% (group 2); after 1 month: 24% of 21 women (group 1) vs. 52% of 19 women (group 2), p <0.05 after 2 months: 6% of 14 women (group 1) vs. 43% of 14 women (group 2), p <0.05

Table 1. Continued

Study	Number of women studied	Study population	Treatment		Outcome
			Lactobacilli	Control	
Reid *et al*. [44]	42	Women with no symptoms of urogenital infection at entry into the study (seven women reported history of BV, 14 women had asymptomatic BV, based on Nugent score)	Group 1 (n = 10): *L. rhamnosus* GR-1/ *L. fermentum* RC-14 8×10^8/day orally for 28 days Group 2 (n = 12): *L. rhamnosus* GR-1/*L. fermentum* RC-14 1.6×10^9/day orally for 28 days Group 3 (n = 11): *L. rhamnosus* GR-1/*L. fermentum* RC-14 6×10^9/day orally for 28 days GG 10^{10}/day for 28 days		Conversion from BV Nugent score[c] (at the start of the study) to normal or intermediate Nugent score (at day 28): seven of 11 (64%) women of groups 1, 2 and 3 Conversion from BV or intermediate flora at the start of the study to normal flora on day 28: significantly greater for group 2 than for group 4, p 0.017 No symptoms of BV for 6 weeks
Reid *et al*. [42]	64	Women with no urogenital infection during the year before the study (16 women had asymptomatic BV, based on Nugent score	*L. rhamnosus* GR-1 + *L. fermentum* RC-14 (>10^9/strain/capsule viable) orally once-daily for 60 days (n = 32)	n = 32 (placebo)	Restoration from BV to normal Nugent score: 37% (lactobacilli-treated) vs. 13% (placebo), p 0.02 Vaginal lactobacilli count 28 and 60 days after start of treatment: lactobacilli-treated > placebo (p 0.01) Subjective improvement of vaginal health (vaginal irritation, pain, odour, discharge, change of mucus 2 months after start of the study: 30% (lactobacilli-treated) vs. 12% (placebo), p 0.17
Reid *et al*. [43]	59	Pre-menopausal women without urogenital infections during the year prior to the study (15 women had BV, based on Nugent score	*L. rhamnosum* GR-1 and *L. fermentum* RC-14 (>10^9/capsule viable) orally once-daily for 60 days (n = 29)	n = 30 (placebo)	Change from BV (Nugent score 7–10) to normal or intermediate vaginal microbiota (Nugent score 0–6) 2 months after start of the study: 7/8 (87.5%) lactobacilli-treated vs. 0/7 (0%) placebo-treated, p <0.05 Subjective improvement of vaginal health 2 months after start of the study (compared with start of the study): no vaginal itchiness, 45% lactobacilli vs. 20% placebo (p <0.01), no vaginal odour or discharge, 27% lactobacilli vs. 20% placebo, no limit to intimate contact, 27% lactobacilli vs. 20% placebo; no hesitancy with regard to having sex, 36% lactobacilli vs. 30% placebo
Ozkinay *et al*. [35]	360	Women aged 17–65 years with vaginal infections (19 women with BV)	One vaginal tablet/day of live *L. acidophilus* (≥10^7 CFU/tablet) and oestriol (0.03 mg/tablet) and lactose (600 mg/tablet) (Gynoflor) for 6 days (for post-menopausal women) (n = 240)[d]	n = 120[d] (placebo)	No significant improvement of vaginal symptoms (discharge, burning, itching, vulvar/vaginal inflammation, dyspareunia) (both lactobacilli- and placebo-treated) Normal flora index[e] increased significantly more in lactobacilli-treated than in placebo-treated (p 0.002 and p 0.006, 3–7 days and 4–6 weeks after end of treatment, respectively; degree of purity of vaginal flora[e] increased significantly more in lactobacilli-treated than in placebo-treated (p <0.0001 and p 0.001, 3–7 days and 4–6 weeks after end of treatment, respectively)
Eriksson *et al*. [40]	187	Women aged 18–53 years with BV (≥3 Amsel's criteria positive, 68.4% (128/187): Nugent score >6)	Tampons with *L. gasseri*, *L. casei* var. *rhamnosus* and *L. fermentum* for ≥5 days (n = 91)[f]	n = 96[f] (placebo)	Cure rate after second menstruation: Amsel's criteria 0/4: 56% (lactobacilli-treated) vs. 62.5% (placebo), p >0.05 Nugent score ≤3 (percentage of women with Nugent score >6 before treatment: 54.4% (lactobacilli-treated) vs. 64.2% (placebo), p >0.05

[a]Amsel's criteria are: vaginal fluid with pH >4.5; thin, homogeneous, greyish-white adherent discharge; fishy odour on addition of potassium hydroxide 10% w/v to the discharge (positive amine test or sniff/whiff test); and clue cells on saline wet mount.
[b]The percentages are estimated approximately based on Figs 1 and 3 of Shalev *et al*. [41].
[c]Points 1 to 4+ are allocated to each of the following morphotypes: lactobacilli (large Gram-positive rods), *Gardnerella vaginalis* (small Gram-variable rods), *Bacteroides* spp. (small Gram-negative rods) and *Mobiluncus* spp. (curved Gram-variable rods). Nugent score after adding the points: 0–3, normal lactobacilli-dominant vaginal microbiota; 4–6, intermediate vaginal microbiota; and 7–10, BV.
[d]Lactobacilli or placebo were administered 2–3 days after the end of anti-infective therapy (oral metronidazole for trichomoniasis and BV; oral fluconazole ± local ketoconazole for candidiasis).
[e]Normal flora index (NFI) was calculated by allocating a value from 0 to 3 to each of the following parameters: number of lactobacilli, number of leukocytes, number of pathogenic microorganisms and pH of vaginal secretion, and then adding the values together. The higher the NFI, the healthier the vaginal flora. The degree of purity of the vaginal flora (lactobacillary grade) was estimated as follows: grade I, dominant lactobacilli, no other bacteria; grade II, lactobacilli and other bacteria; grade III, few or no lactobacilli and other bacteria; and grade IV, no lactobacilli, no other bacteria.
[f]Lactobacilli or placebo were administered during the menstruation period following administration of clindamycin 100 mg/day vaginally for 3 days.
VVC, vulvovaginal candidiasis.

women with BV showed that significantly more women treated with *L. acidophilus* intra-vaginally were cured of BV at both 1 and 2 months after the end of treatment when compared with women treated with acetic acid or given no treatment [33]. Parent *et al*. [34] found that cure was more common, and the number of vaginal lactobacilli was significantly higher, in women with BV at both 2 and 4 weeks after the start of a 6-day treatment with *L. acidophilus* and oestriol, when compared with women with BV who received a placebo.

Ozkinay *et al*. [35] suggested that a healthier vaginal microbiota, consisting of higher numbers of lactobacilli and lower numbers of pathogenic bacteria, was established in women who had received *L. acidophilus* intra-vaginally and oestriol for 6–12 days, compared with those receiving a placebo, 2 or 3 days after the end of treatment of BV or trichomoniasis with oral metronidazole, and of vaginal candidiasis with oral fluconazole with or without local ketoconazole. However, no significant improvement of vaginal symptoms was observed for either group of patients.

A prospective cohort study by Chimura *et al*. [36] also yielded positive results regarding the effect of intra-vaginal administration of yoghurt containing *Lactobacillus* for 11 women with BV. A statistically significant reduction in vaginal inflammation, discharge and vaginal pH was found, and all 14 Gram-negative strains isolated initially had disappeared 3 days after treatment. Bacteriologically, BV was eradicated in six (54.5%) of the 11 women, and was partly eradicated in three (27.3%). Two other prospective cohort studies [37,38] administered *L. acidophilus* intra-vaginally in combination with vitamin B complex or oestriol to women with vaginitis, but it was not mentioned whether the women included in this latter study had BV.

In contrast, several other RCTs have failed to detect a significant difference in the cure rates for women with BV, and have detected no significant change in the number of vaginal lactobacilli, after intra-vaginal treatment with specific strains of lactobacilli, and vaginal metronidazole was found to be significantly more effective than lactobacilli in curing BV in one study. Fredricsson *et al*. [39] found that instillation of *L. acidophilus* into the vaginas of 14 women with BV cured only one (7.1%) subject, while vaginal metronidazole tablets cured 92.9%, and vaginal acetic acid jelly cured 17.6% of the women with BV who were treated with each regimen. Moreover, treatment with *L. acidophilus* did not increase the number of vaginal lactobacilli isolates. Subsequently, Eriksson *et al*. [40] used vaginal clindamycin to treat 187 women with BV, and then administered *L. gasseri*, *Lactobacillus casei* var *rhamnosus* and *Lactobacillus fermentum*, or a placebo, intra-vaginally during the following menstruation cycle. The BV cure rate after the second menstruation, as defined by either Amsel's criteria or Nugent's score, did not differ significantly for the women treated with the tested lactobacilli when compared with those treated with a placebo.

Oral administration of *L. acidophilus*, *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 for 2 months has been found in other RCTs to be more effective than a placebo in preventing recurrences of BV and/or increasing vaginal colonisation with lactobacilli, thus restoring the normal vaginal microbiota. Of 46 women with recurrent vaginitis participating in a study by Shalev *et al*. [41], 28 had BV, but only seven completed the study. The percentage of women with positive *L. acidophilus* vaginal cultures after 1 month and after 2 months increased among women receiving yoghurt containing *L. acidophilus* during the first 2 months of the study, and was significantly higher than for women receiving pasteurised yoghurt during the same period. Moreover, episodes of BV after 1 and after 2 months decreased significantly in women who consumed yoghurt containing *L. acidophilus* during the first 2 months of the study when compared with the episodes of BV among women consuming pasteurised yoghurt during the same period [41].

Reid *et al*. [42] found that the numbers of vaginal lactobacilli showed a significant increase in 32 healthy women receiving *L. rhamnosus* GR-1 and *L. fermentum* RC-14 orally on a daily basis for 2 and 4 weeks compared with 32 healthy women taking a placebo. The vaginal microbiota was restored from asymptomatic BV to a normal Nugent score in significantly more women receiving lactobacilli than in placebo-treated women. A similar result was obtained in a second RCT involving 59 women treated with either the same lactobacilli at the same dosage and for the same period or with a placebo [43]. The dose of lactobacilli required to restore and maintain a normal (based on Nugent score) vaginal microbiota was found to be $>10^8$ viable *L. rhamnosus* GR-1 and *L. fermentum* RC-14 daily in a trial involving 42 healthy women [44].

Finally, *L. acidophilus* was administered intramuscularly in a small prospective cohort study by Pattman *et al*. [45]. Three 0.5-mL injections at 2-weekly intervals were administered to eight women with recurrent BV (more than two episodes during the 6-month period preceding the study) who had been treated with oral metronidazole. Six women were treated concurrently with metronidazole. Four (50%) of these women had no episodes of BV for 6 months, two had a recurrence of BV within 3 months of completing treatment, but had no more episodes after being treated with metronidazole, and one had two recurrences, received one additional injection with *L. acidophilus*, and remained asymptomatic for the following 6 months. Clue cells were replaced by lactobacilli in all women who reported improvement of their symptoms.

CONCLUSIONS

Various in-vitro studies have shown that specific strains of lactobacilli inhibit the growth of bacteria causing BV by producing H_2O_2, lactic acid, and/or bacteriocins, and/or inhibit the adherence of *G. vaginalis* to the vaginal epithelium. Most relevant clinical trials have suggested that oral administration of *L. acidophilus*, or intra-vaginal administration of *L. acidophilus* or *L. rhamnosus* GR-1 and *L. fermentum* RC-14, is able to increase the numbers of vaginal lactobacilli, restore the vaginal microbiota to normal, and cure women of BV, although several trials found that intravaginal instillation of lactobacilli had no significant effect on the treatment of BV. In most of the relevant RCTs, lactobacilli were compared with a placebo, in two RCTs with acetic acid, and in one RCT with no treatment. Interestingly, in only one RCT [39] were lactobacilli compared with metronidazole. However, further RCTs, including larger samples of women with BV, in which lactobacilli are compared either with a placebo or metronidazole, need to be conducted before it will be possible to reach definitive conclusions as to whether probiotics represent an effective and safe method for treating women with BV.

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