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Effectiveness of vaginal administration of Lactobacillus rhamnosus following conventional metronidazole therapy: how to lower the rate of bacterial vaginosis recurrences

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SUMMARY

Eighty-four patients with bacterial vaginosis diagnosed according to Amsel's criteria were randomized to receive either oral metronidazole 500 mg twice a day for seven days, or one vaginal tablet containing freeze-dried *Lactobacillus rhamnosus* once a week at bedtime for two months starting one week after the last antibiotic administration. Follow-up was performed at days 30, 90 and 180. Chi-squared analysis showed a significant difference between the two treatment groups at day 90 (P=0.05). Safe and effective long-term vaginal administration of *Lactobacillus rhamnosus* appears to be a useful complementary approach in the management of bacterial vaginosis.

KEY WORDS: Vaginosis, Lactobacilli, Probiotics

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In healthy women, the vaginal environment is a balanced ecosystem characterized by various species of *Lactobacillus*. These bacteria inhibit the growth of other microorganisms through different mechanisms including production of organic acids such as lactic acid and other antimicrobial substances (hydrogen peroxide and bacteriocins), competition for mannose and glycoprotein receptors and adhesion to the epithelium (Reid and Burton, 2002).

The depletion of vaginal *Lactobacilli* is known to be associated with bacterial vaginosis (BV) (Spiegel 1991; Wang, 2000), a disorder represented by a complex change in vaginal flora and characterized by a reduction in the prevalence and concentration of *Lactobacilli* and an increase in some vaginal anaerobic or facultative anaerobic bacteria, accompanied by loss of the usual vaginal acidity (Hay, 2005).

Since a pool of pathogens is involved and classical signs of inflammation are absent, the older term "Gardnerella vaginitis" was definitively replaced by the new one " bacterial vaginosis" more than twenty years ago (Holmes *et al.,* 1981; Blackwell *et al.,* 1983).

The aetiology of BV is probably multifactorial and the factor initiating the shift is still unknown (Donders *et al.,* 2000).

A body of evidence indicates that a dysregulation in vaginal microecology of lactic acid producing bacteria and an impaired mucosal vaginal immune response play a crucial role in the pathogenesis of the disorder (Cauci *et al.,* 1997). BV is not regarded as a sexually transmitted disease

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(STD), though it is *sexually associated* (Larsson *et al.,* 1991).
In women of reproductive age the prevalence of BV varies between 9% and 29% according to the epidemiologic characteristics of the population subgroups enrolled in the studies conducted worldwide (Lamont *et al.* 2000; Allsworth *et al.* 2007; Evans *et al.* 2007). BV seems to be more common in women with STDs and in those who have recently changed sex partner, but it has been reported even in virgin women. Furthermore, in many studies it is associated with smoking, black race and use of an intrauterine device (IUD) (Schmid 1999; Calzolari *et al.,* 2000).
Some differences in the prevalence of BV may also be explained by the use of different diagnostic criteria.
Furthermore, in about 50% of cases BV appears to be asymptomatic. Although it was previously regarded as a harmless condition, studies in the past decade have highlighted the association of BV with various gynaecologic and obstetric pathologies such as pelvic inflammatory disease, increased risk of infection after gynaecologic surgery, human papilloma virus (HPV) infection and progression of squamous intraepithelial lesion (SIL), chorioamnionitis, postpartum and postabortion endometritis, preterm labour and delivery and spontaneous abortion. BV may also be associated with an increased risk for human immunodeficiency virus (HIV) transmission between adults (Taha *et al.,* 1998; Rauth *et al.,* 2000; Morris *et al.,* 2001; Romero *et al.,* 2002; Beverly *et al.,* 2005; Tolosa *et al.,* 2006).
The social, clinical and economic implications of adverse outcomes associated with BV have led to increased attention to diagnosis and treatment.
Attempts to correct the imbalance in vaginal microecology only with the use of antibiotics have been substantially unsuccessful, especially in the management of recurrences, while recent advances in our understanding of microbiological, immune and metabolic alterations in the vaginal microenvironment of women with BV have raised further interest in the therapeutic use of probiotics (Famularo *et al.,* 2001).
The concept of probiotics dates back more than 100 years and treatment of vaginitis and vaginosis with *Lactobacillus* replacement therapy was first described in the USA in 1933 by Molher and Brown. Since then the attention of the investigators has focused on the role that *probiotics* may play in the treatment and prevention of various urogenital infections (Butler and Beakley, 1960; Reid *et al.,* 1990; Bruce *et al.,* 1992). One current trend is to add or even prefer "natural" products to the usual therapy: the Food and Agriculture Organization of the United Nations and the World Health Organization Working Group recently defined probiotics as *"live microorganisms which when administered in adequate amounts confer a health benefit on the host"* so developing guidelines for what constitutes a true *probiotic.* (FAO/ WHO 2001; FAO/WHO 2002).
According to these criteria *Lactobacillus rhamnosus* can be considered a probiotic. Open clinical studies showed vaginal colonization and beneficial effect on the host following oral intake or vaginal application of the probiotic. *Lactobacillus rhamnosus* was found to be one of the most effective strains with regard to adhesion to squamous and uroepithelial cells, competitive exclusion of pathogens and production of inhibitors of urogenital pathogen growth (Reid *et al.,* 1994; Mc Lean and Rosenstein, 2000; Reid and Burton, 2002; Gardiner *et al.,* 2002).
Various clinical studies seem to provide evidence that in many cases the use of selected *Lactobacillus* strains can effectively recolonize the vagina and lower the rate of BV recurrences, (Rossi *et al.,* 1999; Cadieux *et al.,* 2002; Rossi *et al.,* 2003; Marcone *et al.,* 2003; Anukam *et al.,* 2006).
We carried out a prospective randomized controlled trial to test the effectiveness of vaginal application of *Lactobacillus rhamnosus* in improving the long-term outcome of BV treatment with conventional metronidazole therapy.
We conducted our study at "La Sapienza" University of Rome, Institute of Gynecology, Perinatology and Child Health - Gynecologic Infection and Cervicovaginal Pathology Unit starting in 2004.
Eligible patients were women of childbearing age with a diagnosis of BV defined as meeting all of Amsel's criteria (>20% clue cells, off-white thin homogeneous vaginal discharge, vaginal pH >4.5 and a positive " whiff test") (Amsel *et al*., 1983).
The exclusion criteria were urogenital infections not falling in the above diagnostic criteria or an STD, pregnancy, subjects younger than 18 or older than 40 years, smoking, contraception other than natural methods.

TABLE 1

Therapy day 0	*Healing follow-up day 30*	*Healing follow-up day 90*	*Healing follow-up day 180*
42 Oral Metronidazole	34 81%	30 71%	28 67%
42 Oral metronidazole followed by vaginal lactobacillus rhamnosus	37 88%	37 88%	35 83%
	P=0.36	P=0.05	P=0.07

Eighty-four subjects fulfilling the entry criteria were treated either with oral metronidazole (Group A, n=42) or with oral metronidazole and vaginal *Lactobacillus rhamnosus* (Group B, n=42) following a randomization scheme. Study subjects were not blinded to the treatment they received. Group A received oral metronidazole 500 mg twice a day for seven days according to CDC guidelines. In Group B the treatment with metronidazole was followed by vaginal application (one tablet containing 40 mg , i.e. >40000 CFU) of freeze-dried *Lactobacillus rhamnosus* once a week at bedtime for two months starting one week after the last antibiotic administration. In addition to the study entry criteria, all patients included in statistical analyses met the following requirements:

1) had no antimicrobial therapy for conditions other than BV during the study;
2) started study medication within 48 hours of the baseline visit.

At days 30, 90 and 180, clinical assessment, pH measurement and KOH testing of each patient subject were carried out by a single physician who also collected vaginal swabs. Microscopic evaluation of wet mount was carried out blindly by a skilled physician. Chi square test was applied for the statistical comparison of the outcome in the two groups.

All patients enrolled in this study returned for follow-up visits scheduled 30, 90 and 180 days after the end of metronidazole treatment. At the first reexamination 37 (88%) patients in group B showed a complete remission and none of them had a recurrence at 90 day follow-up.

After 180 days 35 (83%) patients in group B still had a balanced vaginal ecosystem while just 28 (67%) patients in group A had not been treated for recurrences and still had a satisfactory assessment (Table 1).

Chi-squared analyses showed a significant difference between the two treatment groups at day 90 (P=0.05).

The results obtained in the present trial appear to confirm that CDC-recommended therapy of BV with metronidazole was effective in healing BV, and that subsequent vaginal administration of *Lactobacillus rhamnosus* further increased the rate of therapeutic success and prolonged the period without BV relapses. Interestingly, the significant (*P*=0.05) protective effect of *Lactobacillus rhamnosus* vaginal application persisted for at least 30 days after the end of treatment with the probiotic (follow-up day 90). Consistently, a higher percentage of treated patients showed no BV recurrencies at follow-up day 180 compared to controls.

Although the difference was not statistically significant (*P*=0.07), this indicates that vaginal colonization by *Lactobacillus rhamnosus* greatly outlasts the supplementation of probiotic and suggests that treatment with *Lactobacillus rhamnosus* for longer than two months after metronidazole therapy could result in better control of BV recurrencies in patients at risk for BV relapse.

Our data show that vaginal application of *Lactobacillus rhamnosus* significantly prolongs the effectiveness of antibiotic treatment in preventing BV recurrence.

Therefore longer administration of probiotic may be useful for long-term control of BV relapses after conventional therapy with metronidazole. This safe and effective complementary treatment may represent a valuable approach in the management of BV for maintenance of healthy vaginal ecosystem.

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