DOI: 10.5281/zenodo.4527082
UDC: 618.15-002:[579.864+577.175.643]

Lactobacillus rhamnosus, and *Lactobacillus reuteri* with estriol in the treatment of vaginal dysbiosis pathologies

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Manuscript received June 02, 2020; revised manuscript July 27, 2020; published online February 22, 2021

Abstract

Background: A healthy vagina is characterized by hydrogen peroxide and acid-producing lactobacilli, which are crucial to maintain the physiological vaginal ecosystem and their depletion speeds up bacterial overgrowth with pH elevation, salidase and amine production, leading to the observed signs and symptoms of vaginal dysbiosis. An effective treatment should be combined of lactobacillary flora and low doses of estrogen to maintain the physiology of the vaginal epithelium.
Material and methods: This is a prospective randomized study that included 218 patients, who were divided into 2 groups according to the treatment regimen, performed between October 2018 and December 2019 at the Department of Obstetrics and Gynecology of *Nicolae Testemitanu* State University of Medicine and Pharmacy. Patients selected were divided into L_1 (120 patients assigned to treatment with the combination of two microorganisms *Lactobacillus rhamnosus* and *Lactobacillus reuteri* with estriol 0.03 mg vaginal pessaries) and L_2 (98 patients who were given *Lactobacillus rhamnosus* in vaginal capsules). The treatment regimen for both groups was the same – 1 pessary or 1 capsule once in 24 hours, in the evening, for 12 days. Patients were evaluated before treatment (visit 1) and on day 13 of treatment (visit 2) and 1 month after the end of treatment (visit 3).
Results: Of the 120 women included in the study in the first group and 98 in the second group, a significant improvement (normocenosis) according to the research physician and patients was found in 93.3% (112) patients of group 1 and 71.4% (70) patients in group 2, satisfactory improvement (consistent with the intermediate type of biocenosis) in 5% (6) patients of group 1 and 15.3% (14) patients from group 2, unsatisfactory result 1.7% (2) patients from group 1 and 13.3% (13) of patients of group 2 (later relapse was noted in these patients).
Conclusions: Probiotic treatment with vaginal *Lactobacillus rhamnosus, Lactobacillus reuteri* and low doses of estriol seems to be useful in hindering bacteria growth especially after antibiotic therapy; therefore this intervention may be considered a new prophylactic treatment for preventing recurrence of bacterial vaginosis, in particular in high-risk patients.
Key words: bacterial vaginosis; *Lactobacillus rhamnosus*, vaginal flora, estriol.

Cite this article
Profire L, Niguleanu A, Corolcova N, Burac M, Cotelea V. *Lactobacillus rhamnosus,* and *Lactobacillus reuteri* with estriol in the treatment of vaginal dysbiosis pathologies. *Mold Med J.* 2021;64(1):35-40. doi: 10.5281/zenodo.4527082.

Introduction

Vaginal eubiosis is characterised by a beneficial lactobacillus-dominated vaginal microbiota. In contrast, vaginal dysbiosis is characterised by an overgrowth of multiple anaerobes and is associated with an increased risk of adverse urogenital and reproductive health outcomes [1]. A healthy vagina is characterized by hydrogen peroxide and acid-producing lactobacilli, which are crucial to maintain the physiological vaginal ecosystem and their depletion speeds up bacterial overgrowth with pH elevation, salidase and amine production. Lactobacilli, particularly those producing H_2O_2, play a pivotal role in controlling the microenvironment of the vagina and in inhibiting the overgrowth of potentially pathogenic organisms [2]. Possible mechanisms of this protection include inactivation of pathogens by different Lactobacillus products (lactic acid, H_2O_2 and bacteriocins), competition for epithelial cell attachment sites and stimulation of the local immune system [3].

Several preclinical *in vitro* and *in vivo* studies have shown that *Lactobacillus rhamnosus* and *Lactobacillus reuteri* have antibacterial, antifungal and anti-inflammatory properties, as well as anticarcinogenic, antigenotoxic, antimutagenic and immunomodulating properties. *Lactobacillus reuteri* have the ability to produce antimicrobial molecules, such as organic acids, ethanol, reuterin. Due to their antimicrobial properties, *Lactobacillus reuteri* can inhibit the colonization of pathogenic microbes and reconstruct the host microflora [4].

To maintain a healthy vaginal ecosystem or restore its disruption, sufficient estrogen levels, an intact mature vaginal epithelium and physiological lactobacterial microflora are required. Thus, a combination of beneficial lactobacilli and estrogen is a topical treatment [5, 6].

Currently, in European countries there is a tendency to reduce the use of local estrogens [7]. Given the pathogenesis of vaginal atrophy, estrogen therapy is the gold standard in treatment. Of the three natural estrogens of the human body, estriol has the shortest half-life and the least biological activity. Estrogens, estrone (E1), estradiol (E2), and estriol (E3) are female sex hormones that are usually determined in the human body. While E2 and E1 can be reversibly metabolized, E3 cannot. Like all estrogens E3 stimulates the proliferation and maturation of the vaginal epithelium. However, in E3, sensitivity to receptors is lower (about 10 times) than in E2, and thus, it cannot induce the development of estrogenic effects on the endometrium, bone, and breast tissue in physiological concentrations [8-11].

Vaginal estrogens are more effective in eliminating genitourinary symptoms than oral drugs, since lower doses are required due to the lack of hepatic metabolism, and a high level of estrogen induces direct vaginal response [10]. Thus, topical administration of E3 in the treatment of vaginal diseases is, in general, preferable, since this hormone is safer for local administration than other estrogens and has a more pronounced proliferative reaction than when taken orally. This is especially important if systemic hormone replacement therapy with estrogen is not required [9]. Estriol has a positive effect on the microflora of the vagina due to an increase in colonies of lactobacilli and a decrease in enterobacteria [8, 11-14].

Material and methods

A prospective randomized study was performed that included 218 patients. The patients were divided into 2 random groups, according to the treatment they received: L_1 – 120 patients and L_2 – 98 patients. The study was performed at the Department of Obstetrics and Gynecology of *Nicolae Testemitanu* State University of Medicine and Pharmacy between 14.10.2018-16.12.2019.

Patients in L_1 were given a combination of two microorganisms *Lactobacillus rhamnosus* and *Lactobacillus reuteri* with estriol 0.03 mg vaginal pessaries and patients in L_2 were given *Lactobacillus rhamnosus* in vaginal capsules. The treatment regimen for both groups was the same – 1 pessary or 1 capsule once in 24 hours, in the evening, for 12 days. Intravaginal washings or topical use of certain therapeutic or other substances were prohibited during treatment, and patients were advised to abstain from sexual intercourse for 8 hours after administration of the medication. Simultaneous treatment was allowed only in patients with hypertension.

The inclusion criteria in the study were: the age of patients 20-45 years, patients who needed to restore normal vaginal microflora and those who received local or systemic antibacterial therapy for the treatment of infectious diseases of internal or external genitalia; recurrent chronic genital infection; the period from the end of antibacterial therapy till the patient's address for microflora recovery treatment was less than 1 month; informed consent to comply with the requirements of the drug administration protocol and the time of examination.

The exclusion criteria from the study were: hypersensitivity to the medication, benign or malignant tumors of the uterus, vulva and vagina (including intraepithelial neoplasia of the vagina, endometriosis) and mammary glands, vaginal bleeding of indefinite etiology, use of any drugs with a vaginal administration one week before the enrollment in the study (spermicides, intimate gel, vaginal washings); sexually transmitted diseases (trichomoniasis, gonorrhea, ureaplasma, genital herpes, chlamydia, human immunodeficiency virus HIV, AIDS); enrollment in other studies in the last 30 days; pregnancy and lactation; the patient's refusal to participate in the study.

The efficacy treatment was evaluated taking into account the dynamics of clinical symptoms, the gynecological valve examination of the genitalia and the results of the microscopic examination. Clinical efficacy was analyzed according to the dynamics of symptoms on a generally accepted scale in four points (0 points – no complaints, 1 point – insignificant, 2 points – moderately expressed, 3 points – expressed). Dynamics of objective clinical signs (vaginal discharge, hyperemia) according to the results of the vaginal examination (assessment on a scale according to the degree of manifestations: absence – 0, medium – 1, moderate – 2, expressed – 3). Dynamics of the results of smear microscopy (Gram) of the pathological vaginal discharge before treatment (visit 1) and on day 13 of treatment (visit 2) and 1 month after the end of treatment (visit 3), pH assessment of vaginal fluid.

The results of the study were processed using the STATISTICA 7.0 and the Microsoft Office software package, Microsoft Excel (2007), using parametric and nonparametric statistical criteria.

Results and discussion

The mean age of the patients included in the study was 36.6 ± 6.84 years in L_1 and 33.9 ± 6.05 years in L_2. The main criteria for inclusion in the study was the need to restore the vaginal flora after antibacterial therapy (bacterial vaginosis, candidiasis, nonspecific vaginitis). The data are presented in table 1. Before treatment, the patient of the study groups presented the following complaints: vaginal discharge 53.3% (64) in L_1 vs 50% (49) in L_2, $p > 0.05$; vaginal odor 14.2% (17) in L_1 vs 12.2% (12) in L_2, $p > 0.05$; itching and burning 6.7% (8) in L_1 vs 6.1 %(6) in L_2, $p > 0.05$; discomfort 45.2% (54) in L_1 vs 47.95% (47) in L_2, $p > 0.05$; dyspareunia 14.2% (17) in L_1 vs 12.2% (12) in L_2, $p > 0.05$.

During the gynecological examination before treatment, the vaginal mucosa was of a pale pink color, without a vascular pattern in most patients in the first and second group. Moderate hyperemia of the vaginal mucosa and cervicitis were determined in 18.3% (22) patients of the first group and in 18.4% (18) in the second group, ($p> 0.05$). The intensity of hyperemia of the vaginal mucosa at the beginning of the study in L_1 was 0.38 ± 0.78 points and 0.37 ± 0.77 points in patients of L_2. At the second visit, mucosal hyperemia was absent in all patients in the first group, thus a significant

Table 1

Vaginal dysbiosis pathologies of the patients from the studied groups before enrollment in the study

	L_1 n=120		L_2 n=98		P
	n	%	n	%	
Bacterial vaginosis	36	30	31	31.6	>0.05
Vulvovaginal candidias	14	11.7	8	8.2	>0.05
Nonspecific vaginitis	70	58.3	59	60.2	>0.05

decrease in the frequency of this symptom was observed (p<0.05), which was also confirmed by the positive dynamics of clinical symptoms (complaints – lack of itching, burning, normal vaginal discharge or their absence). In 10.2% patients of the second group, mild hyperemia and vascular pattern were observed, a significant decrease in the frequency of this symptom was also observed (p<0.05). However, in L_1, compared with the L_2, a significantly more decrease in cases of vaginal hyperemia was observed (p<0.05). At the 3rd visit in the first group, moderate hyperemia was detected in 4 (3.3%) patients, severe hyperemia – in 2 (1.7%) patients; the total score for the intensity of hyperemia was 0.11 ± 0.51. In dynamics, compared with visit 1, a significant decrease in cases of vaginal hyperemia was observed (p <0.05). In the second group, moderate hyperemia was also detected in 4 (4.1%) patients, severe hyperemia – in 7 (7.1%) patients, the total score characterizing the intensity of hyperemia was 0.29 ± 0.85 (tab. 2). The results (the 1st and the 3rd visits) in the second group showed statistically significant differences (p<0.05). However, in the first group, compared with the 2nd group, a more significant decrease in cases of hyperemia was observed (p<0.05).

In both groups in patients who demonstrated severe vaginal hyperemia, at the third medical appointment the vaginal mucosa was edematous and in those patients a relapse of the previous vaginal dysbiosis was detected and appropriate treatment was prescribed.

The microscopic examination for detecting vaginal biocenosis was performed before and after treatment. In both groups key cells, yeast cells, pseudomycelia, and fungal

Table 2

Hyperemia of the vaginal mucosa in dynamics

Medical appointment	Intensity	L_1 n=120		L_2 n=98		p
		n	%	n	%	
1	Moderate	22	18.3	18	18.4	>0.05
	Absent	98	81.7	80	–	
	Total score	0.38±0.78		0.37±0.77		
2	Absent	120	100	90	91.8	<0.05
	Medium	0	–	10	10.2	
	Total score	0		0.10±0.30		
3	Absent	120	100	87	88.7	<0.05
	Moderate	4	3.3	4	4.1	>0.05
	Expressed	2	1.7	7	7.1	<0.05
	Total score	0.11±0.51		0.29±0.85		

Table 3

The number of leukocytes in the vaginal smear in dynamics under the influence of treatment

Number	L_1 n=120			L_2 n=98		
	Visit 1 n (%)	Visit 2 n (%)	Visit 3 n (%)	Visit 1 n (%)	Visit 2 n (%)	Visit 3 n (%)
£ 10 in f/v	58 (48.3)	110 (91.7)	112 (93.3)	43 (43.9)	64 (63.9)	82 (83.7)
11-20 in f/v	62 (51.7)		2 (1.7)	55 (56.1)	34 (34.7)	4 (4.1)
>20 in f/v	–	–	6 (0.5)	–	–	12 (12.2)

spores were absent in all patients but was detected a leukocyte reaction from medium to moderate severity (tab. 3).

At the first medical appointment, in 58 (48.3%) patients in L_1 were detected up to 10 leukocytes in the field of vision and in 43 (43.9%) women in L_2. In 62 (51.7%) patients in group 1 and 55 (56.1%) patients in group 2 were revealed 11 to 20 cells in the field of view, (p> 0.05). At the second visit, a decrease in the number of white blood cells in the visual field in dynamics was revealed: up to 10 white blood cells in the visual field were observed in 110 (91.7%) patients of group 1 and in 64 (63.9%) patients of group 2. In 10 (8.3%) women in group 1 and 34 (34.7%) in group 2 were revealed 11 to 20 cells in the field of view, (p<0.05). At the 3rd visit, up to 10 leukocytes in the field of vision were observed in 112 (93.3%) patients of group 1 and 82 (83.7%) patients in group 2, (p <0.05). In 2 (1.7%) cases in group 1 and in 4 (4.1%) cases in group 2, 11 to 20 cells were detected in the field of view, (p> 0.05). In 6 (0.5%) cases in group 1 and 12 (12.2%) cases in group 2, the number of leukocytes was more than 20 in the field of view, (p<0.05). Comparison of the results between the groups in dynamics revealed the predominance of patients in the first group with a low white blood cell count compared to the second group.

Microscopy of the vaginal discharge at the first medical appointment revealed a predominance of cases with a large number of superficial epithelial cells in the field of view in both groups: 82 (68.3%) cases in L_1 and 63 (64.3%) cases in L_2. In 38 (31.7%) cases in L_1 and 36 (36.7%) cases in L_2 was revealed a moderate number of epithelial cells. Differences between groups were not statistically significant (p> 0.05). At the second visit, the number of epithelial cells decreased: a small number of cells was detected in 98 (81.7%) patients of group 1 and 69 (70.4%) patients in group 2. In 14 (11.7%) patients in group 1 and 25 (25.5%) patients of group 2 was revealed a moderate number of cells. Differences between groups were statistically significant, (p <0.05). A large number of cells was revealed in 8 (6.7%) participants in group 1 and in 4 (4.1%) patients in group 2. Differences between groups were not statistically significant (p> 0.05). At the 3rd visit, the number of epithelial cells in vaginal smears decreased in most patients and corresponded to the phase of the menstrual cycle. A small number of cells was detected in 102 (85%) patients of group 1 and in 65 (66.3%) patients of group 2, (p <0.05). In 10 (8.3%) participants of the group 1 and in 20 (20.4%) patients of group 2 was revealed a moderate number of cells (p <0.05). A large number of cells was detected in 6 (5%) participants of group 1 and in 13 (13.3%) patients of group 2, differences between the groups were statistically significant (p <0.05). The dynamics of the number of epithelial cells in the vaginal smears under the influence of treatment is presented in table 4.

In the studied groups none of the patients showed normocenosis before treatment – the normal state of the vaginal microbiota (the prevalence of lactobacilli, the absence of gram-negative microflora, the number of leukocytes up to 10 in the f/v, epithelial cells, respectively, the phase of the menstrual cycle). An intermediate type of vaginal biocenosis (moderate or insignificant number of lactobacilli, gram-

Table 4

The number of epithelial cells in the vaginal smear under the influence of treatment

Number	L_1 n=120			L_2 n=98		
	Visit 1 n (%)	Visit 2 n (%)	Visit 3 n (%)	Visit 1 n (%)	Visit 2 n (%)	Visit 3 n (%)
Small (unique)	0	98 (81.7)	102 (85)	0	69 (70.4)	65 (66.3)
Moderate	38 (31.7)	14 (11.7)	10 (8.3)	36 (36.7)	25 (25.5)	20 (20.4)
Considerable	82 (68.3)	8 (6.7)	6 (5)	63 (64.3)	4 (4.1)	13 (13.3)

Table 5

Vaginal biocenosis under the influence of treatment

Type of vaginal biocenosis	L_1 n=120			L_2 n=98		
	Visit 1 n (%)	Visit 2 n (%)	Visit 3 n (%)	Visit 1 n (%)	Visit 2 n (%)	Visit 3 n (%)
Normocenosis	0	108 (90)	112 (93.3)	0	65 (66.3)	70 (71.4)
Transitional	36 (30)	12 (10)	6 (5)	24 (24.5)	29 (29.5)	15 (15.3)
Dysbiosis	84 (70)	0	2 (1.7)	62 (63.3)	4 (4.1)	13 (13.3)

Table 6

The dynamics of the pH of the vaginal discharge under the influence of treatment

pH	L_1 n=120			L_2 n=98		
	Visit 1 n/%	Visit 2 n/%	Visit 3 n/%	Visit 1 n/%	Visit 2 n/%	Visit 3 n/%
3.5-4	0	108 (90)	112 (93.3)	0	65 (66.3)	70 (71.4)
4.1-4.5	36 (30)	12 (10)	6 (5)	24 (24.5)	29 (29.5)	15 (15.3)
>4.5	84 (70)	0	2 (1.7)	62 (63.3)	4 (4.1)	13 (13.3)

positive cocci, gram-negative bacilli; leukocytes, monocytes, macrophages, epithelial cells, complaints and clinical manifestations) was detected in 36 (30%) cases in group 1 and 24 (24.5%) cases in group 2. Vaginal dysbiosis (a significant decrease or complete absence of lactobacilli, abundant polymorphic gram-negative and gram-positive strain and coccal flora, variable white blood cell count – up to 20 in the f/v) was assessed in 84 (70%) cases in group 1 and in 62 (63.3%) cases in group 2, p> 0.05. Vaginal biocenosis before and after treatment is presented in table 5.

The dynamics of the pH of the vaginal discharge corresponded to the dynamics of changes in the biocenosis of the vagina. When comparing the results, a significant increase in the acidity of the pH of the vaginal discharge and an increase in its acidic protective function were observed in group 1 when compared with group 2. The differences between the groups were statistically significant (p <0.05). The dynamics of the pH of the vaginal discharge in the study groups are presented in table 6.

Of the 120 women included in the study in the first group and 98 in the second group, a significant improvement (normocenosis) according to the research physician and patients was found in 112 (93.3%) patients of group 1 and 70 (71.4%) patients in group 2, satisfactory improvement (consistent with the intermediate type of biocenosis) in 6 (5%) patients of group 1 and 14 (15.3%) patients from group 2, unsatisfactory result in 2 (1.7%) patients from group 1 and 13 (13.3%) of patients of group 2 (later relapse was noted in these patients).

Conclusions

Throughout the study period, the clinical symptom monitoring data and the results of microscopic examination allowed us to state the fact of the clinical efficacy of the treatment with the combination of *Lactobacillus rhamnosus*, *Lactobacillus reuteri* and estriol in most patients, expressed in improving the overall well-being of the patients, namely the disappearance or reduction of itching, burning, pathological discharge, discomfort, vaginal hyperemia, pH of the vaginal discharge, as well as reducing the severity of the leukocyte reaction and normalizing the morphological picture of the vaginal smear.

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Authors' contribution
LP – conducted/performed the laboratory work; AN – designed the research, did statistics and interpreted the data; NC – conceptualized the project and revised the manuscript critically; MB – interpreted the data and drafted the manuscript; VC – draf-ted the manuscript. All the authors revised and approved the final version of the manuscript.

Funding
This study was supported by *Nicolae Testemitanu* State University of Medicine and Pharmacy. The trial was the authors' initiative. The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The research was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy, protocol No 14 of March 17, 2017.

Conflict of interests
No competing interests were disclosed.