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CHARACTERISTICS OF MICROORGANISMS LINKED TO VAGINAL INFECTIONS AND THE ANTIMICROBIAL PROPERTIES OF LACTOBACILLI

Sohibnazarov Khonsuluv[1], Ibragimova Shakhnoza[2], Zakirova Darya[3], Reyimbergenova Zumrad[4], Abdunabiev Azimjon[5].Umarova Shakhnoza[6]
[1]Raja Ramanna Center for advanced technologies, xonsuluv91as@gmail.com
[2]Raja Ramanna Center for advanced technologies, nadiya9393@gmail.com
[3]Raja Ramanna Center for advanced technologies, catdasha@mail.ru
[4]Raja Ramanna Center for advanced technologies, zumradreyimbergenova@gmail.com
[5]Raja Ramanna Center for advanced technologies, abdunabiev96a@mail.ru
[6]Raja Ramanna Center for advanced technologies, shakhnozaumarova0@gmail.com

Abstract
The increasing challenge of resistance, in bacteria underlines the importance of implementing successful antimicrobial approaches for addressing recurring vaginal infections specifically. This research examines how vaginal lactobacilli play a role in fighting off vaginal pathogens through their properties. The prevalent species of lactobacillus found in a functioned flora help maintain a harmonious setting that guards against conditions, like bacterial vaginosis (BV) urinary tract infections and various genital ailments. A decrease, in lactobacilli levels is often linked to an increased vulnerability to imbalances and issues in the system; their protective function is crucial in such scenarios. Lactobacilli combat bacteria through the production of substances like acid and hydrogen peroxide as well as bacteriocins. Understanding these microbe interactions could lead to the development of treatments, for preserving vaginal health naturally and resisting antibiotic resistant pathogens. This research emphasizes the role that lactobacilli play in sustaining health and battling infections.

Keywords: lactobacillus, bacterial vaginitis, vaginal infection, antibiotic resistance.

1. INTRODUCTION

The diverse bacterial flora in the female genital tract significantly influences vaginal health and overall flora balance. Vaginal infections are among the most prevalent female health issues, often leading to severe complications and contributing to the high incidence of gynecological disorders. Bacterial vaginosis (BV) is the most frequent cause of abnormal vaginal discharge in women of reproductive age [1, 2]. The composition of microbial flora in the vagina and cervix varies across different stages of health, development, and disease.

Lactobacillus bacteria, which are typically found in the vaginal microflora, play an essential role. A reduction in lactobacilli numbers in patients suffering from various conditions, such as urinary tract infections, bacterial vaginosis, and other genital infections—including cervical cancer—can lead to significant health issues, warranting careful attention [3,4]. Generally, lactobacilli are not linked to disease and are regarded as non-pathogenic members of the intestinal and vaginal flora [5]. They help sustain a healthy vaginal environment by inhibiting the growth of pathogenic bacteria.

Regulatory processes *Lactobacillus* species produce antibacterial compounds such as lactic and other organic acids, hydrogen peroxide (H_2O_2), and bacteriocins [8].

2. MATERIALS AND METHODS

2.1 Isolation of bacterial vaginal infection pathogens (BV).

Vaginal pathogens were isolated from vaginal cultures of women with clinical BV at Aisha (AIWA) medical center (Tashkent) and Karima hospital (Tashkent), whose diagnosis was confirmed by significant BV. Appropriate vaginal fluid samples were cultured on MHA (himedia) soft agar (tryptic soy broth with 0.7% agar, BBL, Microbiology System, Md) under aerobic conditions at 37℃ for 18-24 hours, and pH values were determined.

2.2 Isolation of lactic acid bacteria from various sources

The substrate for isolation of LAB (fermented vegetables - medicinal plants and women swabs sample) placed in MRS broth and cultured at 37 ° C for 48 hours for enrichment. Serial dilutions were prepared from the enriched broth and sown as a continuous lawn on MRS agar, 2 dishes for each dilution. 1 dish from 2 replicates was placed in a thermostat under aerobic cultivation conditions, the second - in an anaerobic jar, where the air was replaced with gaseous nitrogen (under anaerobic conditions). Cultivated at 37 ° C for 48 hours. After cultivation, isolated colonies differing in morphological features were selected from the dishes, then transferred to a dish with MRS agar containing 2-3 drops of an alcohol solution of the bromocresol purple indicator to determine acid formation. The dishes were incubated at 37°C until growth appeared. Isolates that changed the color of the medium from purple to yellow were preliminarily considered to be LAB and used for further studies.

2.3 Bacterial identification and susceptibility testing

The identification of bacteria isolated from urine samples were performed by Maldi-TOF. Initially, the urine samples are cultured on Mueller Hinton agar (Himedia) and were incubated at temperature 35–37°C.

2.4 Study of the sensitivity of isolates to antibiotics

Sensitivity to antibiotics was studied using the method of Da CunhaL R. et al. [5]. The sensitivity of all 4 vaginal isolates to 10 antibiotics was studied – representatives of different groups, most frequently used in clinical practice – Fluconazole (25 μg), chloramphenicol (30 μg), erythromycin (10 μg), penicillin-G (10 unit), cefazolin (30 μg), tetracycline (30 μg), ciprofloxacin (5 μg), cefoperazone/Sulbactam (75/10 μg), nitroxoline (30μg) (HiMedia, India). The diameter of the growth-free zone was measured and the ratio of each culture to antibiotics was assessed as resistant (R), moderately resistant (SR) and sensitive (S) according to the supplier's instructions. The analysis was performed in triplicate. The sensitivity of isolates of pathogens causing dermatological infections was studied using the same method using disks with antibiotics. In addition to the antibiotics listed above, the following disks with antibiotic were used: oxacillin (1 μg), (HiMedia, India).

2.4 Study of antimicrobial activity of isolates

Antagonistic properties of isolates against pathogens in vitro were analyzed by the spot method described by Da Cunha L.R. et al. [6].

3. RESULT

3.1 Lactobacillus isolation

The vaginal samples from 50 healthy and diseased women were diluted and cultured on MRS agar plates. Colonies (80) were mostly obtained from the surface of MRS agar. Most of them showed round, small colonies without pigment and white to cream in color. They were all tested later
Gram staining, catalase production and spore formation are examined. Only 5 of them showed characteristics of gram-positive, catalase-negative and non-sporulating lactobacilli. Finally, 5 potent antimicrobial isolates were selected for further analysis.

Samples taken from 100 patients with vaginal infections yielded both Gram-positive and Gram-negative bacteria. A total of 150 bacterial isolates were identified using MALDI-TOF (Table-1.) five bacterial pathogens were selected using selective media, and pure cultures were maintained on nutrient agar for further investigation. The cultures included *Candida albicans, E. coli, E. faecalis, S. epidermidis.*

Table- 1 Bacteria isolated from vaginal infection

Microorganism	***Overall %***
Gram positive bacteria	
S. epidermidis	6%
Staphylococcus pasteurii	3%
Staphylococcus aureus	8%
Enterococcus faecalis	2%
Enterococcus faecium	2%
Lactobacillus concavus	0.1%
L.paracasei	0.1%
Grame negative bacteria	
E.coli	23%
Klebsiella pneumoniae	2%
Proteus mirabilis	5%
P. aeruginosa	2%
Pseudartrobacter olychromoges	1,6%
Psrevibacillus invocatus	1,6%
Fungi	
Candida albicans	8.3%
Candida tropicalis	3.3%
Candida galabrata	3.3%
Candida krusei	1.6%
Total	**100%**

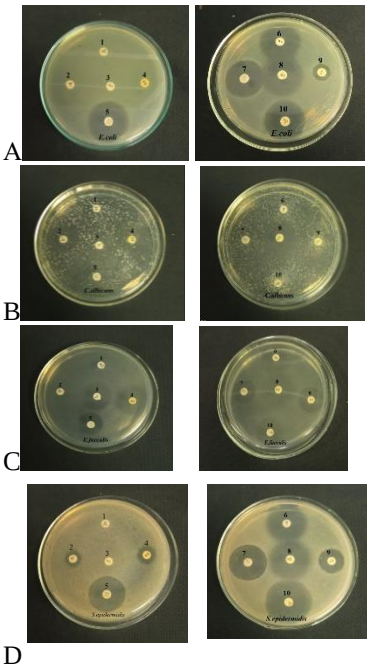

Fig 1. A. E.coli; B.C.albicans;C.E.faecalis; D. S.epidermidis
1.Fluconazole 2. Oxacillin 3. Erythromycin 4. Tetracycline 5. Cefoperazone/ Sulbactam 6. Chloramphenicol 7. Cefazolin 8.Nitroxoline 9.Penicillin-G 10.Ciprofloxacin

The occurrence of Candida infections is increasing with a large number of antibiotic resistant strains in recent years [9]. *Candida albicans* vaginitis, caused by the polymorphic fungus *C. albicans*, is a prevalent gynecological infection among women worldwide. Limited antifungal drug options and a rising rate of opportunistic fungal infections have led to growing drug resistance in fungal pathogens, which presents a significant public health challenge [10]. Recurrent infections are common, even in patients treated with antifungal medications for *C. albicans* vaginitis [11]. New therapeutic agents for *C. albicans* vaginitis are urgently awaiting for development because of the antibiotic resistance of clinical isolated *C. albicans* strains. Antifungal drug resistance has been studied and most extensively with the yeast *Candida albicans* owing to its importance as an opportunistic pathogen and its experimental tractability relative to other medically important fungal pathogens. In our researches the resistance of selected bacteria and yeast to various groups of antibiotics was studied. *E.coli* resistance to beta-lactam and macrolide antibiotics such as penicillin-G,

oxacillin, and erythromycin has been observed. Nevertheless, these cases showed sensitivity to antibiotics of the new class of antibiotics, such as cephalosporins (eg, cefazolin and cefoperozone/sulbactam), sulfonamides, chloramphenicol, and fluoroquinolones (eg, ciprofloxacin) However, *Candida albicans* was found to be sensitive only to tetracycline and cefoperozone/sulbactam, but all others were found to be resistant to the antibiotics studied.

Staphylococcus epidermidis and *Enterococcus faecalis* bacteria showed sensitivity to erythromycin and oxacillin, respectively.

Table 2. Effect of antibiotics on vaginal pathogens

№	**Antibiotics**		**Growth inhibition zone diameter, mm**

Lactobacillus strains		**Lactobacillus strains Diameter of Inhibitory zone (mm)**			
		C. albicans	***E.coli***	***E.faecalis***	***S.epidermidis***
1	*L.plantarum-1*	34±0.5	18±0.28	13±0.	16±0.28
2	*L.plantarum-2*	28±0	15±0.5	18±0.5	15±0.5
3	*L.plantarum-3*	28±0.7	21±0.28	28±0.5	21±0.5
4	*L.delbrueckii*	0	0	0	0
5	*L.paracasei*	0	0	0	0

		Class of antib.	**Conc. µg/disc**	***E.coli***	***C.albicans***	***S. epidermidis***	***E. faecalis***
1	Fluconazole	**I**	25	-	R	-	-
2	Erythromycin		15	R	R	R	S
3	Oxacillin	**II**	1	R	R	SR	R
4	Penicillin-G		10	SR	R	S	S
5	Nitroxoline	**III**	30	S	R	S	S
6	Tetracycline		30	R	S	SR	S
7	Cefazolin	**IV**	30	S	R	S	S
8	Cefoperazone/Sulbactum	**V**	10	S	S	S	S
9	Chloramhenicol	**VI**	30	S	R	S	S
10	Ciprofloxacin	**VII**	5	S	R	S	S

Note: I. Macrolides; II. β-laktam; III.Tetracycline; IV.Cephalosporin; V.Sulfonamides; VI.Chloramphenicol; VII.Fluro-Quinolones: R-resistance; SR-sensitive resistance;S-sensitive; - not determined.

The rises in antibiotic resistance have shifted focus toward alternative treatment options. Probiotic therapy, which involves the oral or intravaginal administration of Lactobacilli to restore healthy microbial balance, has shown promise [7].

Antimicrobial activity of lactobacilli isolated from plants and patients with BV against four types of vaginal opportunistic pathogens was determined.

The findings revealed that the three bacteria isolated from plant sources exhibited varying levels of activity. Considered the studied bacteria, three strains belong to *L. plantarum*. Among them, *L. plantarum*-1 demonstrated a 13 mm activity against *E. faecalis*, which is lower, and a 34 mm activity against *C. albicans*, which is higher compared to the other Lactobacillus strains.

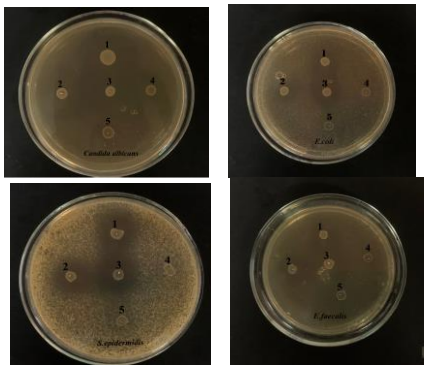

Fig 2. Activity of lactobacilli against vaginal infectious agents

Table 1 Comparison of average inhibitory zones of six lactobacillus strains against four representative BV causing tract infection

Additionally, L. delbrueckii and L. paracasei isolated from the vaginal microflora did not show antimicrobial activity against the studied gram-negative and gram-positive bacteria and Candida albicans belonging to the genus Candida.However, numerous studies have reported that these bacteria exhibit activity against strains isolated from different sources [12]. Consequently, they may prove beneficial for other fields in the future.

4. CONCLUSION

Bacterial vaginosis (BV), a polymicrobial vaginal infection in reproductive-age women, is a significant health concern. BV often disrupts the balance between vaginal microflora and opportunistic bacteria, potentially leading to gynecological complications such as premature birth, miscarriage, and endometritis. The use of lactobacilli offers a promising approach to combat antibiotic resistance and restore the natural balance of the vaginal microbiome Therefore, it is planned to study the proteins produced by lactobacteria and their activity in further studies

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