

CLINICAL REPORT

A Double-blind Treatment Study of Bacterial Vaginosis with Normal Vaginal Lactobacilli after an Open Treatment with Vaginal Clindamycin Ovules

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The expected 4-week cure rate after conventional treatment of bacterial vaginosis are only 65–70%. In an attempt to improve the cure rate by adding probiotic lactobacilli we performed a double-blind placebo-controlled study of adjuvant lactobacilli treatment after an open treatment with vaginal clindamycin ovules. Women with bacterial vaginosis as defined by Amsel's criteria were treated with clindamycin ovules. Vaginal smears were collected and analysed according to Nugent's criteria. During the following menstruation period the women used, as an adjuvant treatment, either lactobacilli-prepared tampons or placebo tampons. The lactobacilli tampons were loaded with a mixture of freeze-dried *L. fermentum*, *L. casei var. rhamnosus* and *L. gasseri*. The cure rate was recorded after the second menstruation period. There was no improvement in the cure rate after treatment with lactobacilli-containing tampons compared to placebo tampons; the cure rates as defined by Amsel's criteria were 56% and 62%, respectively, and 55% and 63%, as defined by Nugent's criteria. This is the first study to report cure rates for women with 'intermediate' wet smear ratings according to Nugent's classification and this group had an overall cure rate of 44%. The cure rate of treatment of bacterial vaginosis was not improved by using lactobacilli-prepared tampons for one menstruation. **Key words:** bacterial vaginosis; treatment; lactobacilli; clindamycin.

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The concept of bacterial vaginosis (BV) had already been introduced by Gardner & Dukes (1) in 1955, but it was not until Amsel et al. (2) introduced the composite diagnosis of BV that the condition was internationally accepted (3).

The diagnosis of BV is somewhat controversial, as two different gold standards exist; Amsel's composite clinical criteria and Nugent's criteria on Gram-stained smears (4). The two methods are not completely

comparable. For diagnosis of BV using Amsel's criteria three of four clinical criteria have to be fulfilled; while BV is diagnosed according to Nugent as a score above six.

To date treatment of BV has not been very satisfying. The 1-week cure rate after oral metronidazole or clindamycin vaginal cream has been reported to be as high as 90% (5) but the 4-week cure rate is no better than 60–70% (6–8). The use of clindamycin vaginal ovules has resulted in the same rate of 68.1% 'improved' (9) and 54% cured (10) after 4 weeks.

Lactic acid-producing bacilli in the normal vaginal flora are considered to protect women from getting vaginal infections (11). Lactobacilli constitute the normal bacterial flora of the vagina and have a physiological role in maintaining a low pH (<4.5) and protecting against invasion by other micro-organisms. However, what kind of lactobacilli are commensal in the vagina is a matter of debate. Traditionally *Lactobacillus acidophilus* has been considered a commensal in the human vagina. Recent studies, using not only traditional methods but also genotyping with rapid amplified polymorphic DNA (RAPD) analysis, have shown the most common vaginal lactobacilli species to be *L. crispatus*, *L. gasseri*, *L. ineri* and *L. jensinii* (12, 13). So far trials that aimed to re-implant lactobacilli as treatment for BV have not been convincing. One clinical double-blind placebo-controlled study showed that women who received ovules with *L. acidophilus* were initially cured, but after the next menstruation BV relapsed giving only an 18% cure rate after 4 weeks (14). The efforts by Reid et al. (15) to restore asymptomatic bacterial vaginosis with oral capsules of *L. fermentum* and *L. casei var. rhamnosus* to lactobacilli flora succeeded in 37% of the women compared with 13% given placebo.

Lactobacilli are sensitive to clindamycin, but not to metronidazole. We chose to investigate additional lactobacillus treatment after traditional treatment with clindamycin. We chose clindamycin to eradicate all original lactobacilli before we introduced the 'new' lactobacilli. The purpose of this study was to improve the cure rate after traditional treatment with clindamycin ovules. As lack of lactobacilli is characteristic of BV,

the addition of new lactobacilli was expected to increase the cure rate.

MATERIALS AND METHODS

Patients from 13 different clinics (11 gynaecological outpatient clinics and two STD clinics) were included. To be included the patients should fulfil three of four of Amsel's criteria: typical or homogeneous discharge, a vaginal pH above 4.5, positive amine test ('sniff test') and presence of clue cells in a wet smear (2). The women should be above 18 years old and menstruating, with or without hormone replacement therapy. Women who were pregnant, planning for pregnancy or breast-feeding were excluded. The women should also be willing to use tampons during their menstruation period. Women undergoing antibiotic treatment during the week preceding the BV diagnosis were excluded, as well as women with ongoing yeast infection and *Chlamydia trachomatis* infection.

The regional ethics committee approved the study in all the countries involved (Finland, Norway and Sweden). The study was accepted by the National Agency for Medicine in Finland, Medical Products Agency in Sweden and the Norwegian Medicines Agency.

After signed informed consent the patients were randomized into two different groups: the placebo group and the lactobacilli group. All patients were first treated with clindamycin ovules (Dalacin® 100 mg ovule, Pharmacia Upjohn, Stockholm, Sweden) vaginally once daily for 3 days during the open part of the study. During the following menstruation they were either using tampons impregnated with lactobacilli or placebo tampons. To be included in the efficacy analysis the patient had to have used at least five tampons. All unused tampons were returned. During the second menstruation the women used their normal menstrual protection.

Lactobacilli used

The study products were tampons impregnated with freeze-dried *L. gasseri*, *L. casei* var *rhamnosus* and *L. fermentum* (Medipharm AB, Kågeröd, Sweden) mixed with coconut fat (Karlshamns AB, Karlshamn, Sweden). The lactobacilli used were originally isolated from healthy women at the Department of Clinical Microbiology at University of Lund and have been purified and cultivated by Medipharm AB. The lactobacilli-impregnated and the placebo tampons are produced and prepared by Rauscher Consumer Products GmbH (Schönau, Austria). Each lactobacilli-impregnated tampon contained 10^8 living bacteria per tampon at the beginning of the study, and when tampon samples from the same batch were analysed at the completion of the study the number of viable bacteria was 10^6 per tampon. The reference tampons are commercially available and are identical to the lactobacilli-impregnated tampons but the lactobacilli tampons had a slight difference in colour.

Samples taken

At the baseline visit patients were evaluated according to Amsel's criteria and a vaginal smear was collected for later Gram staining and diagnosis according to Nugent's classification. A microbiological test for *C. trachomatis* was also done according to the clinical practice at each clinic. Some centres also tested for additional STDs. After the first menstruation (during which the study tampons were used) and after the second menstruation the patients performed self-taken vaginal

smears using cotton-tipped swabs that were introduced 2–3 cm into the vagina. After air-drying of the slide the patients sent the slide by mail to the study coordinator. A questionnaire concerning adverse events and symptoms was also filled out by each patient and posted to the study-coordinator. At the follow up-visit, after the second menstruation, the patients were re-evaluated according to Amsel's criteria for efficacy and another vaginal smear was taken.

Definition of cure according to the Amsel's criteria

The patients had to have at least three of four of Amsel's criteria to enter the study (2). To be regarded as cured none of these criteria should be fulfilled. 'Improved' is defined as having one of Amsel's criteria after two menstruation periods and 'partly improved' was two criteria. When a patient had three or four criteria it was regarded as failure.

Definition of cure according to Nugent's criteria

All vaginal smears were Gram stained at the clinical microbiological laboratory in Linköping and analysed according to Nugent using high power field microscopy with 1000× and oil immersion by one investigator (BC). Her diagnostic abilities have been tested in an international BV workshop (16). A high number of bacteria with a lactobacilli morphotype gave a score of 0, whereas no lactobacilli scored 4. This score was added to the score of small bacteria with gardnerella morphotype scored 0. Finally the score of curved long rods morphotype (*Mobiluncus*) (0–2) was added. A total Nugent's score of 0–3 was considered as normal, 4–6 as intermediate and 7–10 as BV (4). The cure rates are shown only for patients who had a score of >6 at inclusion, i.e. fulfilled the criteria for BV according to Nugent at inclusion. To be regarded as cured the patient had to have a score of 3 or less, and to be regarded as improved a score of 4–6. A score of 7 and above was considered as failure.

Statistics

The expected 4-week cure rate after treatment with clindamycin ovules is 60–70% (9, 10). We expected treatment with human lactobacilli to improve the cure rate by 20%. To detect this difference a study with an 80% power and with a significance level of 0.05 had to include 164 patients. To accomplish this, the study was designed to include 250 patients. The χ^2 test was used to compare improvement and cure rates between different groups. All results are according to per protocol unless otherwise stated, i.e. according to intention to treat (ITT).

RESULTS

During the period from April 2001 to November 2001, 255 patients were included in the study and randomized into the two different groups. Thirty were lost to follow-up. Thirty-eight more were excluded from the 'per protocol' analysis due to violation of the protocol, four because of wrong inclusion (less than 18 years old and no menstruation period), eleven because of incorrect use of the clindamycin or the study products, eight because of infection with *C. trachomatis*, eleven because of other treatments and four because of pregnancy – leaving 187 women in the per protocol analysis.

There were no differences between the treatment group and the placebo group in the background data (Table I).

There was no significant difference in cure rate based on Amsel's criteria between the treatment group and the placebo group, as 56.0% and 62.5% were cured, respectively (Fig. 1). In the ITT analysis of all 224 patients who came back to the follow-up visit, including the protocol violators, the cure rate was 56.8% in the treatment group and 60.2% in the placebo group.

At inclusion, 128 of the 187 patients in the per protocol analysis fulfilled the diagnostic criteria of BV according to Nugent. The analysis of the women with a Nugent's score of more than 6 at inclusion showed no difference in cure rate when using the lactobacilli-impregnated tampons. At the follow-up visit 54.4% were cured in the treatment group and 64.2% in the placebo group (not significant).

The results of the second self-taken smear were very similar to the results of the smears taken by the physician at the follow-up visit. The cure rates of the 43 women with intermediate flora (a Nugent's score of 4–6 at inclusion) did not differ significantly between the treatment and the placebo group. Of all the women who had intermediate flora at inclusion, 44.2% were cured at the follow-up using Nugent's classification.

There was no major significant difference in the severity, nature and frequency of adverse events between the treatment group and the placebo group. The most

Table I. Background data of the treatment group and the placebo group

	Lactobacilli group	Placebo group	Total
Randomized	127	128	255
Lost	15	15	30
Excluded	21	17	38
Per protocol analysis	91	96	187
Age (median; range)	32 (20–52)	32 (18–53)	32 (18–53)
Race:			
Caucasian	86	92	178
Other	5	4	9
Contraception:			
Pill	26	22	48
IUD	27	26	53
'Mechanical'	11	15	26
Other	27	33	60
Amsel's criteria			
3	26.4%	30.2%	28.3%
4	73.6%	69.8%	71.7%
Nugent's criteria			
<4	9.6%	14%	11.8%
4–6	22.2%	14.7%	18.5%
>6	68%	71.3%	69.7%
No. of tampons used (median)	13.5	16	15
Days from clindamycin (mean)	19	16.3	17.6

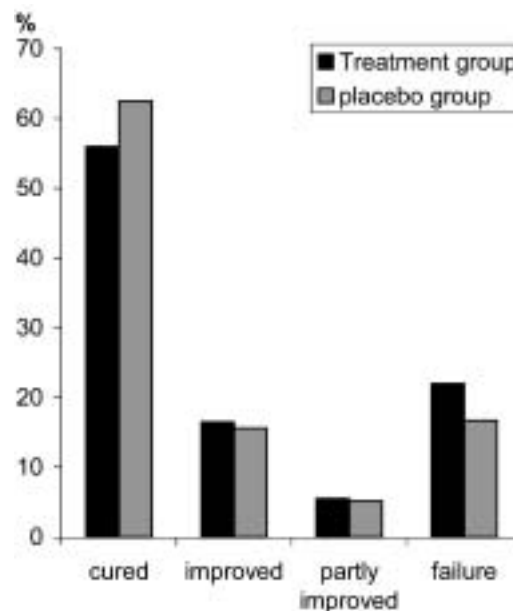


Fig. 1. The percent distribution of various responses after treatment for 3 days with clindamycin ovules followed by lactobacilli-prepared tampons or regular placebo tampons. Per protocol analysis. Cure rates are shown according to Amsel's criteria.

common adverse event was clinical candida infection with an incidence of 14.2% and 13.5% in the lactobacilli group and placebo group, respectively. Four women in the lactobacilli group reported itching and burning versus eight women in the placebo group. No serious adverse events were reported.

DISCUSSION

The study failed to show an increase in the low cure rate after treatment with clindamycin ovules for BV by adding lactobacilli-impregnated tampons. There are several possible explanations for this; for example, the composition of the lactobacilli, the technical handling of the tampons, the duration and number of used tampons, the properties of the tampons, and the dosage of lactobacilli.

The tampons used in the study were prepared with *L. gasseri*, *L. casei* var. *raffinosus* and *L. fermentum*, which originally were isolated from healthy Swedish women and cultivated and lyophilized for application on the tampons. It is possible that the composition of bacteria was not optimal for re-colonization in the vagina. The ability of the lactobacilli in the tampons to re-colonize the vagina was not systematically studied. Recent studies indicate that *L. crispatus* is one of the most commonly found bacteria in healthy women (12, 17) and the tampon formula may have shown improved results if *L. crispatus* had been included. However, so far *L. crispatus* has proven very difficult to cultivate on a large scale, so the possibilities for including it in a product such as a tampon will be limited. Culturing the tampons

shows a concentration of at least 10^6 lactobacilli/tampon. The dose of lactobacilli used might have been too low, even though we calculated that pretreatment with clindamycin would make it easier to introduce a new lactobacilli flora. Another possible explanation for the lack of effect is that the patients were subjected to too short an exposure of the lactobacillus-loaded tampons.

The efficacy did not improve if we looked specifically at the women who had used more than 15 tampons, and even if we controlled for the lag time between the end of clindamycin treatment and the start of treatment with lactobacilli. The exclusion of patients with a hormone-treated intrauterine device did not change the efficacy of the study.

The cure rate differed between the different centres. At one centre the cure rate was 85.7% in the lactobacilli group. Selection bias could explain the varying cure rates at the different centres. The fact that two of the centres are STD clinics with a different population of patients could be another explanation. As Amsel's criteria are subjective there could be differences between the investigators; however, the analysis of the Gram-stained slides by a single investigator would compensate for this.

This study was preceded by a double-blind placebo-controlled pilot study where 16 women were included and treated with clindamycin vaginal ovules for 3 days, followed either by placebo tampons or lactic acid bacteria-loaded tampons for use during the following first menstruation. In this pilot study 86% of the women who used the lactic acid bacteria-loaded tampons were cured or improved when evaluated by Amsel's criteria two menstruation periods after the BV diagnosis. The corresponding cure rate in the placebo group was 68%. However, the main study reported here could not verify this high cure rate.

In BV research there are two different diagnostic systems, two gold standards: Amsel's criteria and Nugent's criteria. The first one is clinical and the other is laboratory-based. The same subset of women will not fulfil the diagnosis of BV when using Amsel's criteria as when using Nugent's criteria. In our study 12% of the patients that were included according to Amsel's criteria had Nugent scores below 4 (normal), 19% had scores between 4 and 6 (intermediate) and 68% had scores above 6 (i.e. fulfilling the diagnosis of BV). The efficacy was similar whichever classification scheme was used.

The advantage of Nugent's scoring system is that one unbiased investigator can analyse all the samples. It can also be used for self-taken smears. One important result from the study is that the patients' self-taken smears were of good quality and comparable to those performed by a physician.

When we reclassified our data according to the Ison & Hay (18) classification – because there are concerns that

microscopic area can interfere with the results (19) – the cure rate increased to 69% in the treatment group and 73% in the placebo group. The failure rate was 23% and 19%, respectively.

The analysis of the treatment efficacy for the women with intermediate flora at inclusion (i.e. a score of 4–6 according to Nugent's classification) is the first treatment study of the Nugent intermediate group to our knowledge. The ITT-analysis of 43 women showed that 44% were cured after treatment with clindamycin and lactobacilli/placebo tampons after the second menstruation period and 40% of the patients still had intermediate flora. Nearly one-fifth changed from intermediate to BV even after treatment with clindamycin.

The reported side effects and adverse events in this study were low and the use of lactobacilli-impregnated tampons was safe.

It would be interesting to study the recolonization rate in a study of this kind. This was not done. It would also be interesting in a future study to follow-up the recolonization rate by currently available methods such as RAPD.

Our study also raised further issues for discussion concerning, among others, lactobacilli species and the concentration of the mixture. Further work has to be done in this field.

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Conflict of interest: P-G.L. has been a scientific advisor to Ellen AB.

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