https://doi.org/10.29326/2304-196X-2024-13-3-261-268

The effect of the nisin-based pharmaceutical formulation used in the treatment plan for cows with subclinical mastitis on the milk microbiota

Mariya N. Isakova, Yana Yu. Lysova
Ural Federal Agrarian Research Center, Ural Branch of the Russian Academy of Sciences, 112a Belinsky str., Ekaterinburg 620142, Russia

ABSTRACT
Due to the growing threat of antimicrobial resistance, the search and development of new drugs to treat infectious mammary gland diseases of high yielding cows is an urgent task. The paper presents data on the microbiota composition of milk from high yielding cows suffering from subclinical mastitis; 144 microbial isolates were recovered from 70 milk samples; with the highest number of *Staphylococcus aureus* and *Streptococcus dysgalactiae* detected (22.2 and 16.0%, respectively). The study showed that a significant number of *Staphylococcus aureus* isolates (53.1%) were resistant to I generation cephalosporins; 52.6% of the isolated *Streptococcus dysgalactiae* strains showed resistance to tetracyclines; 33.3% of *Staphylococcus haemolyticus* isolates were resistant to macrolides. 42.1; 35.3 and 62.5% of *Enterococcus faecium*, *Aerococcus viridans* and coliform bacteria isolates, respectively, were resistant to penicillins. 38.5% of *Staphylococcus epidermidis* isolates were found to be resistant to tetracyclines. *Corynebacterium pseudotuberculosis* isolates showed equal resistance to penicillin and tetracycline antimicrobials (20.0%). The research revealed presence of multi-drug resistant coliform bacteria, *Streptococcus dysgalactiae*, *Aerococcus viridans*, *Staphylococcus aureus* strains. Experiments to study the effect of the new nisin-based pharmaceutical formulation on microbiota of milk from cows with subclinical mastitis were carried out using 35 high yielding cows. A microbiological testing of cow milk on day 14 from the beginning of the treatment showed that the number of microbiota-free samples increased to 88.6%, while in 1.4% of cases *Staphylococcus aureus* isolates were recovered (10^3 CFU/mL). The titers of coliform and *Staphylococcus aureus* bacteria isolated in 1.4% (10^1 CFU/mL) and 2.7% (10^2 CFU/mL) of cases, respectively, were not etiologically significant.

Keywords: cows, subclinical mastitis, antimicrobial resistance, antimicrobials, treatment regimen, bacteriocin nisin, milk microbiota, colony-forming units

Acknowledgements: This work has been supported by the grants the Russian Science Foundation (project No. 22-76-00009, https://rscf.ru/project/22-76-00009).

For citation: Isakova M. N., Lysova Ya. Yu. The effect of the nisin-based pharmaceutical formulation used in the treatment plan for cows with subclinical mastitis on the milk microbiota. *Veterinary Science Today.* 2024; 13 (3): 261–268. https://doi.org/10.29326/2304-196X-2024-13-3-261-268

Conflict of interests: The authors declare no conflict of interests.

For correspondence: Mariya N. Isakova, Cand. Sci. (Veterinary Medicine), Senior Researcher, Department of Reproductive Biology and Neonatology, Ural Federal Agrarian Research Center, Ural Branch of the Russian Academy of Sciences, 112a Belinsky str., Ekaterinburg 620142, Russia, *tmarya105@yandex.ru*

УДК 619:618.19-002:636.2:637.12.04/.07:615.015.8

Влияние композиции на основе бактериоцина низина в схеме лечения коров с субклиническим маститом на микробиоту молока

М. Н. Исакова, Я. Ю. Лысова
ФГБНУ «Уральский федеральный аграрный научно-исследовательский центр Уральского отделения Российской академии наук» (ФГБНУ УрФАНИЦ УрО РАН), ул. Белинского, 112а, г. Екатеринбург, 620142, Россия

РЕЗЮМЕ
В связи с растущей угрозой развития антибиотикорезистентности поиск и разработка новых средств для лечения инфекционных заболеваний молочной железы высокопродуктивных коров является актуальной задачей. В статье представлены данные по изучению состава микробиоты секрета молочной железы высокопродуктивных коров при скрытой форме мастита. Из 70 проб секрета молочной железы было выделено 144 изолята микроорганизмов, наибольшее количество приходилось на *Staphylococcus aureus* и *Streptococcus dysgalactiae* (22,2 и 16,0% соответственно). Исследованиями установлено, что у максимального количества изолятов *Staphylococcus aureus* (53,1%) наблюдали устойчивость к цефалоспоринам I поколения. Выделенные штаммы *Streptococcus dysgalactiae* в 52,6% случаев проявили устойчивость к препаратам группы тетрациклинов; 33,3% изолятов *Staphylococcus haemolyticus* были резистентны к препаратам группы макролидов. Устойчивостью к препаратам групп пенициллинов обладали 42,1; 35,3 и 62,5% изолятов *Enterococcus faecium*, *Aerococcus viridans* и бактерий группы кишечной палочки соответственно. В 38,5% случаев установлена резистентность к препаратам группы тетрациклинов у изолятов *Staphylococcus epidermidis*. Изоляты *Corynebacterium pseudotuberculosis* проявили устойчивость к антимикробным

препаратам групп пенициллинов и тетрациклинов в равной степени (20,0%). Полученные данные показали наличие полирезистентных штаммов бактерий группы кишечной палочки, *Streptococcus dysgalactiae*, *Aerococcus viridans*, *Staphylococcus aureus.* Экспериментальные исследования по изучению влияния разработанной фармацевтической композиции, содержащей бактериоцин низин, на состав микробиоты молока при лечении коров с субклиническим маститом выполнены на 35 высокопродуктивных коровах. Проведенное на 14-й день с начала курса лечения микробиологическое исследование секрета молочной железы коров показало, что число проб с отсутствием микрофлоры увеличилось до 88,6%, при этом количество колониеобразующих единиц, равное 10^3 КОЕ/мл, установлено у 1,4% изолятов *Staphylococcus aureus.* Выделенные в 1,4 (10^1 КОЕ/мл) и 2,7% (10^2 КОЕ/мл) случаев бактерии группы кишечной палочки и *Staphylococcus aureus* соответственно не являлись этиологически значимыми в диагностическом титре.

Ключевые слова: коровы, субклинический мастит, антибиотикорезистентность, антимикробные препараты, схема лечения, бактериоцин низин, микробиота молока, колониеобразующие единицы

Благодарности: Исследование выполнено за счет гранта Российского научного фонда (проект № 22-76-00009, https://rscf.ru/project/22-76-00009).

Для цитирования: Исакова М. Н., Лысова Я. Ю. Влияние композиции на основе бактериоцина низина в схеме лечения коров с субклиническим маститом на микробиоту молока. *Ветеринария сегодня*. 2024; 13 (3): 261–268. https://doi.org/10.29326/2304-196X-2024-13-3-261-268

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Исакова Мария Николаевна, канд. вет. наук, старший научный сотрудник отдела репродуктивной биологии и неонатологии ФГБНУ УрФАНИЦ УрО РАН, ул. Белинского, 112а, г. Екатеринбург, 620142, Россия, *tmarya105@yandex.ru*

INTRODUCTION

Mammary gland inflammation in cows is most often caused by a bacterial infection [1, 2, 3]. The quantity of bacteria detected depends on the form of mastitis and its severity, as well as the pathogen type [4, 5, 6, 7]. The most often detected microorganisms in milk of cows suffering from mastitis are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Escherichia coli*, *Enterococcus faecium*, *Enterococcus faecalis* [1, 2, 3, 4]. The subclinical form of mastitis, which is more difficult to diagnose due to the lack of visible changes in the mammary gland and milk is the major danger for dairy farming, though it gives higher somatic cell counts and total bacterial counts in milk. Microbial metabolites and toxins accumulate in the milk from subclinically mastitic cows affecting milk taste, nutritional value of raw milk and dairy products and decreasing their shelf life [8, 9, 10, 11]. The prevalence of subclinical mastitis in high yielding cows in developing countries is high [12, 13, 14, 15, 16]. That is why to reduce milk rejection and prevent antimicrobial resistance (AMR), the use of antimicrobials is minimized in treatment plans, and vaccines, bacteriophages, phage lysines, bacteriocins are used as an alternative [17, 18, 19, 20, 21, 22, 23, 24]. Pursuant to the "Strategy to Prevent the Spread of Antimicrobal Resistance in the Russian Federation to 2030", approved by the Russian Federation Government Decree on 25 September 2017 No. 2045-r, we tested the drugs based on antimicrobial peptides of microbial origin used to treat infectious mammary gland diseases of high yielding cows.

The work is relevant because the formulation is based on bacteriocin nisin to be used in the treatment regimen for cows with subclinical mastitis as an alternative to known antimicrobial drugs.

The novelty of the work is that for the first time, data on the effect of a new nisin-based formulation used in the treatment regimen of cows with subclinical mastitis on the milk microbiota were obtained.

Practical significance: in order to prevent AMR, the use of the nisin-based formulation makes it possible to reduce the use of antimicrobial drugs for mastitis treatment.

The purpose of this study was to evaluate the effect of the nisin-based pharmaceutical formulation used in the treatment plan for cows suffering from subclinical mastitis on the milk microbiota. For this purpose the following tasks were set: to study the microbiota composition of milk from high yielding cows with subclinical mastitis; to analyze the comparative AMR profiles of microorganisms isolated from milk of subclinically mastitic cows; to study the effect of the nisin-based formulation used in the treatment regimen of cows with subclinical mastitis on the milk microbiota composition.

MATERIALS AND METHODS

Objects of the study: high yielding cows with subclinical mastitis, microorganisms isolated from milk, a nisin-based formulation.

The effect of the nisin-based formulation, used in the treatment regimen of cows with subclinical mastitis, on the milk microbiota composition was studied in 35 high yielding cows with a milk yield of more than 8,000 kg per year, kept at the nucleus farm in the Polevsky Raion of the Sverdlovsk Oblast. According to the treatment regimen of subclinical mastitis, the animals received 10 mL of a new pharmaceutical formulation intra-cisternally into the affected quarter daily for five days.

All experiments were carried out in strict accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123).

During the study in 2023, the following isolates were recovered: *Staphylococcus aureus* (*n* = 32), *Streptococcus dysgalactiae* (*n* = 23), *Staphylococcus haemolyticus* (*n* = 20), *Enterococcus faecium* (*n* = 19), *Aerococcus viridans* (*n* = 17), *Staphylococcus epidermidis* (*n* = 13), coliform bacteria of *Escherichia* and *Enterobacter* genera (*n* = 8), *Corynebacterium pseudotuberculosis* (*n* = 5), *Mucor* (*n* = 4) and *Penicillium* spp. (*n* = 3) fungi.

A previously developed formulation containing nisin and water-based excipients was used with the following weight ratio percentage: nisin A – 0.3; silicon glycerolates

in a 6-mole excess of glycerol $Si(C_3H_7O_3)_4 \times 6C_3H_8O_3$ – 3.0; boron bisglycerolates $H[B(C_3H_6O_3)_2]$ – 2.0; glycerol – 10.0; distilled water – up to 100 [25, 26].

Test methods. The morphology of the recovered isolates was studied by seeding on Hiss growth media with sugars ("motley row"), was referenced by Bergey's Manual of Determinative Bacteriology [27], and the Guide to Clinically Significant Fungi [28], and then studied by MALDI-TOF mass spectrometry (Matrix-assisted laser desorption ionization time-of-flight mass spectrometry) using VITEK® MS (bioMérieux, France). For bacteriological and mycological testing, the milk samples were seeded on liquid and solid nutrient agars: meat peptone, *Streptococcus* Selective Agar, *Enterococcus* Selective Agar, Endo agar, *Staphylococcus* Selective Agar No. 10, Czapek medium, Sabouraud Dextrose Liquid Medium, bismuth sulfite agar, cetrimide agar, Levin medium, Ploskirev medium (State Research Center for Applied Microbiology and Biotechnology, Russia), 5% sheep blood agar (based on Columbia agar; Bio-Rad, France), defibrinated sheep blood (E&O Laboratories Ltd, Scotland), Salt Egg Yolk Agar Base (nutrient agar for microorganism culture GRM-agar, State Research Center for Applied Microbiology and Biotechnology, Russia), Uri*Select*4 nonselective chromogenic agar (Bio-Rad Laboratories, Inc., USA) and Sabouraud 2% Glucose Chloramphenicol Agar (SIFIN diagnostics GmbH, Germany).

The resistance of recovered isolates to 34 antimicrobial drugs from 15 groups (tetracyclines, penicillins, carbapenems, macrolides, lincosamides, ansamycins, amphenicols, I, II, III generation aminoglycosides, I, II, III generation cephalosporins, II, III generation fluoroquinolones) was evaluated by the disc diffusion test [29]. Commercially available discs were used (Scientific Research Center of Pharmacotherapy, Russia). The results were interpreted taking into account the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Statistical data were processed using AMRcloud, Microsoft Excel 2007 and Statistica 6.0. softwares.

RESULTS AND DISCUSSION

The studies were performed in 2023–2024 in the Department of Reproductive Biology and Neonatology, the Laboratory of Microbiological and Molecular Genetic Research of the Ural Federal Agrarian Research Center, Ural Branch of the Russian Academy of Sciences, in the laboratory of the medical center "Quality Med" LLC (Ekaterinburg) funded by the grant of the Russian Science Foundation No. 22-76-00009.

144 microorganisms were isolated from 70 milk samples from cows with subclinical mastitis, among them (Fig. 1): *S. aureus* (22.2%), *S. dysgalactiae* (16.0%), *S. haemolyticus* (13.9%), *E. faecium* (13.2%), *A. viridians* (11.8%), *S. epidermidis* (9.0%), coliforms of *Escherichia* and *Enterobacter* species (5.6%), *C. pseudotuberculosis* (3.5%), as well as *Mucor* (2.8%) and *Penicillium* spp. (2.1%) fungi.

In the present study, 81.4% of cows with subclinical mastitis were co-infected, among them 21.4% were co-infected with two pathogens, 28.6 and 17.1% of cows were infected with three and four pathogens. A complex microbiome consisting of five microorganisms was isolated in 14.3% of the samples.

The proportion of *S. aureus* isolates resistant to I, II and III generation cephalosporins was 53.1, 46.8 and 37.5%, respectively; to macrolides (erythromycin, clarithromycin) – 34.4%. Resistance to tetracyclines and penicillins was found in 31.3 and 28.1% of isolates, respectively. The minimum percentage of resistant *S. aureus* strains was reported for the following groups of antibiotics: II generation fluoroquinolones (12.5%), III generation fluoroquinolones (9.4%), carbapenems (6.3%). Intermediate resistance was established in 25.0% of isolates to amikacin (III generation aminoglycoside antimicrobial drug).

52.6% of *S. dysgalactiae* isolated strains showed resistance to tetracyclines (tigecycline, doxycycline). Non-sensitivity to III generation cephalosporins was established in 42.1% of isolates. Comparatively lower resistance was reported against II generation aminoglycosides (31.6%). 57.9% of *S. dysgalactiae* isolates demonstrated intermediate resistance to II generation cephalosporins (cefuroxim, cefoxitin).

Monitoring of antimicrobial resistance of *S. haemolyticus* isolates recovered from milk showed their resistance to macrolides (erythromycin, clarithromycin) in 33.3% of cases. A few isolates showed resistance to III generation cephalosporins (cefixime, cefoperazone, ceftriaxone) and ansamycins (rifampicin) – 13.3 and 6.7%, respectively. Intermediate sensitivity was revealed in 6.7% of the isolates to tetracyclines (doxycycline).

Resistance to penicillins, I generation aminoglycosides and III generation cephalosporins (42.1, 36.8 and 26.3%,

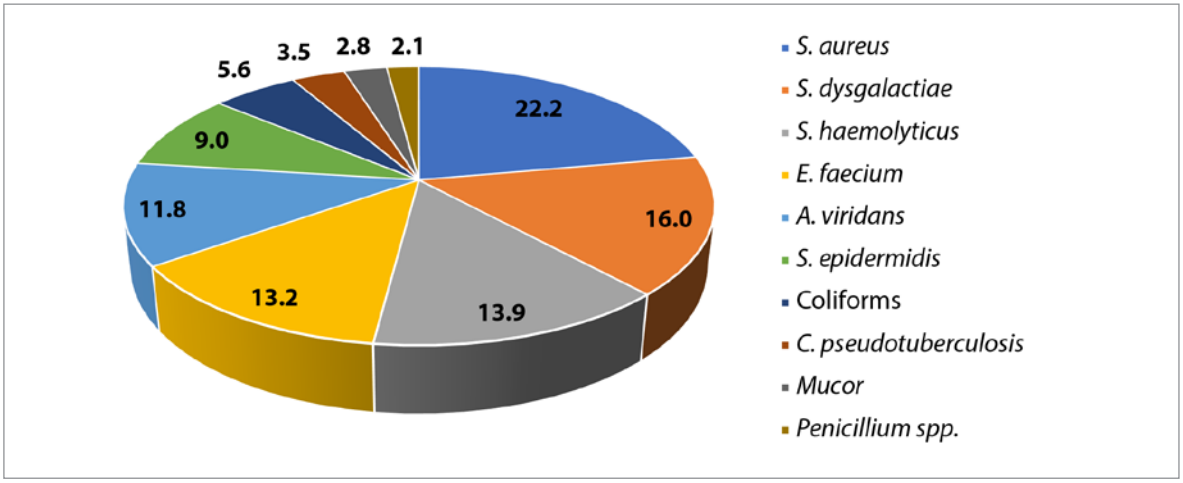

Fig. 1. Composition of the milk microbiota from cows with subclinical mastitis (n = 144)

Table 1
The structure of the microorganism population isolated from milk of cows with subclinical mastitis after using the new nisin-based formulation (*n* = 35)

Microorganism	Start of the experiment		After treatment course (Day 5)		Day 14 (from the beginning)	
	n	%	*n*	%	*n*	%
Monocultures						
S. aureus	8	22.9	4	11.4	3	8.6
S. dysgalactiae	4	11.4	2	5.7	–	–
A. viridans	2	5.7	1	2.9	–	–
S. epidermidis	1	2.9	–	–	–	–
C. pseudotuberculosis	1	2.9	–	–	–	–
S. haemolyticus	1	2.9	–	–	–	–
Coliforms	–	–	–	–	1	2.8
Associations						
S. aureus + coliforms + *E. faecium*	4	11.4	2	5.7	–	–
S. aureus + coliforms + *Streptococcus* spp. + *Penicillium* spp.	3	8.6	–	–	–	–
S. aureus + coliforms + *Streptococcus* spp. + *E. faecium*	3	8.6	–	–	–	–
S. aureus + coliforms + *Mucor*	2	5.7	–	–	–	–
S. aureus + coliforms + *Streptococcus* spp. + *Mucor*	2	5.7	–	–	–	–
S. aureus + coliforms + *Streptococcus* spp. + *E. faecalis* + *Mucor*	2	5.7	–	–	–	–
S. aureus + coliforms + *E. faecium* + *Streptococcus* spp.	1	2.9	–	–	–	–
S. aureus + *Streptococcus* spp. + *Mucor*	1	2.9	–	–	–	–
S. aureus + coliforms	–	–	1	2.9	–	–
E. faecalis + coliforms	–	–	1	2.9	–	–
Total	35	100	11	31.4	4	11.4

respectively) was demonstrated by *E. faecium* isolates. Intermediate sensitivity was found in 21.1% of the isolates to doxycycline from the tetracycline group.

The AMR profile of *A. viridans* isolates recovered from milk demonstrated the highest resistance to penicillins (ampicillin, amoxicillin, penicillin) – 35.3% and I generation aminoglycosides (kanamycin) – 23.5%. Intermediate susceptibility was revealed in 29.4% of the studied isolates to the tetracyclines (tetracycline, doxycycline).

38.5% of *S. epidermidis* isolates showed resistance to tetracyclines (tetracycline, doxycycline). Minimal resistance (15.4%) was reported against II generation aminoglycosides (gentamicin). 23.1% of the isolates demonstrated intermediate resistance to III generation fluoroquinolones (levofloxacin).

Isolated coliform bacteria had the greatest resistance (62.5%) to penicillins. Resistance to the ansamycins (rifampicin) was found in 37.5% of coliform isolates. Intermediate resistance to penicillins and II generation cephalosporins (cefuroxim, cefoxitin) was reported in 25.0 and 12.5% of coliform bacteria, respectively.

Corynebacterium pseudotuberculosis isolates were found to be equally resistant to penicillin and tetracycline antimicrobials (20.0%). Intermediate sensitivity was observed in 40.0% of the isolates to II generation cephalosporins (cefuroxim).

The obtained AMR profile of the milk microbiota from subclinically mastitic cows revealed the presence of multidrug-resistant coliform bacteria, *S. dysgalactiae*, *A. viridans*, *S. aureus* isolates. 62.5 and 47.1% of the tested coliform and *A. viridans* isolates respectively, had resistance to two groups of antimicrobials. Resistance of *S. dysgalactiae* to three antimicrobial groups was found in 43.5% of the isolated strains, to four groups in 26.1% isolates. 62.5% of the tested *S. aureus* isolates were resistant to four groups of antimicrobials, 46.9% of them were resistant to five groups and resistance to more than six groups of antimicrobials was reported in 15.6% of isolates.

The study showed that milk from subclinically mastitic cows has a complex microbiome, and the isolated microbiota are highly resistant to major antimicrobials used to treat mastitis. In this connection, a new pharmaceutical formulation based on bacteriocin nisin was included in the subclinical mastitis treatment regimen.

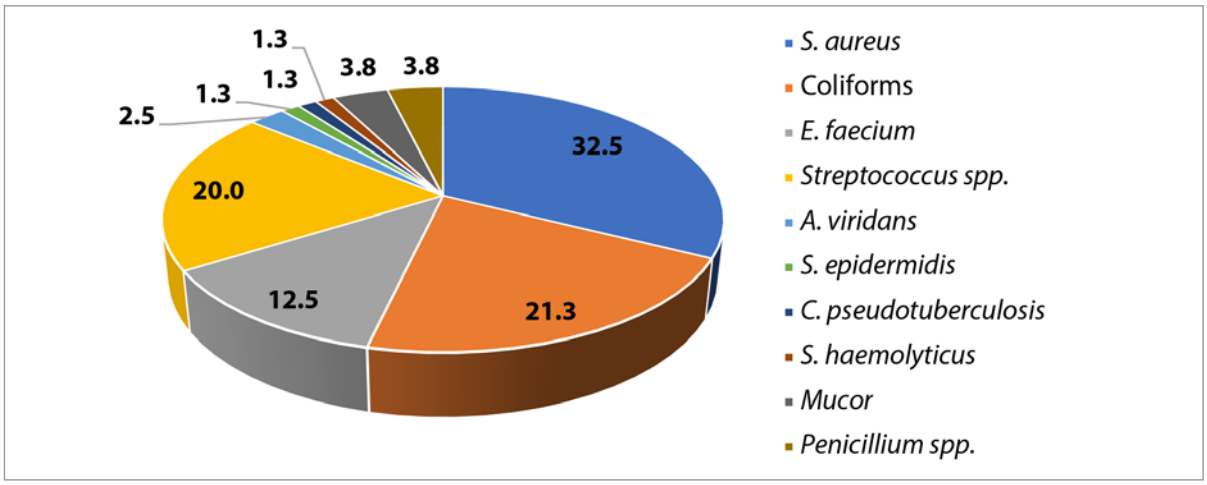

Fig. 2. The structure of microbial population isolated from milk samples from cows with subclinical mastitis at the beginning of the experiment (n = 80)

In milk samples from cows with subclinical mastitis, before using the nisin-based formulation, the isolated microorganisms were found both as a monoculture (48.6%) and as bacteria and fungi associations (51.4%). *S. aureus* (22.9%), *S. dysgalactiae* (11.4%), *A. viridans* (5.7%), *S. epidermidis* (2.9%), *C. pseudotuberculosis* (2.9%), *S. haemolyticus* (2.9%) were recovered as monocultures.

In the structure of bacterial associations, 11.4% of the samples were represented by *S. aureus* + coliforms + *E. faecium*; three-component associations included *S. aureus* + coliforms + *Mucor* (5.7%), *S. aureus* + *Streptococcus* spp. + *Mucor* (2.9%). Moreover, four-component associations were most often isolated from milk samples: *S. aureus* + coliforms + *Streptococcus* spp. + *Penicillium* spp. (8.6%), *S. aureus* + coliforms + *Streptococcus* spp. + *E. faecium* (8.6%), *S. aureus* + coliforms + *Streptococcus* spp. + *Mucor* (5.7%), *S. aureus* + coliforms + *E. faecium* + *Streptococcus* spp. (2.9%). The composition of the five-component associations was represented by *S. aureus* + coliforms + *Streptococcus* spp. + *E. faecalis* + *Mucor* (5.7%). The results are given in Table 1.

Eighty microorganisms in total were isolated from 35 milk samples at the beginning of the experiment, among them 74 bacteria and 6 fungi species (Fig. 2).

Herewith, the number of microbial cells in each sample was different. At the beginning of the experiment, 28.4% of *S. aureus* isolates were etiologically significant for the inflammation development in the mammary gland; the number of colony-forming units per 1 mL of the tested sample equal to 10^3, 10^6 and 10^7 CFU/mL was found in 9.5; 8.1 and 10.8% of the isolates, respectively. All 16 isolated *Streptococcus* spp. cultures (21.6%) were detected in the amount of 10^3 CFU/mL; 13.5% of coliform isolates, which can cause mastitis in animals, were in the amount of 10^5 CFU/mL. 10^3 and 10^5 CFU/mL values were found in 2.7 and 8.1% of *E. faecium* isolates, respectively. *S. epidermidis*, *C. pseudotuberculosis*, and *S. haemolyticus* were detected in the amount of 10^3 CFU/mL in 1.4% of cases. *A. viridans* were detected in a titer of 10^2 CFU/mL and from the beginning of the experiment were not an etiologically significant microorganism for mastitis development in cows (Table 2).

After the treatment course of animals with subclinical mastitis using the new nisin-based formulation, no microflora growth was observed in 68.6% of the samples (Table 1). The microbiota isolated from 11 samples of milk was a monoculture in 20.0% of cases represented by *S. aureus* (11.4%), *S. dysgalactiae* (5.7%), *A. viridans* (2.9%). In other samples, microorganism associations were detected: *S. aureus* + coliforms + *E. faecium* (5.7%); *S. aureus* + coliforms (2.9%); *E. faecalis* + coliforms (2.9%). 10^3 and 10^6 CFU/mL of *S. aureus* microbial cells were revealed in an equal number of isolates (1.4%). In 6.8% of cases, *S. aureus* isolated in a diagnostic titer, were not etiologically significant (10^2 CFU/mL), as well as coliforms, *E. faecium*, *A. viridans*, detected at the level of 10^1–10^2 CFU/mL. In one sample, *E. faecium* was detected in the amount of 10^3 CFU/mL, which accounted for 1.4% in the total structure of isolated microorganisms.

A microbiological testing of milk performed on Day 14 from the beginning of the treatment course showed an increase in the number of microbiota-free samples to 88.6%. In the tested samples, the milk microbiota was represented as a monoculture, where *S. aureus* and coliforms accounted for 8.6 and 2.8%, respectively (Table 1). 1.4% of *S. aureus* isolates were revealed in the amount of 10^3 CFU/mL. Coliforms and *S. aureus* isolated in 1.4% (10^1 CFU/mL) and 2.7% (10^2 CFU/mL) of cases respectively, were not etiologically significant in the diagnostic titer (Table 2).

Table 2
Number of bacteria, isolated from cow milk (n = 74)

Bacteria	CFU/mL	Start of the experiment		After treatment course (Day 5)		Day 14 (from the beginning of treatment)	
		n	%	*n*	%	*n*	%
S. aureus	10^2	5	6.8	5	6.8	2	2.7
	10^3	7	9.5	1	1.4	1	1.4
	10^6	6	8.1	1	1.4	–	–
	10^7	8	10.8	–	–	–	–
Coliforms	10^1	3	4.1	2	2.7	1	1.4
	10^2	4	5.4	1	1.4	–	–
	10^5	10	13.5	–	–	–	–
E. faecium	10^2	2	2.7	2	2.7	–	–
	10^3	2	2.7	1	1.4	–	–
	10^5	6	8.1	–	–	–	–
Streptococcus spp.	10^3	16	21.6	2	2.7	–	–
A. viridans	10^2	2	2.7	1	1.4	–	–
S. epidermidis	10^3	1	1.4	–	–	–	–
C. pseudotuberculosis	10^3	1	1.4	–	–	–	–
S. haemolyticus	10^3	1	1.4	–	–	–	–

In the last decade, intensive studies look at the potential of bacteriocins as next-generation therapeutics against drug-resistant bacteria [30, 31, 32, 33]. Bacteriocins from lactic acid bacteria are being tested as controlling agents for bacterial and viral infections; they can inhibit biofilm synthesis [33, 34, 35]. In a number of experiments, high antimicrobial activity of bacteriocin nisin was established against several species of staphylococci, including *Staphylococcus saprophyticus, S. aureus*, *S. epidermidis*, *S. haemolyticus* [36, 37, 38], including multidrug resistant and methicillin-resistant *S. aureus* [39]. There are studies on clinical isolates of *S. agalactiae* that have demonstrated different susceptibility to nisin [40]. Pérez-Ibarreche M. et al. [41] described the results of using nisin to effectively control biofilm of *S. uberis* strains that cause mastitis in cows. The use of nisin, which has antimicrobial activity against major mastitis-causing pathogens, could offer a potential alternative to antibiotics [36, 42, 43]. The data of our study confirm that it is feasible to include nisin into mastitis treatment regimens. During the experiment, it was found that the mastitis-causing pathogens isolated from milk, such as *S. aureus*, coliforms, *E. faecium*, *Streptococcus* spp., *A. viridans*, *S. epidermidis*, *C. pseudotuberculosis*, *S. haemolyticus*, are susceptible to the nisin-based formulation. Since the discovery of bacteriocins, researchers have mainly focused on testing their antimicrobial activity *in vitro*. However,

for the use of bacteriocins as antimicrobial drugs, it is necessary to study their clinical efficacy [44]. The effect of the nisin-based formulation on microbiota of milk from high yielding cows proved its effectiveness for treating subclinical mastitis: for example, in 88.6% of the samples, no microorganism growth was observed.

CONCLUSION

The study revealed the milk microbiota composition of high yielding cows with subclinical mastitis. It was found that in 81.4% of cases the disease occurs as a co-infection, among them two pathogens were isolated together in 21.4% of cases and three pathogens were isolated in parallel in 28.6% of cases. *S. aureus* (22.2%) and *S. dysgalactiae* (16.0%) were the most frequent isolated species.

A comparative analysis of the AMR of isolates recovered from milk of cows with subclinical mastitis showed the presence of multi-drug resistant strains of coliform bacteria, *S. dysgalactiae*, *A. viridans*, *S. aureus*. 62.5 and 47.1% of the tested coliforms and *A. viridans* isolates respectively, had resistance to two groups of antimicrobials. Resistance of *S. dysgalactiae* to three antimicrobial groups was found in 43.5% of the isolated strains, to four groups in 26.1% isolates. 62.5% of the tested *S. aureus* isolates were resistant to four groups of antimicrobials; 46.9% of them were resistant to five groups and resistance to more than six groups of antimicrobials was reported in 15.6% of isolates.

The study of the effect of the nisin-based formulation on microbiota of milk from cows with clinical mastitis revealed that after treatment 88.6% of the milk samples showed no microorganism growth. The milk microbiota in 8.6% of cases was represented by *S. aureus*, 2.8% were coliform bacteria. Herewith, in 1.4 and 2.7% of the samples, coliforms and *S. aureus* were detected in diagnostic titers equal to 10^1 and 10^2 CFU/mL, respectively, therefore they were not etiologically significant microorganisms for mastitis development.

REFERENCES

1. Fursova K. K., Sokolov S. L., Shchannikova M. P., Nikanova D. A., Artem'eva O. A., Kolodina E. N., et al. Changes in the microbiome of milk in cows with mastitis. *Doklady Biochemistry and Biophysics.* 2021; 497: 75–80. https://doi.org/10.1134/S1607672921020046

2. Firsov G. M., Fomenko S. A., Rezyapkina E. A., Nistratova M. V., Firsova Yu. G. Mikrobiologiya moloka i molochnykh produktov = Microbiology of milk and dairy products. *News of Science and Education*. 2018; 10 (2): 103–105. https://www.elibrary.ru/ycjsxz (in Russ.)

3. Aknazarov B. K., Matiev A. A., Abdymanap U. N., Kyzaibekova S. A., Borbiev B. I., Aitpek U. I. Microbial contamination of the mammary gland in cows with mastitis. *Vestnik Kyrgyzskogo natsional'nogo agrarnogo universiteta im. K. I. Skryabina*. 2023; (2): 84–91. https://www.elibrary.ru/elymnn (in Russ.)

4. Pisarenko N. A., Belugin N. V., Skripkin V. S., Fedota N. V. Animal health appraisal of milk at a subclinical mastitis. *Veterinariya sel'skokhozyaistvennykh zhivotnykh*. 2016; (5): 46–50. https://www.elibrary.ru/yxfpoh (in Russ.)

5. Oikonomou G., Bicalho M. L., Meira E., Rossi R. E., Foditsch C., Machado V. S., et al. Microbiota of cow's milk; distinguishing healthy, sub-clinically and clinically diseased quarters. *PLoS ONE*. 2014; 9 (1):e85904. https://doi.org/10.1371/journal.pone.0085904

6. Gonçalves J. L., Kamphuis C., Vernooij H., Araújo J. P. Jr., Grenfell R. C., Juliano L., et al. Pathogen effects on milk yield and composition in chronic subclinical mastitis in dairy cows. *The Veterinary Journal*. 2020; 262:105473. https://doi.org/10.1016/j.tvjl.2020.105473

7. Malek dos Reis C. B., Barreiro J. R., Mestieri L., Porcionato M. A., dos Santos M. V. Effect of somatic cell count and mastitis pathogens on milk composition in Gyr cows. *BMC Veterinary Research*. 2013; 9:67. https://doi.org/10.1186/1746-6148-9-67

8. Pang M., Xie X., Bao H., Sun L., He T., Zhao H., et al. Insights into the bovine milk microbiota in dairy farms with different incidence rates of subclinical mastitis. *Frontiers in Microbiology*. 2018; 9:2379. https://doi.org/10.3389/fmicb.2018.02379

9. Catozzi C., Sanchez Bonastre A., Francino O., Lecchi C., De Carlo E., Vecchio D., et al. The microbiota of water buffalo milk during mastitis. *PLoS ONE*. 2017; 12 (9):e0184710. https://doi.org/10.1371/journal.pone.0184710

10. Kaczorowski Ł., Powierska-Czarny J., Wolko Ł., Piotrowska-Cyplik A., Cyplik P., Czarny J. The influence of bacteria causing subclinical mastitis on the structure of the cow's milk microbiome. *Molecules*. 2022; 27 (6):1829. https://doi.org/10.3390/molecules27061829

11. Burakova I., Gryaznova M., Smirnova Y., Morozova P., Mikhalev V., Zimnikov V., et al. Association of milk microbiome with bovine mastitis before and after antibiotic therapy. *Veterinary World*. 2023; 16 (12): 2389–2402. https://doi.org/10.14202/vetworld.2023.2389-2402

12. Pigareva G. P. Distribution of mastitis in cows in the conditions of LLC "Don", Khokholsky District, Voronezh Region. *Teoriya i praktika innovatsionnykh tekhnologii v APK: materialy natsional'noi nauchno-prakticheskoi konferentsii (Voronezh, 1 marta – 28 aprelya 2023 g.). Ch. 1 = Theory and practice in innovative technologies in agribusiness: proceedings of the national scientific and practical conference (Voronezh, 1 March – 28 April, 2023). Part 1*. Voronezh: Voronezh SAU; 2023; 62–65. https://www.elibrary.ru/vkugzz (in Russ.)

13. Tsapenkova A. M., Vysotskaya N. V. Rasprostranenie i prichiny vozniknoveniya klinicheskogo i subklinicheskogo mastita u korov vo Vladimirskoi oblasti = Spread and causes of clinical and subclinical mastitis in the Vladimir Oblast. *Agrarnaya nauka v usloviyakh modernizatsii i innovatsionnogo razvitiya APK Rossii: sbornik materialov Vserossiiskoi nauchno-prakticheskoi konferentsii (Ivanovo, 29–30 noyabrya 2021 g.). Tom 1 = Agricultural science in the conditions of the Russian agribusiness modernization and innovative development: proceedings of the All-Russian scientific and practical conference (Ivanovo, 29–30 November, 2021). Vol 1*. Ivanovo: Ivanovo SAA; 2021; 203–206. https://www.elibrary.ru/qynlxb (in Russ.)

14. Abed A. H., Menshawy A. M. S., Zeinhom M. M. A., Hossain D., Khalifa E., Wareth G., Awad M. F. Subclinical mastitis in selected bovine dairy herds in North Upper Egypt: Assessment of prevalence, causative bacterial pathogens, antimicrobial resistance and virulence-associated genes. *Microorganisms*. 2021; 9 (6):1175. https://doi.org/10.3390/microorganisms9061175

15. Ndahetuye J. B., Persson Y., Nyman A. K., Tukei M., Ongol M. P., Båge R. Aetiology and prevalence of subclinical mastitis in dairy herds in peri-urban areas of Kigali in Rwanda. *Tropical Animal Health and Production*. 2019;

51 (7): 2037–2044. https://doi.org/10.1007/s11250-019-01905-2

16. Bari M. S., Rahman M. M., Persson Y., Derks M., Sayeed M. A., Hossain D., et al. Subclinical mastitis in dairy cows in south-Asian countries: a review of risk factors and etiology to prioritize control measures. *Veterinary Research Communications*. 2022; 46 (3): 621–640. https://doi.org/10.1007/s11259-022-09948-x

17. Rainard P., Gilbert F. B., Martins R. P., Germon P., Foucras G. Progress towards the elusive mastitis vaccines. *Vaccines*. 2022; 10 (2):296. https://doi.org/10.3390/vaccines10020296

18. Erskine R. J. Vaccination strategies for mastitis. *Veterinary Clinics of North America: Food Animal Practice*. 2012; 28 (2): 257–270. https://doi.org/10.1016/j.cvfa.2012.03.002

19. Rainard P., Gilbert F. B., Germon P., Foucras G. Invited review: a critical appraisal of mastitis vaccines for dairy cows. *Journal of Dairy Science*. 2021; 104 (10): 10427–10448. https://doi.org/10.3168/jds.2021-20434

20. Schmelcher M., Powell A. M., Becker S. C., Camp M. J., Donovan D. M. Chimeric phage lysins act synergistically with lysostaphin to kill mastitis-causing *Staphylococcus aureus* in murine mammary glands. *Applied and Environmental Microbiology*. 2012; 78 (7): 2297–2305. https://doi.org/10.1128/AEM.07050-11

21. Zduńczyk S., Janowski T. Bacteriophages and associated endolysins in therapy and prevention of mastitis and metritis in cows: current knowledge. *Animal Reproduction Science*. 2020; 218:106504. https://doi.org/10.1016/j.anireprosci.2020.106504

22. Touza-Otero L., Landin M., Diaz-Rodriguez P. Fighting antibiotic resistance in the local management of bovine mastitis. *Biomedicine & Pharmacotherapy*. 2024; 170:115967. https://doi.org/10.1016/j.biopha.2023.115967

23. Raheel I., Mohammed A. N., Mohamed A. A. The efficacy of bacteriocins against biofilm-producing bacteria causing bovine clinical mastitis in dairy farms: a new strategy. *Current Microbiology*. 2023; 80 (7):229. https://doi.org/10.1007/s00284-023-03324-x

24. Ivanov E. V., Kapustin A. V., Laishevtsev A. I., Supova A. V., Aliper T. I., Verkhovsky O. A. The effectiveness of the Kombovak-Endomast vaccine in the fight against infectious mastitis and endometritis in cows. *Veterinariya*. 2023; (11): 10–13. https://doi.org/10.30896/0042-4846.2023.26.11.10-13 (in Russ.)

25. Isakova M. N., Lysova Ya. Yu., Khonina T. G. Development of new medicinal compositions based on Bacteriocin-nisin, with subsequent evaluation of their antimicrobial activity. *Veterinariya*. 2023; (7): 43–49. https://doi.org/10.30896/0042-4846.2023.26.7.43-49 (in Russ.)

26. Isakova M. N., Belousov A. I., Drozdova L. I. Morphofunctional changes in the organs of experimental animals when using pharmaceutical compassions based on nizin. *Agrarian Bulletin of the Urals*. 2023; (8): 48–58. https://doi.org/10.32417/1997-4868-2023-237-08-48-58 (in Russ.)

27. Holt J. G., Krieg N. R., Sneath P. H. A., Staley J. T., Williams S. T. Bergey's Manual of Determinative Bacteriology. 9th ed. Baltimore: Williams & Wilkins; 1994. 787 p.

28. Sutton D. A., Fothergill A. W., Rinaldi M. G. Guide to Clinically Significant Fungi. Baltimore: Williams & Wilkins; 1997. 471 p.

29. Guidelines for Susceptibility Testing of Microorganisms to Antibacterial Agents: Methodical Guidelines MUK 4.2.1890-04. Moscow: Federal Center for State Sanitary and Epidemiological Supervision of the Ministry of Health of the Russian Federation; 2004. 91 p. (in Russ.)

30. Gradisteanu Pircalabioru G., Popa L. I., Marutescu L., Gheorghe I., Popa M., Czobor Barbu I., et al. Bacteriocins in the era of antibiotic resistance: rising to the challenge. *Pharmaceutics*. 2021; 13 (2):196. https://doi.org/10.3390/pharmaceutics13020196

31. Flynn J., Ryan A., Hudson S. P. Synergistic antimicrobial interactions of nisin A with biopolymers and solubilising agents for oral drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*. 2022; 171: 29–38. https://doi.org/10.1016/j.ejpb.2021.12.010

32. Khan F., Singh P., Joshi A. S., Tabassum N., Jeong G.-J., Bamunuarachchi N. I., et al. Multiple potential strategies for the application of nisin and derivatives. *Critical Reviews in Microbiology*. 2023; 49 (5): 628–657. https://doi.org/10.1080/1040841x.2022.2112650

33. Fernandes A., Jobby R. Bacteriocins from lactic acid bacteria and their potential clinical applications. *Applied Biochemistry and Biotechnology*. 2022; 194 (10): 4377–4399. https://doi.org/10.1007/s12010-022-03870-3

34. Heinzinger L. R., Pugh A. R., Wagner J. A., Otto M. Evaluating the translational potential of bacteriocins as an alternative treatment for *Staphylococcus aureus* infections in animals and humans. *Antibiotics*. 2023; 12 (8):1256. https://doi.org/10.3390/antibiotics12081256

35. Daba G. M., Elkhateeb W. A. Ribosomally synthesized bacteriocins of lactic acid bacteria: Simplicity yet having wide potentials – A review. *International Journal of Biological Macromolecules*. 2024; 256 (Pt. 1):128325. https://doi.org/10.1016/j.ijbiomac.2023.128325

36. Cao L. T., Wu J. Q., Xie F., Hu S. H., Mo Y. Efficacy of nisin in treatment of clinical mastitis in lactating dairy cows. *Journal of Dairy Science*. 2007; 90 (8): 3980–3985. https://doi.org/10.3168/jds.2007-0153

37. Bennett S., Ben Said L., Lacasse P., Malouin F., Fliss I. Susceptibility to nisin, bactofencin, pediocin and reuterin of multidrug resistant *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus uberis* causing bovine mastitis. *Antibiotics*. 2021; 10 (11):1418. https://doi.org/10.3390/antibiotics10111418

38. Le M. N., Kawada-Matsuo M., Komatsuzawa H. Efficiency of antimicrobial peptides against multidrug-resistant staphylococcal pathogens. *Frontiers in Microbiology*. 2022; 13:930629. https://doi.org/10.3389/fmicb.2022.930629

39. Wang J., Ma X., Li J., Shi L., Liu L., Hou X., et al. The synergistic antimicrobial effect and mechanism of nisin and oxacillin against methicillin-resistant *Staphylococcus aureus*. *International Journal of Molecular Sciences*. 2023; 24 (7):6697. https://doi.org/10.3390/ijms24076697

40. Hayes K., Field D., Hill C., O'Halloran F., Cotter L. A novel bioengineered derivative of nisin displays enhanced antimicrobial activity against clinical *Streptococcus agalactiae* isolates. *Journal of Global Antimicrobial Resistance*. 2019; 19: 14–21. https://doi.org/10.1016/j.jgar.2019.04.010

41. Pérez-Ibarreche M., Field D., Ross R. P., Hill C. A bioengineered nisin derivative to control *Streptococcus uberis* biofilms. *Applied and Environmental Microbiology*. 2021; 87 (16):e0039121. https://doi.org/10.1128/aem.00391-21

42. Kitazaki K., Koga S., Nagatoshi K., Kuwano K., Zendo T., Nakayama J., et al. *In vitro* synergistic activities of cefazolin and nisin A against mastitis pathogens. *Journal*

of Veterinary Medical Science. 2017; 79 (9): 1472–1479. https://doi.org/10.1292/jvms.17-0180

43. Bennett S., Fliss I., Ben Said L., Malouin F., Lacasse P. Efficacy of bacteriocin-based formula for reducing staphylococci, streptococci, and total bacterial counts on teat skin of dairy cows. *Journal of Dairy Science*. 2022; 105 (5): 4498–4507. https://doi.org/10.3168/jds.2021-21381

44. Guryanova S. V. Immunomodulation, bioavailability and safety of bacteriocins. *Life.* 2023; 13 (7):1521. https://doi.org/10.3390/life13071521

Received 01.04.2024
Revised 07.05.2024
Accepted 02.07.2024

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Mariya N. Isakova, Cand. Sci. (Veterinary Medicine), Senior Researcher, Department of Reproductive Biology and Neonatology, Ural Federal Agrarian Research Center, Ural Branch of the Russian Academy of Sciences, Ekaterinburg, Russia; *https://orcid.org/0000-0001-7130-5627, tmarya105@yandex.ru*

Yana Yu. Lysova, Head of Laboratory of Microbiological and Molecular Genetic Research, Department of Veterinary Laboratory Diagnosis with Testing Laboratory, Senior Researcher, Ural Federal Agrarian Research Center, Ural Branch of the Russian Academy of Sciences, Ekaterinburg, Russia; *https://orcid.org/0000-0001-6797-0659, mikroba.urnivi@mail.ru*

Исакова Мария Николаевна, канд. вет. наук, старший научный сотрудник отдела репродуктивной биологии и неонатологии ФГБНУ УрФАНИЦ УрО РАН, г. Екатеринбург, Россия; *https://orcid.org/0000-0001-7130-5627, tmarya105@yandex.ru*

Лысова Яна Юрьевна, заведующий лабораторией микробиологических и молекулярно-генетических методов исследования отдела ветеринарно-лабораторной диагностики с испытательной лабораторией, старший научный сотрудник ФГБНУ УрФАНИЦ УрО РАН, г. Екатеринбург, Россия; *https://orcid.org/0000-0001-6797-0659, mikroba.urnivi@mail.ru*

Contribution: Isakova M. N. – acquisition of relevant published data; collection of samples; data analysis; data presentation, interpretation and scientific justification of the conclusions; drafting of the paper; Lysova Ya. Yu. – determination of test methods, laboratory tests, presentation of major test results.

Вклад авторов: Исакова М. Н. – сбор литературных данных по тематике исследования, отбор проб патологического материала, анализ данных, концепция представления материалов, формулирование и научное обоснование выводов, оформление ключевых результатов исследования в виде статьи; Лысова Я. Ю. – определение методов исследования, проведение лабораторных исследований, оформление ключевых результатов исследования.