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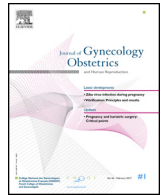
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Review

Efficacy and safety of vaginally administered lyophilized *Lactobacillus crispatus* IP 174178 in the prevention of bacterial vaginosis recurrence



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ABSTRACT

Background. – Bacterial vaginosis (BV) is a recurrent disease in women despite treatment by antibiotics. This study investigated the impact of a vaginal probiotic, *Lactobacillus crispatus* IP174178* (*Lc*), on the rate of recurrence and time to recurrence.

Methods. – A prospective, multi-centre, double blind, randomised phase III trial in women with at least two documented episodes of BV in the previous year (diagnosis confirmed by presence of three Amsel criteria and a Nugent score ≥ 7), and who had been clinically cured (i.e., no Amsel criteria) after oral metronidazole treatment (1 g/day \times 7 days). The patients were randomised to receive vaginal capsules of either *Lc* or placebo, once a day, for 14 days over the first two menstrual cycles and another 14 days of the same treatment for the following two menstrual cycles. The primary efficacy endpoint was the number of patients with at least one bacteriologically confirmed recurrence of BV.

Results. – Out of 98 assessable patients (mean age 35.7 years), 78 women were evaluated (20 patients had missing data). During the treatment period, 16/39 patients (41%) had at least one recurrence in the placebo group versus 8/39 patients (20.5%) in the *Lc* group ($P = 0.0497$). The time to recurrence was longer by 28% in the *Lc* group (3.75 ± 0.16 months) vs. the placebo group (2.93 ± 0.18 months) ($P = 0.0298$). Tolerability and safety were good in both groups.

Conclusion. – In women with recurrent BV after antibiotics, treatment with *Lc* IP 174178 administered over four menstrual cycles, could significantly reduce the rate of recurrence and increase the time to recurrence.

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Bacterial vaginosis (BV) is a common infection estimated to affect approximately 30% of women worldwide [1]. Prevalence varies from region to region with a lower prevalence in Europe–24% in Norway and 19% in Poland [2]–but as high as 68% in Mozambique [2] and exceeding 30% in South East Asia, Australia and New Zealand [2].

BV is the result of a vaginal dysbiosis with the disappearance or rarefaction of the *Lactobacillus* flora and the development of a polymicrobial flora combining predominantly anaerobic bacteria, *Gardnerella vaginalis* (*G. vaginalis*), and/or mycoplasmas [3]. It is therefore not an infection *per se* but rather a multifactorial

imbalance in the vaginal microbiota. Risk factors include: a new sexual partner, a high number of sexual partners [4,5] (male or female [6]), smoking [4–7], vaginal douches [8], and contraception using an intrauterine device [9]. BV is a known risk factor for premature births [10,11], chorioamnionitis and neonatal infection, including in babies born at term [11]. BV is also a risk factor for HIV infection (relative risk = 1.6) [12]. Finally, BV recurrence has an impact on the patient's quality of life [13].

The diagnosis of BV is based on the clinical criteria defined by Amsel in 1983 [14]. Women are diagnosed as having BV if they present three of the following criteria: homogenous greyish leucorrhoea, rotten fish odour (spontaneous or following a potassium hydroxide test), a vaginal pH > 4.5 , or the presence of clue cells by microscopic examination. Clinical diagnosis can be confirmed by microbiology with a Nugent score of > 7 . Treatment

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for BV consists of orally administered metronidazole at a dose of 2×500 mg per day for 7 days [15,16]. The immediate clinical results demonstrate a recovery rate of 70% to 80% [14], but a recurrence rate of 33% at 3 months [17] and of 49 to 66% at 1 year [18].

There are microbiological factors associated with the recurrence of BV: *G. vaginalis* and *Atopobium vaginae*, two of the main bacteria involved in BV, produce a biofilm that adheres firmly to the vaginal wall [19] and which replaces the physiological lactobacillus biofilm. The *G. vaginalis* biofilm has been shown to be resistant to antibiotic treatments such as metronidazole [20].

Several clinical studies [21–23] have shown that courses of probiotics (*Lactobacillus rhamnosus*, *L. fermentum*, *L. plantarum*, *L. salivarius*, *L. brevis*, etc.) reduce the symptoms of BV. Other studies [24,25] have explored *Lactobacillus* supplementation (*L. gasseri*, *L. rhamnosus*, *L. acidophilus*) in the prevention of BV recurrence. However, no studies have investigated the use of *L. crispatus*, despite its beneficial properties. *L. crispatus*, and in particular the *L. crispatus* strain IP 174178, is considered to be a biomarker for vaginal health [3]. It produces lactic acid, microbicide and virucide, which facilitate the exfoliation of glycogen-rich cells in the vaginal epithelium [3]. The aim of our prospective, randomised, double-blind, superiority clinical study (Evaflöre) was to study the efficacy and safety of *L. crispatus* IP 174178 administered vaginally in the prevention of BV recurrence.

Materials and methods

The study was carried out by gynaecologists and general practitioners in 29 centres in France between April 2013 and October 2015. It was coordinated by the Institut Fournier (Paris).

Study procedure

Selection visit (Visit 1)

Patients had to be over 18 years and present with all three of the following Amsel (Fig. 1):

- homogeneous greyish leucorrhoea;
- “rotten fish” odour or positive potassium hydroxide test;
- vaginal pH > 4.5.

Patients had to have two documented episodes of BV (medical records and/or bacteriological examination) within the previous year. A bacteriological sample was taken to confirm BV and to rule out a sexually transmitted infection. All patients signed an informed consent form and were covered by the French social security system.

The exclusion criteria were genital infections, pregnancy and breast feeding.

All patients meeting the inclusion criteria were prescribed metronidazole 500 mg to be taken orally twice a day for 7 days.

Inclusion visit (Visit 2, Day 0)

After completing the treatment with metronidazole, patients with a Nugent score > 7 at Visit 1 and clinically cured at Visit 2 (i.e., no Amsel criteria) were randomised to receive vaginal capsules of a

placebo or of *L. crispatus* IP 174178 (10^9 CFU per gram). The treatment consisted of daily administration of a vaginal capsule for 14 days for two menstrual cycles. The patients were contacted by telephone on Day 28 to ensure treatment compliance.

Follow-up visit (Visit 3, Day 56)

All the patients were clinically examined. If the three Amsel criteria were present, a bacteriological sample was taken and the patient was prescribed a new course of metronidazole.

Compliance, adverse events (AE) and concomitant treatments were evaluated.

Another 14-day course of the same treatment (placebo or *L. crispatus* IP 174178) was then given to all the patients for the next two menstrual cycles.

The patients were contacted by telephone on Day 84 to ensure treatment compliance.

End of treatment visit (Visit 4, Day 112)

All the women were clinically examined for the presence or absence of the three Amsel criteria and a bacteriological sample was taken.

Compliance, AEs and concomitant treatments were evaluated.

End of study visit (Visit 5, Day 196)

The women were clinically examined and a bacteriological sample was taken as for Visit 4.

Recurrence visits

If vaginal symptoms reappeared during the course of the study and outside the scheduled visits, patients were invited to a consultation for a clinical examination and a bacteriological sample was taken.

Objectives

Primary objective

To assess the efficacy of *L. crispatus* IP 174178 in the prevention of BV recurrence by comparing the percentage of patients presenting with a clinical recurrence of BV confirmed by a Nugent score of ≥ 7 at the end of treatment (Visit 4, Day 112).

The primary endpoint was the number of patients in the two treatment groups presenting with at least a bacteriologically confirmed clinical recurrence of BV at Visit 4 (Day 112).

Secondary objectives

- Time to first recurrence of clinical BV and clinically and bacteriologically confirmed BV between Visit 2 (Day 0) and Visit 4 (Day 112).
- Number of patients presenting with at least a clinical recurrence between Visit 2 (Day 0) and Visit 4 (Day 112).
- Number of patients presenting with at least a clinical recurrence, number of patients with at least a bacteriologically confirmed clinical recurrence and time to recurrence between Visit 4 (Day 112) and Visit 5 (Day 196).
- AEs at Visit 5 and overall safety at Visit 4 (Day 112).
- Compliance.



Fig. 1. Study plan.

Additional analyses

Average number of recurrences per patient between Visit 2 (Day 0) and Visit 5 (Day 196).

Statistical method

The initial sample size calculation was carried out on the basis of a two-sided ($\alpha = 0.05$, $\beta = 0.20$) χ^2 (Chi²) test with a power of 80% and a difference in BV recurrence of 20% between the two groups. The percentage of patients without recurrence in the placebo group was set at 50%.

Taking into account patients lost to follow-up (10%), 348 patients had to be selected to include 98 assessable patients in each treatment group giving a total Intent-to-treat (ITT) population of 196 patients.

The analyses were run using the SAS[®] software version 9.3.

We used the χ^2 test, to compare the percentage of patients between the two treatment groups who presented with at least one clinical and bacteriological recurrence at Visit 4 (Day 112).

We used the Kaplan Meier method to estimate the time to clinical recurrence (regardless of bacteriological confirmation) and compared the time to clinical recurrence between the two treatment groups using the log-rank test (PROC LIFETEST) in unilateral expression. Patients lost to follow-up were censored from this analyse.

AEs were coded using the 2014 edition of the WHO Drug Dictionary. The two treatment groups were compared using the χ^2 test.

We used the Wilcoxon rank test to compare overall safety between the two groups at Visit 4 (Day 112).

Both the χ^2 test and the Student *t*-test were used to compare compliance.

Randomisation was stratified by site and treatments were allocated by two blocks.

Results

Study population

Any deviations were reviewed and classified as major or minor during a data review meeting (Fig. 2).

Safety population (SAF)

Safety analyses was based on the safety population which included all randomised patients who had taken at least one treatment capsule ($n = 98$).

Full analysis set (FAS)

The FAS included all randomised patients who had taken at least one treatment capsule ($n = 98$). Any randomisation errors were processed during the blind data review.

Per protocol (PP) population

The PP population included all assessable patients in the FAS who finished the study without any deviation from the protocol ($n = 85$).

The efficacy analyses were carried out in both the FAS and PP populations.

Demographic data at inclusion

The mean age of the women included was 35.7 years. There were no significant differences in percentages between the two treatment groups regarding smoking, use of an intrauterine device or use of vaginal douches.

Primary objectives

Out of the 98 patients in the FAS, nine patients in the placebo group and 11 patients in the *L. crispatus* IP 174178 group could not be analysed due to missing data.

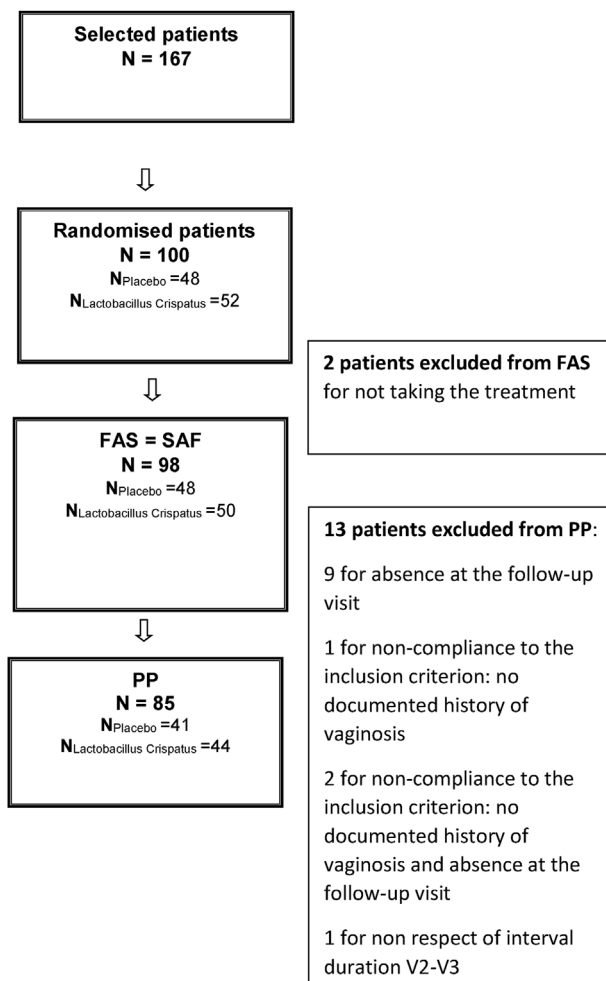


Fig. 2. Flow chart.

In the FAS population, 16 of the 39 patients (41%) in the placebo group presented with at least one recurrence of BV [90% CI 28.1–54] versus eight of the 39 patients (20.5%) in the *L. crispatus* IP 174178 group [90% CI 9.9–31.1] ($P = 0.0497$).

Out of 85 patients in the PP population, four patients in the placebo group and five patients in the *L. crispatus* IP 174178 group could not be analysed due to missing data. In the PP population, 16 of the 37 patients (43.2%) in the placebo group presented with at least one recurrence of BV [90% CI 29.8–56.6] versus eight of the 39 patients (20.5%) in the *L. crispatus* IP 174178 group [90% CI 9.9–31.1] ($P = 0.0331$) (Table 1).

Secondary objectives

Time to first clinical and bacteriological recurrence between Visit 2 (Day 0) and Visit 4 (Day 112)

In the FAS population ($n = 98$), the time to first recurrence was shorter in the placebo group, with a median of 2.93 ± 0.17 months compared to 3.75 ± 0.16 months in the *L. crispatus* IP 174178 group ($P_{\log\text{-rank}} = 0.0298$) (Fig. 3).

Time to first clinical recurrence between Visit 2 (Day 0) and Visit 4 (Day 112)

In the FAS population ($n = 98$), the time to first recurrence was 2.84 ± 0.17 months in the placebo group versus 3.76 ± 0.17 months in the *L. crispatus* IP 174178 group ($P_{\log\text{-rank}} = 0.0149$).

Number of patients experiencing at least one clinical recurrence between Visit 2 (Day 0) and V4 (Day 112)

In the FAS population ($n = 98$), more patients had a clinical recurrence in the placebo group: 47.5% (19/40 patients, 8 patients not assessable due to missing data for this criterion) versus 25% (10/40 patients, 10 patients not assessable for this criterion) in the *L. crispatus* IP 174178 group ($P = 0.0363$).

Post-treatment period Visit 4 (Day 112) to Visit 5 (Day 196)

In the FAS population, the percentage of patients who presented with a clinically and bacteriologically confirmed recurrence was 12.9%, with no significant difference between the two treatment groups ($P = 0.9221$). Likewise, no difference was observed in time to first recurrence of BV between Visit 4 and Visit 5: 2.16 ± 0.09 months in the placebo group versus 2.73 ± 0.08 months in the *L. crispatus* IP 174178 group ($P = 0.8149$).

There was no difference between the groups in the number of patients who had a clinical recurrence and time to recurrence.

In the PP population, no difference was observed between the two treatment groups for the same criteria listed above.

Additional analyses between Visit 2 (Day 0) to Visit 5 (Day 196)

The average number of bacteriologically confirmed clinical recurrences, per patient, was lower in the *L. crispatus* IP 174178 group (0.3 ± 0.7) versus the placebo group (0.7 ± 0.9), without reaching significance ($P = 0.0779$).

Safety and tolerability

In the FAS population, 66.3% of the patients presented with at least one Adverse Event: 70.8% in the placebo group ($n = 34$) versus 62% in the *L. crispatus* IP 174178 group ($n = 31$) ($P = 0.3550$).

Three of 98 patients (3.06%) presented with an AE considered to be linked to the treatment: two in the placebo group (vulvovaginal pruritis and leucorrhoea) and one in the *L. crispatus* IP 174178 group (oral consumption of treatment).

Five patients (two in the placebo group and three in the *L. crispatus* IP 174178 group) discontinued treatment due to an AE: two for metrorrhagia (one in each treatment group), one for diarrhoea (*L. crispatus* IP 174178 group) and two for genital mycosis (one in each treatment group). No serious AEs were observed in *L. crispatus* IP 174178 group whereas 2 were observed in placebo group.

At Visit 4 (Day 112), overall tolerability was assessed by the investigators as being: very good, good, average or poor. The tolerability was deemed to be very good in 92.2% of cases, with no difference between the two treatment groups ($P = 0.6061$).

Compliance

The average number of treatment days was 53.9 ± 11.7 days and the average number of days of treatment interruption was 5.7 ± 7.2 , with a median of 3.0 days, and no difference between the two treatment groups ($P = 0.3075$ and $P = 0.6128$, respectively).

Discussion

Our results show that *L. crispatus* IP174178 (Physioflor[®]) is effective in preventing the recurrence of BV: 59% of the patients in the placebo group had no recurrence at the end of the treatment period versus 79.5% of the patients in the *L. crispatus* group IP 174178 (Table 1).

Sobel et al. [17] treated women suffering from recurrent BV with either two applications per week of a metronidazole vaginal gel or with a placebo vaginal gel for 4 months. Following this treatment, 59% of the patients in the placebo group had no recurrence versus 75% of the patients in the metronidazole group. These results are comparable with ours. However, prolonged use of

antibiotic courses introduces numerous disadvantages, including the risk of resistance.

It is also interesting to note in our results that the first bacteriologically confirmed recurrence of BV occurred around 1 month later in the *L. crispatus* IP 174178 group than in the placebo group (Fig. 3). Likewise, the overall analysis of the average number of recurrences per patient, up to Visit 5, showed a benefit of treatment with probiotics without reaching significance ($P = 0.0779$). This lack of significance may be explained by the lack of study strength due to our small sample of 58 (29 patients in each group) assessable patients (for this criterion).

The safety profile was good in the two groups with only minor AEs. Two cases of vulvovaginal mycosis, one in each treatment group, were reported which is lower than other studies [24].

Our results also confirm the *in vitro* studies by Mc Lean N. W. and Rosenstein I.J. [26], which showed that *L. crispatus* 55730 inhibited pathogenic vaginal bacteria, and by Atassi et al. [27] showing that *L. crispatus* (10^8 CFU/ml) inhibited *G. vaginalis* or *Prevotella bivia* in co-culture for 4 hours.

Some limitations need to be taken into account:

- We were unable to meet our recruitment objective as our inclusion criteria were too selective. The women were required to have at least two bacteriologically confirmed episodes of BV within the previous year, which had to have been documented in their medical records. The choice to include patients with a demonstrated history of BV was to ensure that we recruited patients more likely to suffer a recurrence and therefore to better evaluate the impact of the treatment. This criterion was also applied in Sobel et al.'s study [17] and wasn't in the Larsson et al.'s study [24].

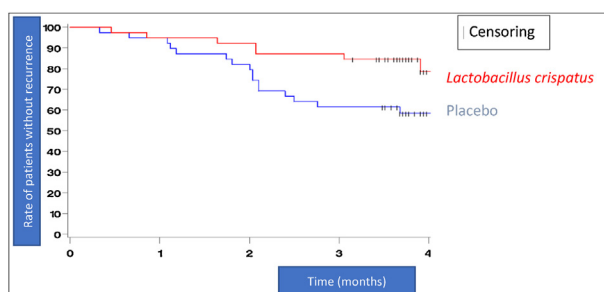
Recruitment stopped after 167 patients were selected in order to remain within a reasonable time frame for the study estimated to be compatible with an informative statistical analysis. Despite this reduction in sample size, the primary efficacy endpoint was positive. However, this might explain why we failed to reach significance for few secondary endpoints.

- It is possible that the length of the therapy (4 months) and follow up (3 months) are responsible for the amount of missing data at the end of trial. At Visit 4, 78 patients were evaluable for the primary objective whereas at Visit 5, less patients were evaluable (number depending on the criterion), with a FAS population $n = 98$. The lack of statistical power due to this small population could explain the non-significance of few criteria evaluated during the follow up.
- BV diagnosis was based on the presence of only three of the four clinical criteria defined by Amsel [14]. The fourth clinical criterion, which involves testing for clue cells, was excluded as most of the investigators did not have the necessary equipment available for this type of examination. Nevertheless, the presence of three criteria out of four represents good diagnostic reliability, with a sensitivity of 70% to 92% [28,29] and a specificity of 94% to 99% [28,29]. Moreover, systematic bacteriological confirmation of BV using the Nugent score reinforced the diagnosis and limited the risk of wrongful inclusion.
- When the protocol was written in 2012, few clinical studies investigating probiotics in the prevention of BV had been published. Furthermore, our study was designed for women with a history of at least two documented recurrences in the past year. We based our study design on Larsson et al.'s study [24] which used a 10-day probiotic treatment schedule over four menstrual cycles and in women who did not have history of recurrences. These elements supported our study

Table 1

Number of patients having at least one (clinical and bacteriological) recurrence of BV between V2 and V4.

Clinical and bacteriological recurrence between V2 and V4	FAS (n=98)		PP (n=85)	
	Placebo (n=48)	<i>Lactobacillus crispatus</i> (n=50)	Placebo (n=41)	<i>Lactobacillus crispatus</i> (n=44)
No, n (%)	23 (59.0)	31 (79.5)	21 (56.8)	31 (79.5)
Yes, n (%)	16 (41.0)	8 (20.5)	16 (43.2)	8 (20.5)
90% CI	[28.1; 54.0]	[9.9; 31.1]	[29.8; 56.6]	[9.9; 31.1]
Total, n (%)	39 (81.3)	39 (78)	37 (90.2)	39 (88.6)
Missing, n (%)	9 (18.8)	11 (22.0)	4 (9.8)	5 (11.4)
	(Chi ² P)=0.0497		(Chi ² P)=0.0331	

**Fig. 3.** Time the first clinical and bacteriological recurrence (between V2 and V4)–Kaplan-Meier plot–FAS (N = 98).

application and the scientific board approved the treatment period of 14 days for four cycles in women with a history of BV recurrence.

- Certain BV risk factors, such as smoking and the use of vaginal douches, were only assessed at inclusion and not throughout the study and this may have contributed to the fact that we were unable to demonstrate long-term efficacy of treatment (i.e., at Visit 5). Likewise, data related to the patients' sexuality, which is also a known risk factor of BV recurrence, were not collected [5–7]. These risk factors may have resulted in vaginal dysbiosis once the probiotic treatment was stopped.
- During the study, some women used unauthorised antibiotics which could induce dysbiosis. These patients were monitored but no significant difference between the two treatment groups was identified. Furthermore, the number of patients with at least one recurrence was not significantly different between the two treatment groups.

The 2010 Cochrane review by Senok [30] concluded that there was insufficient evidence to recommend, or not, probiotics to treat BV. But the authors did state that the Metronidazole with a probiotic regimen appeared promising: microbiological cure was 88% in the Metronidazole and probiotic group versus 40% in the Metronidazole group alone: Odds Ratio 0.09 (95% CI 0.03–0.25). It is important to note that these studies are related to the treatment of BV and not to the prevention of BV recurrence as in our study. Other studies [24], [25] in the prevention of BV recurrence support metronidazole combined with probiotics over metronidazole alone.

Conclusion

Despite the limitations of this study, our results support that repeated courses of vaginally administered *L. crispatus* IP 174178 (Physioflor[®]) is effective in the prevention of BV recurrence, with a time to first recurrence significantly later in the investigational group, with a good safety profile. Our results are comparable with those obtained with prolonged courses of antibiotics with much

fewer environmental risks or the risk of resistance [20]. However, the improvement obtained by using probiotics, must be considered alongside the known risk factors of BV in order to sustain a long-term benefit.

Ethical statement

The study (EUDRACT no.: 2012-002975-33) was approved by the Ethics Committee Île-de-France III and by the French Health Authorities (Agence Nationale de Sécurité du Médicament (ANSM)). The study was carried out in accordance with the current ICH-GCP recommendations, the Declaration of Helsinki and French regulations and laws.

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Disclosure of interest

Dr Bohbot was Principal Investigator and scientific committee's member of the study, sponsored by IPRAD PHARMA Dr Daraï, Dr Bretelle and Dr Cardot were scientific committee's members of the study, sponsored by IPRAD PHARMA Dr Brami and Ms Daniel are employees of the company IPRAD PHARMA.

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