ABSTRACT: To evaluate the efficacy of Lactobacillus plantarum P17630 as vaginal probiotic in preventing recurrence of Candida vulvovaginitis, an observational, multicentre, prospective study on women aged 18-45, with symptomatic non-complicated vulvovaginal candidiasis (VVC), has been conducted: 476 patients were available for analysis: 252 in the study group, i.e. oral fluconazole 150 mg "one-shot" treatment + soft-gel vaginal capsules containing Lactobacillus plantarum P17630, following the menstrual periods; 224 in the control group (fluconazole treatment alone). Clinical data were compared at baseline (T0), after 4 weeks (T4W), and after 4 months (T4M). Statistical analysis was performed using Student's t test, Wilcoxon's test and chi-square test. At T4W the proportion of asymptomatic patients was significantly higher in the lactobacilli group than in the control group (82.9% vs. 71.9%, p=0.003). At T4M follow-up VVC relapse was registered only in 22 cases out of 252 in case group, while 59 out of 224 in control group showed recurrence: OR= 3.74 (2.20-6.34 95% C.I.) and p<0.001. This study suggests that the addition of vaginal Lactobacillus plantarum P17630 to oral fluconazole may enhance therapeutic efficacy in terms of both treatment and prevention of episodes of vulvovaginal candidiasis during a short-term follow-up.

KEY WORDS: Lactobacillus plantarum P17630, Prevention; Recurrence, Soft-gel vaginal capsules, Vulvo-vaginal candidiasis

INTRODUCTION

Vulvovaginitis is a common disorder that affects about 20% of women every year, the frequency of the first diagnosis increases rapidly after the age of 17 and 50% of women have experienced at least one episode by the age of 25 (Geiger et al., 1995). One of the most common causes of vulvovaginitis is vulvovaginal candidiasis (VVC); three-fourths of all women experience at least one episode of VVC in their lifetime; about one half of these women will experience a relapse, while approximately 5% of women with VVC develop a "recurrent vulvo-vaginal candidiasis" (RVVC), which is defined as ≥ 4 episodes of VVC that occur within a 12-month period (Sobel, 2002; Mitchell, 2004). Thus, millions of women suffer from recurrent vulvovaginitis (Sobel, 1985; Spinillo et al., 1993; Foxman et al., 1998) and frequent episodes result in considerable suffering, consumption of healthcare resources, and frequent gynecological examinations: associated symptoms and signs include pruritis, burning, soreness, abnormal vaginal discharge, dyspareunia, vaginal and vulvar erythema and edema, which may cause strain in sexual and marital relations (Foxman et al., 2000).

Diabetes mellitus, immunodeficiency (cell-mediated immunity) associated with subnormal T-lymphocyte response to Candida, frequent antibiotic use associated with decrease in protective vaginal flora, use of spermicidal jellies and creams that alter vaginal flora, use of oral contraceptives, mechanical irritation of the vulvovaginal area (tightly fitted clothing reducing ventilation and stimulating perspiration with increase in local temperature and moisture) are all risk factors for recurrent vulvovaginal candidiasis (Ringdahl, 2000).

Prevention of recurrences of vulvovaginal candidiasis consists in the administration of intermittent maintenance azole treatment. However, insufficient evidence is available to recommend it as standard prophylactic therapy (Ringdahl, 2000; Marrazo, 2002). In a recent prospective cohort study on women receiving maintenance antifungal...
therapy Patel and coll. found that significant risk factors for RVVC were younger age (<40), history of bacterial vaginosis, and behavioural factors, such as wearing pantyliners or pantyhose, consuming cranberry juice or acidophilus-containing products (Patel et al., 2004).

An important alteration of the vaginal flora that promotes recurrent infections is the reduction in lactobacilli (Marelli et al., 2004). Lactobacilli, which are the main component of the vaginal microflora, represent a natural defence against infections via a number of activities: reduction of pH following production of organic acid (mainly lactic acid), production of hydrogen peroxide, competition with pathogens for adherence to vaginal epithelium (Boris and Barbés, 2000). There are a number of lactobacilli species, which differ in their ability to adhere to various kinds of mucosal cells and in other properties. Ideally, besides possessing the abilities described above, strains for protection adhere to various kinds of mucosal cells and in other properties. Ideally, besides possessing the abilities described above, strains for protection against recurrence of vulvovaginal candidiasis, should also be able to produce biofilm, which is a robust defence against pathogen adhesion to the vaginal wall, and be resistant to antibiotic treatment and to changes in local conditions (Marelli et al., 2004).

*Lactobacillus plantarum* P17630 is a selected and patented strain isolated from healthy vagina and deposited with code "P 17630" at LMG-Culture (Laboratorium voor Microbiologie) in Gent, Belgium, organization appointed for preservation of patented strains, in accordance with Treaty of Budapest. *Lactobacillus plantarum* P17630 has proved to be able to thrive in a broad range of environments differing in pH and temperature and to be one of the few lactobacillus strains that are resistant to many commonly use antibiotics, including vancomycin and a number of azoles (Dho et al., 2003). Moreover, it is able to produce biofilm and has been shown to adhere well to human vaginal cells (Bonetti et al., 2003), interfering with the adhesion of *Candida albicans* in a competitive manner (Culici et al., 2004).

The aim of this study was to test the efficacy of *Lactobacillus plantarum* P17630 vaginal capsules, in combination with oral fluconazole treatment, for prevention of recurrence of vulvovaginal candidiasis.

**METHODS**

An observational, multicentre, prospective study has been conducted, during 2003, involving 17 gynecological outpatient clinics in Italy: Venice, Modena, Bologna, Rome (3 centres), Velletri, Latina, Benevento, Trani, Bari (4 centres), Altamura, Putignano, Catania.

Women aged from 18 to 45 years, suffering from symptomatic vulvovaginal candidiasis (VVC), were recruited, whenever they did not meet any of the following exclusion criteria: previous episode of vulvovaginal candidiasis in the last 6 months, pregnancy or lactation, HIV seropositivity, type I diabetes mellitus, concomitant infection due to Chlamydia or Gonococcus with ongoing therapy, concomitant cancer chemotherapy, systemic antibiotics or immunosuppressant therapy. All women gave their written informed consent regarding collection of personal data and aims of the study.

Out of the total observed women, 476 completed the trial: 252 in the study group and 224 in the control group. For all observed patients the standard treatment, for a non-complicated Candida vaginitis, has been oral fluconazole 150 mg cphs “one-shot” therapy. Moreover, the study group (252 pts) received also a soft-gel capsule containing *Lactobacillus plantarum* P17630, each containing 10^8 unit CFU, intravaginally every evening for a period of 6 consecutive days at the end of the menstrual period for three consecutive months. The control group (224 pts.) did not receive any additional therapy besides fluconazole. The same fluconazole therapy was administered to symptomatic male partners, while no therapy was indicated for asymptomatic ones, according to the European STD guidelines (Sherrard, 2001).

A patient history was collected at baseline, including age of first sexual intercourse, parity, number of sexual partners, previous antifungal treatment, and contraceptive method adopted. The investigator collected medical history and clinical data related to vulvovaginal infection, noting the severity of signs and symptoms, such as itching, burning, dyspareunia, leukorrhea, erythema, oedema, according to a semiquantitative rating scale: 0 = absent, 1 = mild, 2 = moderate, 3 = severe. In addition, the investigator noted whether "fishy odor" was present or absent, measured vaginal pH (which was considered normal < 4.5) and collected a specimen of vaginal secretions for a wet-smear examination with phase-contrast microscopy, evidencing spores and/or pseudohyphae of Candida and other recognizable vaginal pathogens, and expressing the “Lactobacillary grade” (LBG) of vaginal ecosystem, according to Donders and coll. (Donders et al., 2000). For homogeneous evaluation also LBG has been converted into the same semiquantitative rating scale from 0 to 3: 0 = grade I, 1 = grade IIa, 2 = grade IIb, 3 = grade III.

Patients were controlled twice, after 4 weeks (T4W) and after 4 months (T4M), when the investigator re-evaluated the same clinical and bacteriological parameters of the first visit. If clinical evidence of VVC was found at 4 weeks follow-up, oral fluconazole treatment was repeated, in both lactobacilli and control group patients, and these subjects were not excluded from the study.

Tolerability and safety were evaluated by putting a non leading question to the patient to ascertain whether any adverse events had occurred; if any had occurred, additional information was to be collected, i.e. its time of onset, nature, duration, outcome, relation to treatment, severity and any action taken.

The changes in the severity of signs and symptoms, vaginal pH and bacteriological evaluation were assessed at 4 weeks (T4W) and 4 months (T4M) vs. baseline (T0).

**Statistical analysis**

The analysis was performed using Student's t-test, Wilcoxon's test and the chi-square test, as appropriate according to the nature of the data. The tests were two-sided and the significance level was set at p = 0.01. The analysis of adverse event was to be performed comparing their frequency and type, setting the significance level at p=0.10.

**RESULTS**

**Patient population**

There were no important differences at baseline (T0) between the test group and the control group (see Table 1), except in terms of mean age at first intercourse, which was lower on average by 1 year in the control group (p=0.0006).
Results of Cultures

In our study, based on a clinical setting, diagnosis of VVC has been made by symptoms, signs and microscopic examination of vaginal fluid (wet-smear), according to European guidelines, and culture has not been assumed as gold standard. Nevertheless, to avoid bias due to different Candida species between cases and controls, the available cultures have been considered and compared in term of albicans vs. non-albicans species (see Table 1) and no significant difference between groups has been found: 147 cultures were available at baseline (T0), 69 out of 82 (84.1%) in case group and 56 out of 65 (86.2%) in control group were with Candida albicans, the remaining were non-albicans species (Fisher’s exact test two-sided p value = 0.8183 considered not significant). Maffei and coll. have demonstrated by genetic analysis that recurrent vaginal candidiasis is caused by the persistence of a single yeast genotype that undergoes morphological and behavioral changes in the presence of antifungal agents due to the selective pressure to which it is submitted (Maffei et al., 1997). Considering the predominant role of C. albicans both in sporadic and recurrent vaginitis, with the homogeneity of Candida species in both groups, and the short time of follow-up studied, it can be excluded in the present study that the variability of Candida species in recurrent vaginitis could have been a confounding factor.
Efficacy
After 4 weeks (T4W) the proportion of asymptomatic patients was significantly higher in the lactobacilli group than in the control group (82.9% vs. 71.9% p=0.003).

After 4 months (T4M) the proportion of asymptomatic patients continued to be significantly higher in the lactobacilli group (91.3% vs. 73.7% p=0.001). The mean value of the rating scale (0-3) in reporting each sign/symptom was recorded in both treatment groups (Figure 1). Table 2 reports the statistical analysis of each variable, using Wilcoxon Two-Sample (rank sums) test, two-sided p value significance level set at 0.01. For the Fishy odor test, classified as “positive”, chi-square statistics were made by Fisher’s exact test, two-sided p value significance level set at 0.01.

The reduction of severity of signs and symptoms was evident after 4 weeks and persisted in both groups after 4 months, but the difference between the two treatment groups became significant in terms of all the signs and symptoms taken into consideration. Moreover, normalization of vaginal pH was achieved in a significantly greater proportion of patients: 75% vs. 56.7%, p<0.01 (Figure 2).

Prevention of recurrence
Clinical relapse of vulvovaginitis has been defined by the presence of symptoms and signs and the wet-smear examination of vaginal fluid, according to the methods used for initial diagnosis and following the European STD Guidelines (Sherrard, 2001) (see Figure 3). After 4 weeks (T4W) 43/252 in case group and 63/224 in control group showed clinical recurrence, with OR= 1.90 (1.23 to 2.94 at 95% C.I.) and p=0.0038 (Cochran-Mantel-Haenszel). Due to short time follow-up, this event may be considered as treatment failure: in fact all patients with relapse repeated fluconazole treatment, but were not excluded from the study.

At 4 months follow-up VVC relapse was registered only in 22 cases out of 252 in case group (8.7%), while 59 out of 224 (26.3%) in control group showed recurrence: OR= 3.74 (2.20 to 6.34 at 95% C.I.) and p<0.0001 (Cochran-Mantel-Haenszel).
research since then has shown that certain lactobacillus Canadian urologist Andrew Bruce in the early 1970s. Extensive microbiota as a barrier to prevent infection was first conceived by by producing antimicrobial compounds (Boris and Barbés, with other microorganisms for adherence to epithelial cells and to contribute to the control of vaginal microbiota by competing to infection is of considerable interest. These organisms are believed D

Safety and tolerability
No adverse events worthy of note were reported.

DISCUSSION
The role of lactobacilli in the female urogenital tract as a barrier to infection is of considerable interest. These organisms are believed to contribute to the control of vaginal microbiota by competing with other microorganisms for adherence to epithelial cells and by producing antimicrobial compounds (Boris and Barbés, 2000).

The concept of restoring the lactobacilli content of the vaginal microbiota as a barrier to prevent infection was first conceived by Canadian urologist Andrew Bruce in the early 1970s. Extensive research since then has shown that certain lactobacillus strains are able to colonize the vagina following vaginal suppository use and reduce the risk of urinary tract infection, yeast vaginitis, and bacterial vaginosis (Reid et al., 2004).

To assess the efficacy of lactobacilli as vaginal probiotics, there are some characteristics that must be verified: capability to adhere to vaginal cells and, thus, to displace well-known vaginal pathogens (Boris et al., 1998), capability to persist and colonize the vaginal environment, self-aggregating and forming a well equilibrated vaginal flora, production of bactericidal compounds including organic acid, which lowers the vaginal pH, hydrogen peroxide, bacteriocin-like substances and possibly biosurfactants, inhibiting the growth in vitro of pathogens, such as Escherichia coli and Streptococcus agalactiae (Boris and Barbés, 2000). After vaginal instillation, some strains of lactobacilli have demonstrated to persist up to 21 days, even in postmenopausal women, as proven by means of PCR-denaturing gradient gel electrophoresis (DGGE) and sequencing of the V2-V3 region of the rRNA gene (Burton et al., 2003).

In terms of bacterial vaginosis, recent studies have shown that oral or vaginal intake of lactobacilli can prevent overgrowth of anaerobic organisms combined with a loss of the protective resident vaginal flora normally found in the healthy vagina (Reid and Bruce, 2003). Nevertheless, for yeast vaginitis very few lactobacilli are able to kill or inhibit adhesion to vaginal cells; at


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<th>Presence of clue-cells(wet smear)</th>
<th>Fisher’s exact test</th>
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<tr>
<td><strong>T0</strong> Cases Controls</td>
<td>OR = 1.27 (0.81-1.98)</td>
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<td>In VVC relapses:</td>
<td>p = 0.31</td>
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<tr>
<td><strong>T4W</strong> Cases Controls</td>
<td>OR = 0.53 (0.21-1.35)</td>
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<td>p = 0.26</td>
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<tr>
<td><strong>T4M</strong> Cases Controls</td>
<td>OR = 0.62 (0.16-2.40)</td>
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<td>p = 0.75</td>
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Another larger trial has been published on the use of lactobacilli in the prevention of vulvovaginal candidiasis. It was a randomised, placebo-controlled, double-blind clinical trial with a factorial 2x2 design (Lactobacillus rhamnosus and Bidifobacterium longum by oral route; oral placebo; Lactobacillus rhamnosus, delbrueckii and acidophilus in a vaginal pessary; placebo pessary) in the prevention of episodes of vulvovaginal candidiasis in 235 women after antibiotic treatment (Pirota et al., 2004). Study treatment was given for 6 days during antibiotic treatment and for 4 days afterwards. A total of 55 women developed post-antibiotic vulvovaginitis. Results were negative: among the women taking oral lactobacilli, the vulvovaginitis rate was 24% both in the group taking also vaginal lactobacilli and in those taking vaginal placebo, whereas among the women allocated to oral placebo, the vulvovaginitis rate was 29% in the group taking vaginal lactobacilli and 17% in the group taking placebo i.e. both placebos. These results are not relevant for the interpretation of our study for two
reasons: one, post-antibiotic vulvovaginal candidiasis is a particular form of the infection that may differ from our patient population with different selection criteria; second, different strains of lactobacilli may have different properties and exhibit different degrees of susceptibility to antibiotic treatment, so results related to other lactobacilli cannot be extrapolated to Lactobacillus plantarum (Dho et al., 2003; Marelli et al., 2004).

Falagas and coll. in a recent review on the use of probiotics to prevent vulvovaginal candidiasis report that most of the relevant clinical trials on the effectiveness of lactobacilli in preventing the colonization and infection of the vagina by C. albicans have methodological problems such as small sample size, no control group (placebo) and include women without confirmed recurrent VVC, and they are not reliable for drawing definitive conclusions, and therefore, the available evidence for the use of probiotics for prevention of recurrent VVC is limited. Thus, they conclude that, despite the promising results of some studies, further research is needed to prove the effectiveness of probiotics in preventing the recurrences of VVC and to allow their wide use for this indication (Falagas et al., 2006). Our study tried to address some of these bias, including in the analysis a large number of subjects, by a multicentre setting, and considering a control group. Patients included were affected by a first-episode uncomplicated VVC, to assess the efficacy of the probiotic in preventing relapse of vaginal colonization and infection of yeast, minimizing the influence of other pathogenetic characteristics that are usually linked to patient with already confirmed recurrent candidosis, such as immunological factors andazole-resistant strains.

In conclusion, our experience suggests that the addition of vaginal Lactobacillus plantarum P17630 to oral fluconazole may enhance therapeutic efficacy in terms of both treatment and prevention of episodes of vulvovaginal candidiasis, at least considering a short-term follow-up.

Further work is warranted to demonstrate its efficacy in the long-term prevention of Candida recurrences. Nevertheless, it may be assumed that, when Lactobacillus plantarum is introduced vaginally, there is an impact on the subject's microflora: if this is dominated by yeast, Gram negative coliforms and anaerobes, or vaginally, there is an impact on the subject's microflora: if this is dominated by yeast, Gram negative coliforms and anaerobes, or Gram positive cocci, then the outcome might significantly benefit the patient.

CONFLICT-OF-INTEREST STATEMENT
Authors have no conflict of interest and there are no financial or personal relationships that could inappropriately influence the author’s manuscript preparation and submission.

REFERENCES


