

Thymol, eugenol and lactobacilli in a medical device for the treatment of bacterial vaginosis and vulvovaginal candidiasis

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SUMMARY

The aim of this non-interventional, observational, multicentre, open-label study was to assess the effectiveness of a vaginal gel containing extracts of *Thymus vulgaris* and *Eugenia caryophyllus* in conjunction with two specific lactobacilli strains (*Lactobacillus fermentum* LF10 and *Lactobacillus plantarum* LP02) specifically formulated in slow-release vaginal capsules, in treating bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) or recurrent vulvovaginal candidiasis disease (RVVC) [Estromineral Probiogel (EPB) in Italy, or Saugella Probiogel; Meda Pharma - Mylan Group]. There was a statistically significant improvement in pruritus, burning, vulvovaginal oedema and erythema, dyspareunia and vaginal secretions in all diagnostic groups. At the end of the study, the microbiological evaluation was normal in 80.0% of cases with BV, 62.5% of cases with VVC and 100.0% with RVVC. The clinical data allow EPB to be recommended in the acute treatment of VVC and BV, suggesting that EPB is a useful maintenance treatment if there are recurrent episodes.

Controlled studies are needed to confirm the efficacy of EPB in the treatment of recurrences and to identify the most appropriate dosage regimen.

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INTRODUCTION

It is estimated that 70% of episodes of vaginitis in premenopausal women are caused by bacterial vaginosis (BV) or vulvovaginal candidiasis (VVC) (Anderson *et al.*, 2004). For most women, episodes of vaginitis will resolve without any difficulty or long-term sequelae. On the other hand, many women with vulvovaginal symptoms remain undiagnosed or their symptoms either fail to improve or recur after treatment. They often self-medicate with various over-the-counter and alternative medicines, and sometimes these treatments in turn exacerbate symptoms and make the overall problem worse (Nyirjesy, 2014).

BV affects approximately 30% of women and is considered the most common form of vaginitis (Anderson *et al.*, 2004). The infection can be considered a “vaginal ecological disaster” in which the vaginal microflora is characterized by a lack of the normal hydrogen peroxide-producing lactobacilli, and an overgrowth of primarily anaerobic organisms. Initially BV was thought to be caused by *G. vaginalis*, but more recent studies, using standard culture and DNA-based technologies, have shown that a broad

range of bacteria are found in vaginal samples of women affected by BV, including *Atopobium vaginae*, *Bacteroides* species, *Prevotella* species, and *Mobiluncus* species (Fredricks *et al.*, 2007).

Meanwhile, approximately 75% of women will develop symptomatic VVC at least once in their lives. Fifty percent of women will experience sporadic recurrence, and perhaps 8-10% will suffer from four or more episodes every year, which is the current definition for recurrent vulvovaginal candidiasis disease (RVVC) (Nyirjesy, 2014). Most women with recurrent disease develop a pattern in which the infection clears with antifungal therapy only to recur within a few weeks or months, usually with the same strain of yeast.

Health care professional standard treatment for BV and VVC consists of oral or intravaginal antibiotics (metronidazole or clindamycin) or azole drugs. Treatment results in symptomatic relief and negative cultures in 80%-90% of patients who complete the prescribed therapy, although these medicines are unable to spontaneously restore the normal flora, which is characterized by a high concentration of lactobacilli (Nyirjesy, 2014). Their main limitation is the inability to offer a long-term defensive barrier, thus facilitating relapses and recurrences. Furthermore, pathogen resistance to multiple medicines is a health problem which means that alternative treatments need to be developed.

The aim of this study was to assess the effectiveness of a vaginal gel containing extracts of *Thymus vulgaris* and *Eugenia caryophyllus* in conjunction with two specific lactobacilli strains (*Lactobacillus fermentum* LF10 and *Lactobacillus plantarum* LP02) specifically formulated in

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slow-release vaginal capsules, in treating BV, VVC and RVVC. The new product was investigated *in vivo* for its ability to create and maintain a vaginal microenvironment that does not encourage the establishment, propagation or persistence of these vaginal infections.

METHODS

This non-interventional, observational, multicentre, open-label study enrolled women of childbearing age who presented symptoms suggesting BV, VVC or RVVC. Exclusion criteria were pregnancy or lactation, allergy to the study medication and use of antibacterial or antimycotic therapy (topical or systemic) in the previous 10 days. Women with any sexually transmitted disease or genital skin disorder were also excluded. The study was reviewed and approved by the local Ethics Committee and women were enrolled into the study after they provided written informed consent.

Symptomatic VVC was diagnosed by Sobel Score ≥ 2 , which is a tool for measuring the severity of vulvovaginal symptoms such as pruritus, burning, erythema and oedema with semi-quantitative scores of 0 (absent), 1 (mild), 2 (moderate) or 3 (severe) (Sobel *et al.*, 2001), and by the presence of blastospores or hyphae on microscopic examination of a fresh sample. Bacterial vaginosis was diagnosed if at least two of the following Amsel's diagnostic criteria were present: whitish or greyish green homogeneous discharge on the vaginal walls, vaginal pH >4.53 , positive whiff test, and presence of clue cells on fresh wet mount microscopy of vaginal fluid.

Each participant was instructed to use a vaginal gel containing extracts of *Thymus vulgaris* and *Eugenia caryophyllus*, lactic acid, xanthan gum and ketoglutaric acid, and a capsule for vaginal use containing the probiotic *Lactobacillus fermentum* LF10 (0.5 billion), the probiotic *Lactobacillus plantarum* LP02 (0.5 billion) and the prebiotic galacto-oligosaccharides (GOS), with the load guaranteed throughout its 2-year shelf life [Estromineral Probiogel (EPB) in Italy, or Saugella Probiogel; Meda Pharma - Mylan Group]. The dosage regimen was one vaginal application of EPB in the evening for six consecutive evenings, without other specific concomitant treatments. After 2 weeks, the doctor carried out a telephone consultation and if troublesome symptoms were still present, EPB was administered twice a week for three more weeks.

Clinical and microbiological assessments took place at baseline and 20-30 days after the initial visit. The case report form recorded clinical symptoms, any adverse events

(attributable to the treatment), physical vulvovaginal examination and any microscopic examination using fresh wet mount microscopy of vaginal secretions. At the start and end of the study, clinical symptoms (pruritus, burning and dyspareunia) and objective signs (vulvovaginal oedema and erythema, pH and vaginal secretion) were evaluated. The severity of the parameters was assessed using a semi-quantitative scale from 0 to 3. The microbiological assessment consisted of microscopic examination of a fresh sample, using three categories:

- *normal vaginal microflora* with numerous pleomorphic lactobacilli, no other bacteria or clue cells or hyphae;
- *partially altered vaginal microflora* in which the proportion of lactobacilli is decreased due to increased numbers of other bacteria or clue cells or hyphae are present;
- *very altered vaginal microflora* in which lactobacilli are severely depressed or absent because of overgrowth of other bacteria or clue cells ($>20\%$) or hyphae are present (Gilbert G.G. Donders, 2007).

At the end of the treatment, a final overall assessment was provided by both the doctor (i.e. resolution of clinical features, temporary resolution with short-term recurrence or non-response) and the patient (i.e. none, poor, fair, good or excellent).

Statistical analysis was performed using the Wilcoxon test on non-parametric data, the Student's t-test on pH values and the χ^2 test with Yates' correction for comparing rates.

RESULTS

Thirty-eight centres took part and each one enrolled between four and 11 subjects, giving a total of 209 women. Out of these, 100 had BV, 82 had VVC and 27 had RVVC (recurrent VVC). Mean age was 35.8 years, mean weight was 61.6 kg and BMI was 22.8 kg/m². Diagnosis details are reported in Table 1. About 60% of candidiasis cases required a second cycle of EPB treatment compared with 36% of bacterial vaginosis cases, with a very statistically significant difference between diagnoses ($p=0.001$). Figures 1, 2 and 3 summarize the results in terms of signs and symptoms present before and after treatment. There was a statistically significant improvement in pruritus, burning, vulvovaginal oedema and erythema, dyspareunia and vaginal secretions in all diagnostic groups. Vaginal pH was significantly reduced in the BV group. At the end of the study, the microbiological evaluation was normal in 80.0% of cases with BV, 62.5% of cases with VVC and 100.0% with RVVC. The flora was partially altered in the remaining cases.

Table 1 - Patients recruited into the study.

	BV (n.=100)	VVC (n.=82)	RVVC (n.=27)	Total (n.=209)
Age (years)	37.7 \pm 11.0	34.5 \pm 8.7	31.6 \pm 7.1	35.8 \pm 10.3
Weight (kg)	62.6 \pm 14.7	60.9 \pm 9.0	59.5 \pm 12.8	61.6 \pm 12.3
BMI (kg/m ²)	23.0 \pm 5.3	22.6 \pm 3.1	22.4 \pm 4.6	22.8 \pm 4.4
Intermediate visit (days)	14.6 \pm 8.3	13.0 \pm 5.8	11.9 \pm 6.2	13.7 \pm 7.1
2 nd cycle (% cases)	36.0	57.3	63.0	44.8
Final visit (days)	32.9 \pm 11.9	29.4 \pm 11.2	32.8 \pm 7.9	32.2 \pm 11.4

BV= bacterial vaginosis. VVC= vulvovaginal candidiasis. RVVC= recurrent VVC. Data are expressed as mean \pm SD.

Figure 1 - Symptoms before and after Estromineral Probiogel treatment.

BV = bacterial vaginosis. VVC = vulvovaginal candidiasis. RVVC = recurrent VVC.

The parameters were assessed using a semi-quantitative scale from 0 to 3. Data are expressed as mean.

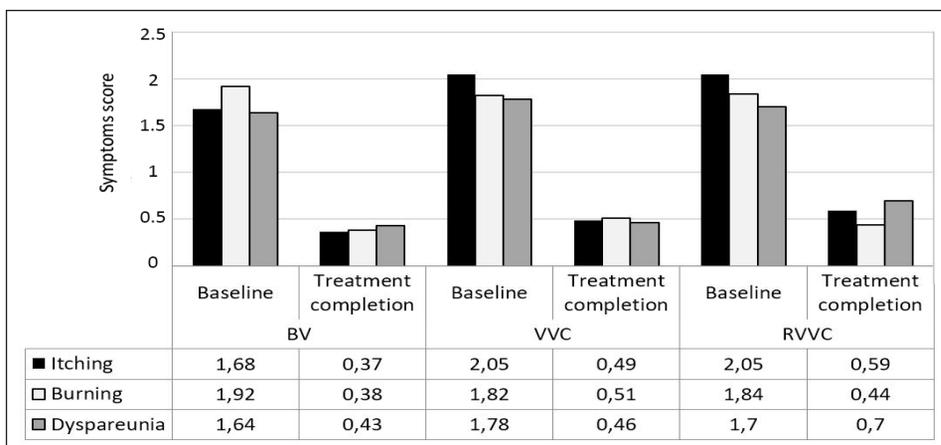


Figure 2 - Signs before and after Estromineral Probiogel treatment.

BV = bacterial vaginosis. VVC = vulvovaginal candidiasis. RVVC = recurrent VVC.

The parameters (erythema, oedema and vaginal discharge) were assessed using a semi-quantitative scale from 0 to 3. Data are expressed as mean.

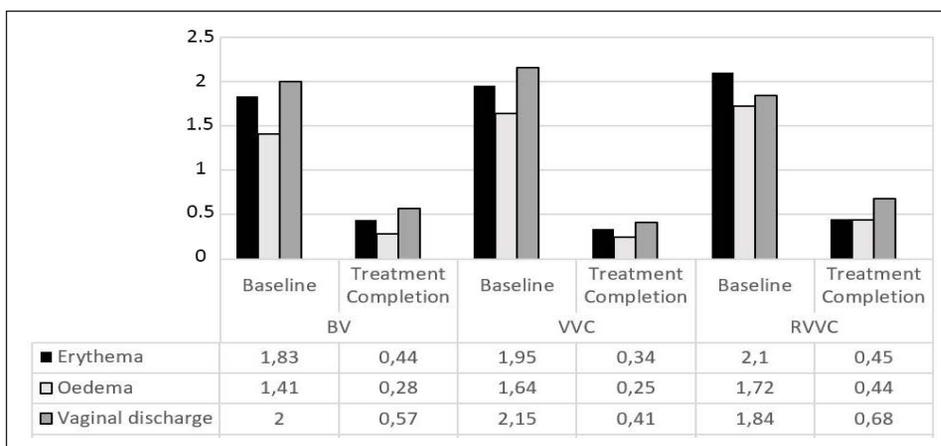
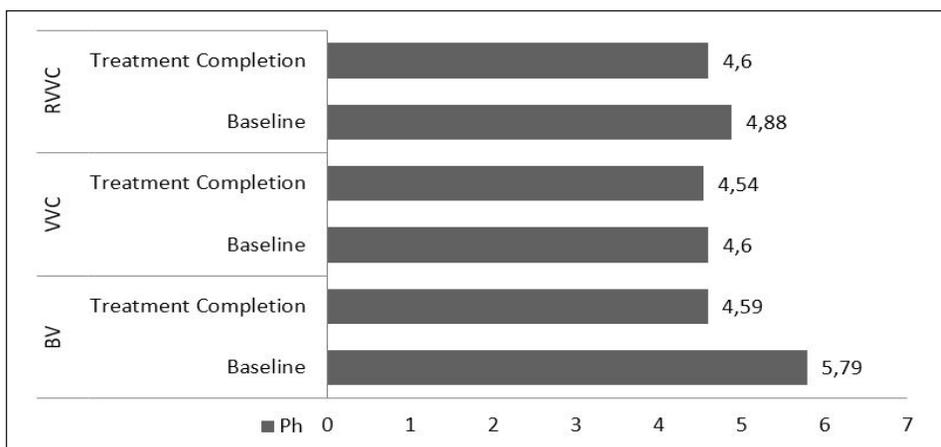


Figure 3 - Vaginal ph before and after Estromineral Probiogel treatment.

BV = bacterial vaginosis. VVC = vulvovaginal candidiasis. RVVC = recurrent VVC.

Data are expressed as mean.



The doctor's final overall assessment of the treatment indicated resolution of the clinical features in 83.9% of cases with BV, 81.6% with VVC and 63.0% with RVVC. No response was seen in 6.5% of cases with BV, 7.9% with VVC and 11.1% with RVVC. The patient's assessment was good or excellent in 84.8% of cases with BV, 85.0% with VVC and 70.3% with RVVC. The therapy was well tolerated no dropouts relating to the EPB treatment were observed throughout the study.

DISCUSSION

In terms of therapeutic efficacy, defined as clinical cure (resolution of signs and symptoms) and microbiological cure, women had cure rates of about 80% with the EPB treatment regimen. There was similar efficacy between the patients affected by BV and VVC.

The data from our study show that in most cases the product used (Estromineral Probiogel or Saugella Pro-

biogel; Meda Pharma - Mylan Group) was able to treat BV and VVC as a single therapeutic approach. The concomitant use of an antimycotic or antibacterial agent was necessary only in 2% of cases with BV and 8.5% of cases with VVC.

Antibiotics and antimycotics generally work well against bacterial and mycotic infections but the potential side effects and progressive increase in bacterial and fungal resistance has stimulated the search for innovative therapeutic approaches. This is even more important for recurrent vaginal infections as epidemiological studies reveal that almost all women diagnosed with fluconazole-resistant *C. albicans* had experienced considerable previous exposure to fluconazole (Marchaim *et al.*, 2012).

In our study, we used a novel therapy that included the use of essential oils such as *Thymus vulgaris* and *Eugenia caryophyllus*. These essential oils are well known and used for their biological, antibacterial, antifungal and antioxidant properties. Thymol, the active substance in *T. vulgaris* extract, has a pronounced, selective, antibacterial action on Gram-positive and Gram-negative bacteria, and an antifungal and anti-inflammatory action (Braga, 2005). Eugenol, the main component of *E. caryophyllus* extract, has antioxidant, anti-inflammatory, antifungal and antibacterial activity and acts synergistically with thymol to inhibit microbial growth and induce morphostructural changes in microbial membrane (Didry *et al.*, 1994). The healthy vaginal epithelium is generally dominated by lactobacilli that produce hydrogen peroxide and lactic acid, acting as a protective layer. This leads to an acidic pH, which inhibits the adhesion and growth of other bacteria, resulting in "good" protective biofilm growth (Terraf *et al.*, 2012).

Biofilms are defined as microbial communities encased in a self-synthesized extracellular polymeric matrix, growing on a biotic or abiotic surface (Ventolini, 2015). A "bad" biofilm is a strategy allowing some organisms to persist in harsh environments, and its physical removal is pivotal for eradication of the infection. Some studies have found that 90% of women with BV and 10% without BV have a complex polymicrobial biofilm, which can be demonstrated with electron microscopy of vaginal biopsies (Verstraelen *et al.*, 2013). The tendency of *Candida albicans* to develop biofilms is also clinically relevant because biofilm-associated fungal cells are much more resistant to the conventional antifungal agents, that act against their planktonic counterparts, and they resist host immune factors (Chandra *et al.* 2001).

The effects of thymol on bacterial and fungal adhesion to human vaginal epithelial cells have been investigated. Tests on *Candida albicans* showed that thymol interferes with the starting phases of biofilm production as well as with mature *Candida albicans* biofilms (Braga *et al.*, 2008). Braga *et al.* have also studied the effect of thyme essential oil on *Gardnerella vaginalis* biofilm (Braga *et al.*, 2008). In this study, thymol, with its small molecule, was shown to interact with the lipid bilayer of cell membranes, thus affecting their structure and surface electrostatics and generating asymmetric membrane tension and leading to a loss of integrity and the leakage of ions, ATP and nucleic acid.

According to our results, it is conceivable that EPB may interfere with pathogen biofilm formation following the drastic reduction of bacterial and fungal concentrations induced by *Thymus vulgaris* and *Eugenia caryophyllus*.

It may then maintain the "positive" biofilm through the concomitant use of the lactobacilli. In fact, the vaginal gel used in the study, in addition to *Thymus vulgaris* and *Eugenia caryophyllus* extracts, contains the probiotic *Lactobacillus fermentum* LF10 and the probiotic *Lactobacillus plantarum* LP02.

Conventional antibiotic therapy produces holes in the microbial biofilm but is not able to eradicate the microorganisms. Thymol and eugenol can easily penetrate the microbial biofilm and facilitate the action of the antibiotic therapy. Lactobacilli, in turn, can interfere with an abnormal vaginal microbiota, strengthening the rationale for a combination of probiotics and antimicrobials for better eradication of pathogenic biofilms (McMillan *et al.*, 2011). Thymol, eugenol, *Lactobacillus fermentum* and *Lactobacillus plantarum* have a complementary action on the microbial biofilm. The natural essential oil extracts alter its morphological structure and function, while the two species of lactobacilli in turn produce a biofilm that prevents the formation of the pathogenic biofilm.

EPB also contains a prebiotic component, galacto-oligosaccharides (GOS), which are the dietary source of lactobacilli. They facilitate the growth and activity of lactobacilli physiologically present in the vaginal flora in an environment low in sources of carbohydrate. The majority of yeasts, including *Candida albicans*, are unable to utilize GOS as an energy source, thereby allowing selective multiplication of the lactobacilli at the expense of the pathogenic species (Di Bartolomeo *et al.*, 2013).

Our study has certain limitations that must be acknowledged, such as the lack of randomization and the open-label nature of the trial. Therefore, a placebo effect cannot be ruled out. Moreover, the sample size for RVVC is small (23 patients). However, a large number of women are well aware of the symptoms and signs of BV and VVC infections after treatment with EPB, and results are consistent across the centres where patients were examined.

In conclusion, the presented clinical data allow EPB to be recommended in the acute treatment of VVC and BV. The pharmacological and microbiological characteristics of EPB suggest that this medical device may be a useful maintenance treatment if there are recurrent episodes. Controlled studies are now needed to confirm the efficacy of EPB in the treatment of recurrences and to identify the most appropriate dosage regimen.

Declaration of interest

F. Murina and F. Vicariotto have received lecture fees from Mylan. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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