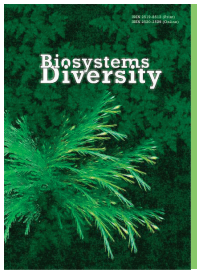

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Prophylactic effect of lactobacilli and bifidobacteria probiotic strains on experimental bacterial vaginitis

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The objective of the study was determining the prophylactic effect of *Lactobacillus casei* IMV B-7280, *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *Bifidobacterium animalis* VKL and *B. animalis* VKB probiotic strains on experimental vaginitis in BALB/c mice induced by *Staphylococcus aureus* 8325-4. The infection with *S. aureus* 8325-4 caused an imbalance of microbiota in the vagina and intestine, as evidenced by an increase in the number of opportunistic microorganisms and a decrease in the amount of lactobacilli and bifidobacteria. *L. casei* IMV B-7280, *B. animalis* VKL and *B. animalis* VKB probiotic strains altered the microbiota spectrum of the vagina and intestine of *Staphylococcus*-infected mice: the amount of *Lactobacillus* and bifidobacteria increased with the reduction of the number of opportunistic microorganisms. Also under the influence of these strains, the normalization of the microbiota spectrum typical for vagina and intestine was observed in different periods of observation – in the intestines of mice the number of coliform bacteria increased, the number of microscopic fungi, streptococci and staphylococci decreased; in the vagina, the number of coliform bacteria and microscopic fungi decreased, the number of streptococci normalized. Rapid elimination of *S. aureus* 8325-4 from the vagina and prevention of the spread of infection to the intestine were observed after use of probiotics. Preventive effect of *L. acidophilus* IMV B-7279 and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 for bacterial vaginitis in mice was less effective. So, the target probiotic strains *L. casei* IMV B-7280, *B. animalis* VKL and *B. animalis* VKB are promising for the creation of highly effective novel probiotic drugs that can be used for directed prevention of infectious and inflammatory diseases of the genitourinary system caused by pathogenic and opportunistic microorganisms.

Keywords: *Lactobacillus*; *Bifidobacterium*; *Staphylococcus*; microbiota; vagina; gut; mice

Introduction

Development and improvement of means for the prevention and treatment of genitourinary system infectious diseases caused by pathogenic and opportunistic bacteria, viruses and microscopic fungi are some of the priority public health needs. Despite the constant increase in the number of antibiotics and antiviral drugs and expansion of their spectrum of action and therapeutic efficacy, the number of infectious diseases of the genitourinary system, according to statistics, not only does not decrease, but on the contrary rapidly increases (Kenyon & Osbak, 2014). In addition, in recent years, a significant increase in the number of chronic and recurrent infections of the genitourinary system, the occurrence of concomitant pathologies in the form of dysbiotic disorders or the accession of other pathogens to the primary infectious process were noted (Cianci et al., 2018).

Frequent recurrent uncomplicated urinary tract infections, which arise primarily in patients with immunosuppression who received high doses of immunosuppressive drugs, antibiotics, hormones, irradiation with radiotherapy etc., can be a cause of serious illness that involves not only organs of genitourinary system, but the organism as a whole. So, prolonged bacterial vaginosis caused by opportunistic bacteria is associated with a high risk of developing of infectious diseases, infertility, autoimmune and neuroendocrine diseases, and may increase the risk of late miscarriage (Martin et al., 1999; Schwebke, 2003; Hay, 2004).

Combined therapy of patients with bacterial vaginitis and urinary system infections including, in particular, the use of antibiotics, resulted in more disorders of the natural balance of microbiota of various cavities and leads to the "vicious circle" of constantly recurrent infectious pathologies and of new courses of antibiotic therapy. In addition, the widespread use of chemotherapeutic agents of various origins, including the newest antibiotics, has led to the selection of resistant strains of opportunistic microorganisms (Podgorskij et al., 2004). Therefore, at the present stage, the use of complex antibiotic therapy with the simultaneous treatment of patients with several antibacterial agents with different mechanisms of action gradually is being introduced to the therapeutic practice, which has negative consequences not only for microbiota of different body cavities, but also for organs and systems of macroorganism.

Therefore, the development of alternative means for prevention and treatment of patients with uncomplicated urinary tract infections and vaginosis that involve the use of natural products with an antagonistic effect on infectious agents and the ability to balance the immune response is relevant. Such means are the latest probiotic preperetions, created on the basis of representatives of normal microbiota – non-pathogenic lactic acid bacteria with antibacterial, anti-inflammatory and immunomodulatory properties (Reid, 2001; MacPhee et al., 2013).

Previously we found that *Lactobacillus acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *L. casei* IMV B-7280, *Bifidobacterium animalis* VKL and *B. animalis* VKB probiotic strains have a wide range of antagonistic effects against pathogenic and opportunistic microorganisms *in vitro* and *in vivo*, as well as immunomodulatory and anti-inflammatory properties aimed at changing the cytokine profile of organism and activating the factors of innate immune response (Lazarenko et al., 2012; Babenko et al., 2015). Probiotic strains *L. casei* IMV B-7280, *B. animalis* VKL and *B. animalis* VKB had an effective curative effect on the model of bacterial vaginitis of BALB/c line mice: under their influence, the elimination of pathogenic and opportunistic bacteria from the vagina, kidneys and intestinal contents was observed. In these mice we detected normalization of microbiota of different biotopes, as well as indicators of immunoreactivity (activity of phagocytic system cells, cellular immunity indexes and production of cytokines – interferon-γ, interleukin (IL)-12, IL-4) (Mokrozub et al., 2015; Lazarenko et al., 2017). However, the potential prophylactic effect

of probiotic strains of lactobacilli and bifidobacteria and their ability to prevent the development of bacterial infection of the genitourinary system was not identified.

The novelty of this study is to implement the latest approaches to the production of probiotic preparations based on the representatives of commensal microbiota of different biotopes with simultaneously declared antibacterial, immunomodulatory and anti-inflammatory properties for the prevention of infectious and inflammatory diseases and treatment of patients. Therefore, the objective of this work was determining the prophylactic action of *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL and *B. animalis* VKB probiotic strains in case of experimental vaginitis in BALB/c line mice induced by *Staphylococcus aureus* 8325-4.

Materials and methods

Objects of the study were five potentially probiotic strains of the genus *Lactobacillus* and *Bifidobacterium*, such as *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL and *B. animalis* VKB. These strains were previously isolated from clinically healthy donors from associated cultures during laboratory studies of fermented biological material. The bacteria that freeze dried using the Cuddon Freeze Dryer FD1500 (New Zealand). Before each experiment, the viability of strains was checked by controlling their growth on selective media for lactobacilli – Man-Rogosa-Sharpe (MRS) and bifidobacteria – Bifido Agar (BA) ("Merck", Germany) at 37 °C for 24–48 hours in aerobic and anaerobic conditions, respectively. In this work, the probiotic preparation Labilact® (SPA Ariadna, Odessa, Ukraine), which includes a mixture of freeze-dried strains of lactobacilli and bifidobacteria, placed in the capsule for intravaginal administration, was used for comparison.

Experimental studies were carried out on female mice of the BALB/c line of 6–8 weeks old, 17–22 g weight, synchronized according to the estral cycle. The animals were kept in standard conditions in plastic cells in a separate room at a constant air temperature (22–25 ºC). The animals received a good meal and had free access to water. Before the experiment, all animals were kept at quarantine for two weeks and had no signs of the disease. All studies were conducted taking into account the norms of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (18.03.1986, Strasbourg) and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment" (Reznikov, 2001).

Animals were randomly assigned to groups of 15 individuals in each (n = 15). A suspension of probiotic bacteria (each strain individually) in 0.15 M NaCl was injected into vagina in the volume of 0.025 mL and the dose of 5 x 10^6 cells/animal once per day during 7 days. Under the same scheme, a separate group of mice was intravaginally administered with the Labilact® probiotic preparation. The control group included intact mice receiving 0.15 M NaCl intravaginally.

In order to simulate vaginitis in mice we used *Staphylococcus aureus* strain 8325-4. This strain was grown on a selective medium for staphylococci BAIRD-PARKER-Agar (Merck, Germany) at 37 °C for 24 hours. Daily culture of *S. aureus* 8325-4 in suspension with 0.15 M NaCl was administered into vagina of mice in a dose of 5 x 10^7 cells per animal after completing the course of prophylactic administration of probiotic strains. *S. aureus* 8325-4 contains the plasmid of resistance to gentamicin, therefore it can be separated from other microorganisms on the growth medium containing this antibiotic (15 μg/mL).

Vaginal discharges were collected on the 1st, 3rd, 6th, 9th and 12th days after infecting mice using standard sterile cotton swabs that were placed in test tubes containing 1 mL of sterile 0.15 M NaCl. At the same time, feces were collected, weighed, placed in a test tube and filled with a volume of 0.15 M NaCl to form a suspension of 0.1 g of feces per mL (De Jongh et al., 1968).

To determine the spectrum of microbiota, aliquots from the vagina and feces were plated on eight nutrient media, namely: meat-peptone agar (MPA) – medium for cultivation of aerobic and optional anaerobic microorganisms; BAIRD-PARKER-Agar (Merck, Germany) – selective medium for staphylococci; BAIRD-PARKER-Agar with gentamicin in concentration of 15 μg/mL – selective medium for isolation of *S. aureus* 8325-4 strain; KF-*Streptococcus* agar (Merck, Germany) – selective medium for streptococci, MRSA (HiMedia, India) – selective medium for lactobacilli; BA (HiMedia, India) – selective medium for bifidobacteria; ENDO (HiMedia, India) – selective medium for coliform bacteria; Sabouraud agar (HiMedia, India) – selective medium for microscopic fungi. After cultivation at 37 °C for 24 hours, the number of colonies per petri dish was calculated, given that one such colony was grown out of one bacterium (Brown & Perry, 1992).

All biological material, as well as all the tools and laboratory utensils used during the research, were decontaminated by autoclaving. Remains of animals were utilized in accordance with the recommendations (Niwayama, 1971).

All received digital data were processed using the computer program Epi Info (version 8.0) by the method of variation statistics. Numerical data was presented in the form of arithmetic average and standard deviations (x ± SD). Null hypothesis for the comparison groups was verified using non-parametric Wilcoxon-Mann-Whitney (U) and Kolmogorov-Smirnov criteria. Differences between the groups were considered statistically significant at $P < 0.05$.

Results

Microbiota of the vagina of mice that received probiotic strains intravaginally as a prophylactic. In the vagina of infected mice that did not receive probiotic strains of bacteria (control group) *S. aureus* 8325-4 was detected in a constant amount throughout the observation period (Fig. 1). At the same time from the vagina of infected mice that received probiotic strains of bacteria *S. aureus* 8325-4 were collected in a smaller quantity. It was completely eliminated from the vagina of infected mice after prophylactic use of *L. casei* IMV B-7280 (on the 6th day), *B. animalis* VKB (on the 9th day) and *B. animalis* VKL, *L. acidophilus* IMV B-7279 or *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (on the 12th day). The number of *S. aureus* 8325-4 did not decrease in the vagina of mice throughout the observation period after prophylactic use of the Labilact® probiotic preparation.

In the vagina of mice of control group, there was a smaller amount of lactobacilli on the 6–12th days and bifidobacteria on the 3–12th days compared to intact mice. The number of lactobacilli increased in the vagina of infected mice that received *L. casei* IMV B-7280 or *B. animalis* VKB on the 1–12th days, *B. animalis* VKL on the 3–12th days, *L. delbrueckii* subsp. bulgaricus IMV B-7281 on the 6–12th days, *L. acidophilus* IMV B-7279 on the 6–9th days, and the Labilact® probiotic preparation only on the 6th day compared to the control group (Fig. 2*a*).

The number of bifidobacteria in the vagina was also higher during the observation period after administration of probiotic bacteria than in the control group, and after prophylactic use of the Labilact® probiotic preparation – only on the 6th and 12th days (Fig. 2*b*).

The imbalance of vaginal microbiota of staphylococcus infected mice against the background of decreasing the amount of lactobacilli and bifidobacteria was also confirmed by a significant increase in the number of opportunistic microorganisms (aerobic and optional anaerobic microorganisms, streptococci and staphylococci). The number of these opportunistic microorganisms at different periods of observation varied in the vagina of mice that received probiotic bacteria and the Labilact® probiotic preparation as a prophylactic (Table 1). Thus, the number of coliform bacteria in the vagina of mice decreased after prophylactic use of *L. casei* IMV B-7280 on the 1–6th and 12th days, *L. acidophilus* IMV B-7279 or *B. animalis* VKB on the 1st, 3rd and 12th days, *B. animalis* VKL or *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 on the 1st and 12th days. In the vagina of infected mice microscopic fungi were detected in a smaller number after prophylactic use of *L. casei* IMV B-7280 or *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 on the 1–12th days, *B. animalis* VKL on the 6–9th days or *B. animalis* VKB on the 6–12th days. However, the number of microscopic fungi increased in the vagina of infected mice after prophylactic use of *L. acidophilus* IMV B-7279 on the 1–12th days, *B. animalis* VKL – on the 3rd and 12th days, *B. animalis* VKB – on the 3rd day, and Labilact® probiotic preparation – on the 3–6th days.

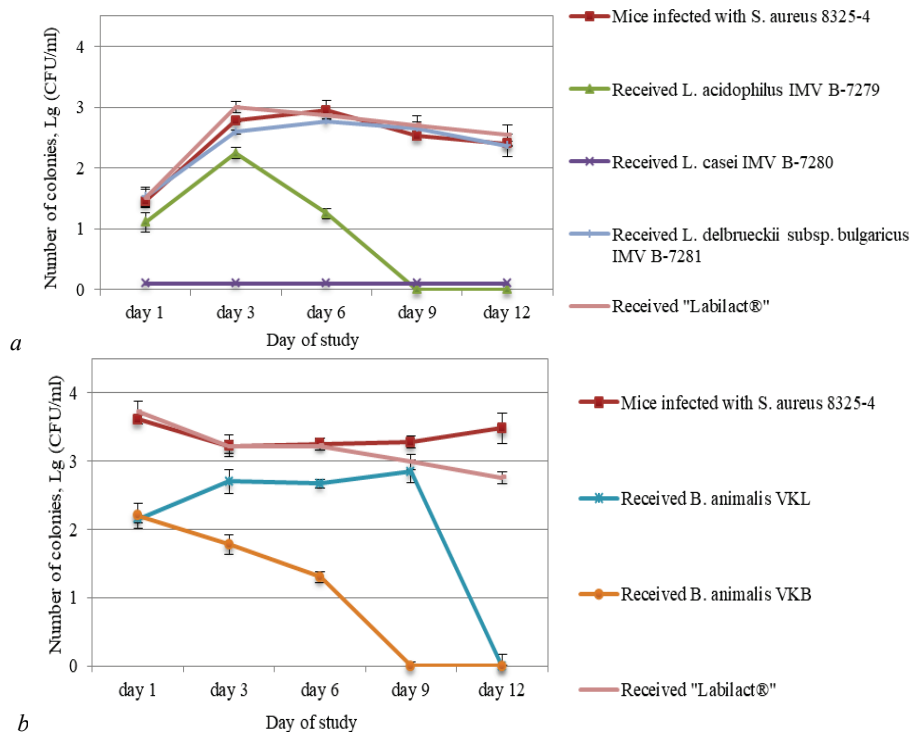

Fig. 1. The number of *S. aureus* 8325-4 colonies in the vagina of infected mice, who had previously received probiotic strains of lactobacilli (*a*) and bifidobacteria (*b*) as a prophylactic: x ± SD, n = 15

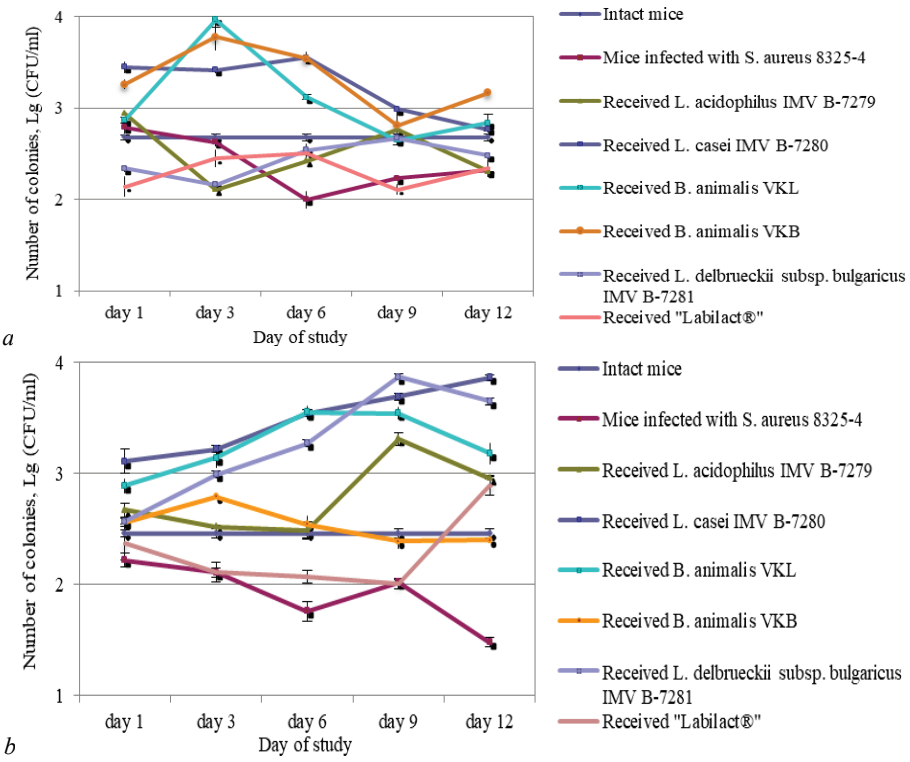

Fig. 2. The number of lactobacilli (*a*) and bifidobacteria (*b*) in the vagina of infected mice, who had previously received probiotic strains as a prophylactic: x ± SD, n = 15

Microbiota of the intestines of mice that received probiotic strains intravaginally as a prophylactic. It was shown that the number of lactobacilli in the intestine of infected mice was lower than in intact mice during the entire period of observation, and bifidobacteria – on the 6–12th days (Fig. 4). In the intestinal contents of infected mice the *S. aureus* strain 8325-4 was detected from the 1st day to 12th day, the total number of staphylococci also increased (Fig. 3, Table 2).

The number of aerobic and optional anaerobic microorganisms was lower in the intestine of infected mice than in intact animals on the 3rd and 9–12th days, and the number of streptococci was lower only on the 6th day. The number of coliform bacteria was lower than in control on the 3–12th days, and the number of microscopic fungi was increased on the 1–6th and 12th days. The obtained data confirm total spread of the infectious process from the genitourinary system on the gastrointestinal tract in infected animals of control group.

S. aureus 8325-4 strain was not detected in the intestine of infected animals during the observation period after prophylactic use of *L. casei* IMV B-7280, *B. animalis* VKB or *B. animalis* VKL. Prophylactic use

of *L. acidophilus* IMV B-7279 reduced the number of *S. aureus* 8325-4 colonies on the 6–9th days and resulted in complete elimination of the pathogen on the 12th day. *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, as well as the Labilact® probiotic preparation, did not affect the number of *S. aureus* 8325-4 colonies in the intestinal contents of infected mice (Fig. 3).

Table 1

Spectrum of opportunistic microorganisms in the vagina of infected mice, that previously received probiotic strains as a prophylactic (x ± SD, n = 15)

Groups of animals	Day	Number of microorganisms, Lg CFU/mL				
		MPA	Baird-Parker-agar	KF-Streptococcus agar	ENDO	Sabouraud agar
Intact mice	–	2.24 ± 0.12	2.51 ± 0.08	2.08 ± 0.04	1.08 ± 0.05	2.07 ± 0.04
Mice infected with *S. aureus* 8325-4	1	3.78 ± 0.13*	4.54 ± 0.08*	3.53 ± 0.05*	1.60 ± 0.04*	2.63 ± 0.05*
	3	3.82 ± 0.09*	4.30 ± 0.05*	3.45 ± 0.03*	1.48 ± 0.04*	1.48 ± 0.02*
	6	3.83 ± 0.11*	4.25 ± 0.07*	3.34 ± 0.07*	1.48 ± 0.06*	2.18 ± 0.08
	9	3.51 ± 0.08*	4.22 ± 0.11*	3.26 ± 0.11*	1.30 ± 0.00*	2.34 ± 0.05*
	12	3.36 ± 0.08*	4.19 ± 0.09*	3.22 ± 0.08*	1.48 ± 0.04*	1.90 ± 0.02
Received *L. acidophilus* IMV B-7279	1	3.00 ± 0.09*•	3.11 ± 0.06*•	2.38 ± 0.02*•	< 0.1*•	2.77 ± 0.04*
	3	3.23 ± 0.04*•	3.02 ± 0.07*•	4.60 ± 0.11*•	1.11 ± 0.02•	3.14 ± 0.06*•
	6	4.78 ± 0.03*•	4.48 ± 0.12*	4.70 ± 0.09*•	2.12 ± 0.03*•	3.55 ± 0.11*•
	9	3.09 ± 0.08*•	3.16 ± 0.09*•	3.36 ± 0.06*	1.21 ± 0.01	2.65 ± 0.03*•
	12	3.28 ± 0.11*	3.41 ± 0.11*•	2.95 ± 0.02*•	1.30 ± 0.02*•	2.12 ± 0.02•
Received *L. casei* IMV B-7280	1	3.08 ± 0.04*•	3.00 ± 0.03*•	3.08 ± 0.07*•	< 0.1*•	1.30 ± 0.03*•
	3	3.30 ± 0.06*•	3.30 ± 0.02*•	3.48 ± 0.10*	< 0.1*•	1.45 ± 0.01*
	6	4.95 ± 0.08*•	4.92 ± 0.09*•	4.64 ± 0.14*•	< 0.1*•	0.76 ± 0.02*•
	9	4.68 ± 0.13*•	4.58 ± 0.10*•	4.53 ± 0.06*•	1.32 ± 0.03*	< 0.1*•
	12	2.75 ± 0.02*•	3.62 ± 0.07*•	3.76 ± 0.06*•	< 0.1*•	< 0.1*•
Received *B. animalis* VKL	1	2.95 ± 0.05*•	2.34 ± 0.01•	3.09 ± 0.03*•	< 0.1*•	2.56 ± 0.03*
	3	2.34 ± 0.06•	3.96 ± 0.07*•	4.86 ± 0.09*•	2.00 ± 0.05*•	2.04 ± 0.07•
	6	4.60 ± 0.16*•	4.60 ± 0.09*•	3.90 ± 0.03*•	2.34 ± 0.06*•	1.28 ± 0.08*•
	9	4.54 ± 0.14*•	4.23 ± 0.14*	4.43 ± 0.09*•	1.78 ± 0.03*•	1.75 ± 0.06*•
	12	3.45 ± 0.08*	3.20 ± 0.03*•	3.26 ± 0.07*	1.30 ± 0.02*•	2.19 ± 0.02•
Received *B. animalis* VKB	1	2.38 ± 0.03•	2.60 ± 0.03•	2.90 ± 0.03*•	< 0.1*•	2.48 ± 0.03*
	3	3.04 ± 0.07*•	2.38 ± 0.04•	2.90 ± 0.02*•	< 0.1*•	2.64 ± 0.02*•
	6	3.06 ± 0.08*•	2.48 ± 0.01•	2.38 ± 0.04*•	1.43 ± 0.01*	2.20 ± 0.01
	9	1.78 ± 0.00*•	2.70 ± 0.04•	3.10 ± 0.06*	1.23 ± 0.02*	1.60 ± 0.03*•
	12	2.30 ± 0.05•	2.56 ± 0.07•	2.38 ± 0.02*•	< 0.1*•	< 0.1*•
Received *L. delbrueckii* subsp. *bulgaricus* IMV B-7281	1	1.60 ± 0.00*•	3.05 ± 0.09*•	1.60 ± 0.01*•	< 0.1*•	< 0.1*•
	3	3.04 ± 0.07*•	3.09 ± 0.11*•	3.15 ± 0.02*•	2.38 ± 0.07*•	< 0.1*•
	6	3.78 ± 0.08*	2.62 ± 0.02•	3.48 ± 0.09*	2.60 ± 0.03*•	1.43 ± 0.02*•
	9	3.28 ± 0.05*	2.79 ± 0.04*•	2.34 ± 0.07*•	1.78 ± 0.02*•	1.10 ± 0.01*•
	12	2.73 ± 0.02*•	2.56 ± 0.03•	2.48 ± 0.12*•	1.32 ± 0.03*•	< 0.1*•
Received "Labilact®"	1	3.79 ± 0.07*	3.99 ± 0.07*•	3.64 ± 0.07*	2.02 ± 0.05*•	2.34 ± 0.04*•
	3	3.54 ± 0.06*	4.15 ± 0.09*	3.89 ± 0.11*•	1.98 ± 0.03*•	1.76 ± 0.01*•
	6	3.17 ± 0.04*•	4.08 ± 0.08*	2.75 ± 0.08*•	1.94 ± 0.04*•	2.55 ± 0.05*•
	9	3.96 ± 0.10*•	3.97 ± 0.09*	2.45 ± 0.04*•	1.55 ± 0.06*•	2.07 ± 0.02•
	12	3.82 ± 0.09*•	4.12 ± 0.12*	2.96 ± 0.05*•	1.67 ± 0.03*•	1.96 ± 0.01

Notes: * – P < 0.05 in comparison with indices of intact mice; • – P < 0.05 in comparison with indices of mice of control group.

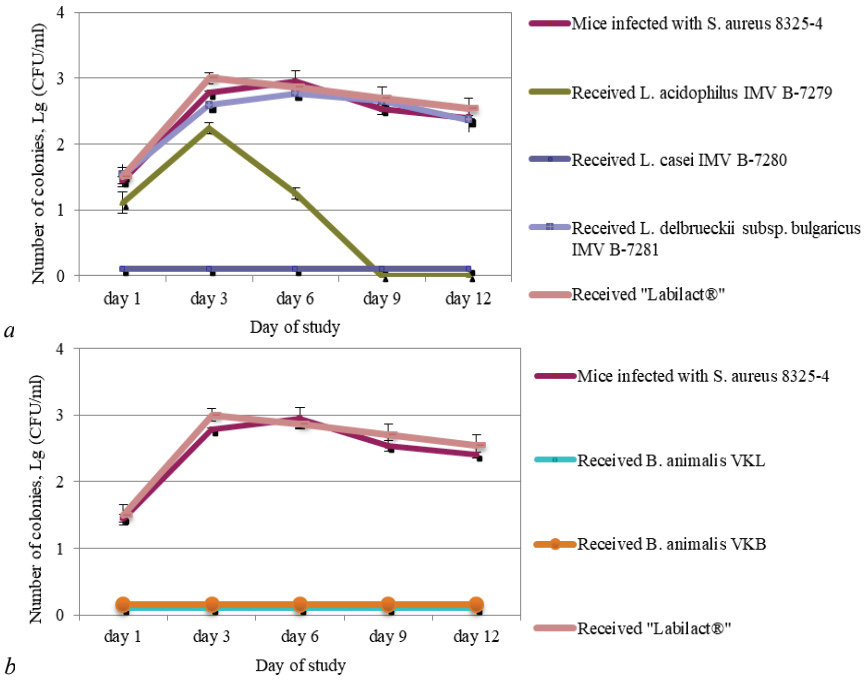

Fig. 3. The number of *S. aureus* 8325-4 colonies in the feces of infected mice, who had previously received probiotic strains of lactobacilli (*a*) and bifidobacteria (*b*) as a prophylactic (x ± SD, n = 15)

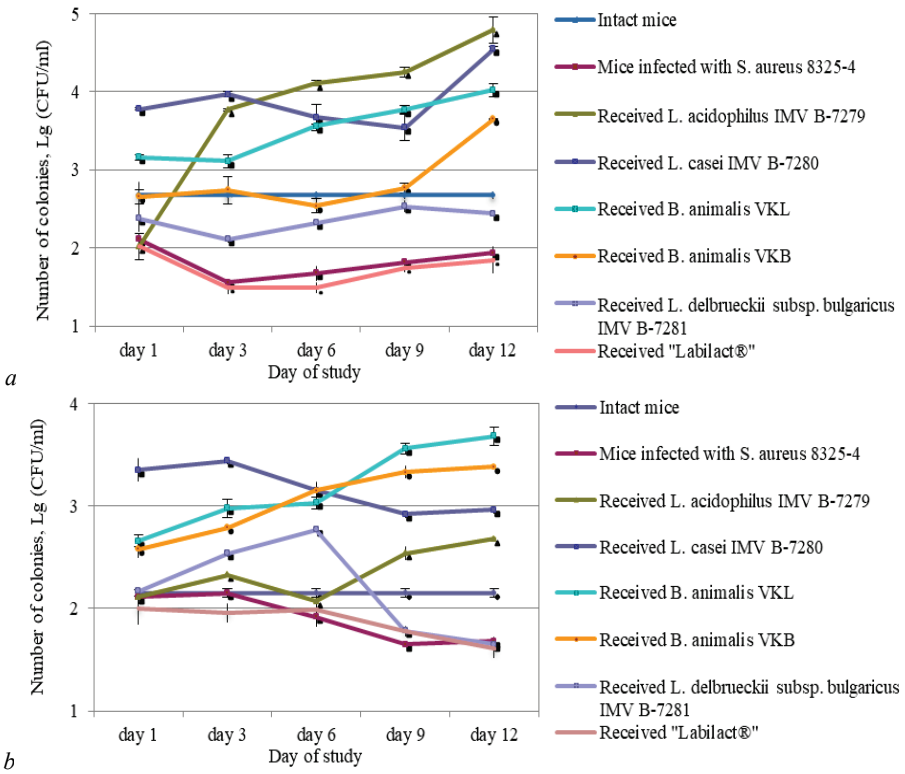

Fig. 4. The number of lactobacilli (*a*) and bifidobacteria (*b*) in the feces of infected mice, who had previously received probiotic strains as a prophylactic (x ± SD, n = 15)

Table 2

Spectrum of opportunistic microorganisms in the feces of infected mice, that previously received probiotic strains as a prophylactic (x ± SD, n = 15)

Groups of animals	Day	Number of microorganisms, Lg CFU/mL				
		MPA	Baird-Parker-agar	KF-Streptococcus agar	ENDO	Sabouraud agar
Intact mice	–	4.75 ± 0.08	2.87 ± 0.04	3.25 ± 0.05	4.15 ± 0.09	2.77 ± 0.05
Mice infected with *S. aureus* 8325-4	1	4.65 ± 0.06	3.98 ± 0.06*	3.12 ± 0.06	4.11 ± 0.07	2.98 ± 0.04*
	3	4.32 ± 0.04*	3.65 ± 0.08*	3.05 ± 0.07	3.64 ± 0.07*	3.11 ± 0.06*
	6	4.98 ± 0.07	3.78 ± 0.11*	2.96 ± 0.02*	3.38 ± 0.08*	3.14 ± 0.08*
	9	4.13 ± 0.05*	3.42 ± 0.06*	3.09 ± 0.05	3.43 ± 0.02*	2.76 ± 0.08
	12	4.25 ± 0.10*	3.56 ± 0.08*	3.13 ± 0.04	3.58 ± 0.03*	3.04 ± 0.06*
Received *L. acidophilus* IMV B-7279	1	4.51 ± 0.09	3.55 ± 0.05*•	3.78 ± 0.03*•	4.17 ± 0.07	2.78 ± 0.03
	3	4.29 ± 0.06*	3.78 ± 0.06*	4.25 ± 0.04*•	3.25 ± 0.04*•	3.56 ± 0.02*•
	6	4.77 ± 0.07	4.22 ± 0.08*•	4.63 ± 0.07*•	3.61 ± 0.09*	4.12 ± 0.05*•
	9	5.12 ± 0.08*•	3.65 ± 0.04*	4.77 ± 0.08*•	3.45 ± 0.02*	4.55 ± 0.05*•
	12	5.66 ± 0.04*•	3.17 ± 0.05*	4.82 ± 0.05*•	4.05 ± 0.03•	4.59 ± 0.08*•
Received *L. casei* IMV B-7280	1	4.56 ± 0.03	3.15 ± 0.07*•	3.15 ± 0.03	4.05 ± 0.05	2.55 ± 0.07•
	3	4.73 ± 0.04•	3.46 ± 0.09*	3.02 ± 0.04	3.28 ± 0.06*•	2.87 ± 0.04
	6	4.81 ± 0.05	3.21 ± 0.08*•	2.98 ± 0.06*	3.45 ± 0.08*	2.43 ± 0.03*•
	9	4.92 ± 0.03•	2.89 ± 0.03•	3.17 ± 0.02	3.79 ± 0.07*•	2.27 ± 0.02*•
	12	4.98 ± 0.03•	2.86 ± 0.05	3.24 ± 0.07	4.07 ± 0.12•	2.11 ± 0.05*•
Received *B. animalis* VKL	1	4.65 ± 0.07	2.65 ± 0.03•	3.26 ± 0.04	4.48 ± 0.04*•	2.61 ± 0.04•
	3	4.12 ± 0.07*	3.11 ± 0.06*•	3.74 ± 0.05*•	3.45 ± 0.03*	2.12 ± 0.07*•
	6	3.86 ± 0.09*•	3.22 ± 0.05*	3.24 ± 0.03•	4.20 ± 0.06•	2.10 ± 0.03*•
	9	3.80 ± 0.04*•	2.98 ± 0.08•	3.12 ± 0.07	4.22 ± 0.07•	1.65 ± 0.01*•
	12	3.44 ± 0.05*•	2.96 ± 0.07	2.54 ± 0.02*•	3.98 ± 0.02•	1.87 ± 0.01*•
Received *B. animalis* VKB	1	4.68 ± 0.07	3.22 ± 0.04*•	3.67 ± 0.04*•	4.21 ± 0.03	2.99 ± 0.02
	3	4.21 ± 0.08*	3.65 ± 0.06*	3.10 ± 0.06	3.77 ± 0.03*	3.15 ± 0.03*
	6	4.78 ± 0.06	3.19 ± 0.05*•	2.65 ± 0.06*•	3.24 ± 0.03*	2.54 ± 0.05•
	9	3.91 ± 0.04*	2.86 ± 0.07•	2.84 ± 0.02*•	3.96 ± 0.05•	2.25 ± 0.04*•
	12	3.65 ± 0.05*•	2.65 ± 0.08	2.33 ± 0.03*•	4.11 ± 0.07•	2.11 ± 0.04*•
Received *L. delbrueckii* subsp. *bulgaricus* IMV B-7281	1	3.28 ± 0.09*•	3.27 ± 0.03*•	3.89 ± 0.06*•	4.22 ± 0.09	2.47 ± 0.06*•
	3	4.16 ± 0.02*	3.77 ± 0.02*	4.14 ± 0.08*•	3.98 ± 0.06•	3.69 ± 0.07*•
	6	3.75 ± 0.07*•	3.86 ± 0.08*	4.26 ± 0.09*•	3.74 ± 0.07*•	4.10 ± 0.08*•
	9	4.79 ± 0.09•	4.17 ± 0.09*•	4.51 ± 0.05*•	3.45 ± 0.09*	5.47 ± 0.17*•
	12	5.27 ± 0.05*•	4.25 ± 0.09*	4.89 ± 0.07*•	3.23 ± 0.04*•	6.17 ± 0.29*•
Received "Labilact®"	1	4.52 ± 0.09	3.83 ± 0.07*	3.24 ± 0.05	4.23 ± 0.07	3.22 ± 0.12*
	3	4.59 ± 0.12	3.77 ± 0.08*	3.27 ± 0.03	3.72 ± 0.04*	3.17 ± 0.08*
	6	4.57 ± 0.12	3.86 ± 0.05*	3.14 ± 0.08	3.49 ± 0.05*	3.10 ± 0.09*
	9	4.22 ± 0.08*	3.58 ± 0.12*	3.22 ± 0.11	3.56 ± 0.08*	2.92 ± 0.06*
	12	4.39 ± 0.11*	3.65 ± 0.06*	3.33 ± 0.09	3.67 ± 0.03*	3.25 ± 0.07*

Note: see Table 1.

After prophylactic use of *L. casei* IMV B-7280, *B. animalis* VKL and *B. animalis* VKB the number of lactobacilli in the intestinal contents of infected animals increased on the 1–12th days, and after use of *L. acidophilus* IMV B-7279 and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 – on the 3–12th days (Fig. 4*a*). The Labilact® probiotic preparation did not affect the amount of lactobacilli in the intestines of infected mice.

The amount of bifidobacteria in the intestine of infected mice increased after use of *L. casei* IMV B-7280, *B. animalis* VKL and *B. animalis* VKB on the 1–12th days, *L. acidophilus* IMV B-7279 – on the 9–12th days, and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 – on the 3–6th days (Fig. 4*b*). The Labilact® drug did not affect the amount of bifidobacteria in the intestines of infected mice.

The number of aerobic and optional anaerobic bacteria in the intestine of infected mice increased after administration of *L. casei* IMV B-7280 on the 3rd and 9–12th days and *L. acidophilus* IMV B-7279 or *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 on the 9–12th days, but decreased after use of *B. animalis* VKL on the 6–12th days, *B. animalis* VKB on the 12th day and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 on the 6th day. *L. casei* IMV B-7280 or *B. animalis* VKB reduced the number of staphylococci on the 1st and 6–9th days, *B. animalis* VKL – on the 1–3rd and 9th days, and *L. acidophilus* IMV B-7279 or *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 – on the 1st day, whereas *L. acidophilus* IMV B-7279 and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 increased the number of staphylococci on the 6th and 9th days, respectively. The number of streptococci increased in different periods of observation after use of *L. acidophilus* IMV-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-728, *B. animalis* VKL or *B. animalis* VKB.

The number of coliform bacteria in the intestine of infected mice after administration of *B. animalis* VKL on the 1st and 6–12th days, *L. casei* IMV B-7280 or *B. animalis* VKB on the 9–12th days, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 – on the 3–6th days, and *L. acidophilus* IMV B-7279 – only on the 12th day was increased, but was decreased after administration of *L. casei* IMV B-7280 or *L. acidophilus* IMV B-7279 on the 3rd day and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 – on the 12th day.

Discussion

Currently medical and scientific community considers vaginal microbiota as a complete "ecosystem" (Pascual et al., 2008) which, apart from many other functions, causes direct or indirect antagonistic action against pathogens that cause genitourinary tract infections, and also affects the development of the immune response (Mumtaz et al., 2008; Verstraelen, 2008; Frey Tirri, 2011; Thomas-White et al., 2015). In addition, according to the latest data, the imbalance of vaginal microbiota can cause a range of diseases, including functional disorders of the pelvic organs, pathology of immune, neuroendocrine, gastrointestinal and nervous systems (Brubaker & Wolfe, 2017; Wood & Anger, 2014).

While some doctors and researchers are still skeptical about the use of probiotics as preparations in monotherapy for infectious diseases of different nature, there is increasing evidence of using probiotics for therapeutic, and especially for prophylactic purpose (Barbés & Boris, 1999). So, recent studies by Cianci et al. (2018) have proven the possibility of preventing the development of vaginitis in women receiving systemic antibiotic therapy, with the prophylactic use of the *L. plantarum* P 17630 probiotic strain. Recine et al. (2015) demonstrated the promising use of the *L. rhamnosus* BMX 54 strain to prevent the development of bacterial vaginosis. Murina et al. (2014) had similar conclusions after studying the effectiveness of *L. fermentum* LF10 and *L. acidophilus* LA02 strains to prevent the development of candidiasis.

There are also comprehensive meta-analyzes that systematize dozens of similar studies and demonstrate the promising use of representatives of saprophytic microbiota to prevent the development of infectious diseases of the urogenital tract and bacterial vaginitis (Grin et al., 2013) in clinical practice, in particular during the preparation for the planned pregnancy. Our results are consistent with the conclusions given by other researchers and confirm the promising use of probiotic strains of lactobacilli and bifidobacteria for the prevention and treatment of infectious and inflammatory diseases of the genitourinary system. In the present study, probiotic strain *L. casei* IMV B-7280, *B. animalis* VKL and *B. animalis* VKB were most effective for prevention of staphylococcal vaginitis as far as they significantly accelerate the elimination of *S. aureus* 8325-4 strain from vagina of infected mice, prevent the spread of the pathogen to the intestines and caused normalization of vaginal and intestinal microbiota by increasing the amount of lactobacilli and bifidobacteria and reducing the number of opportunistic bacteria.

According to the latest data, even a minor violation of vaginal microbiota should be considered as a complete illness (Reid, 2017) with a significant spectrum of potentially adverse variants of development, the importance of creation of new tools for prevention and treatment of this condition at an early stage is difficult to overestimate. In addition, the fact that the disorder in the composition of normal vaginal microbiota and the decrease in the number of saprophytic lactobacilli and bifidobacteria significantly increases the risk of infection with sexually transmitted diseases, including HIV, is already proven, therefore the vaginal use of probiotic strains of bacteria can be considered as direct an effective prophylactic tool to prevent the spread of these diseases (McMillan et al., 2015).

Therefore, the further development of modern probiotics based on highly active strains of lactobacilli and bifidobacteria with a wide range of therapeutic and prophylactic actions, in particular on the basis of *L. casei* IMV B-7280, *B. animalis* VKL and *B. animalis* VKB probiotic strains, may become a new direction not only in the treatment of infectious and inflammatory diseases of the genitourinary system, but also one of the elements of an integrated approach to improving the quality and life expectancy of people.

Conclusions

It was found that infection of mice with *S. aureus* 8325-4 strain caused an imbalance of vaginal and intestinal microbiota, as evidenced by an increase in the number of opportunistic microorganisms and a decrease in the amount of lactobacilli and bifidobacteria. Administration of *L. casei* IMV B-7280, *B. animalis* VKL and *B. animalis* VKB probiotic strains before infection with *S. aureus* 8325-4 had a significant effect on the microbiota spectrum of the vagina and intestine: an increase in the amount of lactobacilli and bifidobacteria was observed with a decrease in the number of opportunistic microorganisms. Rapid elimination of *S. aureus* 8325-4 from the vagina and prevention of the spread of infection to the intestine was also observed after use of these probiotic strains. Preventive action of *L. acidophilus* IMV B-7279 and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 for bacterial vaginitis in mice was less effective. Target probiotic strains *L. casei* IMV B-7280, *B. animalis* VKL and *B. animalis* VKB are promising for the creation of highly effective novel probiotic drugs that can be used for directed prevention of infectious and inflammatory diseases of the genitourinary system caused by pathogenic and opportunistic microorganisms.

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