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Antibiotic resistance of bacterial pathogens circulating on a dairy farm in Sverdlovsk Oblast

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ABSTRACT

Introduction. Currently, there is a need to develop a unified strategy for rational antibiotic therapy, including monitoring the sensitivity of microorganisms, medicinal product rotation, and the use of alternative treatment methods to reduce the spread of antibiotic-resistant bacterial isolates.

Objective. Identification of bacterial pathogens that cause mastitis in cows, with an assessment of their resistance to antimicrobial medicinal products used at a livestock farm located in Sverdlovsk Oblast, for subsequent rotation of antimicrobial agents and the development of individual recommendations.

Materials and methods. The research was conducted in 2022–2024 on the basis of an agricultural farm located in Sverdlovsk Oblast. The identification of grown colonies was performed using MALDI-ToF mass spectrometry, susceptibility to antimicrobials medicinal products was determined by the disk diffusion method, and antibiotic resistance genes were detected by qPCR.

Results. In 2022, test results showed the presence of *Streptococcus* spp. (70.6%), *Escherichia coli* (52.9%), *Staphylococcus aureus* (35.3%), and *Streptococcus agalactiae* (23.5%) in mammary gland secretions. Isolates of *Escherichia coli* and *Staphylococcus aureus* were resistant to several groups of antimicrobials: aminoglycosides, penicillins, tetracyclines and fluoroquinolones (ciprofloxacin), and vancomycin. Resistance genes were identified: *bla*DHA, *bla*CTX-M, and *bla*OXA-10 in *Escherichia coli* (5%); *Erm*B in the group of bacteria *Staphylococcus* and *Streptococcus* (4%); *Mec*A in *Staphylococcus aureus* (isolated cases). Upon repeated testing in 2023, it was observed that all isolated bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Enterobacter* spp., *Streptococcus* spp., *Enterococcus faecalis/faecium*) were sensitive to all antimicrobials medicinal products. The *bla*VIM and *bla*NDM genes were detected in one *Pseudomonas aeruginosa* isolate. The test results obtained in 2024 showed the predominance of *Escherichia coli* and *Staphylococcus* spp. (100%), *Klebsiella pneumonia* (30%), *Enterobacter* spp. (20%), *Enterococcus faecalis/faecium* (10%) in mammary gland secretion samples. Eight different antimicrobial resistance genes were identified, along with the detection of carbapenem-resistant bacteria and vancomycin-resistant *Enterococcus* spp. (*Van*B gene). Based on laboratory tests conducted in 2022–2024 at a livestock farm in Sverdlovsk Oblast, measures to control antimicrobial resistance in bovine mastitis pathogens have been developed and tested.

Conclusion. Replacement of outdated treatment regimens (tetracyclines, aminoglycosides, cephalosporins of the II generation) with cephalosporins of the I/III/IV generations and fluoroquinolones temporarily reduced resistance. However, returning to the previous protocols in 2024 caused a sharp increase in multidrug resistance. Therefore, recommendations have been provided. These include continuous monitoring of pathogen resistance, strict adherence to antibiotic rotation schedules, long-term application of the revised treatment protocols, and the implementation of additional molecular genetic methods to detect bacterial resistance genes. These measures are aimed at controlling the situation at the livestock farm.

Keywords: monitoring, antibiotic resistance, antimicrobials, antimicrobial rotation, laboratory testing, cattle, disinfectants

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Антибиотикорезистентность бактериальных патогенов, циркулирующих на молочнотоварном предприятии Свердловской области

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РЕЗЮМЕ

Введение. В настоящее время возникла необходимость разработки единой стратегии рациональной антибиотикотерапии, включающей мониторинг чувствительности микроорганизмов, ротацию препаратов и использование альтернативных методов лечения, позволяющих сократить распространение антибиотикорезистентных изолятов бактерий.

Цель исследования. Определение бактериальных патогенов, вызывающих мастит у коров, с оценкой их устойчивости к антимикробным препаратам, применяемым на животноводческом предприятии, расположенном на территории Свердловской области, для последующей ротации антимикробных средств и разработки индивидуальных рекомендаций.

Материалы и методы. Исследования проведены в 2022–2024 гг. на базе сельскохозяйственного предприятия Свердловской области. Идентификацию выросших колоний производили методом MALDI-ToF масс-спектрометрии, чувствительность к антимикробным препаратам определяли диско-диффузионным методом, гены резистентности к антибиотикам выявляли с помощью полимеразной цепной реакции в режиме реального времени.

Результаты. В 2022 г. результаты исследований показали наличие в секрете молочной железы *Streptococcus* spp. (70,6%), *Escherichia coli* (52,9%), *Staphylococcus aureus* (35,3%), *Streptococcus agalactiae* (23,5%). Изоляты *Escherichia coli* и *Staphylococcus aureus* обладали резистентностью к нескольким группам антимикробных препаратов: аминогликозидам, пенициллинам, тетрациклинам и фторхинолонам (ципрофлоксацину), ванкомицину. Установили гены устойчивости: *bla*DHA, *bla*CTX-M и *bla*OXA-10 – у *Escherichia coli* (5%); *Erm*B – у группы бактерий *Streptococcus* (4%); *Mec*A – у *Staphylococcus aureus* (единично). При повторном исследовании в 2023 г. наблюдали, что все изолированные бактерии (*Staphylococcus aureus*, *Escherichia coli*, *Enterobacter* spp., *Streptococcus* spp., *Enterococcus faecalis/faecium*) были чувствительны ко всем антимикробным препаратам. У одного изолята *Pseudomonas aeruginosa* выявлены гены *bla*VIM, *bla*NDM. Результаты, полученные в 2024 г., показали преобладание в пробах секрета молочной железы *Escherichia coli* и *Staphylococcus* spp. (100%), *Klebsiella pneumonia* (30%), *Enterobacter* spp. (20%), *Enterococcus faecalis/faecium* (10%). Были выявлены 8 различных генов резистентности к антимикробным препаратам, также обнаружены карбапенем-устойчивые бактерии и ванкомицин-устойчивый *Enterococcus* spp. (ген *Van*B). На основе лабораторных исследований, проведенных в 2022–2024 гг. на животноводческом предприятии Свердловской области, разработаны и апробированы меры контроля антимикробной резистентности возбудителей мастита у коров.

Заключение. Замена устаревших схем лечения (тетрациклины, аминогликозиды, цефалоспорины II поколения) на цефалоспорины I/III/IV поколений и фторхинолоны временно снизила резистентность. Возврат к прежним схемам в 2024 г. вызвал резкий рост полирезистентности. В связи с чем даны рекомендации, включающие непрерывный мониторинг резистентности возбудителей, строгое соблюдение ротации антибиотиков, долгосрочное применение схем лечебных мероприятий, внедрение дополнительных молекулярно-генетических методов для детекции генов устойчивости бактерий в целях контроля ситуации на животноводческом предприятии.

Ключевые слова: мониторинг, антибиотикорезистентность, антимикробные препараты, ротация препаратов, лабораторная диагностика, крупный рогатый скот, дезинфицирующие средства

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INTRODUCTION

The irrational use of antimicrobials in animal husbandry has led to livestock becoming a reservoir of antibiotic-resistant bacteria. Resistant strains of microorganisms pose a threat not only to animal health, but also to human health as they can also enter the human body with products of animal origin (meat, eggs, and dairy products). There is now a pressing need to develop a unified strategy for the rational use of antimicrobials, which includes monitoring of microbial susceptibility, rotation of veterinary medicinal products and use of alternative methods that allow reducing their use. Important measures also include a transition to extensive farming systems, reducing animal stress, and maintaining high hygiene standards. Scientists worldwide emphasize the global nature of the antimicrobial resistance (AMR) problem and the importance of international cooperation in solving it [1, 2, 3, 4]. Foreign authors stress the need for coordinated global, regional, and national strategies, based on the "One World, One Health" approach, to reduce the use of antimicrobials and find alternatives [5, 6, 7]. The World Health Organization and the World Organization for Animal Health have developed lists of critically important antibiotics for human medicine and veterinary medicine in order to limit their irrational use [3].

Russian scientists have experimentally established that the repeated use of the same antibiotics in treatment and prevention protocols both in cattle and in poultry leads to the AMR development in pathogenic microflora. This reduces efficacy of veterinary medicinal products, negatively impacts productivity, and increases risks to animal health [8, 9, 10].

Experience from leading international medical researchers indicates that the periodic rotation of antibiotics can help reduce the risk of AMR development. Rotation of veterinary medicinal products can significantly increase the susceptibility of antibiotic-resistant bacterial strains. Modified treatment protocols, routinely applied in practice, can yield positive results even after several years. The authors have also conducted multi-center studies to confirm these findings and to optimize both the frequency and rotation options of antibiotics [11, 12].

Experts agree that an effective countermeasure against AMR necessitates an integrated approach, combining optimized antibiotic therapy, stringent infection control, innovative methods (such as rapid resistance diagnostics), and AMR monitoring to achieve maximum effect [13, 14].

Modern Russian publications also take into account the ecological status of the territories of the Russian Federation when developing measures to control AMR. Authors discuss enhanced monitoring of radionuclides and heavy metals in feed, as well as antibiotic resistance on farms in industrial zones, alongside the development of adaptive livestock farming technologies to reduce animal stress in polluted areas [15]. Researchers emphasize the need for widespread application of alternative methods, such as vaccination, probiotics, phytobiotics, bacteriophages, bacteriocins, rotation of antibiotics, and controlled application of these alternative methods in industrial livestock and poultry farming [1, 16, 17]. However, despite promising results from using these methods, most of them require additional research, particularly within the context of specific agricultural farms [17, 18, 19, 20].

Research aimed at identifying antibiotic resistance in bacterial pathogens is highly relevant due to the complex AMR situation in animal husbandry, which poses a serious threat to both animal and human health through the food chain. The irrational use of antimicrobials has led to the emergence and spread of resistant microbial strains, significantly reducing treatment efficacy and necessitating new approaches to managing infectious diseases in livestock. In Sverdlovsk Oblast, a region with developed livestock sectors, the AMR problem is particularly significant, underscoring the need for localized monitoring and development of tailored recommendations for specific farms.

The novelty of this study is twofold. First, it provides a comprehensive analysis of the dynamics of the microbial landscape and the resistance profiles of mastitis pathogens on the operational farm in Sverdlovsk Oblast. Second, it develops and validates a practical algorithm for rotating antimicrobials, based on regular molecular genetic monitoring, which has proven effective in a commercial herd.

This study aimed to identify the primary bacterial pathogens responsible for mastitis in cows on the farm in Sverdlovsk Oblast and assess their resistance to antimicrobials. The findings provide a basis for implementing an antimicrobial rotation strategy and delivering tailored farm-specific recommendations.

MATERIALS AND METHODS

This research was conducted as part of the Russian Ministry of Science and Higher Education's state assignment "Development of Methodological Approaches for Monitoring, Controlling, and Containing Antibiotic Resistance of Opportunistic Microorganisms in Animal Husbandry" (No. 0532-2021-0004). The work was carried out across several departments of the Ural Federal Agrarian Scientific Research Centre, Ural Branch of the Russian Academy of Sciences: the Department of Genomic Research and Animal Selection, the Laboratory of Microbiological and Molecular Genetic Research Methods, and the Laboratory of Biological Technologies within the Department of Veterinary Laboratory Diagnostics and its testing facility.

The study involved monitoring circulation of pathogenic and opportunistic microorganisms, determining their susceptibility to standard antibiotics and the antimicrobials/disinfectants in use, identifying resistance genes, and developing recommendations for rotating antimicrobials used in treatment of bovine mastitis. This was implemented and evaluated over a three-year period (2022, 2023, 2024) on the dairy farm located in Sverdlovsk Oblast.

Sampling was conducted as follows: in 2022, 10 samples of mammary-gland secretion were collected from cows with clinical mastitis; in 2023, 3 composite samples were collected from 15 cows with subclinical mastitis on the same farm; and in 2024, 16 samples were collected.

Microbiological tests were performed in accordance with the "Methodological Guidelines for the Bacteriological Examination of Milk and Udder Secretions from Cows" (No. 115-69, approved by the Main Veterinary Directorate of the USSR Ministry of Agriculture on December 30, 1983)[1].

The following nutrient media were used in this study: "Columbia Blood Agar Base" (Bio-Rad Laboratories, Inc., France), defibrinated sheep blood (EKOlab, Russia), dry nutrient medium for accumulation of *Salmonella* (magnesium medium), bismuth sulfite agar, Ploskirev's agar, GRM nutrient agar for microorganism cultivation (State Research Center for Applied Microbiology and Biotechnology, Russia), Sabouraud Dextrose agar with 2% glucose and chloramphenicol, Mueller – Hinton agar (SIFIN diagnostics GmbH, Germany), and trypticase soy broth with 20% glycerol (Condalab, Spain).

Grown colonies were identified using MALDI-ToF mass spectrometry (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) on a Vitek® MS device (bioMérieux, France). For this purpose, bacterial biomass was applied to a target slide spot, then covered by 1 µL of matrix (*α*-cyano-3-hydroxycinnamic acid), and air-dried at room temperature, and its ribosomal mass spectra were read with a special device and were compared with ones from the database using the MYLA® software (bioMérieux, France).

Antibiotic susceptibility was determined by a disk-diffusion test on Mueller – Hinton agar (Bio-Rad Laboratories, Inc., France) following European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard guidelines and disks impregnated with preparations of a specific concentration (Bio-Rad Laboratories, Inc., France). Antibiotic susceptibility patterns were read by an ADAGIO automatic analyzer (Bio-Rad Laboratories, Inc., France). Interpretation of susceptibility categories was performed following EUCAST criteria: Clinical breakpoints-bacteria (v 10.0).

The antibiotic disks used in the study included: amoxicillin / clavulanic acid, gentamicin, oxytetracycline, tigecycline, levofloxacin, norfloxacin, cefepime, cefixime, cefoperazone, cefotaxime, cefpodoxime, ceftazidime, ceftriaxone, ciprofloxacin, and ceftiofur (Bio-Rad Laboratories, Inc., France). Microbiological tests also included determining susceptibility to combined antibacterials used on the farm for treating bovine mastitis (2023–2024), which contained antibiotics from the following classes: cephalosporins, aminoglycosides, tetracyclines, and polypeptide antibiotics.

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[1] https://base.garant.ru/72125912/?ysclid=mguhhtg7xh175440448 (in Russ.)

Table 1
Antibiotic resistance and the presence of AMR genes in bacterial pathogens isolated from cow mammary gland secretions, 2022 (*n* = 10)

Bacterium species	Resistance of the isolated bacteria to the following antimicrobials	AMR genes
E. coli	Aminoglycosides, penicillins, tetracyclines	*bla*DHA, *bla*CTX-M, *bla*OXA-10 (in 5% of cases); resistance to *β*-lactams (cephalosporins and protected penicillins)
S. aureus	Fluoroquinolones (ciprofloxacin), vancomycin, tetracyclines	*Mec*A (in a single case); resistance to cephalosporins of the II generation
Streptococcus spp.	Susceptible to antimicrobials	*Erm*B (in 4% of cases); resistance to macrolides, lincosamides, streptogramines

The isolated microbial cultures were frozen at –20 °C in tubes containing trypticase soy broth with 20% glycerol as a cryoprotectant.

Real-time polymerase chain reaction was performed using the Diatom™ DNA Prep 200 kit (Laboratory Isogene, Russia) for DNA extraction from biological materials and the “COMPLEX RESISTOM ESKAPE-V” reagent kit (Lytech, Russia) – for detecting pathogen DNA and antibiotic resistance genes. Amplification was performed in real time using a QuantStudio 5 system (Thermo Fisher Scientific Inc., USA).

Based on the laboratory findings, tailored recommendations for antibacterial therapy of bovine mammary gland diseases were developed. Antibiotic selection followed established methodological guidelines [21], ensuring a scientifically grounded approach to rotation of antibiotics, and complied with Order No. 771 of the Ministry of Agriculture of the Russian Federation (November 18, 2021)[2] on restrictions governing use of antimicrobials in veterinary medicine.

The obtained data were processed using Microsoft Excel software (Microsoft Office Pro 19).

RESULTS AND DISCUSSION

In 2022, microbiological tests using MALDI-ToF mass spectrometry of the collected biological materials (10 samples of mammary gland secretion from cows) revealed the following bacterial isolates: *Streptococcus* spp. (present in 70.6% of samples), *Streptococcus agalactiae* (23.5%), *Staphylococcus aureus* (35.3%), and *Escherichia coli* (52.9%).

Data on the antibiotic resistance and the presence of AMR genes in these bacterial pathogens are presented in Table 1.

When determining antibiotic resistance by a disco diffusion test, it was found that all identified *E. coli* isolates were resistant to several groups of antimicrobials (aminoglycosides, penicillins, tetracyclines) and sensitive to cefoxitin (cephalosporin of the II generation), ciprofloxacin (fluoroquinolone of the II generation). *S. aureus*, isolated from all 10 samples, was resistant to ciprofloxacin (fluoroquinolone of the II generation), vancomycin (a glycopeptide antibiotic), tetracyclines and was sensitive to chloramphenicol, cefoxitin, and in single cases it was sensitive to tobramycin (aminoglycoside) and linezolid (oxazolidinone). Streptococci isolates were susceptible to all tested antimicrobials. Notably, isolates of *S. aureus* and *E. coli* also exhibited resistance to chlorhexidine- and iodine-based disinfectants used for pre- and post-milking udder hygiene.

Key resistance genes were detected by qPCR: *bla*DHA, *bla*CTX-M, *bla*OXA-10 genes (conferring resistance to *β*-lactams – cephalosporins and protected penicillins) were detected in 5% of *E. coli* isolate; *Erm*B gene (responsible for resistance to macrolides, lincosamides, and streptogramins) was found in 4% of *Streptococcus* spp. isolates; *Mec*A gene (regulating resistance to cephalosporins of the II generation) was identified in one *S. aureus* isolate.

Based on these findings, the following evidence-based recommendations were developed to enhance therapeutic efficacy and curb the further spread of antibiotic resistance. Priority antimicrobials for mastitis treatment were recommended: cefazolin, ceftiofur, cefquinome (representing cephalosporins of the I, III, and IV generation, respectively), and ciprofloxacin (fluoroquinolone of the II generation). Previously used multi-component medicinal products containing tetracyclines, aminoglycosides, macrolides, and cephalosporins of the II generation were recommended for removal from treatment protocols. It was recommended to use antimicrobials of penicillin group with caution. With regard to hygiene and monitoring it was recommended to implement regular disinfection control of milking equipment and conduct a semi-annual (every 6 months) AMR monitoring program of detected pathogens.

In 2023, microbiological tests performed by the disk diffusion test on 3 pooled samples of mammary gland secretion obtained from 15 cows with subclinical mastitis revealed that single *E. coli* and *S. aureus* isolated from the biological material by MALDI-ToF mass spectrometry, possessed resistance to ciprofloxacin. Other bacterial isolates (*S. aureus, Escherichia, Enterobacter, Streptococcus* spp., *Enterococcus faecalis/faecium*) were susceptible to all tested antimicrobials. It should be noted that the *E. coli* and *S. aureus* isolates exhibited susceptibility to chlorhexidine- and iodine-based agents used for udder disinfection before and after milking. Using the qPCR method, the *bla*VIM and *bla*NDM genes, responsible for resistance to carbapenems, were detected in a single *Pseudomonas aeruginosa* isolate. The other

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[2] https://fsvps.gov.ru/files/prikaz-minselhoza-rossii-ot-18-nojabrja-2021-2/?ysclid=mgqesh36jf335708795 (in Russ.)

Table 2
Antibiotic resistance and the presence of AMR genes in bacterial pathogens isolated from cow mammary gland secretions, 2024 (*n* = 16)

Bacterium species	Resistance of the isolated bacteria to the following antimicrobials	AMR genes
E. coli	Cephalosporins, carbapenems (100%)	*bla*OXA-10 (in 30% of cases), *bla*CTX-M (sporadic); resistance to cephalosporins
S. aureus	Cephalosporins, carbapenems (100%)	Not detected
Staphylococcus spp.	Cephalosporins, carbapenems (100%)	*Mec*A (in 50% of cases); resistance to *β*-lactams
K. pneumoniae	Susceptible to antimicrobials	*bla*KPC, *bla*OXA-48-like (in 50% of cases); resistance to carbapenems
Enterobacter spp.	Susceptible to antimicrobials	*bla*Ges, *bla*DHA (in 30% of cases); resistance to carbapenems, protected penicillins and cephalosporins
E. faecalis/faecium	Susceptible to antimicrobials	*Van*B (in a single case); resistance to glycopeptides (vancomycin)

bacterial isolates exhibited no genetic mutations, indicating rational use of antibacterials on the farm during the study period and the future possibility of using a broader spectrum of antimicrobials in the treatment of inflammatory diseases of the mammary gland in cows, taking into account their identification of the phenotypic antibiotic susceptibility.

Throughout 2022–2023, it was established that the detected isolates were resistant to the agents used for treatment after milking. So, the use of combinations of disinfectants with different mechanisms of action was recommended to optimize hygienic measures during milking. A product based on a polyvinylpyrrolidone-iodine complex was proposed as the disinfectant of choice for post-milking teat treatment.

Microbiological tests conducted on the same farm in 2024 showed the predominance of *E. coli* and *Staphylococcus* spp. (100% of samples) in 16 samples of mammary gland secretions collected from cows with mastitis; in contrast, *K. pneumoniae* (30%), *Enterobacter* spp. (20%) and *E. faecalis/faecium* (10%) were less frequently detected.

Data on antibiotic resistance and the presence of AMR genes in bacterial pathogens isolated from cow mammary gland secretions in 2024 are presented in Table 2.

The disk diffusion test revealed that all *E. coli*, *S. aureus*, and *Staphylococcus* spp. isolates exhibited resistance to cephalosporins and carbapenems. The *bla*OXA-10 genes, conferring resistance to cephalosporins, were detected in 30% of *E. coli* isolates by qPCR, and in single cases the *bla*CTX-M genes were detected. The *bla*KPC and *bla*OXA-48-like genes responsible for carbapenem resistance were identified in 50% of *K. pneumoniae* isolates. The *Mec*A gene, conferring *β*-lactam resistance, was confirmed in 50% of *Staphylococcus* spp. isolates. 30% of the *Enterobacter* spp. isolates harbored resistance genes (*bla*Ges, *bla*DHA) that confer resistance to carbapenems protected by penicillins and cephalosporins. *E. faecalis/faecium* carrying the *Van*B gene, associated with glycopeptide (vancomycin) resistance, were detected in single cases. Thus, microbial cultures isolated in 2024 from bovine mammary gland secretions exhibited 8 distinct AMR genes. The findings demonstrate a high prevalence of multi-drug resistance in the bacterial flora of mammary secretions, including resistance to reserve antibiotics.

All isolates detected in 2024 demonstrated susceptibility to the post-milking teat disinfectant containing polyvinylpyrrolidone-iodine complex that was recommended in 2023.

Based on the research findings, the following recommendations were provided to the farm: revision of mastitis treatment protocols with mandatory susceptibility testing of identified pathogens, enhanced biosafety measures (equipment disinfection, animal quarantine), implementation of regular antibiotic resistance monitoring. The recommendations emphasized that critically important antibiotics (cephalosporins and fluoroquinolones of the III and IV generation) should be strictly restricted to use as a last-line therapy in exceptional cases only, to preserve their efficacy.

CONCLUSION

Microbiological tests and MALDI-ToF mass spectrometry identified the following dominant bacterial pathogens in bovine mammary gland secretions: in 2022 – *Streptococcus* spp. (70.6%), *S. agalactiae* (23.5%), *S. aureus* (35.3%), and *E. coli* (52.9%) isolates; in 2023 – antimicrobial-susceptible *S. aureus*, *Escherichia coli, Enterobacter* spp., *Streptococcus* spp., *E. faecalis/faecium*, and *P. aeruginosa* isolates, and in single cases – ciprofloxacin-resistant *E. coli* and ciprofloxacin-resistant *S. aureus*; in 2024 – *E. coli* and *Staphylococcus* spp. were detected in 100% of samples, alongside newly emerging pathogens: *K. pneumoniae* (30%), *Enterobacter* spp. (20%), and *E. faecalis/faecium* (10%).

In 2022, *E. coli* exhibited resistance to aminoglycosides, penicillins, tetracyclines, with 5% of isolates carrying several resistance genes *bla*DHA, *bla*CTX-M and *bla*OXA-10 conferring resistance to cephalosporins and protected penicillins; *S. aureus* demonstrated resistance to fluoroquinolones, vancomycin, tetracyclines, and the *Mec*A gene resistant to cephalosporins of the II generation was identified in a single isolate; 4% of *Streptococcus* spp. group bacteria had the resistance gene to macrolides, lincosamides, streptogramins. In 2023, no AMR genes were detected in the tested isolates, except for one *P. aeruginosa* isolate, which carried the carbapenem resistance genes *bla*VIM and *bla*NDM. In 2024, *bla*-OXA-10 genes were identified in 30% of *E. coli* isolates, while *bla*CTX-M genes, conferring resistance to cephalosporins, were identified in a single isolate. In 50% of *K. pneumoniae* isolates *bla*KPC/OXA-48-like carbapenem resistance genes were identified, while the *Mec*A gene conferring *β*-lactam resistance was detected in *Staphylococcus* spp.; the *bla*Ges/DHA resistance genes to carbapenems, protected penicillins, and cephalosporins were detected in 30% of *Enterobacter* spp. A few single isolates of *E. faecalis/faecium* that harbored the *Van*B gene, which confers resistance to glycopeptides, were reported.

In 2022 it has been established that multicomponent veterinary medicinal products based on tetracyclines, aminoglycosides, macrolides and cephalosporins of the II generation should be excluded from the treatment protocols used in the bovine mastitis treatment. As an alternative, the use of cefazolin, ceftiofur, cefquinome (cephalosporins of the I, III, and IV generation) and ciprofloxacin (fluoroquinolone of the II generation) was recommended. The implementation of an antibiotic rotation system based on monitoring made it possible to temporarily reduce resistance levels in 2023. However, the subsequent return to previous treatment protocols in 2024 provoked a sharp increase in multi-drug resistance among bacterial mastitis pathogens. The obtained results confirm the need for continuous monitoring of antibiotic resistance, strict adherence to recommendations for the rotation of antimicrobials, and the integration of molecular genetic methods into the veterinary control system as a tool for tracking the occurrence of AMR genes in bacteria.

In 2022–2023, an increase in resistance of bacterial isolates to the disinfectants used on the dairy farm was identified. A veterinary medicinal product based on a polyvinylpyrrolidone-iodine complex was proposed as the disinfectant of choice for post-milking teat treatment. Control studies in 2024 confirmed the effectiveness of this measure: no resistance to the disinfectant was detected, justifying its continued use at the farm.

The results of the work are of practical importance for the veterinary service of the farm and can be used in the development of regional programs for AMR control in animal husbandry.

REFERENCES

1. Khoroshevskaya L. V., Khoroshevsky A. P., Slozhenkina M. I., Mosolov A. A. Problems of antibiotic resistance in the modern world. *Agrarian-and-food innovations*. 2021; 16 (4): 47–54. http://doi.org/10.31208/2618-7353-2021-16-47-54 (in Russ.)

2. Zabrovskaia A. V. Prevention of the emergence and spread of strains of microorganisms resistant to antimicrobials. *Hippology and Veterinary Medicine.* 2018; (2): 64–70. https://elibrary.ru/xtugux (in Russ.)

3. Kiseleva E. V., Tunikov G. M. Efficiency of the use of modern antimicrobial preparations for treatment of mastitis in cows in "IP Chapter K(F)X Kalenich V. V." Kolomenskoy District of Moscow Region. *Herald of Ryazan State Agrotechnological University named after P. A. Kostychev.* 2017; (4): 40–44. https://elibrary.ru/ykhlkt (in Russ.)

4. Bezborodova N. A., Kozhukhovskaya V. V., Sokolova O. V., Zaitseva O. S., Krivonogova A. S., Zubareva V. D. Genetic markers of antibiotic resistance of *Streptococcus* spp. and *Staphylococcus* spp. isolated from various biotopes of livestock production objects. *Proceedings of the Kuban State Agrarian University.* 2022; (94): 195–202. https://doi.org/10.21515/1999-1703-94-195-202 (in Russ.)

5. Kasimanickam V., Kasimanickam M., Kasimanickam R. Antibiotics use in food animal production: escalation of antimicrobial resistance: Where are we now in combating AMR? *Medical Sciences*. 2021; 9 (1):14. https://doi.org/10.3390/medsci9010014

6. Endale H., Mathewos M., Abdeta D. Potential causes of spread of antimicrobial resistance and preventive measures in One Health Perspective – A Review. *Infection and Drug Resistance*. 2023; 16: 7515–7545. https://doi.org/10.2147/IDR.S428837

7. Pinto Jimenez C. E., Keestra S., Tandon P., Cumming O., Pickering A. J., Moodley A., Chandler C. I. R. Biosecurity and water, sanitation, and hygiene (WASH) interventions in animal agricultural settings for reducing infection burden, antibiotic use, and antibiotic resistance: a One Health systematic review. *The Lancet Planetary Health*. 2023; 7 (5): e418–e434. https://doi.org/10.1016/S2542-5196(23)00049-9

8. Zubareva V. D., Sokolova O. V., Bezborodova N. A., Shkuratova I. A., Krivonogova A. S., Bytov M. V. Molecular mechanisms and genetic determinants of resistance to antibacterial drugs in microorganisms (review). *Agricultural Biology*. 2022; 57 (2): 237–256. https://doi.org/10.15389/agrobiology.2022.2.237eng

9. Ivanova O. E., Panin A. N., Karabanov S. Yu., Makarov D. A., Akhmetzyanova A. A., Gergel M. A. Veterinary monitoring of antimicrobial resistance in the Russian Federation. *Agricultural Science*. 2021; (4S): 7–11. https://doi.org/10.32634/0869-8155-2021-347-4-7-11 (in Russ.)

10. Sokolova O. V., Shkuratova I. A., Bezborodova N. A., Kozhukhovskaya V. V. Antibiotic resistance of microbiota of mamary gland and reproductive tract of cows. *Veterinariya*. 2021; (9): 10–15. https://doi.org/10.30896/0042-4846.2021.24.9.10-15 (in Russ.)

11. Gruson D., Hilbert G., Vargas F., Valentino R., Bui N., Pereyre S., et al. Strategy of antibiotic rotation: long-term effect on incidence and susceptibilities of Gram-negative bacilli responsible for ventilator-associated pneumonia. *Critical Care Medicine*. 2003; 31 (7): 1908–1914. https://doi.org/10.1097/01.CCM.0000069729.06687.DE

12. Van Duijn P. J., Bonten M. J. Antibiotic rotation strategies to reduce antimicrobial resistance in Gram-negative bacteria in European intensive care units: study protocol for a cluster-randomized crossover controlled trial. *Trials.* 2014; 15:277. https://doi.org/10.1186/1745-6215-15-277

13. Van Duijn P. J., Verbrugghe W., Jorens P. G., Spöhr F., Schedler D., Deja M., et al. The effects of antibiotic cycling and mixing on antibiotic resistance in intensive care units: a cluster-randomised crossover trial. *The Lancet Infectious Diseases*. 2018; 18 (4): 401–409. https://doi.org/10.1016/S1473-3099(18)30056-2

14. Mora-Gamboa M. P. C., Rincón-Gamboa S. M., Ardila-Leal L. D., Poutou-Piñales R. A., Pedroza-Rodríguez A. M., Quevedo-Hidalgo B. E. Impact of antibiotics as waste, physical, chemical, and enzymatical degradation: use of laccases. *Molecules*. 2022; 27 (14):4436. https://doi.org/10.3390/molecules27144436

15. Krivonogova A. S., Loginov E. A., Isaeva A. G., Bespamyatnukh E. N., Lysova Ya. Yu., Moiseeva K. V. Antibiotic resistance of opportunistic pathogenic bacteria in livestock enterprises in areas with different levels of anthropogenic pollution. *Veterinaria Kubani*. 2024; (2): 13–17. https://elibrary.ru/eondxa (in Russ.)

16. Pleshakova V. I., Leshcheva N. A., Koshkin I. N. Phenotypic and molecular genetic methods to determine antibiotic resistance of microorganisms in veterinary medicine. *Bulletin of KSAU*. 2023; (8): 106–115. https://doi.org/10.36718/1819-4036-2023-8-106-115 (in Russ.)

17. Isakova M. N., Sivkova U. V., Ryaposova M. V., Shkuratova I. A., Lysov A. V. Quality profile of milk from high producing dairy cows vaccinated against mastitis. *Veterinary Science Today*. 2020; (4): 255–260. https://doi.org/10.29326/2304-196X-2020-4-35-255-260

18. Zubareva V. D., Sokolova O. V., Bytov M. V., Krivonogova A. S., Volskaya S. V. Alternative treatment methods for bovine mastitis: prospects and limitations (review). *Veterinary Science Today*. 2024; 13 (3): 203–213. https://doi.org/10.29326/2304-196X-2024-13-3-203-213 (in Russ.)

19. Krivonogova A. S., Donnik I. M., Isaeva A. G., Loginov E. A., Petropavlovskiy M. V., Bespamyatnykh E. N. Antibiotic resistance of *Enterobacteriaceae* in microbiomes associated with poultry farming. *Food Processing: Techniques and Technology*. 2023; 53 (4): 710–717. https://doi.org/10.21603/2074-9414-2023-4-2472 (in Russ.)

20. 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

21. Sokolova O. V., Shkuratova I. A., Bezborodova N. A., Zubareva V. D., Pechura E. V., Shilova E. N., et al. Rational antibiotic therapy of inflammatory diseases of the reproductive system and mammary gland in cows on livestock farms in Sverdlovsk Oblast: methodological recommendations. Ekaterinburg: Ural Federal Agrarian Scientific Research Center, Ural Branch of the Russian Academy of Sciences; 2022; 38 p. https://elibrary.ru/fssmyf (in Russ.)

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