

# The role of *Lactobacillus casei rhamnosus* Lcr35 in restoring the normal vaginal flora after antibiotic treatment of bacterial vaginosis

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**Objective** To evaluate the efficacy of additional topical *Lactobacillus casei rhamnosus* (Lcr35) subsequent to antibiotic treatment of bacterial vaginosis (BV) to restore the normal vaginal flora.

**Study design** Single-centre, randomised, observerblinded study.

**Setting** Population-based study in Vienna over 1 year.

**Sample** 190 women were enrolled in the study.

**Methods** Women with Nugent scores between 7 and 10 on initial vaginal swab were randomised to the one of two groups. All women were treated with standard antibiotic therapy for 7 days. Only women in the intervention group received vaginal capsules containing  $10^9$  colony-forming units of live Lcr35 for 7 days after antibiotic treatment. Final vaginal swabs for Nugent scoring were taken 4 weeks after the last administration of the study medication.

**Main outcome measures** The primary efficacy variable was a change in the Nugent score between the baseline and the end of the study of at least 5 grades in each individual woman.

**Results** Sixty-nine of the 83 women (83%) in the intervention group and 31 of the 88 women (35%) in the control group showed a reduction of the Nugent score by at least 5 grades. The difference in the number of women with improvement was highly significant ( $P < 0.001$ ). The median difference in Nugent scores between initial and final swabs was 6.61 in the intervention group and 4.13 in the control group ( $P < 0.001$ ).

**Conclusion** Our data show that the restoration of the vaginal flora after antibiotic treatment of BV can be significantly enhanced by exogenously applied lactobacilli.

**Keywords** Bacterial vaginosis, lactobacilli, vaginal flora.

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## Introduction

Common reproductive tract infections and the associated inflammatory responses are the most frequent gynaecological complaints, representing a central problem in modern clinical care. Vaginitis is usually characterised by vaginal discharge, vulval itching, irritation, or malodour. Bacterial vaginosis (BV), vulvovaginal candidiasis, and trichomoniasis are regarded as the most common vaginal infections worldwide.<sup>1,2</sup>

BV is a condition of the female genital tract characterised by a malodorous vaginal discharge, a vaginal pH of  $>4.5$ , and depletion of *Lactobacillus* spp. BV does not follow Koch's postulate, whereby a single pathogen is responsible for a specific disease but is characterised by an overgrowth of diverse aerobic, anaerobic, and micro-aerophilic species, such as *Gardnerella vaginalis*, *Prevotella* spp., *Peptostreptococcus* spp., *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Mobiluncus*

spp.<sup>3</sup> Metronidazole and clindamycin have been shown to be effective treatments for BV and to promote similar levels of restoration of vaginal lactobacilli.<sup>4,5</sup> Although anti-infective treatment is available and usually highly efficient in eradicating pathogenic microorganisms, its long-term efficiency is often limited by relapses, which are most likely, due to an inability to re-establish the normal lactobacillus-dominated vaginal flora.<sup>4-7</sup>

To understand the treatment failures frequently occurring in vaginal infections, it is important to consider the vaginal ecosystem in health and disease. The normal microflora is dominated by lactobacilli capable of inhibiting the adhesion and growth of pathogens, depleting nutrients otherwise available to pathogens, and modulating the host immune response and microenvironment.<sup>8,9</sup> There are three proposed mechanisms that elucidate the protective role of lactobacilli. First, they help to produce lactic acid as a by-product of glycogen

metabolism in the cells of the vaginal vault, thus acidifying the healthy vagina to a pH of 4.0–4.5, a level at which many pathogenic microbes cannot flourish. Second, many species of *Lactobacillus* produce hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which further inhibits microbial growth.<sup>10–12</sup> Third, lactobacilli compete with pathogenic microorganisms for adherence on epithelial cells.<sup>13,14</sup>

There is a considerable geographical variation in the composition of the normal vaginal *Lactobacillus* flora. Several species of lactobacilli have been described to populate the vagina to varying degrees. Vasquez *et al.*<sup>15</sup> found that the vaginal flora of most participants was dominated by a single *Lactobacillus* species among others, while another study showed that the most frequent *Lactobacillus* species were *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus jensenii*, and *Lactobacillus rhamnosus*.<sup>16</sup>

*Lactobacillus casei rhamnosus* has been successfully exploited commercially as a pharmaceutical product for more than 20 years. Its beneficial effects include the treatment and prevention of diarrhoea. Thus, Forestier *et al.*<sup>17</sup> showed that this strain has probiotic activities, such as the ability to adhere to intestinal cells and also exert antibacterial activity against several pathogens. Furthermore, Lcr35 has been shown to be effective in treating children with chronic constipation.<sup>18</sup> Also, a pilot study by Forestier *et al.* showed that oral administration of a probiotic delayed respiratory tract colonisation by and infection with by *Pseudomonas aeruginosa*.<sup>19</sup> In addition to its positive probiotic activity, *L. rhamnosus* is known to produce the bacteriocin lactocin 160, which has been shown to inhibit growth of *G. vaginalis*.<sup>20</sup> Lactocin 160 is highly specific in its antimicrobial action. It disturbs the cellular membrane and induces ATP efflux, most likely because of pore formation, in this way killing microorganisms associated with BV but leaving the healthy vaginal microflora intact.<sup>21</sup> Lactocin 160 causes minimal irritation and has a good potential for intravaginal application.<sup>22</sup>

While depletion of lactobacilli and an overgrowth of pathogens are distinct features of BV, antibiotic therapy further lowers the numbers of lactobacilli.<sup>23</sup> The topical administration of lactobacilli preparations in addition to antibiotic therapy may help restore the normal vaginal flora and avoid the relapses of infection. Therefore, the aim of this study was to evaluate the efficacy of topical *L. casei rhamnosus* Lcr35 in restoring the normal vaginal flora after antibiotic treatment of BV.

## Materials and methods

This single-centre, randomised, observer-blinded study was performed with the approval of the ethics committee of the Medical University of Vienna and in accordance with the Declaration of Helsinki and the guidelines of Good Clinical Practice. Between May 2005 and April 2007, we enrolled non-pregnant women aged 18–45 years without vaginal bleeding

and neoplasia from among outpatients of the Department of Obstetrics and Gynaecology. To determine the effect of lactobacilli in the absence of estrogen, women receiving oral contraceptives or other estrogen-containing treatments were excluded from the study, as were women having received antibiotic treatment in the past 4 weeks prior to study entry.

Participants and Gram-stain readers were blinded to the treatment assignment. The diagnosis of BV was established by the investigator at the initial examination based on each woman's history and clinical findings, such as a whitish-grey, homogeneous discharge, or fishy smell. The final decision about participation in the study was based on microscopic detection of BV.

From each potential participant, an initial vaginal smear was taken and smeared on microscopy slide. Smears were Gram-stained and evaluated at a central laboratory with staff experienced in the use of the Spiegel criteria.<sup>24</sup> To detect even minor treatment effects, scoring was performed using the entire 10-grade Nugent scale.<sup>25</sup> Only women with evident BV as evidenced by a Nugent score between 7 and 10 were invited to participate in the study after giving written informed consent and were randomised to one of two study groups using a computer-generated randomisation list. All women were treated orally for BV with a standard antibiotic regime, that is 2 × 300 mg clindamycin for 7 days.

Participants assigned to the intervention group also received a vaginal *Lactobacillus* capsule (Gynophilus; Laboratoires Lyocentre, Aurillac Cedex, France) for 7 days after antibiotic treatment. Each capsule contained at least 10<sup>9</sup> colony-forming units of live *L. casei rhamnosus* (Lcr35), 5.59 mg lactose, and 3.41 mg magnesium stearate. Women in the control group did not receive Lcr35.

Final vaginal swabs for Nugent scoring were taken 4 weeks after the last administration of the medication in both groups. Compliance was assessed by interview and by having women return the capsule packs.

## Statistics

The primary efficacy variable was a change in the Nugent score between the baseline and the end of the study of at least 5 grades in each individual woman. In addition, we assessed whether treatment improved the Nugent score grade, regardless of the extent of change.

Assuming a response rate of 50% in the control group, including drop-outs, 95 women had to be randomised to each group to detect a difference in response rates of 20% with a statistical power of 80% at a two-sided significance level of 5%.

The statistical analysis software used is a validated, proprietary development written in IBM APL 2, version 2, Service level 6. Rates between groups were compared in 2 × 2 contingency tables using the chi-square test, and average score

ratings between groups were compared using the *t* test for independent samples. The Bowker test for symmetry in score changes was used for the intraindividual pre- versus post-treatment comparisons.

## Results

Of the 1550 women screened for enrolment in the study, 1360 did not meet the eligibility criteria. Therefore, 95 women were randomised to the intervention group and 95 women were randomised to the control group (Figure 1). All women were of Caucasian origin. In the intervention and control groups, 12 and 7 women were lost to follow up, so that outcome data were available from 83 women in the intervention group and 88 women in the control group. The mean age of the study participants was 32.6 (SD 8.8) years, mean weight was 59.6 (SD 8.2) kg, and mean height was 164.6 (SD 7.5) cm, with no significant differences between the study groups. Likewise, there were no between-group differences in terms of smoking habits, alcohol consumption, or the numbers of previous pregnancies or deliveries. Only 5% of women in both groups had reported a recurrent BV.

The distributions of initial and final Nugent scores in the intervention and control groups are shown in Table 1. The post-treatment comparison of the proportions of women with a normal vaginal flora (83% in the intervention group

and 35% in the control group) using the chi-square test yielded a *P* value of <0.001 ( $\chi^2_{df=1} = 40.37$ ).

In women with an intermediate vaginal flora (11% in the intervention group and 55% in the control group), the same procedure showed a *P* value of <0.001 ( $\chi^2_{df=1} = 36.71$ ). Finally, for the proportions of women with BV, no statistically significant difference between study groups was found ( $P < 0.32$ ,  $\chi^2_{df=1} = 1$ ).

The median difference in Nugent scores between initial and final swabs was 6.61 (SD 2.44) in the intervention group and 4.13 (SD 2.37) in the control group, a highly significant difference between study groups ( $P < 0.001$ ). Table 2 shows the shifts in Nugent scores between initial and final swabs in individual women. Sixty-nine of the 83 women (83%) in the intervention group and 31 of the 88 women (35%) in the control group showed a reduction in the Nugent score by at least 5 grades. In both study groups, the intraindividual improvements evaluated using Bowker's symmetry test were found to be highly significant ( $P < 0.001$ ). By the end of the study, Nugent scores had decreased by even 8 grades in 60% of women in the intervention group and in 14% of women in the control group, this difference again being significant ( $P < 0.001$ ).

## Discussion

The results of this study suggest that the restoration of the vaginal flora after antibiotic treatment of BV can be

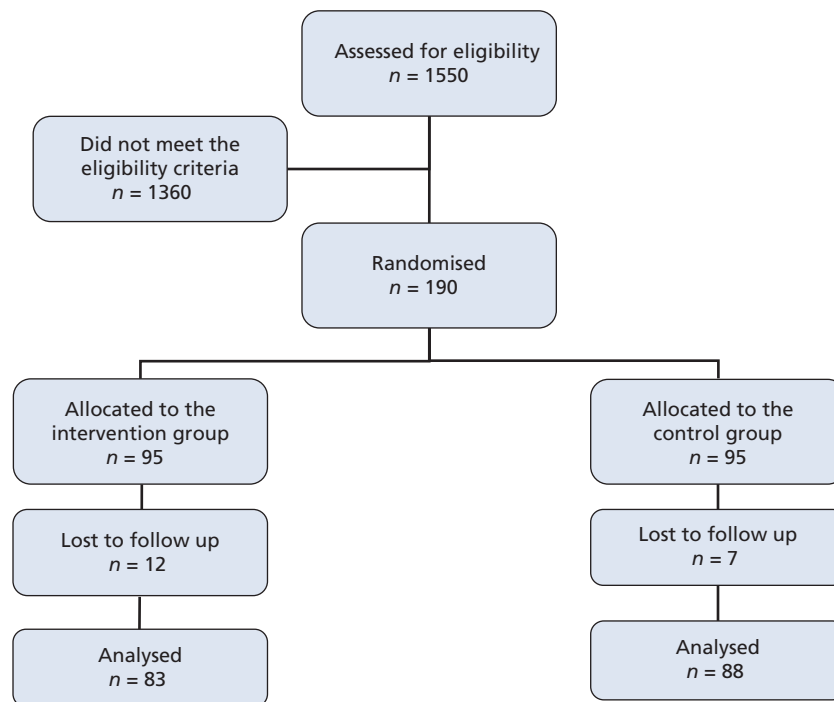


Figure 1. Disposition of women.

**Table 1.** Distribution of the Spiegel grades and Nugent scores for initial and final swabs in the intervention and control groups (chi-square test)

Spiegel grade	Nugent score	Intervention group (n = 83) (100%)		Control group (n = 88) (100%)		P value
		Initial swab, n (%)	Final swab, n (%)	Initial swab, n (%)	Final swab, n (%)	
1 (normal vaginal flora)	0	—	51 (61.4)	—	12 (13.6)	<0.001
	1	—	12 (14.5)	—	6 (6.8)	
	2	—	6 (7.2)	—	8 (9.1)	
	3	—	0 (0)	—	5 (5.7)	
	Total	—	69 (83.1)	—	31 (35.2)	
2 (intermediate vaginal flora)	4	—	3 (3.6)	—	26 (29.6)	<0.001
	5	—	2 (2.4)	—	6 (6.8)	
	6	—	4 (4.8)	—	16 (18.2)	
	Total	—	9 (10.8)	—	48 (54.6)	
3 (BV)	7	5 (6.1)	0 (0.0)	2 (2.3)	0 (0)	<0.32
	8	78 (93.9)	5 (6.1)	86 (97.7)	9 (10.2)	
	9	—	—	—	—	
	10	—	—	—	—	
	Total	—	5 (6.1)	—	9 (10.2)	

significantly enhanced by exogenously applied live lactobacilli. The decision to use Lcr35 as a test preparation was based on the multiple beneficial effects of *L. casei rhamnosus*. Besides its proven positive role as a probiotic,<sup>17</sup> it is one of four lactobacilli frequently isolated from the vagina.<sup>16</sup> Also, it produces proteinaceous substances exhibiting antibacterial activity, such as lactocin 160, a bacteriocin that is highly specific in its antimicrobial action, eliminating microorganisms associated with BV but leaving the healthy vaginal microflora intact.<sup>21</sup> Furthermore, lactocin 160 causes minimal irritation and has a good potential for intravaginal application.<sup>22</sup>

Although modern anti-infective treatment is usually highly efficient in eradicating pathogenic microorganisms, numerous authors have reported treatment failures in BV, particularly in terms of long-term success,<sup>6,7</sup> with BV and an 'intermediate

flora' not adequately responding to standard anti-infective therapy.<sup>6,7,23,26</sup> In our study, a normal vaginal flora could not be restored in 65% of women in the control group.

Although metronidazole is recommended as first-line treatment of BV, clinical practice at our department has shown that clindamycin is better tolerated by our women. Metronidazole and clindamycin have been shown to be effective treatments for BV and to promote similar levels of restoration of vaginal lactobacilli.<sup>4,5</sup> Nyirjesy *et al.* found that clindamycin eradicated an abnormal vaginal flora in 90% of pregnant women. Also, clindamycin has been shown to be more effective than metronidazole at reducing vaginal *Mobiluncus* morphotypes in women with BV, correlating with higher cure rates.<sup>27</sup>

On the downside, treatment of BV with clindamycin is associated with marked evidence of antimicrobial resistance among vaginal anaerobic bacteria.<sup>7</sup>

One limitation of this study is that we did not obtain vaginal swabs for assessment of the vaginal flora after treatment with clindamycin and before administration of lactobacilli. Based on results reported by Aroutcheva *et al.*,<sup>28</sup> clindamycin most likely had the same effect in both study groups, not only eliminating pathogenic microorganisms but also lowering the number of lactobacilli. Therefore, the vaginal flora in the intervention group after antibiotic treatment and before administration of lactobacilli is likely to have been similar to that in the control group.

At the end of the study, there was a significant difference between study groups with respect to both grades 1 and 2 ( $P < 0.001$ ; Table 1). Thus, 11% of women in the intervention group had an intermediate flora compared with 55% in the control group. This finding is important in that approximately

**Table 2.** Difference in Nugent scores between initial and final swabs in the intervention and control groups (chi-square test)

	Shift in Nugent score	Intervention group (n = 83) (100%), n (%)	Control group (n = 88) (100%), n (%)
No improvement	0 or worse	5 (6.1)	9 (10.2)
	-1	0 (0)	0 (0)
	-2	4 (4.8)	16 (18.2)
	-3	3 (3.6)	6 (6.8)
	-4	2 (2.4)	26 (29.6)
Improvement	-5	1 (1.2)	6 (6.8)
	-6	5 (6.1)	8 (9.1)
	-7	13 (15.6)	5 (5.7)
	-8	50 (60.2)	12 (13.6)

half of the women with an intermediate flora have been found to develop BV, suggesting that in the control group, the likelihood for women to progress to BV is higher than in the intervention group.

Although lactobacilli have been shown to dominate the healthy vaginal flora,<sup>29</sup> data on the role of lactobacilli in the treatment or prevention of urogenital infection are scarce. In 2005, Ozkinay *et al.*<sup>30</sup> reported that restoration of the vaginal flora can be significantly enhanced by the administration of live lactobacilli in combination with low-dose estriol. Uehara *et al.*<sup>31</sup> found that local application of lactobacilli may help prevent recurrent urinary tract infections.

Because estrogen encourages the vaginal colonisation with lactobacilli, which metabolise glycogen to produce lactic acid and maintain a low vaginal pH that inhibits the growth of many pathogens, we excluded women receiving oral contraceptives or other estrogen-containing medications to determine the effect of lactobacilli in the absence of estrogen. In our study, local application of Lcr35 after antibiotic treatment of BV significantly improved the vaginal flora as demonstrated on the basis of Nugent scores, a scoring system generally considered an adequate and objective method for the evaluation of the vaginal flora.<sup>25</sup>

Local Lcr35 restored a normal flora in 83% of women in the intervention group. In the control group, 35% of women had a normal vaginal flora at the end of the study. This difference between groups was highly significant (Table 2). Moreover, every single woman in the intervention group had a significant shift in the Nugent score of at least 5 grades between the baseline and the end of the study. Therefore, the results of our study suggest that topical *L. casei rhamnosus* LCR 35 may help restore the normal vaginal flora after antibiotic therapy of BV.

## Disclosure of interests

The paper is an original research article based on a clinical study approved by the ethics committee of Vienna Medical University (EK Nr. 099/2005) and supported by the head of the institute. This is the first presentation of the research results of the study, and there are no relevant financial, personal, political, intellectual, or religious conflicts of interest regarding this paper. The study did not receive any financial or political support from third parties, such as a government organisation or a commercial company. All authors fulfilled all conditions required for authorship.

## Contribution to authorship

L.P.: Organisation and preparation of the research, and writing the paper. A.W.: Organisation of the research and writing the paper. Other non-author individuals: Kurt Neumann, statistical analyses; Birgit Füssl, providing the test preparation.

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