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THE EFFECT OF LACTO- AND BIFIDOBACTERIA IN MONOCULTURE ON THE VAGINAL MICROFLORA IN NORM AND IN CASES OF INTRAVAGINAL STAPHYLOCOCCOSIS

The influence of probiotic strains of Lactobacillus casei IMV B-7280, Lactobacillus acidophilus IMV B-7279, Bifidobacterium animalis VKL, Lactobacillus delbrueckii subsp. bulgaricus IMV B-7281 and Bifidobacterium animalis VKB, each strain separately, to the range of the urogenital tract microflora in physiological norm and in cases of experimental intravaginal staphylococcosis of mice induced by Staphylococcus aureus 8325-4. It was found that all these strains had different efficiency in Staphylococcus aureus 8325-4 growth suppression in the vagina of infected mice and affect the spectrum of microorganisms. Lactobacillus casei IMV B-7280 strain had effective antistaphylococcal effect and reduced the number of coliform bacteria and fungi in the vagina in cases of intravaginal staphylococcosis of mice. Lactobacillus casei IMV B-7280 strain is promising to create probiotic drugs effective in treating intravaginal staphylococcosis.

K e y w o r d s : Lactobacillus, Bifidobacterium, Staphylococcus, vagina, microflora, mice.

It is known that uncomplicated infections of urinary tract and vaginosis are often caused by opportunistic commensal bacteria of different genera [2, 5, 8]. Staphylococcosis usually develops in people with reduced non-specific immunological resistance, as well as in people who received high doses of immunesuppressants, antibiotics, hormones, X-rays, etc. Comprehensive treatment of such patients includes administration of antibacterial drugs and specific immune drugs, sanitation of suppurative foci. However, widespread use of chemical drugs of various origins, including the newest antibiotics, has led to selection of resistant strains of staphylococci, and increase in severity and extent of spreading of staphylococcal lesions [7, 10]. There is a tendency to the increase of secondary staphylococcal diseases. Frequent regressive uncomplicated urinary tract infection can cause serious diseases, such as nephritis, kidney damage, etc. Long-lasting bacterial vaginosis caused by staphylococci is associated with a high risk of development of sexually transmitted infectious diseases, which may increase the risk of late miscarriage [1, 4, 5].

Despite the great importance, approaches to the therapy of these diseases did not change significantly in recent years. Thus, the main agents in the treatment of urogenital infections are antibiotics and antimycotics, but with the growth of antibiotic resistance, an effectiveness of these drugs is reducing, and their use in pregnancy is not always possible [9, 12, 14].

The concept of the protective role of normal microflora of the vagina (e.g., lactobacilli) became the basis for the treatment of urogenital infections by probiotics. Later it was shown that *Lactobacillus* strains can colonize the vagina after the use of vaginal suppositories [1, 7], reduce the risk of urinary tract infections and fungal vaginitis [8, 15, 19] and bacterial vaginosis [14]. The advantage of probiotic therapy, in addition to the lack of adverse drug reactions, is the possibility of their use in daily diet. The disadvantage of the concept is the lack of the results of controlled studies

of the effect of probiotics on the human body. Nevertheless, a number of microorganisms are widely used for this purpose at present [3, 10, 17].

The mechanism of the impact of probiotics on the vaginal mucosa in cases of urogenital infections presumably is multifactorial in nature and is caused by the production of lactic acid, microbicides and hydrogen peroxide, a modification of the immune response, the production of biosurfactant and collagen-binding proteins (inhibition of adhesion of pathogenic bacteria), the synthesis of the specific molecules, that are capable to reduce the virulence of pathogens and other factors [2, 4, 13], which requires further study on models of both *in vitro*, and *in vivo*.

The most promising strains of lactobacilli or bifidobacteria for creating urogenital probiotics are those that survive well in the urogenital tract, show high antagonistic activity against pathogens and opportunistic microorganisms specific for the urogenital tract, and have immunomodulatory properties associated with activation of innate immunity factors and the balancing of the cytokine network, affecting the development of specific immune responses [6, 16, 18]. Therefore, to identify optimal combinations of probiotic strains for a designated purpose, it is expedient to conduct comprehensive studies of their biological effects.

We have previously characterized the following strains of lactobacilli and bifidobacteria: *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *B. animalis* VKL and *B. animalis* VKB. It was found that these strains had *in vitro* antagonistic effects in relation to a wide range of pathogenic and opportunistic microorganisms, including causative agents of infectious diseases of the urogenital tract. Furthermore, it was shown on the model of intact mice, that *in vivo* they effectively induced production of endogenous interferon and activated cells of the phagocytic system, without affecting the production of the proinflammatory cytokine tumor necrosis factor-α [11].

However, the comprehensive studies, which also include the determining of the effects of these probiotic strains on opportunistic microorganisms *in vivo,* are needed for creation of a full-fledged drug, since it is known from literature [3, 5, 15] that under the influence of some probiotic strains of lactic acid bacteria the growth of opportunistic pathogens may be even enhanced.

The aim of this study was to determine the effect 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, each strain separately, on the spectrum of opportunistic microorganisms in the vagina in norm and in cases of intravaginal staphylococcosis of mice.

Materials and Methods. Experimental studies were performed on six-week-old female BALB/c mice that were kept in the vivarium in standard conditions during the experiment. All studies were performed taking into account the rules of the European Convention for the protection of vertebrate animals.

Five strains of lactic acid bacteria – *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL and *B. animalis* VKB were used. Before each experiment the viability of the probiotic cultures was tested by monitoring their growth on the Man-Rogosa-Sharpe (MRS) agar medium at 37 °C for 24-48 h. The study was performed using bacteria lyophilized in Cuddon Freeze Dryer FD1500 (New Zealand).

Staphylococcus aureus 8325-4 strain, that had plasmid-based resistance to gentamicin, was used in the study. Before injection into mice vagina, *S. aureus* 8325-4 was grown on selective medium for staphylococci (BAIRD-PARKER-Agar, Merck, Germany) containing gentamicin (15 μg/ml) at 37 °C for 24 h. Staphylococcosis was modeled through intravaginal administration of the *S. aureus* 8325-4 daily culture to mice, in doses of 5×10^7 cells per animal [18].

Twenty-four hours after infection, mice were given an intravaginal injection of a suspension of lyophilized lacto- or bifidobacteria cells in saline solution at a dose of 1×10^6 cells per animal, once a day for 7 days [5, 20].

Individual groups consisted of intact mice that were given an intravaginal injection of probiotic strains in the same way, and infected mice that received saline intravaginally. The control group consisted of intact mice.

On the 1, 3, 6, 9 and 12th days after the injection of lacto- or bifidobacteria strains material was collected from the vagina and plated onto seven nutrient mediums: MRSA (Man-Rogosa-Sharpe agar medium for lactobacilli), Meat-Peptone Agar (MPA, selective medium for aerobic and

facultative anaerobic organisms), BAIRD-PARKER-Agar (Merck, Germany; selective medium for staphylococci), BAIRD-PARKER-Agar with gentamicin in concentration 15 μg/ml (selective medium for gentamicin-resistant staphylococci), KF-Streptococcus agar (Merck, Germany; selective medium for streptococci), ENDO (NSCAMB, Obolensk, Russia; selective medium for coliform bacteria) and Sabouraud agar (selective medium for fungi). The material was collected using standardized sterile cotton tampons. Swabs from each tampon were performed with 1 mL of saline. After cultivation at 37 °C for 24 h, the number of colony forming units (CFU) was counted, given that one such colony corresponds to one bacterium.

All digital data received were processed with the help of the Origin Pro 8.5. software through analysis of variance. Numerical data were represented as arithmetic average and standard error ($M \pm m$). The null hypothesis for the control and experimental comparative groups was checked using Wilcoxon-Mann-Whitney (U) and Kolmogorov-Smirnov nonparametric criteria. The differences between the groups were considered statistically meaningful at $P < 0.05$ [11].

Results and Discussion. It was found that intravaginal administration of *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL or *B. animalis* VKB, each strain separately, to intact mice led to significant changes in the microflora of the vagina (Table 1).

Thus, the number of lactobacilli that were seeded out from the vagina of intact mice increased under the influence of *L. casei* IMV B-7280 or *B. animalis* VKB throughout the experimental period, *B. animalis* VKL – on the 3, 6, 9 and 12th days. The data suggest that these probiotic strains of bacteria are probably well accustomed to the vagina of intact mice. Instead, after the injection of *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 to the intact mice, the increasing in the number of lactobacilli in the vagina was observed only on the 3 and 6th days, and *L. acidophilus* IMV B-7279 – on the 6th day.

The number of aerobic and facultative anaerobic microorganisms increased in the vagina of intact mice injected with *L. acidophilus* IMV B-7279 or *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (during the whole period of observation), *B. animalis* VKL or *B. animalis* VKB (on the 3, 6, 9 and 12th days) and *L. casei* IMV B-7280 (on the 6 and 9th days) compared with intact mice that did not receive probiotic culture (control group).

The number of staphylococci in the vagina of intact mice increased after the injection of *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 or *B. animalis* VKL (throughout the period of observation), *L. casei* IMV B-7280 (on the 3 and 6th days) and *B. animalis* VKB (only on the 6th day).

The number of streptococci in the vagina of intact mice increased throughout the period of observation under the influence of *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL or *L. casei* IMV B-7280 – on the 3, 6 and 9th days. It should be noted that *B. animalis* VKB did not affect the number of streptococci that were seeded out from the vagina of intact mice.

However, it was found a decrease in the number of coliform bacteria that were seeded out from the vagina of intact mice under the influence of *L. casei* IMV B-7280 (on the 3, 6, 9 and 12th days), *L. acidophilus* IMV B-7279 (on the 3 and 6th days) or *B. animalis* VKB (on the 9 and 12th days). Instead, after the injection of *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 into the intact mice the number of coliform bacteria in the vagina increased in the process of observation. *B. animalis* VKB induced the increase of the number of coliform bacteria in the vagina of intact mice only on the 1 and 3rd day.

The number of fungi in the vagina increased after the injection of *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 or *B. animalis* VKB, each strain separately, throughout the period of observation, but decreased in the vagina of mice intravaginally injected with *L. casei* IMV B-7280 (on the 3, 6, 9 and 12th days). However, *B. animalis* VKL did not influence the number of fungi in the vagina of intact mice.

Thus, *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL or *B. animalis* VKB strains differently affect the qualitative and quantitative composition of vaginal microflora of intact mice. Thus, under the influence of *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 or *B. animalis* VKL

the number of staphylococci and streptococci increased in the vagina of intact mice throughout the period of observation. After the injection of *L. casei* IMV B-7280 the increase in the number of streptococci in the vagina of intact mice was observed on the 3, 6 and 9th days, and staphylococci – on the 1, 3 and 6th days. Instead, *B. animalis* VKB increased the number of staphylococci only on the 6th day, but did not affect the number of streptococci. It should be noted that only under the influence of *L. casei* IMV B-7280 in the vagina of intact mice the number of fungi decreased (on the 3, 6, 9 and 12th days). *B. animalis* VKL did not influence the number of these microorganisms in the vagina.

However, other probiotic strains: *L. acidophilus* IMV B-7279, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 or *B. animalis* VKL throughout the period of observation induced the increasing of fungal flora in the vagina of intact mice. Therefore the influence of *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL or *B. animalis* VKB, individually, on the vaginal microflora in cases of experimental staphylococcosis of mice was determined in further research.

The results of detecting the influence of *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL or *B. animalis* VKB, each strain separately, on the range of vaginal microflora of mice with experimental infection are shown in Table 2. It should be noted that the significant changes in the vaginal microflora were detected in the vagina of mice infected with staphylococcus, which did not receive probiotic strains.

Thus, the number of aerobic and facultative anaerobic bacteria, staphylococci and streptococci separately and coliform bacteria increased in the vagina of mice after they were infected with staphylococcus throughout the period of observation. However, the number of fungi increased on the 1 and 9th days, and the number of lactobacilli, however, decreased from the day 6 and throughout the following observation period. *S. aureus* 8325-4 strain was seeded out from the vagina of the infected mice in large numbers during the entire period of observation (Fig. 1).

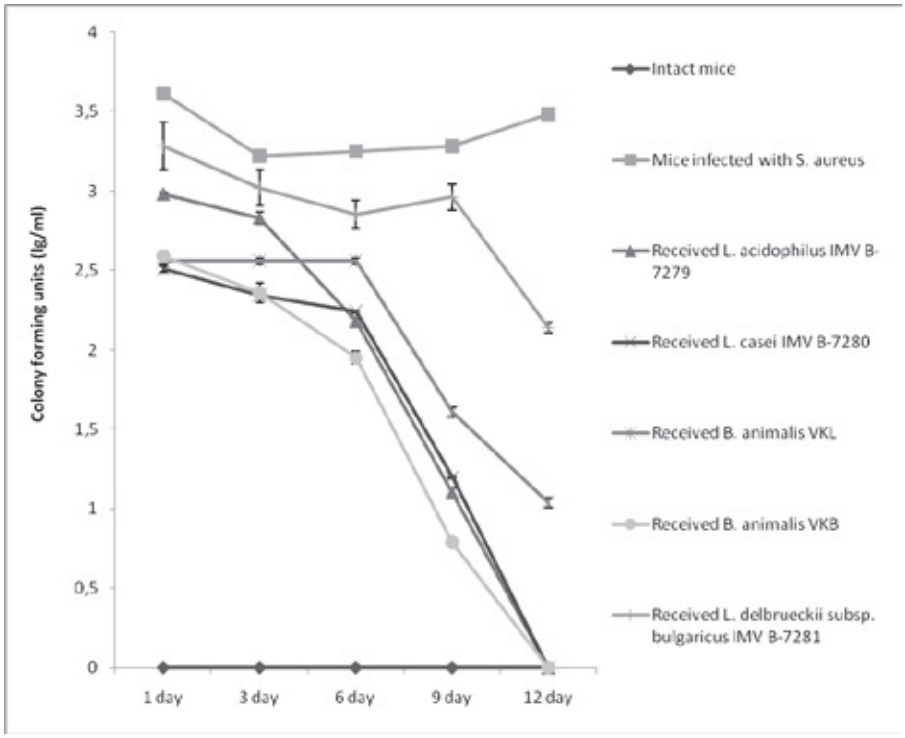

Fig. 1. **The number of colonies of *S. aureus* 8325-4 that were sowed from the vagina of the infected mice after receiving intravaginal injection of probiotic strains of lacto- or bifidobacteria, each of them separately**

It was found that the number of lactobacilli in the vagina of infected mice increased under the influence of *L. acidophilus* IMV B-7279 or *B. animalis* VKL throughout the period of observation, *L. casei* IMV B-7280 – on the 3, 6, 9 and 12th days, *B. animalis* VKB – on the 6, 9 and 12th days, and

L. delbrueckii subsp. *bulgaricus* IMV B-7281 only on the 6th day. It should be noted, that the number of lactobacilli in the vagina became lower after infected mice were intravaginally injected with *B. animalis* VKB (on the 3rd day) and *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (on the 1 and 3rd days) compared with infected mice which did not receive probiotic culture.

The obtained data showed that *S. aureus* 8325-4 was seeded out from the vagina of infected mice in much smaller numbers throughout the observation period after the injection of *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *B. animalis* VKL or *B. animalis* VKB, each strain separately. *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 had antistaphylococcal effect only on the 6, 9 and 12th days. *S. aureus* 8325-4 was not seeded out from the vagina of infected mice injected with *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279 or *B. animalis* VKB on the 12th day.

However, the number of aerobic and facultative anaerobic microorganisms changed in the vagina of infected mice that received these probiotic strains. Thus, under the influence of *L. casei* IMV B-7280 the number of microorganisms decreased during the entire period of observation. There was a reduction of aerobic and facultative anaerobic microorganisms in the vagina of infected mice injected with *L. acidophilus* IMV B-7279 on the 1, 3 and 6th days, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 – on the 1, 3 and 12th days, *B. animalis* VKL – only on the 6th day. Instead, after the infected mice were intravaginally injected with *B. animalis* VKB, the number of microorganisms of this group was the same as in the vagina of infected mice that did not receive probiotic strains.

The number of staphylococci from the vagina of infected mice that were plated onto the selective agar medium for staphylococci without gentamicin reduced under the influence of *L. casei* IMV B-7280, *B. animalis* VKL, *B. animalis* VKB or *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 during the entire period of observation. However, after the injection of *L. acidophilus* IMV B-7279 the number of staphylococci in the vagina of infected mice reduced only on the 1 and 3rd days.

The number of streptococci in the vagina of infected mice under the influence of *L. casei* IMV B-7280 decreased on the 1 and 3rd days, but in terms of further observation was the same as in the vagina of infected mice that did not receive probiotic strains. After the injection of *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 the number of streptococci in the vagina of infected mice reduced on the 1, 3, 9 and 12th days. However, it was observed that the number of streptococci in the vagina of infected mice increased under the influence of *B. animalis* VKL during the period of observation and *B. animalis* VKB – on the 6, 9 and 12th days. *L. acidophilus* IMV B-7279 did not affect the number of streptococci that were seeded out from the vagina of infected mice.

Coliform bacteria were seeded out from the vagina of infected mice in smaller numbers under the influence of *L. acidophilus* IMV B-7279 or *L. casei* IMV B-7280 for the period of observation, *B. animalis* VKB – on the 3 and 6th days, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 – on the 1 and 12th days, *B. animalis* VKL – only on the 3rd day. There was an increase in the number of coliform bacteria in the vagina after the infected mice were injected with *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 (on the 3, 6 and 9th days), *B. animalis* VKL or *B. animalis* VKB (on the 9 and 12th days).

The number of fungi in the vagina of infected mice decreased during the entire period of observation only under the influence of *L. casei* IMV B-7280 or *L. delbrueckii* subsp. *bulgaricus* IMV B-7281. After the injection of *B. animalis* VKB the number of fungi in the vagina increased on the 3 and 12th days, and on the 6th day – reduced as compared with mice that did not receive probiotic strains. However, the number of fungi, that were seeded out from the vagina of infected mice, increased under the influence of *L. acidophilus* IMV B-7279 or *B. animalis* VKL in the course of the observation.

Thus, it was determined that *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL or *B. animalis* VKB, each separately, after their intravaginal injection to intact or *S. aureus* infected mice increased the number of vaginal lactobacilli and influenced the range of saprophyte, pathogenic or opportunistic microflora in the vagina.

These strains had an antistaphylococcal effect on the model of experimental intravaginal staphylococcosis of mice: the growth of *S. aureus* 8325-4 strain, which is used to model this pathological process in the vagina, was inhibited under their influence. It should be noted that on the 12th day a complete elimination of *S. aureus* 8325-4 strain from the vagina of mice injected with *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280 or *B. animalis* VKB took place.

Table 1

Number of colonies of opportunistic microorganisms, that were sowed from the vagina of intact mice after receiving intravaginal injection of probiotic strains of lacto- and/or bifidobacteria, each of them separately

Groups of mice / time of observation, day		Colony forming units (lg/ml)					
		Meat-Peptone Agar	BAIRD-PARKER-Agar	KF-Streptococcus agar	Man-Rogosa-Sharpe	ENDO	Sabouraud agar
Intact mice	-	2.24 ± 0.12	2.51 ± 0.08	2.08 ± 0.04	2.68 ± 0.03	1.08 ± 0.05	2.07 ± 0.04
Received *L. acidophilus* IMV B-7279	1 day	3.22 ± 0.04*	2.92 ± 0.02*	4.56 ± 0.17*	2.71 ± 0.09	1.00	3.14 ± 0.11*
	3 day	3.11 ± 0.11*	3.35 ± 0.14*	5.01 ± 0.15*	2.81 ± 0.07	0*	3.27 ± 0.07*
	6 day	3.14 ± 0.07*	3.45 ± 0.12*	4.94 ± 0.09*	3.55 ± 0.04*	0*	2.94 ± 0.05*
	9 day	3.42 ± 0.15*	3.28 ± 0.09*	4.92 ± 0.14*	2.62 ± 0.08	1.20 ± 0.14	4.01 ± 0.11*
	12 day	2.96 ± 0.03*	3.01 ± 0.06*	4.22 ± 0.13*	2.52 ± 0.09	1.08 ± 0.12	2.99 ± 0.06*
Received *L. casei* IMV B-7280	1 day	2.31 ± 0.11	2.67 ± 0.11	2.65 ± 0.19	4.22± 0.06*	1.00 ± 0.10	2.02 ± 0.11
	3 day	2.42 ± 0.07	2.97 ± 0.03*	3.12 ± 0.07*	4.14 ± 0.11*	0*	1.36 ± 0.02*
	6 day	2.99 ± 0.04*	3.11 ± 0.06*	3.65 ± 0.11*	3.85 ± 0.09*	0*	1.44 ± 0.03*
	9 day	2.78 ± 0.03*	2.65 ± 0.14	2.86 ± 0.16*	3.61 ± 0.07*	0*	1.32 ± 0.04*
	12 day	2.30 ± 0.06	2.51 ± 0.07	2.17 ± 0.12	2.98 ± 0.04*	0*	1.10 ± 0.11*
Received *B. animalis VKL*	1 day	2.41 ± 0.04	3.14 ± 0.09*	3.12 ± 0.11*	2.86 ± 0.05	1.10 ± 0.03	2.23 ± 0.09
	3 day	2.97 ± 0.02*	3.55 ± 0.12*	3.04 ± 0.07*	3.14 ± 0.11*	1.25 ± 0.04	2.04 ± 0.09
	6 day	3.12 ± 0.08*	3.62 ± 0.14*	2.96 ± 0.11*	3.58 ± 0.08*	1.39 ± 0.06	2.12 ± 0.12
	9 day	3.02 ± 0.09*	3.12 ± 0.06*	2.74 ± 0.04*	3.92 ± 0.12*	1.25 ± 0.07	2.33 ± 0.08
	12 day	2.86 ± 0.03*	2.98 ± 0.04*	2.65 ± 0.07*	3.65 ± 0.04*	1.12 ± 0.11	2.15 ±0.09
Received *B. animalis VKB*	1 day	2.45 ± 0.11	2.62 ± 0.09	2.14 ± 0.11	3.25 ± 0.08*	2.14 ± 0.09*	2.67 ± 0.04*
	3 day	3.14 ± 0.08*	2.79 ± 0.14	2.22 ± 0.09	3.43 ± 0.07*	2.17 ± 0.07*	2.81 ± 0.07*
	6 day	3.55 ± 0.09*	3.01 ± 0.04*	2.34 ± 0.12	3.24 ± 0.03*	1.10 ± 0.07	2.91 ± 0.05*
	9 day	3.28 ± 0.04*	2.67 ± 0.09	2.12 ± 0.04	3.12 ± 0.05*	0*	2.98 ± 0.07*
	12 day	3.04 ± 0.03*	2.11 ± 0.17	2.22 ± 0.09	3.06 ± 0.11*	0*	2.69 ± 0.05*
Received *L. delbruckei* IMV B-7281	1 day	3.45 ± 0.06*	3.77 ± 0.11*	3.47 ± 0.11*	2.62 ± 0.06	2.44 ± 0.11*	2.86 ± 0.04*
	3 day	3.89 ± 0.11*	4.03 ± 0.21*	4.20 ± 0.23*	3.54 ± 0.04*	2.52 ± 0.09*	3.11 ± 0.22*
	6 day	4.12 ± 0.14*	4.12 ± 0.16*	4.45 ± 0.12*	3.22 ± 0.03*	2.63 ± 0.12*	3.22 ± 0.12*
	9 day	4.26 ± 0.09*	4.33 ± 0.15*	4.30 ± 0.09*	2.54 ± 0.11	2.11 ± 0.11*	3.44 ± 0.09*
	12 day	3.98 ± 0.12*	4.14 ± 0.11*	4.22 ± 0.14*	2.45 ± 0.12	1.54 ± 0.02*	3.04 ± 0.07*

Note: Significant differences with the control is represented by * ($P < 0.05$) while differences with the indicators of the infected mice who did not receive probiotic strains are represented by • ($P < 0.05$)

Table 2

Number of colonies of opportunistic microorganisms, that were sowed from the vagina of *S. aureus* infected mice after receiving intravaginal injection of probiotic strains of lacto- and/or bifidobacteria, each of them separately

Groups of mice / time of observation, day		Colony forming units (lg/ml)					
		Meat-Peptone Agar	BAIRD-PARKER-Agar	KF-Streptococcus agar	Man-Rogosa-Sharpe	ENDO	Sabouraud agar
Intact mice	-	2.24 ±0.12	2.51 ± 0.08	2.08 ± 0.04	2.68 ± 0.03	1.08 ± 0.05	2.07 ± 0.04
Mice infected with *S. aureus*	1 day	3.78 ± 0.13*	4.54 ± 0.08*	3.53 ± 0.05*	2.79 ± 0.13	1.60 ± 0.04*	2.63 ± 0.05*
	3 day	3.82 ± 0.09*	4.30 ± 0.05*	3.45 ± 0.03*	2.63 ± 0.04	1.48 ± 0.03*	1.48 ± 0.02*
	6 day	3.83 ± 0.11*	4.25 ± 0.07*	3.34 ± 0.07*	2.00 ± 0.01*	1.48 ± 0.05*	2.18 ± 0.08
	9 day	3.51 ± 0.08*	4.22 ± 0.11*	3.26 ± 0.11*	2.23 ± 0.02*	1.30 ± 0.01	2.34 ± 0.05*
	12 day	3.36 ± 0.08*	4.19 ± 0.09*	3.22 ± 0.08*	2.32 ± 0.03*	1.48 ± 0.04*	1.90 ± 0.02
Received *L. acidophilus* IMV B-7279	1 day	3.07 ± 0.13*•	3.33 ± 0.04*•	3.26 ± 0.10*	4.29 ± 0.13*•	1.30 ± 0.02*•	3.29 ± 0.07*•
	3 day	3.13 ± 0.11*•	3.15 ± 0.11*•	3.29 ± 0.14*	4.44 ± 0.19*•	0*•	2.62 ± 0.02*•
	6 day	3.38 ± 0.02*•	4.51 ± 0.11*	3.67 ± 0.12*	3.51 ± 0.13*•	0*•	3.51 ± 0.05*•
	9 day	3.28 ± 0.26*	4.50 ± 0.07*	3.62 ± 0.13*	3.45 ± 0.16*•	1.00 ± 0.01•	3.67 ± 0.02*•
	12 day	3.22 ± 0.19*	4.22 ± 0.07*	3.42 ± 0.16*	3.28 ± 0.08*•	0*•	3.32 ± 0.05*•
Received *L. casei* IMV B-7280	1 day	2.34 ± 0.04•	2.11 ± 0.02*•	2.72 ± 0.07*•	3.11± 0.09*	1.00 ± 0.02•	2.72 ± 0.03*
	3 day	2.15 ± 0.09•	1.60 ± 0.03*•	2.26 ± 0.20•	3.33 ± 0.08*•	0*•	2.81 ± 0.01*•
	6 day	3.38 ± 0.06*•	1.90 ± 0.04*•	3.32 ± 0.09*	3.49 ± 0.05*•	2.62 ± 0.04*•	3.89 ± 0.07*•
	9 day	3.05 ± 0.08*•	2.08 ± 0.01*•	3.22 ± 0.14*	2.62 ± 0.02•	2.23 ± 0.02*•	3.49 ± 0.07*•
	12 day	2.75 ± 0.06*•	2.43 ± 0.05•	2.91 ± 0.15*	2.65 ± 0.03•	2.34 ± 0.03*•	3.09 ± 0.05*•
Received *B. animalis VKL*	1 day	3.79 ± 0.03*	3.03 ± 0.10*•	3.81 ± 0.11*•	3.20 ± 0.07*•	1.90 ± 0.15*	2.96 ± 0.05*•
	3 day	3.91 ± 0.18*	2.98 ± 0.08*•	3.93 ± 0.11*•	3.40 ± 0.08*•	0*•	3.32 ± 0.07*•
	6 day	3.31 ± 0.09*•	2.96 ± 0.09*•	4.25 ± 0.12*•	4.10 ± 0.10•*	1.48 ± 0.02*	2.71 ± 0.03*•
	9 day	3.48 ± 0.10*	2.94 ± 0.03*•	4.26 ± 0.09*•	4.05 ± 0.10*•	1.60 ± 0.02*•	2.64 ± 0.08*•
	12 day	3.30 ± 0.05*	2.79 ± 0.04•	4.15 ± 0.08*•	3.92 ± 0.09*•	2.04 ± 0.04*•	2.30 ± 0.09•
Received *B. animalis VKB*	1 day	3.82 ± 0.05*	3.85 ± 0.08*•	3.49 ± 0.05*	2.71 ± 0.04	1.48 ± 0.05*	2.68 ± 0.05*
	3 day	3.93 ± 0.10*	3.82 ± 0.10*•	3.32 ± 0.10*	2.20 ± 0.03*•	0*•	2.85 ± 0.07*•
	6 day	3.72 ± 0.09*	3.81 ± 0.13*•	3.88 ± 0.08*•	3.60 ± 0.09*•	0*•	1.90 ± 0.06•
	9 day	3.66 ± 0.07*	3.78 ± 0.12*•	3.72 ± 0.07*•	3.49 ± 0.11*•	1.60 ± 0.02*•	2.49 ± 0.04*
	12 day	3.59 ± 0.05*	3.48 ± 0.16*•	3.64 ± 0.07*•	3.21 ± 0.10*•	2.08 ± 0.04*•	2.28 ± 0.02*•
Received *L. delbruckei* IMV B-7281	1 day	1.60 ± 0.03*•	3.04 ± 0.10*•	1.60 ± 0.02*•	1.89 ± 0.03*•	0*•	0*•
	3 day	3.04 ± 0.07*•	3.09 ± 0.11*•	3.15 ± 0.05*•	2.15 ± 0.08*•	2.38 ± 0.07*•	0*•
	6 day	3.77 ± 0.08*	2.62 ± 0.07•	3.47 ± 0.08*	2.44 ± 0.02*•	3.60 ± 0.04*•	1.30 ± 0.02*•
	9 day	3.30 ± 0.02*	2.80 ± 0.08•	2.30 ± 0.07•	2.38 ± 0.06*	1.80 ± 0.03*•	1.60 ± 0.02*•
	12 day	2.70 ± 0.04*•	2.60 ± 0.04•	2.50 ± 0.07*•	2.22 ± 0.04*	0*•	0*•

Note: Significant differences with the control is represented by * ($P < 0.05$) while differences with the indicators of the infected mice who did not receive probiotic strains are represented by • ($P < 0.05$)

According to the literature data, the other probiotic strain – *L. paracasei* CRL 1289 prevent vaginal colonization by uropathogen *S. aureus* strain on the mice model of intravaginal staphylococcosis [20] and probiotic strains *Lactobacillus* GR-1 and B-54 or RC-14 reduced the risk of urogenital tract infectious diseases progress by normalization of vaginal microflora of mice [14, 15].

In our researches the influence of *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL or *B. animalis* VKB, each separately, on the saprophyte and pathogenic microflora of the vagina in cases of physiological norm and experimental staphylococcosis was differently directed. After the injection of *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL or *B. animalis* VKB, each strain separately, to intact mice in different periods of observation an increase of the total number of aerobic and facultative anaerobic bacteria, including staphylococci and streptococci was observed. It is known [2, 14] that optionally pathogenic and saprophytic staphylococci and streptococci, representatives of normal flora of vagina may have antagonistic action against pathogenic microorganisms. So, we may assume that the increasing of the total number of aerobic and facultative anaerobic bacteria after the injection of probiotic strains in case of physiological norm should consider their positive influence on the microflora spectrum of the vagina of intact mice.

In cases of staphylococcosis, in contrast, the number of aerobic and facultative anaerobic microorganisms decreased under the influence of the majority of strains that we studied.

In the vagina of mice infected with staphylococcus *L. casei* IMV B-7280 reduced effectively the number of aerobic and facultative anaerobic microorganisms, the number of staphylococci reduced under the influence of *L. casei* IMV B-7280, *B. animalis* VKL, *B. animalis* VKB or *L. delbrueckii* subsp. *bulgaricus* IMV B-7281. *L. casei* IMV B-7280 or *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 induced the decrease of the number of streptococci in the vagina of infected mice in different periods of observation. For the interpretation of our data it is expedient to study the species range of staphylococci and streptococci in cases of vaginal staphylococcosis, because their range may significantly differ from that in norm (unpublished data).

It is shown that the number of coliform bacteria decreased or increased in the vagina of intact or staphylococcus infected mice under the influence of some of probiotic strains we studied. In particular, in cases of physiological norm the number of coliform bacteria in the vagina of mice decreased under the influence of *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *B. animalis* VKB in different times of observation. It should be noted that *L. acidophilus* IMV B-7279 or *L. casei* IMV B-7280 also effectively suppressed the growth of coliform bacteria in the vagina of staphylococcus infected mice. According to the literature data, other probiotic strain – *L. fermentum* L23 also suppressed the growth of *E. coli* in a model of experimental intravaginal infection in mice caused by this pathogen [13]. It was found in our research that the number of coliform bacteria, however, increased in the vagina of intact mice injected with *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 or *B. animalis* VKB. There was an increase in the number of this group of bacteria in the vagina of staphylococcus infected mice that were injected with *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL or *B. animalis* VKB.

It is known that lactobacilli also suppressed the growth of fungi of the *Candida* genus in the reproductive tract of mice [8]. Of all the strains of probiotic bacteria we studied, only *L. casei* IMV B-7280 caused the reduction of fungi in the vagina of both intact and staphylococcus infected mice. Under the influence of *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 the number of fungi in the vagina reduced only in the group of staphylococcus infected mice. *B. animalis* VKL in cases of physiological norm had no effect on fungi in the vagina of mice. Other probiotic strains – *L. delbrueckii* subsp. *bulgaricus* IMV B-7281 or *B. animalis* VKB caused, on the contrary, the increasing of the number of fungi in the vagina of both intact and infected mice in different periods of observation.

Thus, *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280, *L. delbrueckii* subsp. *bulgaricus* IMV B-7281, *B. animalis* VKL or *B. animalis* VKB, individually, suppressed the growth of *S. aureus* 8325-4 in the vagina of staphylococcus infected mice, but differently influenced the saprophyte and pathogenic microflora. Analyzing the obtained data, we can conclude the results of investigation of each probiotic strain alone influence on the microflora of the infected mice vagina: *L. casei* IMV

B-7280 strain is the most promising for further research and development of probiotic drug for correcting the microflora of the urogenital tract. This strain suppressed the growth of *S. aureus* 8325-4, fungi and coliform bacteria in the vagina. Therefore, the therapy using *L. casei* IMV B-7280 strain may be an effective alternative therapy for infectious and inflammatory diseases of the urogenital tract. This strain can be used alone or as a part of different probiotic composition for prevention of infectious and inflammatory diseases of urogenital tract, but further studies are required.

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ВПЛИВ ПРОБІОТИЧНИХ ШТАМІВ ЛАКТО-ТА БІФІДОБАКТЕРІЙ НА СПЕКТР МІКРОФЛОРИ ПІХВИ ЗА УМОВ ФІЗІОЛОГІЧНОЇ НОРМИ ТА ПРИ ЕКСПЕРИМЕНТАЛЬНІЙ СТАФІЛОКОКОВІЙ ІНФЕКЦІЇ

Р е з ю м е

Визначено вплив пробіотичних штамів *Lactobacillus casei* IMB B-7280, *Lactobacillus acidophilus* IMB B-7279, *Bifidobacterium animalis* VKL, *Lactobacillus delbrueckii* subsp. *bulgaricus* IMB B-7281 та *Bifidobacterium animalis* VKB, кожний штам окремо, на спектр мікрофлори урогенітального тракту за умов фізіологічної норми та експериментальної інтравагінальної стафілококової інфекції у мишей, індукованої *Staphylococcus aureus* 8325-4. Встановлено, що усі ці штами з різною ефективністю пригнічували ріст *Staphylococcus aureus* 8325-4 у піхві інфікованих мишей, а також впливали на спектр її мікрофлори. Штам *Lactobacillus casei* IMB B-7280 мав ефективнішу антистафілококову дію за експериментальної стафілококової інфекції, затримував ріст у піхві бактерій групи кишкової палички, а також грибної флори. Штам *Lactobacillus casei* IMB B-7280 є перспективним для створення пробіотичних препаратів, ефективних при лікуванні інтравагінальної стафілококової інфекції.

К л ю ч о в і с л о в а: *Lactobacillus, Bifidobacterium, Staphylococcus, піхва, мікрофлора, миші*

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ВЛИЯНИЕ ПРОБИОТИЧЕСКИХ ШТАММОВ ЛАКТО- И БИФИДОБАКТЕРИЙ НА СПЕКТР МИКРОФЛОРЫ ВЛАГАЛИЩА В УСЛОВИЯХ ФИЗИОЛОГИЧЕСКОЙ НОРМЫ И ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ СТАФИЛОКОККОВОЙ ИНФЕКЦИИ

Р е з ю м е

Определено влияние пробиотических штаммов *Lactobacillus casei* IMB B-7280, *Lactobacillus acidophilus* IMB B-7279, *Bifidobacterium animalis* VKL, *Lactobacillus delbrueckii* subsp. *bulgaricus* IMB B-7281 и *Bifidobacterium animalis* VKB, каждого отдельно, на спектр микрофлоры урогенитального тракта мышей в условиях физиологической нормы и экспериментальной интравагинальной инфекции, индуцированной *Staphylococcus aureus* 8325-4. Установлено, что все эти штаммы с различной эффективностью угнетали рост *Staphylococcus aureus* 8325-4 в урогенитальном тракте мышей, а также влияли на спектр вагинальной микрофлоры. Штамм *Lactobacillus casei* IMB B-7280 имел более эффективную антистафилококковую активность при экспериментальной стафилококковой инфекции, угнетал рост во влагалище бактерий группы кишечной палочки и грибов. Штамм *Lactobacillus casei* IMB B-7280 является перспективным для создания пробиотических препаратов, эффективных при лечении интравагинальной стафилококковой инфекции.

К л ю ч е в ы е с л о в а: *Lactobacillus, Bifidobacterium, Staphylococcus, влагалище, микрофлора, мыши*

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