J Gynecol Obstet Hum Reprod xxx (2017) xxx–xxx

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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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ARTICLE INFO

*Article history:*
Received 7 April 2017
Received in revised form 9 November 2017
Accepted 22 November 2017
Available online xxx

*Keywords:*
Bacterial vaginosis
Vaginal probiotic
*Lactobacillus crispatus*
Vaginal infection
Recurrence bacterial vaginosis
Metronidazole

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 $\geq 7$), 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 $\pm$ 0.16 months) vs. the placebo group (2.93 $\pm$ 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.



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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https://doi.org/10.1016/j.jogoh.2017.11.005
2468-7847/

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 (Evaflore) 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 (109 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).

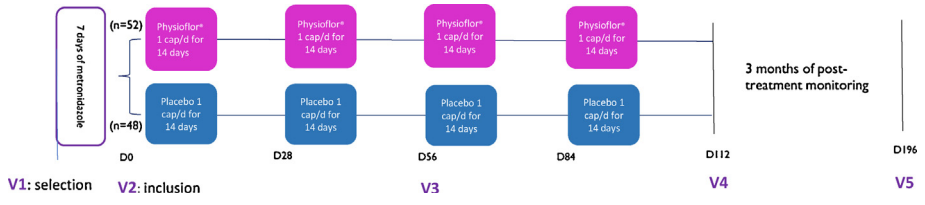


**Fig. 1.** Study plan.

Please cite this article in press as: Bohbot JM, et al. Efficacy and safety of vaginally administered lyophilized *Lactobacillus crispatus* IP 174178 in the prevention of bacterial vaginosis recurrence. J Gynecol Obstet Hum Reprod (2018), https://doi.org/10.1016/j.jogoh.2017.11.005