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Efficacy of vaginal preparation containing Lactobacillus acidophilus, lactic acid and deodorized garlic extract in treatment and prevention of symptomatic bacterial vaginitis: result from a single-arm pilot study

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ABSTRACT

Several studies indicate that the re-establishment of a physiological ecosystem by the re-colonization of vaginal mucosa with lactobacilli is an effective strategy for treating and preventing vaginal dysbiosis. We aimed to analyse the effects of a vaginal probiotic preparation containing Lactobacillus acidophilus, lactic acid and deodorized garlic extract and its ability to restore physiological conditions in women with symptomatic vaginitis. We conducted a uncontrolled clinical trial on 60 heathy and regularly menstruated women aged 18 to 45 with symptomatic bacterial vaginitis and a positive history for recurrences. All enrolled women were directed to apply a vaginal tablet containing a probiotic preparation of Lactobacillus acidophilus LA14, lactic acid and deodorized garlic extract according to the following schedule: 1 tablet/day for 14 consecutive nights. Overall a significant restoration ($p<0.05$) of the eubiosis condition was observed when data detected at baseline (T0) were compared with those detected 14 days after the start of treatment (T1) and 4 weeks after the end of treatment (T2). In only 9 cases (15%) a clinical and microbiological diagnosis of vaginitis was confirmed at T2. The relapse occurred in only 2 cases (3%). Vaginally administered probiotic preparation containing Lactobacillus acidophilus, lactic acid and deodorized garlic extract is safe and effective in treatment and prevention of symptomatic bacterial vaginitis.

Key words: vaginal dysbiosis, vaginal microbiota, vaginitis, Lactobacillus, probiotics.

SOMMARIO

Il mantenimento di un normale microbiota vaginale, dato dalla presenza qualitativa e quantitativa di un adeguato numero di lattobacilli, rappresenta un'efficace strategia terapeutica per il trattamento e la prevenzione delle disbiosi vaginali.

Obiettivo di questo studio è quello di valutare l'effetto della somministrazione di un probiotico vaginale contenente lattobacilli acidophilus LA14, acido lattico ed estratto deodorizzato di aglio, nel ristabilire il regolare microbiota vaginale in donne con vaginite sintomatica. Abbiamo condotto uno studio prospettico su una corte di 60 donne sane, normalmente mestruate, con età compresa tra 18 e 45 anni e diagnosi di vaginite batterica recidivante. A tutte è stato somministrato il probiotico sotto forma di compresse vaginali con posologia di 1 compressa al giorno per 14 giorni. I risultati evidenziano un significativo ripristino della condizione di eubiosi vaginale ($p<0.05$) dopo il trattamento.

La diagnosi clinica e microbiologica di vaginite è stata confermata alla fine del trattamento in soli 9 casi (15%) mentre la recidiva si è verificata in soli 2 casi (3%). In conclusione, l'utilizzo di prodotti vaginali a base di probiotici contenente lattobacilli acidophilus LA14, acido lattico ed estratto deodorizzato di aglio sono considerati sicuri ed efficaci nel trattamento e prevenzione delle vaginiti batteriche sintomatiche.

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INTRODUCTION

The human vaginal environment is a complex ecosystem where, under physiological conditions, a balance is established between the host and both fungal and bacterial microorganisms, which normally coexist in a tightly regulated manner [1]. In this mutualistic relationship, the host provides benefit to the microbial communities in the form of nutrients needed to support bacterial growth, and the vaginal microbiota plays a protective role in preventing colonization by potential pathogens. Nevertheless, under certain circumstances this passive coexistence can turn into a pathologic state, followed by symptomatic disease [2,3].

Healthy vaginal microbiota is generally dominated by Lactobacillus spp, especially Lactobacillus crispatus, Lactobacillus jensenii, Lactobacillus iners and Lactobacillus gasseri that account for the 95% of the vaginal bacterial flora [4-10]. These microorganisms act by different mechanisms such as the production of lactic acid, resulting in a low pH (3.5–4.5) [11,12]; enhancement on the host's innate immune system [13]; competition with uropathogens in adhering to epithelial cells [14,15] and the active production of antimicrobial compounds, including target-specific bacteriocins [16,17] and hydrogen peroxide (H2O2) [18]. However, the composition of the vaginal ecosystem is not static and several physiological (i.e. age, hormonal state) or pathological factors, are able to induce qualitative/quantitative modifications that increase the sensitivity for mycotic, protozoan or bacterial vaginal infections [19-26]. From a microbiological standpoint, vaginal dysbiosis is characterized by the depletion of Lactobacillus spp. and increased microbial diversity [27,28].

In bacterial vaginosis (BV) [29,30], a substantial reduction in vaginal lactobacilli together with an increasing in a wide variety of anaerobe commonly occur. Clinical consequences include impairment of vaginal pH of 4.5–7.0 and vaginal discharge with a "fishy" odour as consequence of the high trimethylamine levels arising from the degradation of carnitine and choline, produced by many BV-associated bacteria, including Gardnerella, Atopobium, Bacteroides, Mobiluncus, Prevotella, Peptostreptococcus, and Mycoplasma hominis [31-36]. Donders et al. [37] have identified another clinical entity defined with the term "aerobic vaginitis" (AV) on the basis of bacterial, immunologic and clinical characteristics. AV differs from BV because of the dominance of aerobic bacteria from the rectal reservoir (especially Escherichia coli and Streptococcus Agalactiae) [19,38,39].

Several antibiotics agents such as metronidazole (classically targeted to infection by anaerobic microorganisms) and clindamycin (also including good activity against Gram-positive microorganisms) are commonly employed in clinical practice for treatment of gynaecological conditions. However, it is apparent how the use of conventional antibiotics therapy not only often results aspecific and therefore, not long-term resolutive, but also it could cause further imbalances within a complex ecosystem and determining a shift from one pathologic state to another [40-44]. These evidences, together with the susceptibility of lactobacilli to clindamycin and the growing problems of recurrence and drug resistance highlight the need for the development of new management approach with the goal of an effective treatment and adequate recurrences prevention [45, 46].

A number of clinical studies indicate that the re-establishment of the physiological ecosystem by the re-colonization of vaginal mucosa by lactobacilli is an effective strategy for treating and preventing different forms of vaginal dysbiosis [47-50].

The present study was designed to analyse the effects of vaginal probiotic preparation containing Lactobacillus acidophilus, lactic acid and deodorized garlic in women with symptomatic bacterial vaginitis and a positive history for previous recurrences. In particular, we aimed to assess the clinical and microbiological effects and the ability to restore physiological conditions in vaginal microbiota.

MATERIALS AND METHODS

This single-arm pilot study was conducted between January to November 2017 at the Department of General Surgery and Medical Surgical Specialties, University of Catania (Italy). The study protocol was approved by the Ethics Committee of the Department and conformed to the ethical guidelines of the Helsinki Declaration (as revised in Tokyo 2004). Each woman who accepted to participate in this study was well informed regarding the procedures that she would undergo and signed a consent form for data collection for research purposes. Patient anonymity was preserved and no remuneration was offered to be included in this study.

All eligible participants attending to our Gynaecological Service were submitted to a preliminary assessment including an accurate

anamnesis as well as pelvic examination and transvaginal ultrasound. We enrolled all healthy and regularly menstruated women, who voluntarily accepted to participate in the study and who met the following inclusion criteria: age between 18 and 45 years, presence of at least one mild to moderate self-reported symptom (itching, burning, leucorrhoea, subjective vulvar discomfort) and a history of recurrent vaginitis (at least 4 documented episodes in the last 12 months). The exclusion criteria were: severe symptoms, specific cervico-vaginitis due to Chlamydia, Neisseria gonorrhoeae, or Trichomonas vaginalis, clinically apparent herpes simplex infection or defined diagnosis of human papillomavirus, herpes simplex virus type 1 or 2, or human immunodeficiency virus infection; use of antibiotic/antifungal, probiotic or immunosuppressive drugs in the previous 3 months, use of mechanical contraceptives (diaphragms, intrauterine device, hormonal rings) and any others physiological or pathological conditions that could interfere with the results of the study (pregnancy or breastfeeding, diabetes, chronic inflammatory diseases, neoplastic disease, genital tract bleeding of unknown nature).

All eligible women underwent a baseline evaluation (T0) which included: assessment of presence and intensity of vaginal symptoms, measurement of vaginal pH value, assessment of Amsel criteria [51], Nugent score [52], Lactobacillary Grade (LBG) according to Donders classification [37,53-55] and microbiological count through vaginal discharge sampling.

Clinical signs and symptoms (leucorrhoea, burning, itching, and subjective vulvar discomfort) were evaluated through a severity score on a scale of 0 (absent or normal) to 3 (severe).

Vaginal fluid pH value was measured using pH test strips (McKesson, San Francisco, CA, USA).

Vaginal discharge samples were obtained from the lateral vaginal wall and the posterior vaginal fornix using sterile cotton-tipped swabs, then the collected samples were immediately transferred, under refrigerated conditions, to the Laboratory of Microbiology of the Department of Agriculture, Food and Environment, University of Catania (Catania, Italy) for the examination.

For each participant, three vaginal swabs were collected: two vaginal swabs were used to assess Amsel criteria and Nugent score: the first one was used for microscopic examination of the fresh smear (detection of clue cells and Gram staining) and the second one was subjected to Nugent score determination and whiff-amine test on two different glass slides (the presence of a 'fish odor', was evaluated by the researcher's after adding a drop of 10% KOH directly to the glass surface); another swabs, filled with transport medium, was used for microbiological counts.

The Nugent score was assessed on a 10-point scale, performing a Gram stain followed by optical microscopic observation under oil immersion. Large Gram-positive bacilli were assumed to be the Lactobacillus morphotype, smaller Gram-variable bacilli were assumed to be the Gardnerella morphotype, and other organisms were categorized by morphology only, e.g. Gram-negative bacilli, curved rods, Gram-positive cocci in chains, and fusiform.

Microbiological analysis of vaginal discharge, collected using a sterile synthetic swab tip Transystem Amies Medium Clear (BiolifeSrl, Milan, Italy), was analysed as follows. After dislodging the cells in sterile phosphate-buffered saline (PBS), serial dilutions were made and plated on the following agar media and conditions: Rogosa SL agar (Biolife) incubated at 35-37°C for 40-48 h for Lactobacillus counts; Streptococcus Selective Agar (Biolife) incubated at 32°C for 24 h for Streptococci; Gardnerella vaginalis Selective Medium (Oxoid, Milan, Italy) incubated at 37°C for 40-48 h for Gardnerella vaginalis; MacConkey Agar Mug (Biolife) incubated at 37° C for 16-18 h for Escherichia coli; Mannitol Salt Agar (Oxoid) incubated at 32 °C for 48 h for staphylococci; Slanetz Bartley Agar (Biolife) incubated at 37°C for 48 h for Enterococci; Chromogenic Candida Agar (Biolife) incubated at 35°C-37°C for 48 h for C. albicans, C. tropicalis, C. krusei. All analysis was performed in duplicate.

For the purposes of results interpretation: the presence of at least three of the Amsel criteria was assumed for BV. They included the presence of a thin, greyish-white, homogeneous leucorrhoea, vaginal pH > 4.5, presence of clue cells (epithelial cells covered by bacteria), and positive whiff-amine test.

A Nugent score of 0–3 was interpreted as Lactobacillus-predominant normal vaginal microbiota, a score of 4–6 was considered as intermediate, and a score of 7–10 was assumed as BV-like conditions, with the dominance of small Gram-negative and Gram-variable straight and curved rods [52].

According to Donders classification [35,51,53],

lactobacillary grades (LBG) was assessed: a LBG I was assumed for a normal flora with predominantly lactobacillary morphotypes, LBG II corresponded to a diminished lactobacillary flora mixed with other bacteria, and grade III was defined as an abnormal flora which consists of numerous other bacteria with absence of lactobacillary flora.

After baseline evaluation, only those patients in whom a clinical and microbiological diagnosis of bacterial vaginitis was confirmed through above described methods were enrolled to the subsequent phases of the study, while symptomatic patients, without any clinical or microbiological evidence, were excluded.

All enrolled women were directed to apply a vaginal tablet containing a probiotic preparation of Lactobacillus acidophilus LA14, lactic acid and deodorized garlic extract according to the following schedule: 1 tablet/day for 14 consecutive nights.

Examination of each patient were scheduled in three appointments: baseline, 14 days after the start of treatment (treatment: T1), and 4 weeks after the end of treatment (post-treatment: T2).

Women were advised not to take topical vaginal or other antifungal, antibiotics or other probiotic agents, even if taken orally, throughout the duration of the study. Moreover, they were asked to accurately record in a personal 'patient's diaries' any potential adverse reaction or any use of medication during the observational period, which were carefully analysed and documented at each follow-up. In addition, they have been warned to promptly report to the investigator any adverse reactions or any worsening of symptomatology. In cases of significant discomfort, worsening of self-reported symptoms, as well as any clinical evidences of worsening, the subject was immediately excluded from the experimental observation.

For the purposes of this study, the main end-points for resolution of the pathological condition were defined as: absence of vaginal symptoms, negative results for at least 2 Amsel criteria, Nugent score < 7, LBG <3, negative microbiological culture and presence of >105 c.f.u. of Lactobacillus in the vaginal flora per swab.

STATISTICAL ANALYSIS

Patients' baseline characteristics were reported as mean and standard deviation (SD) and percentages for continuous and categorical variables, respectively. Diagnostic clinical and microbiological parameters were compared by Fisher Exact Test. Analysis was performed using the statistical software R. A p-value < 0.05 was considered as statistically significant.

For the comparison of results obtained from microbiological count performed on vaginal swabs, analysis of variance (ANOVA) was carried out. A p-value < 0.05 was considered as statistically significant.

RESULTS

Initially, 120 participants were selected to be eligible for satisfying inclusion criteria but, after completing clinical and microbiological baseline assessment, 14 women were excluded because of an unconfirmed microbiological diagnosis of vaginal dysbiosis. All the participants who used at least one vaginal capsule (intent to treat, ITT) were subjected to safety analysis. Of the 106 participants (ITT, safety assessment), only 63 completed the treatment by adhering to the therapeutic regimes, others (43 participants) came out of the study for different reasons (**Fig. 1**).

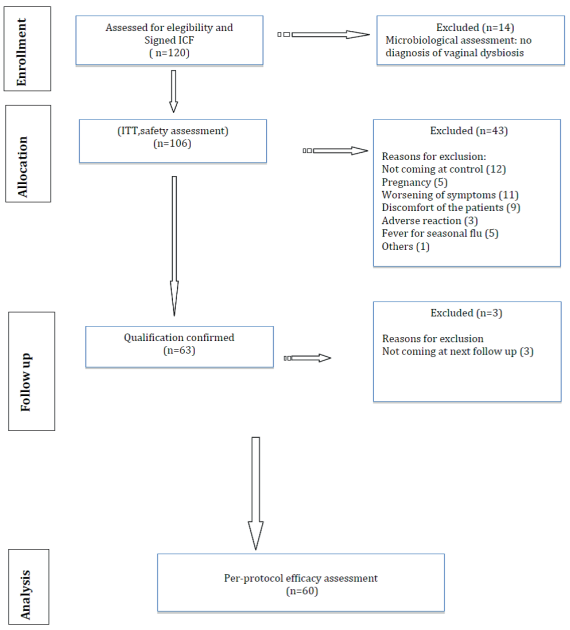

Figure 1.
Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

Adverse reactions were reported in about 5% (3 participants) of the population, including the onset of irritation and hypersensitivity reactions.

No serious adverse events were recorded during the observational period and nobody among participants was excluded from the study for significant adverse events.

Totally, 60 women, aged from 19 to 42 years (mean age of 28.83 ± 6.50 years), underwent the full observational period designed for the study protocol and were therefore subjected to efficacy analysis. The demographic characteristics, BMI, sexual activity, smoking and contraceptives use, as well as the clinical and microbiological finding resulting from the analysis of the total sample are reported in **table 1**. The most complained symptoms were leucorrhoea (100% of cases) and subjective vulvar discomfort (93%), while burning and itching were reported in 37% and 32% of cases respectively.

The baseline microbiological analysis of vaginal swabs (T0) demonstrated that, in most of cases, clinical symptoms were due to the presence of a mixed microbial flora with the dominance of one or more species. The majority of examined patients showed an absence or very low amounts of lactobacilli (LBG III= 53%), or an intermediate flora (LBG II= 47%) with low density (< 1.0×105 CFU/mL) of lactobacilli. In 65% of cases, the examined vaginal fluids had a pH value > 4.5. Dominant species were identified in each of examined swab, they were represented as follows: G. Vaginalis (n = 33; 55%), C. Albicans (n = 21; 35%) and less frequently S. Agalactiae (n = 3; 5%), E. coli (n = 2; 3%) and Enterococcus spp. (n = 1; 2%) (**Tab. 1**).

Results of both clinical and microbiological criteria, assumed for symptomatic vaginitis at baseline (T0), 14 days after the start of treatment (T1) and 4 weeks after the end of treatment (T2) are shown in **table 2**. The evaluation of vaginal samples after treatment, showed a very good colonization as assessed by microbiological analysis. In fact, among the 60 women presenting at baseline with an absence (53%) or a low number of lactobacilli (47%), 46 (77%) had a normal flora at the first follow-up visit (T1). 4 weeks after the end treatment (T2) 50 women had a normal flora (83%), 6 showed intermediate colonization (10%), and 4 remained unchanged (7%).

In only 10% and 8% of participants, at least three Amsel criteria were satisfied at T1 and T2 respectively. Moreover, treatment significantly reduced the Nugent score to below the threshold of 7 in 55 participants (92%) at T1. Few shifts were detected after 4 weeks from the end of treatment (T2): in fact, the majority of women (95%) showed a Nugent score below the threshold of 7.

Table 1.
Baseline demographics, clinical and microbiological characteristics of the study sample (n=60).

Demographic characteristics		
Age		28.83±6.50
Sexual activity		52 (87%)
Smoking		31 (52%)
Body mass index (kg/m2)		24,26±2,67
18.5-24.9		2 (3%)
25-29.9		36 (60%)
> 30		22 (37)
Contraceptive use		44 (73%)
Oral		8 (13%)
Barrier		23 (38%)
Others		13 (22%)
Clinical and microbiological characteristics		
Vulvovaginal signs and symptoms	Itching	19 (32%)
	Burning	22 (37%)
	Leucorrhoea	60 (100%)
	Subjective vulvar discomfort	56 (93%)
Amsel Criteria	Homogenous vaginal discharge	43 (72%)
	Clue cell presence	41 (68%)
	Positive amine test	31 (52%)
	Vaginal pH > 4.5	39 (65%)
Nugent score	0–3	0
	4–6	11 (18%)
	7–10	49 (82%)
Lactobacillary Grade (LBG)	I	0
	II	28 (47%)
	III	32 (53%)
Microbiological species	*Gardnerella vaginalis*	33 (55%)
	Streptococcus agalactiae	3 (5%)
	Escherichia coli	2 (3%)
	Enterococcus spp.	1 (2%)
	Candida spp	21 (35%)

Table 2.
Clinical and microbiological parameters for total sample (n=60); baseline (T0), 15th day after the start of Treatment (T1) and 4 weeks after maintenance treatment (T2).

Diagnostic parameters		**Baseline**	**T1**	**T2**	**P value for trend**
Amsel Criteria	Homogenous vaginal discharge	43 (72%)	15 (25%)	10 (16%)	P<0.05
	Clue cell presence	41 (68%)	6 (10%)	6 (10%)	P<0.05
	Positive amine test	31 (52%)	2 (3%)	2 (3%)	P<0.05
	Vaginal pH > 4.5	39 (65%)	6 (10 %)	5 (8%)	P<0.05
Nugent score	0–3	0	46 (77%)	50 (83%)	P<0.05
	4–6	11 (18%)	9 (15%)	7 (12%)	P>0.05
	7–10	49 (82%)	5 (8%)	3 (5%)	P<0.05
Lactobacillary Grade (LBG)	I	0	46 (77%)	50 (83%)	P<0.05
	II	28 (47%)	10 (16%)	6 (10%)	P<0.05
	III	32 (53%)	4 (7%)	4 (7%)	P<0.05
Microbiological species	*Gardnerella vaginalis*	33 (55%)	4 (7%)	3 (5%)	P<0.05
	Streptococcus agalactiae	3 (5%)	0	0	P>0.05
	Escherichia coli	2 (3%)	0	0	P>0.05
	Enterococcus spp.	1 (2%)	0	0	P>0.05
	Candida spp	21 (35%)	9 (15%)	6 (10%)	P<0.05

Moreover, only 10% and 8% of participants at T1 and T2 sampling times, respectively, had a vaginal pH > 4.5.

Patients' symptoms before and after treatment are reported in **figure 2**. A significant improvement of self-reported symptoms was observed by the comparison of the enrolment visit with those after 14 days of treatment (T0 versus T1) and after 4 weeks from the end of treatment (T0 vs T2). Moreover, all of the still-symptomatic women, upon further examination, showed a reduction in the symptoms' score.

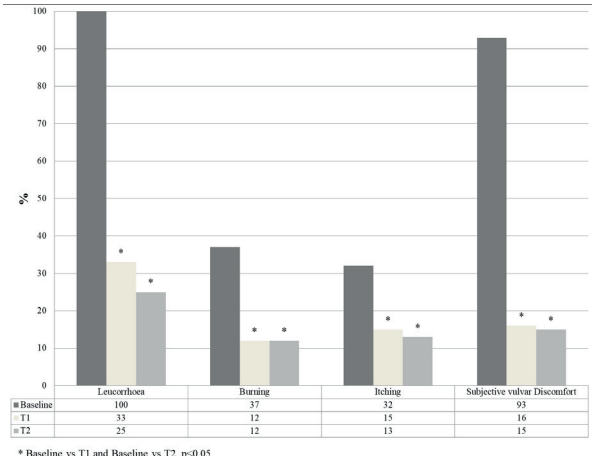

Figure 2.
Patients' self-reported symptoms at baseline (T0), 15th day after the start of treatment (T1) and 4 weeks after maintenance treatment (T2).

Microbial counts, expressed as the mean and standard deviation of log cfu/ml of the main microbial groups detected during the whole of the study, are reported in **Table 3**.

Table 3.
Microbial counts and significance (ANOVA) for total sample (n=60); baseline (T0), 15th day after the start of treatment (T1) and 4 weeks after maintenance treatment (T2).

Microbial count (log cfu/ml)							
Microbial group	**T0**	**T1**	**T2**	**P value T0 vs T1**	**P value T0 vs T2**	**P value T1 vs T2**	**P value for trend**
Gardnerella spp.	10.8 ± 3.0	4.8 ± 2.5	6.3 ± 2.4	$3.4x10^{-24}$	$4.5x10^{-16}$	0.001	$8.4x10^{-24}$
Candida spp.	7.3 ± 3.4	4.9 ± 1.7	5.7 ± 1.6	$2.7x10^{-6}$	0.001	0.012	$1.05x10^{-3}$
Streptococcus spp.	3.2 ± 2.7	4.1 ± 2.9	3.5 ± 2.4	0.107	0.564	0.249	0.261
Escherichia Coli	2.5 ± 2.6	2.1 ± 2.1	2.8 ± 2.3	0.433	0.532	0.124	0.302
Enterococcus spp.	4.1 ± 2.7	4.2 ± 2.7	4.3 ± 2.6	0.767	0.656	0.886	0.903
Lattobacillus spp.	4.5 ± 2.0	10.1 ± 3.2	11.7 ± 2.1	$9.2x10^{-24}$	$7.3x10^{-26}$	0.002	$2.7x10^{-27}$

At baseline (T0), patients had a complex microbiota dominated by potentially pathogenic bacteria with a low cell density of lactobacilli. Treatment reduced the cell density of all the studied microbial groups notwithstanding the statistical significance (p>0.05) was observed only for Gardnerella and Candida spp, these results are probably influenced by the small sample size. Moreover, a significant increase in the count of lactobacilli was observed at T1 follow-up (p < 0.005) and this trend was also observed post-treatment (T2).

Overall a significant restoration (p<0.05) of the eubiosis condition (presence of >105 c.f.u. of Lactobacillus in the vaginal flora per swab) was observed when data detected at T0 were compared with those detected at T1 and T2 follow-up. In only 9 cases (15%) a clinical and microbiological diagnosis of vaginitis was confirmed at the T2 follow-up. The relapse, diagnosed on the basis of microbiological criteria (presence of >105 c.f.u./ swab of any bacterial species in patient with a negative microbiology at the previous follow-up) occurred in only 2 cases (3%).

DISCUSSION

Vaginal symptoms are the most common reasons for women seeking medical care. In fact, it is estimated that about 75% of women will have at least one episode of lower genital tract infection in their life and that about half of them will present new occurrences [56-59].

In recent years, the field of research on vaginal microbiota has made great progress providing a better understanding and care of the vaginal conditions related to its pathological modification[60].

Moreover, vaginal dysbiosis has been recognised as an important factor for the increased susceptibility to sexually transmitted infection, including HIV, pelvic inflammatory disease, sexual dysfunctions, preterm birth and maternal and neonatal infections [61-63].

Therefore, a correct diagnostic definition and an effective treatment are essential goals for women health. Some of the most common management strategies have involved the wide use of antibiotics. This approach is often ineffective because it leads to ecosystem disturbances, difficulties in adapting to treatment, adverse effects and selection of resistant strains [64].

Numerous evidences have recognised that the predominance of lactobacilli is responsible for the balance and maintenance of the vaginal ecosystem so new approaches, that look at the restoration of vaginal microflora's balance, rather than modify its components have been promoted, both in therapeutic and preventive settings [65- 68].

In our study we have observed that probiotic

preparations containing Lactobacillus acidophilus LA14, lactic acid and deodorized garlic extract, have a significant impact on symptomatic vaginitis, whatever its nature, notwithstanding different subgroups may be affected in different ways and at several levels if the various clinical and microbiological aspects of response are analysed.

Although the reported percentage of treatment failure (≈20%) may appear significant, it should be noted that all symptomatic patients, for the presence of at least one mild to moderate self-reported symptom (itching, burning, leucorrhoea, subjective vulvar discomfort), were recruited in the study, so the baseline group was quite heterogeneous, including paucisymptomatic subjects and symptomatic subjects with moderate symptoms.

Different species of lactobacilli have been previously evaluated for the treatment of vaginal dysbiosis. Mastromarino et al. [69] in a double-blind, placebo-controlled clinical trial, demonstrated that that the intravaginal administration of exogenous selected strains of lactobacilli is effective in restoring a normal vaginal microbiota and can be used for treating BV. Similarly, Rossi et al. [70] in a prospective clinical trial demonstrated that long-term administration of vaginal tablets containing Lactobacillus Rhamnosus represents an effective and safe treatment for restoring the physiological vaginal pH and controlling BV symptoms.

In women with lactobacillus-dominated microbiota, the lactic acid concentration is inversely related with pH, indicating that lactic acid is a primarily responsible for acidification of the vagina. As consequences, Lactobacillus spp., naturally or administered as probiotics, may establish vaginal eubiosis through an increment in lactic acid. They may also release other antimicrobial factors such as bacteriocins [71,72]. While many lactobacillus-based probiotics have been previous selected on the basis of hydrogen peroxide (H2O2) production, recent studies demonstrate that lactic acid is the main antimicrobial factor produced by lactobacilli [73].

Our study demonstrated that combined probiotic treatment resulted in an effective restoration of physiological pH, accompanied by remission or attenuation of clinical signs and symptoms. Notably, restoration of a vaginal physiological pH was maintained at the second follow-up (T2) indicating a significant control of recurrences. Moreover, the microbiological analysis of vaginal swabs demonstrated a significant efficacy in reduction of viable cells for all the microbial groups investigated including Candida spp and aerobic bacteria.

Previously, Heczko et al. [66] have found that an orally administered probiotic mixture of three viable strains: L.gasseri, L.fermentum, and L.plantarum significantly delayed the clinical relapse of BV and AV in patients who used a targeted antibiotic therapy. However, it is important to highlight how a therapeutic approach based on oral administration of antibiotics have long-term negative effects on the vaginal milieu, so it should be considered only for short courses and to control acute symptoms in complicated and severe cases of AV which represent rare and specific subcategories [74, 75].

Finally, the establishment of a healthy vaginal microbiota might be a supportive and preventive measure also against vulvovaginal candidiasis (VVC), although it has not yet been clearly identified the mechanisms underlying the antifungal activity of Lactobacillus species [40,42]. Moreover, in vitro studies have shown that garlic has fungistatic properties at temperatures below 37°C and fungicidal properties at 37°C [76]. Garlic and its bioactive components have the ability to suppress hyphae production and to affect the expression level of SIR2 gene which are essential virulence determinant of C. albicans for invasive infections [77]. So, the presence of deodorized garlic extract, could weaken or prevent the formation of biofilm, making pathogens more sensitive to the therapeutic action of the antimicrobial agents [78, 79]. No others studies have previous examined the use of probiotic preparations containing odourless garlic extract.

Few previously published studies have specifically addressed the qualitative and quantitative aspects of the flora of women with VVC. Vaginal colonization with Candida spp. seems more common in women with a lactobacilli-dominated vaginal microbiota than in women with lactobacillary depletion [80-83]. Hillier et al. [80], in a large cross-sectional study of predominantly asymptomatic pregnant women, observed that cases of asymptomatic Candida infection were more frequent in women having only H2O2 negative lactobacilli. These observations suggest that H2O2- producing lactobacilli might play a greater role in inhibiting C. Albicans growth in vivo [81]. Moreover, Wagner et al. [83] have reported that C. Albicans infection are able to induces a pro-inflammatory immune response in vaginal epithelial cells, on the contrary lactobacilli can inhibit NF-kB-associated inflammatory genes also inducing IL-1a and IL-1b expression through

an alternative signal transduction pathway which modulates vaginal epithelial cell cytokine production [84]. The modulation of the immune response seems to be an important way for control of chronic and recurrent vaginal infections as well as the inflammatory mechanisms related to preterm delivery [85-87].

In conclusion, an imbalance of vaginal environment can result in extremely heterogeneous microbiological frameworks in which the overlap between different microbial species (bacteria, fungi, protozoa) gives rise to different forms of mixed vaginitis. Therefore, conventional antibiotic therapy often results aspecific and not long-term resolutive. Results of our study suggest that a restoration of the physiological conditions, through the recolonization of the mucosa by lactobacilli is an effective strategy for treatment and prevention of vaginal dysbiosis.

Nevertheless, several limitations of the study should be taken into account in the interpretation of our preliminary data: first of all, the sample size is limited, as well as the follow-up, and we were unable to determine whether the treatment's effects will last for longer than the 4-weeks study period; second, it was not possible to ascertain the contribution of each component of the vaginal preparation to the improvement in vaginal health and symptoms relief; third, the study design does not have a control arm (placebo or no treatment). Despite these limitations, results of our research are important to draw a preliminary trail in this field. In fact, most of the previous clinical trial have evaluated the efficacy of the probiotic-antibiotic association in the treatment and prevention of vaginitis.

No previous studies have analysed the effects of this combined probiotic preparation comparing different microbiological and clinical patterns.

Given the limitations of this single-arm pilot study, the new combined vaginal preparation should be tested in a randomised controlled trial using a placebo or current standard of care treatment. We aim to address all these aspects in our future investigations.

CONCLUSIONS

Vaginally administered probiotic preparation containing Lactobacillus acidophilus, lactic acid and deodorized garlic extract are safe and effective in treatment and prevention of symptomatic bacterial vaginitis. Multiple mechanisms can synergistically act to restore natural balance of the vaginal microbiota: lactobacillus acidophilus LA14 limits the colonization and growth of pathogens by restoring normal vaginal microflora; lactic acid, lowering the vaginal pH makes the vaginal environment unfavourable to pathogens; the presence of deodorized garlic extract as an ancillary substance, weakens or prevents the formation of biofilm, making pathogens more sensitive to the action of the two main components of the product.

For optimal management of vaginal dysbiosis, it is important to increase awareness of the vaginal ecosystem, good vaginal hygiene and lifestyle.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. No specific funding was obtained.

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