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## **THE EFFECT OF LACTO- AND BIFIDOBACTERIA COMPOSITIONS ON THE VAGINAL MICROFLORA IN CASES OF INTRAVAGINAL STAPHYLOCOCCOSIS**

*The effect of intravaginal injection of Lactobacillus casei IMV B-7280, Lactobacillus acidophilus IMV B-7279, Bifidobacterium animalis VKL and Bifidobacterium animalis VKB strains in various compositions on the range of microflora of the urogenital tract on the model of experimental intravaginal staphylococcosis of mice was determined. It was established that under the influence of various compositions of these strains changes in microflora spectrum occurred: the number of representatives of normoflora increased and the number of pathogenic microorganisms, including staphylococci, streptococci, coliform bacteria and fungi, significantly decreased. It was determined that strains of lacto- and bifidobacteria, that were studied, are prospective components of future probiotic drugs efficient in treating staphylococcosis.*

*Keywords:* Lactobacillus, Bifidobacterium, Staphylococcus, vagina, microflora, mice

Urogenital infection- inflammatory bacterial diseases are common in modern clinical practice. One of the basic causes of the urogenital infections is a violation of vaginal microecology, which is very sensitive to various factors such as hormonal status, sexual activity, use of oral contraceptives, the glycogen content, the pH of the vagina, therapy with glucocorticoids, immunosuppressive treatment, etc. [2, 5, 6].

Despite the great importance, approaches to the therapy of these diseases did not changed 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, the effectiveness of these drugs is reducing, and their use in pregnancy is not always possible [9, 12].

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 [1, 11] 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 [6, 15].

The mechanism of the impact of probiotics on the vaginal mucosa in cases of urogenital infections is presumably 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 both *in vitro*, and *in vivo*.

Previously [10, 13] we have characterized the strains of lacto- and bifidobacteria: *Lactobacillus casei* IMV B-7280, *L. acidophilus* IMV B-7279, *Bifidobacterium animalis* VKL and *B. animalis* VKB. It was established that these probiotic strains in monoculture and in various compositions have antistaphylococcal effect on the model of experimental intravaginal staphylococcosis of mice. It was found that under the influence of these strains and their various compositions, the *in vitro* growth of *S. aureus* was inhibited, and the number of colonies of *S. aureus* plated from the vagina of infected mice was significantly reduced [10], but the most effective elimination of *S. aureus in vivo* was observed under the influence of these probiotic strains in different compositions. However, the changes in the range of other pathogenic bacteria, that can also cause the infectious-inflammatory diseases of the urogenital tract, have not been studied yet.

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Therefore, the aim of this study was to determine the effect of probiotic strains of lactobacilli and/or bifidobacteria in various compositions to the range of opportunistic vaginal microflora on the model of experimental 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 [14].

Four strains of lactic acid bacteria – *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *B. animalis* VKL and *B. animalis* VKB were used in various compositions. 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). “Labilakt®” (Ariadna, Odessa, Ukraine), that includes a composition of lyophilized strains of lacto- and bifidobacteria was used at work for comparison.

*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  $5 \times 10^7$  cells per animal.

Twenty-four hours after infection, mice were given an intravaginal injection of a suspension of lyophilized lacto- and/or bifidobacteria cells in saline solution at a dose of  $1 \times 10^6$  cells per animal, once per day for 7 days. Strains were injected in the following compositions: *L. casei* IMV B-7280 - *L. acidophilus* IMV B-7279; *L. casei* IMV B-7280 - *B. animalis* VKL; *L. casei* IMV B-7280 - *B. animalis* VKB; *L. acidophilus* IMV B-7279 - *B. animalis* VKL; *L. acidophilus* IMV B-7279 - *B. animalis* VKB; *B. animalis* VKL - *B. animalis* VKB; *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB; *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279; *L. acidophilus* IMV B-7279 - *B. animalis* VKL - *B. animalis* VKB; *L. casei* IMV B-7280 - *B. animalis* VKB - *L. acidophilus* IMV B-7279; *L. casei* IMV B-7280 - *B. animalis* VKB - *B. animalis* VKL - *L. acidophilus* IMV B-7279 in equal proportion.

Other separate groups were intact and infected mice that did not receive probiotic strains, but intravaginally received the same volume of saline, and infected mice that received “Labilakt®” in the same way as the other compositions of probiotic strains.

On the 1, 3, 6, 9 and 12<sup>th</sup> days after the injection of lacto- and/or bifidobacteria strains in various composition or “Labilakt®” material was collected from the vagina and plated onto six nutrient mediums: MRSa (Man-Rogosa-Sharpe agar medium for lactobacilli), Meat-Peptide Agar (MPA, selective medium for aerobic and facultative anaerobic organisms), BAIRD-PARKER-Agar (Merck, Germany; selective medium for 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 obtained 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  $P < 0.05$ .

**Results and Discussion.** The results of the investigation of intravaginal injection of probiotic strains in the compositions of two cultures to the range of microflora of the urogenital tract are presented in Table 1. It should be noted that in mice infected with *S. aureus*, that did not receive probiotic strains, the significant changes in the microflora of the urogenital tract was observed. Thus, after injection of the infectious agent, the number of aerobic and facultative anaerobic microorganisms, as well as staphylococci and streptococci increased during the observation period, and coliform bacteria and fungi – on the 1 and 9<sup>th</sup> days. On the contrary, the number of lactobacilli decreased from the 6<sup>th</sup> day and during the subsequent period of observation.

Table 1

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

Groups of mice / time of observa- tion, day		Colony forming units (lg/ml)					
		Meat-Pep- tone Agar	Baird-Park- er-Agar	KF-Strepto- coccus agar	Man-Rogosa- Sharpe	ENDO	Sabouraud agar
Intact mice	-	2.24 ± 0.10	2.51 ± 0.09	2.08 ± 0.10	2.68 ± 0.03	1.08 ± 0.02	2.07 ± 0.09
Mice infected with <i>S. aureus</i>	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 <i>L. casei</i> IMV B-7280 - <i>B. animalis</i> VKB	1 day	2.45 ± 0.07*	2.98 ± 0.09**	2.40 ± 0.09**	4.16 ± 0.18**	1.00 ± 0.04*	2.28 ± 0.08*
	3 day	2.18 ± 0.10*	3.00 ± 0.13**	2.79 ± 0.13**	3.72 ± 0.09**	0*	2.51 ± 0.05**
	6 day	2.81 ± 0.04**	2.88 ± 0.07**	2.72 ± 0.04**	3.80 ± 0.02**	0*	1.90 ± 0.04*
	9 day	2.90 ± 0.09**	2.73 ± 0.05*	2.69 ± 0.05**	3.81 ± 0.11**	1.00 ± 0.02*	2.74 ± 0.09**
	12 day	2.86 ± 0.07**	2.68 ± 0.04*	2.73 ± 0.04**	3.49 ± 0.12**	0*	2.08 ± 0.05*
Received <i>L. casei</i> IMV B-7280 - <i>B. animalis</i> VKL	1 day	3.11 ± 0.07**	3.11 ± 0.08**	2.96 ± 0.09**	1.70 ± 0.03*	1.11 ± 0.02*	0*
	3 day	3.26 ± 0.11**	3.06 ± 0.04**	2.75 ± 0.08**	2.56 ± 0.09	0*	0*
	6 day	3.05 ± 0.12**	2.21 ± 0.05*	2.64 ± 0.04**	3.23 ± 0.10**	1.75 ± 0.03**	1.00 ± 0.02**
	9 day	2.48 ± 0.09*	2.55 ± 0.04*	2.22 ± 0.03*	4.32 ± 0.12**	0*	0*
	12 day	3.27 ± 0.08*	2.28 ± 0.03*	2.33 ± 0.08*	2.02 ± 0.04**	1.11 ± 0.01*	0*
Received <i>L. aci- dophilus</i> IMV B-7279 - <i>B. ani- malis</i> VKB	1 day	4.12 ± 0.14*	3.99 ± 0.11**	3.44 ± 0.04*	3.03 ± 0.07*	1.45 ± 0.02**	1.75 ± 0.03*
	3 day	3.99 ± 0.13*	4.03 ± 0.03**	3.54 ± 0.08*	3.50 ± 0.05**	0*	2.12 ± 0.02*
	6 day	3.68 ± 0.09*	3.85 ± 0.09**	2.78 ± 0.04**	3.05 ± 0.04**	1.75 ± 0.03**	2.15 ± 0.03
	9 day	3.35 ± 0.04*	3.79 ± 0.10**	2.66 ± 0.08**	2.60 ± 0.07*	0*	2.44 ± 0.04*
	12 day	3.22 ± 0.08*	3.85 ± 0.09**	2.65 ± 0.05**	1.60 ± 0.10**	0*	1.55 ± 0.08**
Received <i>L. aci- dophilus</i> IMV B-7279 - <i>B. ani- malis</i> VKL	1 day	3.26 ± 0.07**	3.77 ± 0.08**	2.40 ± 0.11*	4.23 ± 0.14**	1.11 ± 0.01*	2.44 ± 0.03**
	3 day	3.48 ± 0.05**	3.55 ± 0.05**	2.35 ± 0.03*	4.13 ± 0.08**	0*	1.17 ± 0.02**
	6 day	3.66 ± 0.08*	3.44 ± 0.04**	2.20 ± 0.02*	3.66 ± 0.07**	0*	2.10 ± 0.04
	9 day	3.73 ± 0.04*	3.37 ± 0.07**	2.15 ± 0.04*	2.81 ± 0.04*	1.45 ± 0.03	1.66 ± 0.02**
	12 day	3.62 ± 0.04*	3.54 ± 0.09**	2.10 ± 0.03*	2.18 ± 0.04*	0*	1.00 ± 0.01**
Received <i>B. ani- malis</i> VKB - <i>B. ani- malis</i> VKL	1 day	4.08 ± 0.09*	2.08 ± 0.02**	4.24 ± 0.11**	4.14 ± 0.11**	1.30 ± 0.03*	2.88 ± 0.08**
	3 day	4.13 ± 0.12*	1.90 ± 0.01**	4.26 ± 0.07**	4.51 ± 0.13**	2.23 ± 0.04**	3.91 ± 0.07**
	6 day	3.92 ± 0.05*	2.11 ± 0.08**	4.18 ± 0.01**	4.08 ± 0.14**	2.93 ± 0.05**	2.83 ± 0.04**
	9 day	3.98 ± 0.14**	2.32 ± 0.03*	4.12 ± 0.11**	3.79 ± 0.09**	2.72 ± 0.04**	2.88 ± 0.09**
	12 day	3.94 ± 0.09**	2.23 ± 0.04*	4.08 ± 0.13**	3.77 ± 0.11**	2.69 ± 0.05**	2.58 ± 0.08**
Received <i>L. aci- dophilus</i> IMV B-7279 - <i>L. casei</i> IMV B-7280	1 day	4.21 ± 0.16*	3.15 ± 0.13**	3.66 ± 0.09*	2.11 ± 0.05**	1.15 ± 0.02*	2.67 ± 0.09*
	3 day	3.77 ± 0.14*	3.11 ± 0.08**	3.71 ± 0.11*	2.22 ± 0.07**	0*	3.14 ± 0.02**
	6 day	3.65 ± 0.04*	2.95 ± 0.04**	3.55 ± 0.05**	3.14 ± 0.05**	1.98 ± 0.03**	1.16 ± 0.02**
	9 day	3.14 ± 0.04**	2.44 ± 0.03*	3.61 ± 0.11**	2.94 ± 0.07**	0*	1.92 ± 0.04*
	12 day	3.12 ± 0.02*	2.06 ± 0.04**	3.12 ± 0.11*	2.16 ± 0.08**	1.11 ± 0.01*	1.36 ± 0.03**

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 \*\* ( $P < 0.05$ )

It was found that the number of lactobacilli in vagina increased throughout the period of observation under the influence of compositions *L. casei* IMV B-7280 - *B. animalis* VKB or *B. animalis* VKB - *B. animalis* VKL compared with the infected mice that did not receive probiotic cultures. The increase of the number of lactobacilli in the vagina of infected mice was observed under the effect of *L. acidophilus* IMV B-7279 - *B. animalis* VKL (on the 1, 3, 6 and 9<sup>th</sup> days), *L. acidophilus* IMV B-7279 - *B. animalis* VKB (on the 3, 6 and 9<sup>th</sup> days) or *L. casei* IMV B-7280 - *B. animalis* VKL

(on the 6 and 9<sup>th</sup> days). However, the number of lactobacilli in the vagina appeared lower than in the infected mice not treated with probiotic cultures on the 1 and 12<sup>th</sup> days under the influence of compositions *L. casei* IMV B-7280 - *B. animalis* VKL or *L. acidophilus* IMV B-7279 - *B. animalis* VKB and also under the effect of *L. casei* IMV B-7280 - *L. acidophilus* IMV B-7279 (on the 1 and 3<sup>rd</sup> days). After injection of “Labilakt®”, the number of lactobacilli in the vagina of infected mice increased on the 3, 6, 9 and 12<sup>th</sup> days. The drug “Labilakt®” induced the increasing of the number of lactobacilli in the vagina of infected mice on the 3, 6, 9 and 12<sup>th</sup> days. It should be noted that after the injection of almost all the compositions of two probiotic cultures, the number of *lactobacilli* in the vagina of the infected mice appeared to be even greater than in the control (intact mice). The number of lactobacilli in the vagina of mice treated with *L. casei* IMV B-7280 - *B. animalis* VKL (on the 3<sup>rd</sup> day), *L. acidophilus* IMV B-7279 - *B. animalis* VKB (on the 9<sup>th</sup> day), *L. acidophilus* IMV B-7279 - *B. animalis* VKL (on the 9<sup>th</sup> day) or “Labilakt®” (throughout the period of observation) was at the level of the control group.

It was found that in the vagina of mice infected with *S. aureus*, the number of aerobic and facultative anaerobic microorganisms was reduced under the influence of *L. casei* IMV B-7280 - *B. animalis* VKB during the whole experimental period, *L. casei* IMV B-7280 - *B. animalis* VKL – on the 1, 3, 6 and 9<sup>th</sup> days, *L. acidophilus* IMV B-7279 - *B. animalis* VKL – on the 1 and 3<sup>rd</sup> days and *L. casei* IMV B-7280 - *L. acidophilus* IMV B-7279 – only on the 9<sup>th</sup> day compared with the infected mice that did not receive probiotic cultures. But the number of aerobic and facultative anaerobic microorganisms in the vagina of mice treated with composition *B. animalis* VKB - *B. animalis* VKL even increased on the 9 and 12<sup>th</sup> days. Instead, after the injection of the composition *L. acidophilus* IMV B-7279 - *B. animalis* VKB, the number of these microorganisms in the vagina during the whole observation period was the same as in the infected mice that did not receive probiotic cultures. “Labilakt®” reduced the number of aerobic and facultative anaerobic microorganisms in the vagina of mice infected with *S. aureus* during the term of the experiment. Under the influence of only two compositions: *L. casei* IMV B-7280 - *B. animalis* VKB (on the 1 and 3<sup>rd</sup> days) or *L. casei* IMV B-7280 - *B. animalis* VKL (on the 9<sup>th</sup> day) the number of aerobic and facultative anaerobic microorganisms in the vagina of infected mice decreased to the level of the control group.

As shown in Table 1, the number of staphylococci appeared lower after the injection of all the compositions of two probiotic strains throughout the observation period compared with the infected mice that did not receive probiotic compositions. The number of staphylococci in the vagina of infected mice decreased to the level of control under the influence of only three compositions: *L. casei* IMV B-7280 - *B. animalis* VKB (on the 9 and 12<sup>th</sup> days), *L. casei* IMV B-7280 - *B. animalis* VKL (on the 6, 9 and 12<sup>th</sup> days), *L. acidophilus* IMV B-7279 - *L. casei* IMV B-7280 (on the 9<sup>th</sup> day).

The number of staphylococci in the vagina of infected mice appeared smaller than in the control group (intact mice) after the administration to the infected mice *B. animalis* VKB - *B. animalis* VKL (on the 1, 3 and 6<sup>th</sup> days) and on the 9 and 12<sup>th</sup> days – remained at the level of the control group (intact mice). Under the influence of *L. acidophilus* IMV B-7279 - *L. casei* IMV B-7280 the number of staphylococci in the vagina of infected mice on the 12<sup>th</sup> day was also lower than in the control. The number of staphylococci also reduced to the control level under the influence of “Labilakt®” on the 6, 9 and 12<sup>th</sup> days.

The number of streptococci decreased after injection of *L. casei* IMV B-7280 - *B. animalis* VKB, *L. casei* IMV B-7280 - *B. animalis* VKL or *L. acidophilus* IMV B-7279 - *B. animalis* VKL throughout the period of observation in comparison with the infected mice that did not receive probiotic cultures. Under the influence of *L. acidophilus* IMV B-7279 - *B. animalis* VKB a decreased level of streptococci was observed on the 6, 9 and 12<sup>th</sup> days.

After intravaginal injection of *L. acidophilus* IMV B-7279 - *L. casei* IMV B-7280 composition the number of streptococci in the vagina of infected mice did not change significantly, but under the influence of *B. animalis* VKL - *B. animalis* VKB their number increased throughout the period of observation. The “Labilakt®” injection was characterized by increasing the number of streptococci in the vagina of infected mice on the 1 and 3<sup>rd</sup> days, but on the 6, 9 and 12<sup>th</sup> days their number was the same as in the infected mice not treated with probiotic cultures. The number of streptococci decreased to the control level only under the influence of *L. acidophilus* IMV B-7279 - *B. animalis* VKL during the period of observation and *L. casei* IMV B-7280 - *B. animalis* VKL – on the 9 and 12<sup>th</sup> days.

The number of coliform bacteria in the vagina decreased under the influence of *L. casei* IMV B-7280 - *B. animalis* VKB (throughout the period of observation), *L. casei* IMV B-7280 - *B. animalis* VKL (on the 1, 3, 9 and 12<sup>th</sup> days), *L. acidophilus* IMV B-7279 - *B. animalis* VKB (on the 1, 3, 9 and 12<sup>th</sup> days), *L. acidophilus* IMV B-7279 - *L. casei* IMV B-7280 (on the 1, 3, 9 and 12<sup>th</sup> days), *L. acidophilus* IMV B-7279 - *B. animalis* VKL (on the 1, 3, 6 and 12<sup>th</sup> days), *B. animalis* VKL - *B. animalis* VKB (on the 1<sup>st</sup> day) in comparison with the infected mice not treated with probiotic cultures. The reduced number of coliform bacteria under the influence of the “Labilakt®” drug was observed only on the 6<sup>th</sup> day.

However, after the injection of some of these compositions to the infected mice, the number of coliform bacteria in the vagina appeared to be even greater than in the control group and in the vagina of mice infected with *S. aureus*, that did not receive probiotic cultures. It was observed after the injection of *L. casei* IMV B-7280 - *B. longum* VK, *L. acidophilus* IMV B-7279 - *B. animalis* VKB and *L. acidophilus* IMV B-7279 - *L. casei* IMV B-7280 (on the 6<sup>th</sup> days), the “Labilakt®” drug (on the 1 and 9<sup>th</sup> days) or *B. animalis* VKL - *B. animalis* VKB (on the 3, 6, 9 and 12<sup>th</sup> days).

It was shown that administration of different compositions of two probiotic strains into the vagina of the infected mice changed the number of fungi in their vaginal flora. Their number decreased throughout the period of observation under the influence of the composition *L. casei* IMV B-7280 - *B. animalis* VKL compared with the infected mice not treated with probiotic cultures and the control group (intact mice). The composition *L. acidophilus* IMV B-7279 - *B. animalis* VKL induced the reduction of fungal flora on the 1, 3, 9 and 12<sup>th</sup> days, *L. acidophilus* IMV B-7279 - *B. animalis* VKB – on the 1 and 12<sup>th</sup> days, *L. acidophilus* IMV B-7279 - *L. casei* IMV B-7280 – on the 9 and 12<sup>th</sup> days, *L. casei* IMV B-7280 - *B. animalis* VKB – on the 1<sup>st</sup> day. However, the number of fungi increased under the influence of the last composition on the 3, 6, 9 and 12<sup>th</sup> days. The composition *L. acidophilus* IMV B-7279 - *B. animalis* VKB also caused the increase of the number of fungi on the 3<sup>rd</sup> day, and *L. acidophilus* IMV B-7279 - *L. casei* IMV B-7280 – on the 3 and 6<sup>th</sup> days. It should be noted that the number of fungi in the vagina of infected mice increased under the influence of composition *B. animalis* VKL - *B. animalis* VKB on the 1, 3, 6, 9 and 12<sup>th</sup> days. The drug “Labilakt®” increased the number of fungi on the 3, 9 and 12<sup>th</sup> days. In other terms of observation under the influence of this drug the number of fungi was the same as in the infected mice that did not receive probiotic cultures. The increase of the number of fungi in the vagina of the infected mice took place under the influence of *L. casei* IMV B-7280 - *B. animalis* VKB (on the 3 and 9<sup>th</sup> days), *L. acidophilus* IMV B-7279 - *B. animalis* VKB (on the 9<sup>th</sup> day), *L. acidophilus* IMV B-7279 - *B. animalis* VKL (on the 1<sup>st</sup> day), *L. acidophilus* IMV B-7279 - *L. casei* IMV B-7280 (on the 1 and 3<sup>rd</sup> days) or *B. animalis* VKL - *B. animalis* VKB (throughout the period of observation) in comparison with the control group (intact mice). The drug “Labilakt®” induced the increase of the number of fungi in the vagina of infected mice on the 1, 3 6 and 9<sup>th</sup> days (in comparison with the control group).

The compositions of the two strains being compared, their antistaphylococcal action was equal. The number of coliform bacteria effectively reduced under the influence of *L. casei* IMV B-7280 - *B. animalis* VKB, and fungi – under the influence of *L. casei* IMV B-7280 - *B. animalis* VKL.

The results of studying the influence of compositions of three or four strains on the vaginal microflora spectrum are presented in Table 2.

The number of lactobacilli in the vagina of mice infected with *S. aureus* during the observation period increased under the influence of the compositions *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279, *L. acidophilus* IMV B-7279 - *B. animalis* VKL - *B. animalis* VKB or *L. casei* IMV B-7280 - *B. animalis* VKB - *B. animalis* VKL - *L. acidophilus* IMV B-7279 as compared with the infected mice not treated with probiotic cultures. The number of lactobacilli increased also on the 3, 6, 9 and 12<sup>th</sup> days after the injection of *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB or *L. casei* IMV B-7280 - *B. animalis* VKB - *L. acidophilus* IMV B-7279. On the 1<sup>st</sup> day after the injection of the composition *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB to the infected mice, the number of lactobacilli was the same as in the vagina of the infected mice, but higher than in the control (intact mice). However, in the vagina of infected mice treated with the composition *L. casei* IMV B-7280 - *B. animalis* VKB - *L. acidophilus* IMV B-7279, the number of lactobacilli temporarily decreased on the 1<sup>st</sup> day.

The number of aerobic and facultative anaerobic microorganisms in the vagina of infected mice decreased under the influence of compositions *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB and *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279 during the observation period in comparison with the infected mice not treated with probiotic cultures. However, their number increased after the injection of *L. acidophilus* IMV B-7279 - *B. animalis* VKL - *B. animalis*

VKB and *L. casei* IMV B-7280 - *B. animalis* VKB - *B. animalis* VKL - *L. acidophilus* IMV B-7279 throughout the period of observation. Under the influence of composition *L. casei* IMV B-7280 - *B. animalis* VKB - *L. acidophilus* IMV B-7279, the number of these bacteria decreased on the 3, 6 and 9<sup>th</sup> days, and in other terms of observation it was the same as in the vagina of the infected mice that did not receive probiotic cultures.

Table 2

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

Groups of mice / time of observation, day		Colony forming units (lg/ml)					
		Meat-Peptide Agar	Baird-Parker-Agar	KF-Streptococcus agar	Man-Rogosa-Sharpe	ENDO	Sabouraud agar
Intact mice	-	2.24 ± 0.03	2.51 ± 0.07	2.08 ± 0.03	2.68 ± 0.05	1.08 ± 0.05	2.07 ± 0.07
Mice infected with <i>S. aureus</i>	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 <i>L. casei</i> IMV B-7280 - <i>B. animalis</i> VKL - <i>B. animalis</i> VKB	1 day	2.28 ± 0.02*	3.06 ± 0.11*	2.88 ± 0.04*	3.10 ± 0.10*	1.90 ± 0.05*	1.48 ± 0.03*
	3 day	3.09 ± 0.12*	3.34 ± 0.07*	2.23 ± 0.05*	2.88 ± 0.05*	0*	1.60 ± 0.09*
	6 day	2.54 ± 0.02*	2.08 ± 0.02*	2.57 ± 0.05*	3.14 ± 0.07*	2.20 ± 0.04*	1.30 ± 0.01*
	9 day	2.72 ± 0.07*	2.96 ± 0.02*	2.65 ± 0.07*	3.32 ± 0.12*	1.30 ± 0.01	1.70 ± 0.02*
	12 day	2.67 ± 0.08*	2.52 ± 0.05*	2.73 ± 0.01*	3.22 ± 0.11*	1.48 ± 0.02*	1.30 ± 0.05*
Received <i>L. casei</i> IMV B-7280 - <i>B. animalis</i> VKL - <i>L. acidophilus</i> IMV B-7279	1 day	2.34 ± 0.09*	2.58 ± 0.07*	1.90 ± 0.01*	3.26 ± 0.10*	1.00 ± 0.01*	1.30 ± 0.07*
	3 day	2.40 ± 0.07*	3.23 ± 0.03*	2.18 ± 0.11*	3.49 ± 0.10*	0*	0*
	6 day	3.27 ± 0.05*	2.34 ± 0.07*	1.12 ± 0.02*	3.53 ± 0.10*	0*	1.60 ± 0.04*
	9 day	2.86 ± 0.07*	2.49 ± 0.04*	1.60 ± 0.01*	3.45 ± 0.14*	1.30 ± 0.03	1.00 ± 0.01*
	12 day	2.71 ± 0.03*	2.28 ± 0.07*	2.40 ± 0.05*	3.38 ± 0.11*	1.00 ± 0.01*	1.00 ± 0.01*
Received <i>L. acidophilus</i> IMV B-7279 - <i>B. animalis</i> VKL - <i>B. animalis</i> VKB	1 day	4.19 ± 0.07*	3.39 ± 0.04*	4.08 ± 0.11*	3.22 ± 0.01*	1.48 ± 0.02*	2.15 ± 0.07*
	3 day	4.20 ± 0.10*	3.51 ± 0.07*	4.11 ± 0.11*	3.45 ± 0.05*	0*	2.40 ± 0.08*
	6 day	4.29 ± 0.09*	3.32 ± 0.08*	3.99 ± 0.07*	3.54 ± 0.07*	0*	1.00 ± 0.05*
	9 day	4.13 ± 0.11*	3.24 ± 0.07*	4.04 ± 0.08*	3.33 ± 0.08*	1.60 ± 0.01*	1.90 ± 0.07*
	12 day	4.08 ± 0.10*	2.96 ± 0.05*	3.80 ± 0.09*	3.50 ± 0.03*	1.00 ± 0.02*	1.95 ± 0.04
Received <i>L. casei</i> IMV B-7280 - <i>B. animalis</i> VKB - <i>L. acidophilus</i> IMV B-7279	1 day	3.21 ± 0.12*	3.62 ± 0.04*	3.98 ± 0.09*	2.25 ± 0.03*	1.00 ± 0.02*	1.65 ± 0.02*
	3 day	3.14 ± 0.03*	3.32 ± 0.12*	3.76 ± 0.04*	3.11 ± 0.08*	0*	2.11 ± 0.04*
	6 day	3.13 ± 0.05*	3.30 ± 0.08*	3.88 ± 0.09*	3.15 ± 0.08*	1.17 ± 0.02*	2.03 ± 0.03
	9 day	3.22 ± 0.07*	3.16 ± 0.09*	3.68 ± 0.08*	3.55 ± 0.08*	0*	1.85 ± 0.04*
	12 day	3.36 ± 0.09*	2.89 ± 0.08*	3.55 ± 0.04*	3.11 ± 0.09*	1.36 ± 0.03	1.17 ± 0.02*
Received <i>L. casei</i> IMV B-7280 - <i>B. animalis</i> VKB - <i>B. animalis</i> VKL - <i>L. acidophilus</i> IMV B-7279	1 day	4.28 ± 0.10*	4.27 ± 0.12*	4.13 ± 0.13*	3.41 ± 0.11*	3.76 ± 0.05*	3.46 ± 0.14*
	3 day	4.23 ± 0.12*	4.28 ± 0.12*	4.08 ± 0.11*	2.98 ± 0.15*	3.78 ± 0.07*	3.57 ± 0.13*
	6 day	4.32 ± 0.11*	3.83 ± 0.07*	3.23 ± 0.12*	4.11 ± 0.05*	3.54 ± 0.09*	2.65 ± 0.05*
	9 day	4.22 ± 0.12*	3.86 ± 0.02*	3.46 ± 0.08*	4.05 ± 0.15*	3.51 ± 0.05*	2.80 ± 0.05*
	12 day	4.18 ± 0.08*	3.50 ± 0.09*	3.93 ± 0.07*	3.98 ± 0.07*	3.32 ± 0.03*	2.62 ± 0.02*

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 • ( $P < 0.05$ )

The number of staphylococci in the vagina of the infected mice decreased throughout the period of observation under the influence of the compositions *L. casei* IMV B-7280 - *B. animalis* VKB - *L. acidophilus* IMV B-7279, *L. acidophilus* IMV B-7279 - *B. animalis* VKL - *B. animalis* VKB, *L. ca-*

*sei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279 or *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB as compared with the infected mice not treated with probiotic cultures. But under the influence of the composition *L. casei* IMV B-7280 - *B. animalis* VKB - *B. animalis* VKL - *L. acidophilus* IMV B-7279 on the 1 and 3<sup>rd</sup> days, the number of staphylococci in the vagina was the same as in the vagina of the infected mice, but on the 6, 9 and 12<sup>th</sup> days it decreased. Under the influence of composition *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279 the number of staphylococci in the vagina of infected mice decreased to the level of control (intact mice) on the 1, 6, 9 and 12<sup>th</sup> days, and under the influence of *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB – only on the 12<sup>th</sup> day.

Throughout the period of observation the number of streptococci in the vagina of the infected mice decreased under the influence of compositions *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB or *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279, but increased under the influence of *L. acidophilus* IMV B-7279 - *B. animalis* VKL - *B. animalis* VKB, *L. casei* IMV B-7280 - *B. animalis* VKB - *L. acidophilus* IMV B-7279 in comparison with the infected mice that did not receive probiotic cultures. The composition *L. casei* IMV B-7280 - *B. animalis* VKB - *B. animalis* VKL - *L. acidophilus* IMV B-7279 increased the number of streptococci on the 1, 3 and 12<sup>th</sup> days. The number of streptococci in the vagina of infected mice decreased to the level of the control group only under the influence of compositions the *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279 (on the 1 and 3<sup>rd</sup> days) or *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB (on the 3<sup>rd</sup> day).

The number of coliform bacteria in the vagina of the infected mice decreased after the injection of compositions *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279 (on the 1, 3, 6 and 12<sup>th</sup> days), *L. casei* IMV B-7280 - *B. animalis* VKB - *L. acidophilus* IMV B-7279 (on the 1, 3, 6 and 9<sup>th</sup> days), *L. acidophilus* IMV B-7279 - *B. animalis* VKL - *B. animalis* VKB (on the 3, 6 and 12<sup>th</sup> days) or *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB (on the 3<sup>rd</sup> day) as compared with the infected mice. However, the composition *L. acidophilus* IMV B-7279 - *B. animalis* VKL - *B. animalis* VKB increased the number of bacteria on the 9<sup>th</sup> day and *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB – on the 1 and 6<sup>th</sup> days.

There was the increase in the number of fungi in the vagina of the infected mice under the influence of *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB or *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279 during the observation period as compared with the infected mice that did not receive probiotic strains and the control group (intact mice). The composition *L. casei* IMV B-7280 - *B. animalis* VKB - *L. acidophilus* IMV B-7279 induced the reduction of the number of fungi on the 1, 9 and 12<sup>th</sup> days, and *L. acidophilus* IMV B-7279 - *B. animalis* VKL - *B. animalis* VKB – on the 1, 6 and 9<sup>th</sup> days. The number of fungi also increased on the 3<sup>rd</sup> day after the infected mice were injected with *L. casei* IMV B-7280 - *B. animalis* VKB - *L. acidophilus* IMV B-7279 or *L. acidophilus* IMV B-7279 - *B. animalis* VKL - *B. animalis* VKB.

The number of fungi in the vagina of the infected mice decreased under the influence of these two compositions on the 1 and 6<sup>th</sup> days, respectively, in comparison with the control group. In the vagina of infected mice treated with the composition *L. casei* IMV B-7280 - *B. animalis* VKB - *B. animalis* VKL - *L. acidophilus* IMV B-7279 the number of fungi and coliform bacteria increased as compared with a group of infected animals not treated with probiotic cultures.

The results of the comparative analysis of the effectiveness of compositions of three or four probiotic strains that we studied showed that the antistaphylococcal activity during the observation period was observed in four compositions of the three strains: *L. casei* IMV B-7280 - *B. animalis* VKB - *L. acidophilus* IMV B-7279, *L. acidophilus* IMV B-7279 - *B. animalis* VKL - *B. animalis* VKB, *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279 and *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB. However, the number of streptococci and fungi in the vagina of the infected mice was reduced more effectively by the compositions *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB or *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279. The number of coliform bacteria was effectively reduced under the influence of *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279.

Thus, the obtained data shows that *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *B. animalis* VKL and *B. animalis* VKB in various compositions of two, three or four strains had an impact on the number of pathogenic bacteria in the vagina of staphylococcus infected mice. It should be noted that after the injection of probiotic cultures in different compositions the number of lactobacilli in the vagina increased. All the compositions of probiotic bacteria induced the reducing of the number of staphylococci in the vagina. The number of streptococci decreased effectively under the influence

of the composition *L. acidophilus* IMV B-7279 - *B. animalis* VKL. The number of fungi was effectively reduced in the infected mice vagina under the influence of the compositions *L. casei* IMV B-7280 - *B. animalis* VKL, *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB or *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279. The number of coliform bacteria was effectively reduced under the influence of *L. acidophilus* IMV B-7279 - *B. animalis* VKL, *L. casei* IMV B-7280 - *B. animalis* VKB, *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279 or *L. casei* IMV B-7280 - *B. animalis* VKB - *L. acidophilus* IMV B-7279.

When testing compositions of two strains *in vivo* [10], *L. casei* IMV B-7280 - *B. animalis* VKL composition was the most effective on the model of intravaginal staphylococcus infection in mice. This composition appeared to be a more active antagonist of staphylococci than *L. casei* IMV B-7280 or *B. animalis* VKL separately. The composition of three strains (*L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB) also had high antistaphylococcal activity *in vivo*. The antistaphylococcal activity of the of *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB composition on the 3, 6, and 9<sup>th</sup> days appeared to be higher than of all these monocultures used separately, and on the 12<sup>th</sup> day it was higher than that of *B. animalis* VKL. The antistaphylococcal activity of the two-strain compositions (*L. casei* IMV B-7280 - *B. animalis* VKL or *L. casei* IMV B-7280 - *B. animalis* VKB), was the same as that of the *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB composition on the 3 and 6<sup>th</sup> days. However, on the 9<sup>th</sup> day *S. aureus* 8325-4 was still recovered from the vagina after the injection of the *L. casei* IMV B-7280 - *B. animalis* VKL or *L. casei* IMV B-7280 - *B. animalis* VKB compositions, but was completely eliminated from the vagina of mice treated with the *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB composition. *L. casei* IMV B-7280, *B. animalis* VKL and *B. animalis* VKB, which were part of this composition, had high adhesive activity in respect of epithelial cells (unpublished data), and *B. animalis* VKB and *L. casei* IMV B-7280 demonstrated the best antistaphylococcal activity when injected into mice separately in comparison with the other studied strains of bacteria. It should be noted that after the injection of a composition of four strains into the infected mice, *S. aureus* 8325-4 was recovered from the vagina of mice in larger amounts than after the injection of some probiotic cultures or their compositions.

We have not investigated the mechanisms of lacto- and bifidobacteria influence on the vaginal microflora. However, the literature data showed some mechanisms of antimicrobial action of lactic acid bacteria. So, it is considered that [8] the main mechanism of probiotics action against urogenital infections is the production of biosurfactants and collagen-binding proteins, which leads to suppression of the pathogenic bacteria adhesion, that was shown for the following pathogens: *E. coli* 67, *E. coli* Hu734, *E. faecalis* 1131, *E. faecalis* 1396, *P. mirabilis* 28cii, *P. aeruginosa* AK1, *S. epidermidis* 3059, *K. pneumoniae* 3a [3], as well as for some fungi. As it was established earlier [3, 8] *L. acidophilus* or *L. rhamnosus* GR-1 and *L. fermentum* RC-14 for 2 months led to the recovery from the bacterial vaginosis or to reduction of the incidence of the bacterial vaginosis recurrences and / or [11, 15] to increase of the number of lactobacilli in the vagina and restoring of the normal microflora of the vagina significantly more often in comparison with the group which was not treated or treated with placebo. The positive impact on the microflora of the urogenital tract and the ability to prevent the development of bacterial vaginosis by reducing the number of pathogens has been shown for the strain *Lactobacillus crispatus* CTV-05 [11].

Since the protective mechanisms could include not only inhibition of growth and adhesion of pathogens, but also immune modulation, in our recent studies we have investigated the immunomodulatory properties of probiotic strains of *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *B. animalis* VKB and *B. animalis* VKL. It was established that the phagocytic cells were activated and the parameters of cellular immune response normalized under their influence [10, 16]. The production of interferons [10] and other Th1-type cytokines also increased (unpublished data).

Thus, the strains *L. casei* IMV B-7280, *L. acidophilus* IMV B-7279, *B. animalis* VKB and *B. animalis* VKL in various compositions are all promising for the development of probiotic drugs with antistaphylococcal activity and for correction of the urogenital tract microflora, but the most successful compositions are *B. animalis* VKB - *B. animalis* VKL, *L. casei* IMV B-7280 - *B. animalis* VKL - *L. acidophilus* IMV B-7279, *L. casei* IMV B-7280 - *B. animalis* VKL - *B. animalis* VKB, that caused not only *S. aureus* elimination, but also normalization of the vaginal microflora. It should be noted that for the final selection of the most effective composition of probiotic microorganisms it is important to give attention not only to the effects of probiotic strains on the microflora, but also to their immunomodulatory properties.



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## ВПЛИВ КОМПОЗИЦІЙ ЛАКТО- ТА БІФІДОБАКТЕРІЙ НА СПЕКТР ВАГІНАЛЬНОЇ МІКРОФЛОРИ ПРИ ІНТРАВАГІНАЛЬНІЙ СТАФІЛОКОКОВІЙ ІНФЕКЦІЇ

### Резюме

Визначено вплив інтравагінального введення штамів *Lactobacillus casei* IMV B-7280, *Lactobacillus acidophilus* IMV B-7279, *Bifidobacterium animalis* VKL та *Bifidobacterium animalis* VKB у складі різних композицій на спектр мікрофлори урогенітального тракту на моделі експериментальної інтравагінальної стафілококової інфекції у мишей. Встановлено, що під впливом різних композицій цих штамів відбувалася зміна спектру мікрофлори, збільшення кількості представників нормофлори та суттєво знижувалась кількість умовно-патогенних мікроорганізмів, зокрема стафілококів, стрептококів, бактерій групи кишечної палички та грибів. Визначено, що штами лакто- та біфідобактерій, які досліджувались, є перспективними для створення пробіотичних препаратів, ефективних при лікуванні інтравагінальної стафілококової інфекції.

Ключові слова: *Lactobacillus*, *Bifidobacterium*, *Staphylococcus*, піхва, мікрофлора, миші

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## ВЛИЯНИЕ КОМПОЗИЦИЙ ЛАКТО- И БИФИДОБАКТЕРИЙ НА СПЕКТР ВАГИНАЛЬНОЙ МИКРОФЛОРЫ ПРИ ИНТРАВАГИНАЛЬНОЙ СТАФИЛОКОККОВОЙ ИНФЕКЦИИ

### Резюме

Определено влияние интравагинального введения штаммов *Lactobacillus casei* IMV B-7280, *Lactobacillus acidophilus* IMV B-7279, *Bifidobacterium animalis* VKL и *Bifidobacterium animalis* VKB в составе различных композиций на спектр микрофлоры урогенитального тракта на модели экспериментальной интравагинальной стафилококковой инфекции у мышей. Установлено, что под влиянием различных композиций этих штаммов происходило изменение спектра микрофлоры, увеличение количества представителей нормофлоры и существенно снижалась количество условно-патогенных микроорганизмов, в частности стафилококков, стрептококков, бактерий группы кишечной палочки и грибов. Определено, что штаммы лакто- и бифидобактерий, которые исследовались, являются перспективными для создания пробиотических препаратов, эффективных при лечении интравагинальной стафилококковой инфекции.

Ключевые слова: *Lactobacillus*, *Bifidobacterium*, *Staphylococcus*, вагина, микрофлора, мыши

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