

Distribution features of resistant strains of *Staphylococcus aureus* on dairy farms

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Abstract—Research of opportunistic microbiocenosis in livestock enterprises of the Ural region was conducted. Dairy farms located in areas with different levels of technogenic contamination of agrobiocenoses with metals Cu, Cd, Fe, Zn, Hg, As, Pb and radionuclides ⁹⁰Sr, ²³⁷Cs, ²¹⁰Pb were studied. The proportion of *S. aureus* strains in the opportunistic microbiocenosis of different farm departments was analyzed, and the selected strains were also tested for sensitivity to the antibiotics most frequently used in the industry. It was found that on all the studied farms predominate *S. aureus* strains with reduced sensitivity to antibiotics, the most common in animal husbandry practice. It was discovered that the proportion of strains of *S. aureus* resistant to rifampicin, cefazolin and tetracycline is increasing in areas with discernible technogenic contamination of agrobiocenosis. The share of multi-resistant strains of *Staphylococcus aureus*, resistant to 3 or more classes of antibiotics, was also maximum in ecologically unfavorable areas.

Keywords— dairy farming, opportunistic microflora, *Staphylococcus aureus*, resistance to antibiotics.

I. INTRODUCTION

The spread of microbial resistance to antibiotics is currently one of the dominant problems in medicine, veterinary medicine, animal husbandry and ecology [1, 2]. In conditions of deteriorating environmental conditions, permanent pollution of farmland with industrial pollutants – the natural immune resistance of organism is decreasing [3, 4]. These factors lead to an increase in the infectious diseases incidence in animals, an increase in the frequency of purulent septic pathologies of various organs and systems. Therefore, the use of antibiotics is currently necessary to control the livestock morbidity, as antibiotic therapy today is the most accessible and effective way to combat pathogenic and opportunistic bacteria. In such conditions, there is a rapid adaptation of strains to the antibiotic drugs, with such typical representatives of opportunistic bacteria as *S. aureus*, *P. aeruginosa*, *E. coli* that are adapting actively [1, 5, 6]. Most antibiotics are used both in veterinary and in medical practice for the treatment of infectious, surgical and purulent septic pathology in humans [7,8]. In this connection, the identification of strains of pathogenic and opportunistic bacteria, including *S. aureus*, resistant to these antibiotics, and the study of the mechanisms of their formation in animal husbandry is important not only for animal, but also for human health.

II. OBJECTIVE

The objective was to study the proportion of *S. aureus* in the opportunistic microbiocenosis of dairy farms, located in areas with different levels of technogenic contamination of agrobiocenoses, as well as an analysis of the sensitivity of the isolated strains to the most frequently used antibiotics in this industry.

III. METHODS

The genus and specie composition of the microbiota on dairy farms located in the Ural region in areas with varying degrees of anthropogenic pollution and contamination of agrobiocenoses were investigated. Zone 1 had the lowest, comparable to background, indicators of the content of heavy metals Cu, Cd, Fe, Zn, Hg, As, Pb, ⁹⁰Sr, ²³⁷Cs, ²¹⁰Pb in soil, fodder plants, feed and water. Zone 2 was characterized mainly by contamination of agrobiocenosis with metal pollutants Cu, Cd, Fe, Zn, Pb. Zone 3 is located in the least wealthy region, in the northern part of the East Ural Radioactive Trace, and is characterized by the highest level of contamination of agrobiocenosis with Cd, Fe, Zn, Hg, Cu, As and radionuclides Pb, ⁹⁰Sr, ²³⁷Cs, ²¹⁰Pb. A total of 15 of dairy farms were examined. Sampling was performed on each farm in the maternity ward, calf barn and dairy herd. We collected samples from mucous membranes, wool cover, from udder in cows and calves, from equipment (feeders, drinkers), fences, surfaces, livestock equipment (buckets, funnels, teats, tools, cleaning rags); we took samples of litter, manure, drinking water, feed, air. Then, the selected samples were analyzed by the standard microbiological methods, including seeding on culture media, cultivation, isolation of a clean line, identification of microorganisms, determination of their sensitivity to antibiotics by the disk diffusion test (minimum inhibitory concentration) and serial dilution method [9]. We studied the sensitivity of *Staphylococcus aureus* to the most frequently used classes of antibiotics: rifampicin (ansamycin), meropenem (carbapenem, beta-lactam), ampicillin, amoxicillin (semisynthetic penicillin), cefazolin (cephalosporin of the I generation), enrofloxacin (fluoroquinolones), azithromycin (macrolide and azalide), tetracycline (tetracycline) and vancomycin (glycopeptide). In the presence of susceptibility of *Staphylococcus aureus* strains to antibiotics, the cases of dose-dependent action, the native moderate and weak sensitivity of the microorganism to the antibiotic are not taken into account. An analysis of the blood of animals for an immunological analysis is also carried out at all surveyed enterprises. We also studied data

on the livestock morbidity; collected control samples of feed and water, analyzed the content of metallic pollutants of natural and technogenic origin in order to assess the degree of technogenic pollution of the environment.

The plan, research methods and data processing algorithms are approved by the ethical commission and the metrology and standardization council.

IV. RESULTS AND DISCUSSION

Microbiological analysis of samples taken from different dairy farms in the Sverdlovsk region showed that the genus and species composition of the microbiome is relatively the same, but the percentage ratio of microorganisms varies depending on the company and the particular department. Typical representatives of pathogenic and opportunistic microorganisms, which most often detected in samples taken were: *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus durans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Proteus vulgaris*, *Proteus*

mirabilis, *Echerichia coli*, *Bacillus subtilis*, *Enterobacter* spp., *Citrobacter farmeri*, *Klebsiella* spp., Also found significant amounts of *Candida albicans*, *Aspergillus* spp., *Mucor* spp., *Penicillium* spp., *Fusarium* spp.

On farms that are located in Zone 1, *Enterococcus faecium* was found (in 100% of samples), as well as *Staphylococcus aureus* (in 75% of samples). The fungal flora was represented by *Aspergillus* spp. and *Candida albicans* (more than 75% and 70%, respectively). In general, we established the following ratio of isolated strains of microorganisms in a descending order: *Enterococcus faecium*, *Staphylococcus aureus*, *Aspergillus* spp., *Candida albicans*, *Pseudomonas aeruginosa*, *Enterobacter non-pathogenic*, *Echerichia coli*, *Proteus* spp., *Staphylococcus saprothryphros*, a pathogenic, *Echerichia coli*, *Proteus* spp., *Staphylococcus saprothryphros*, a pathogenic, *Echerichia coli*, *Proteus* spp., *Staphylococcus saprothrophosis*, pathogenic, pathogenic, *Echerichia coli*, *Proteus* spp., *Staphylococcus saprophros*, a pathogenic, non-pathogenic, pathogenic, *Echerichia coli*, *Proteus* spp. (Fig. 1).

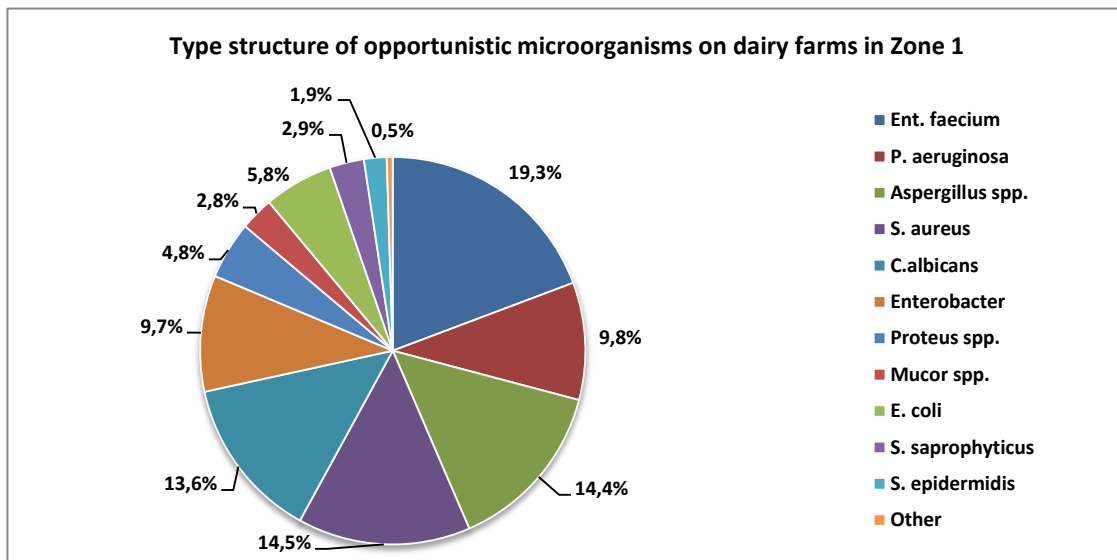


Fig. 1. Type structure of opportunistic microbiocenosis of dairy farms that are located in Zone 1 (2018)

The largest number of strains of *Staphylococcus aureus* on farms in Zone #3 was detected in samples of milk for calves, samples taken from the mucous membranes of mouth and nose of calves, as well as samples taken from surfaces and equipment of maternity wards, calf barns and dairy herd sections. At the same time, strains of *S. aureus*, found in maternity wards and calf barns in half of the cases had the same antibiotic sensitivity profile. This fact suggests that the contamination of a newborn calf with strains of opportunistic microbiome occurs when it comes into contact with the litter, equipment and surfaces in the maternity ward. When there is a circulation of resistant strains in the maternity wards, the risk of their colonization is high.

The group of livestock enterprises in Zone 2 included several large and small dairy farms interconnected by economic, technological and logistical ties. The following microbiological image was observed on these objects: dominant strains of *Ent. faecium* (more than 85% of positive samples), *S. aureus* (in 85% of samples), *P. aeruginosa* (in 70% of samples), *Ent. faecalis* (more than 65% of samples), *Enterobacter* spp. (about 70% of samples). Typical structure of opportunistic microbiocenosis in descending order: *Ent. faecium*, *S. aureus*, *P.aeruginosa*, *Ent. faecalis*, *Enterobacter*, *C. albicans*, *Aspergillus* spp., *Mucor* spp., *S. epidermidis*, *Proteus* spp., *B. subtilis*, and other microorganisms (Fig. 2).

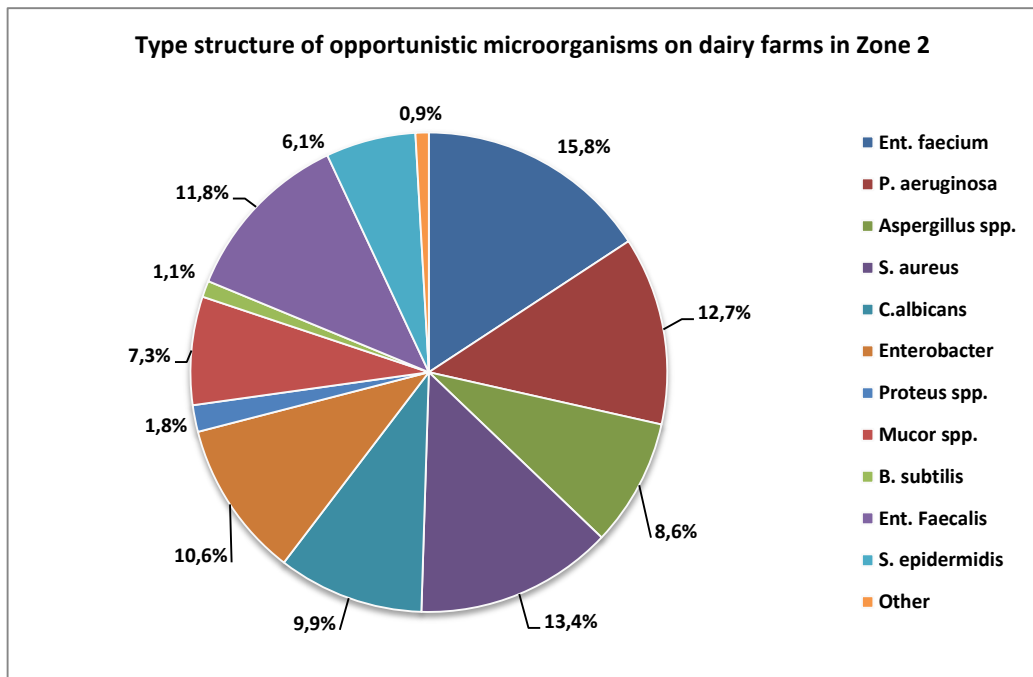


Fig. 2. Type structure of opportunistic microbiocenosis of dairy farms that are located in Zone 2 (2018)

Livestock agglomeration of the dairy branch, located in Zone 3, includes a number of enterprises with a total livestock of about 18.000 of cattle. This agrarian zone, along with the northern one, is characterized by an unfavorable ecological situation associated with discernible contamination of agrobiocenosis with heavy metals, radionuclides and other xenobiotics of anthropogenic origin. The structure of the microbiota in the surveyed enterprises of this zone varied depending on the specific farm, however, in general, it was observed that more strains of different microorganisms were present in the samples than in similar samples from Zone 1 and 2. Samples included: Ent. faecium (in 79% of the total number of samples), P.aeruginosa (63%),

S. aureus (15.4%), Proteus spp. (more than 42% of samples), Ent. faecalis (41% of samples), Enterobacter (39%). Also, strains of fungal microbiome were often encountered: in each examined department of a dairy farm, an average of 55-57 samples of C. albicans were sown in 18 cases, Aspergillus spp. in 21, Mucor spp. in 21, Penicillium spp. in 9, and Fusarium in 8 cases, indicating a high contamination of objects with mold fungi and yeast spores. The typical structure of the opportunistic microbiocenosis of a dairy farm in this zone is shown in Fig. 3.

Attention is drawn to the fact that strains of Staphylococcus aureus prevailed in maternity wards, while the share was relatively small at dairy herd section.

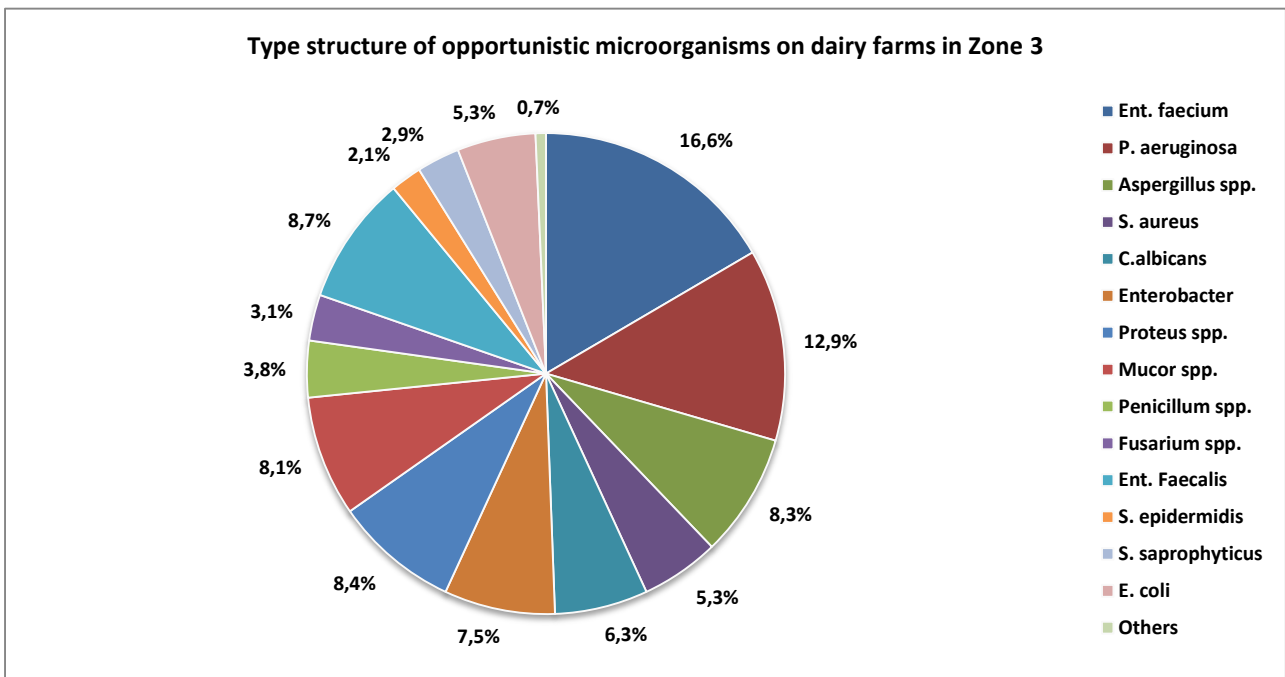


Fig. 3. Type structure of opportunistic microbiocenosis of dairy farms that are located in Zone 3 (2018)

The study of antibiotic susceptibility of microorganism strains selected on dairy farms in Zone 1 showed a high level of spread of resistant strains of *Staphylococcus aureus*. 35.7% of the isolated strains were insensitive to ampicillin, 28.5% to amoxicillin, which indicated that the microorganism had acquired resistance to semi-synthetic penicillin. More than 7% of the strains were resistant to meropenem and other drugs from the group of capapenem, presumably due to the production of beta-lactamase. 15% of the strains were resistant to tetracycline, 21% to azithromycin, 6.5% to vancomycin, cefazolin, 7.1% to enrofloxacin, 6.8% to rifampicin. Multi-resistant strains (full resistance to antibiotics of three or more classes) of *S. aureus* accounted for 11% of all isolated and were resistant to semi-synthetic penicillin, tetracycline, cephalosporin of I generation, fluoroquinolones, macrolides and capbapenems. A significant number of multi-resistant strains of staphylococci were isolated from samples taken from the equipment for calf care, from milk for calves, litter in the maternity wards. In samples taken from the oral mucosa in calves on these farms, strains of *Staphylococcus*, *Pseudomonas bacillus*, *Escherichia coli* that are resistant to one antibiotic, were detected in more than 25% of cases.

As a result of microbiological studies of dairy farms from Zone 2, mono- and multi-resistant strains of *S. aureus* were detected as well. In 22% of cases, strains were resistant to combinations of two antibiotics of different classes (most often semi-synthetic penicillin + macrolide, tetracycline + macrolide, tetracycline + glycopeptide). Multi-resistance was detected in 4.8% of isolated strains of *Staphylococcus aureus*. The cases of multiple resistance of *S. aureus* were found in the samples of litter, samples taken from the equipment of the maternity wards, rags for cleaning milking cows, vaginal mucosa and from manure samples from pits on various dairy farms. At the same time, the strains were resistant to semi-synthetic penicillin, carbapenem, fluoroquinolones, tetracycline.

In samples taken at enterprises in Zone 3, the largest number of mono-resistant strains was found in *Ent. faecium* (more than 50% of all isolated strains), multi-resistant – *S. aureus* (28% of isolated strains had resistance to antibiotics of 3 and more classes). *Staphylococcus aureus* was most often resistant to semi-synthetic penicillin, cephalosporin, macrolides, glycopeptides, ansamycin.

The highest number of rifampicin-resistant strains was found in Zone 3 (Fig. 4).

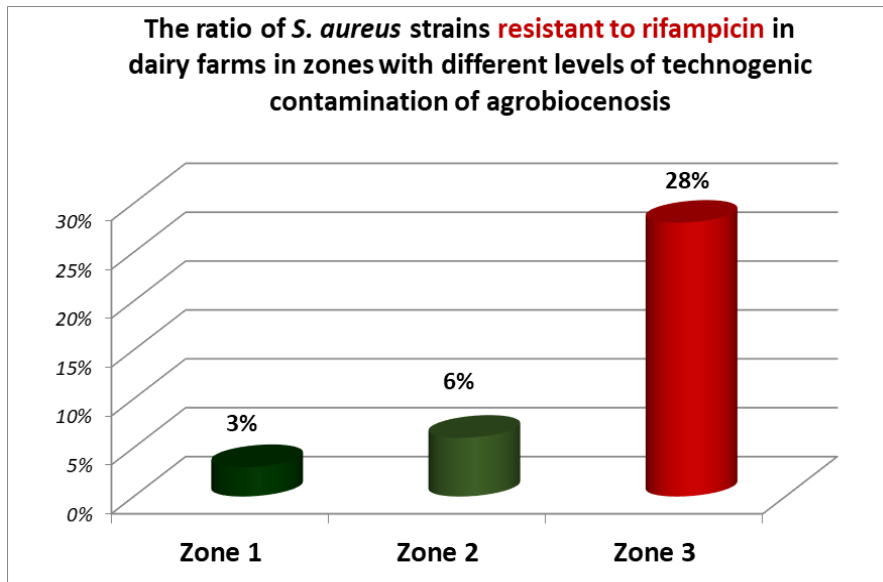


Fig. 4. The ratio of *S. aureus* strains resistant to rifampicin from all isolated strains on farms in zones with different levels of technogenic contamination of agrobiocenosis (2018)

Zonal dynamics were also detected in the number of tetracycline, cefazolin and ampicillin resistant strains of *Staphylococcus aureus* (Fig. 5). In Zone 3, on farms with the highest level of technogenic contamination of

agrobiocenoses and fodder with heavy metals and radionuclides, the highest level of *S. aureus* resistance to these antibiotics was noted.

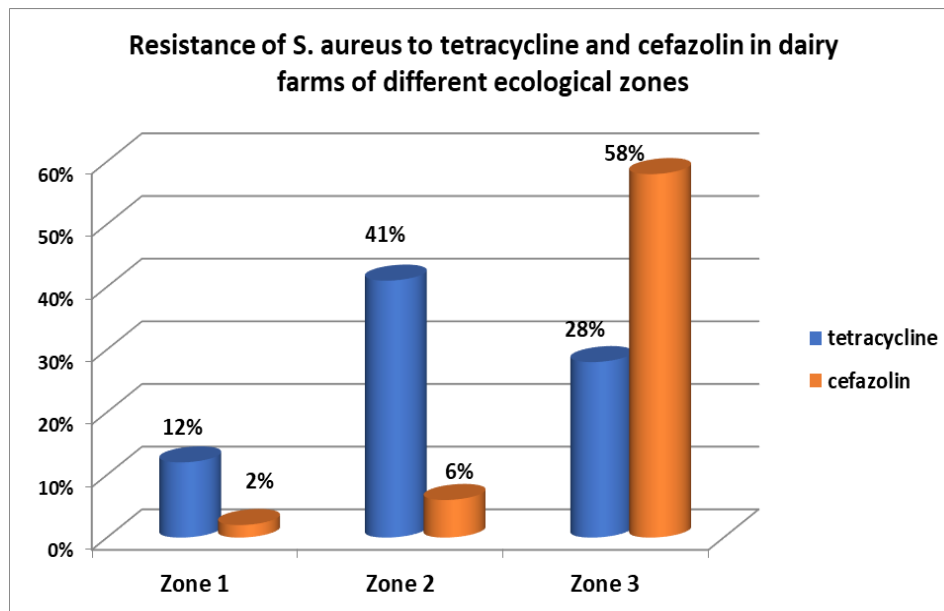


Fig. 5. The ratio of *S. aureus* strains resistant to tetracycline and cefazolin from all isolated strains on farms in zones with different levels of technogenic contamination of agrobiocenosis (2018)

In total, antibiotic classes revealed resistance to semi-synthetic penicillin in 21% of strains, to carbapenem – in 6.4%, to tetracycline – in 11.5%, to cephalosporin of I generation – in 3.8%, to fluoroquinolones – in 2.5%, to macrolides – in 9.6%, to glycopeptides in 8.3%, to ansamycin – in 1.9% of strains of microorganisms found in samples throughout the entire surveyed region (all three zones).

In addition, we analyzed the dynamics of changes in the sensitivity of individual strains of *S. aureus* to antibiotics, conducted a comparative analysis of indicators of microbial resistance in previous years for specific objects (maternity wards of 4 dairy farms. Analysis and synthesis of the obtained data led to the conclusion about the acquired resistance of strains *S. aureus*, *S. saprophyticus*, *S. epidermidis*. Polyresistant strains of *S. aureus* were subjected to additional analysis to determine the likely resistance mechanisms (tests with oxacillin, inhibitors of beta-lactamase). In almost 65% of the test cases, resistance was detected. This fact indicated that *S. aureus* has classical resistance to methicillin (oxacillin) because of PSB-2a penicillin-binding protein [10, 11]. In 35% of the tests, *Staphylococcus* was sensitive to oxacillin, which indicated the presence of a plasmid beta-lactamase of class A in the microorganism and another mechanism of resistance – mass production of beta-lactamase, which was confirmed by positive tests with beta-lactamase inhibitors. The mono-resistance of *Staphylococcus* spp. to macrolides, most likely, was caused by the presence of enzyme inactivation mechanisms in the strains (production of macrolide phosphotransferase, etc.) or active elimination [6,11]. In general, the obtained results indicate that the isolated strains have an acquired resistance of a plasmid or chromosomal character, which is realized through various mechanisms or their combinations.

Analysis of the results of cattle blood tests from Zone 1 showed that, in general, the immune-hematological status of the examined animals corresponds to physiological parameters. However, all animals showed an increase in the functional activity of cells of the monocyte-macrophage link

by 6-7%, the level of circulating immune complexes (CIC) on average exceeded the physiological parameters by 15-16% (88.5-116.5 units). The leukocyte – T lymphocyte ratio index (LTI) and immunoregulatory index (IRI) characterize the morpho-functional usefulness of a specific cellular unit. In the examined animals, they corresponded to the normoergic state of the immune system. When analyzing the results of studies of biological samples from cattle from Zone 2 and 3, it was found that in 82% of cases there was an increase in the relative number of eosinophils by 1.8-2.0 times. We noted a decrease in the absorptive capacity of cells of the monocyte-macrophage link by 1.5-2 times and an increase in the level of the CIC by an average of 25-30%. Changes in the LTI and IRI in the examined cattle population were not statistically significant ($p > 0.05$). The obtained results indirectly testified to the violation of mucosal resistance and the reduction of mucosal immunity in animals.

To assess the impact of technogenic contamination of agrocenoses on the structure of the microbiota and the distribution of microbial resistance, we conducted a study of content of heavy metals in feed. A study of cattle feed samples showed similar trends, and it was noted that the feed from different areas differs in the concentration of cadmium, lead, iron and radionuclides – radiogenic strontium and cesium. The highest level of activity of the feed for ^{90}Sr was detected on dairy farms located in the territory of the East Ural Radioactive Trace in Zone 3. The average content of radiogenic strontium was 8.50 ± 0.21 Bq/kg, and the peak (at one of the enterprises) was 13.60 ± 0.24 Bq/kg. In Zone 1, the radiogenic strontium activity averaged 2.52 ± 0.06 Bq/kg. Thus, the difference in the degree of technogenic contamination of vegetable feed in agrobiocenoses was confirmed. Chronic toxic load caused by contamination of feed with heavy metals and other xenobiotics of anthropogenic origin leads to impaired metabolism and weakened immune resistance of animals [3, 12]. This, in turn, leads to an increase in the incidence of purulent septic, bacterial and viral diseases, including those that are caused by opportunistic microorganisms [13, 14]. The use of antibiotics for the treatment of livestock is often based on the cost of the medication, whereas a preliminary analysis of the

pathogen susceptibility to the antibiotic is not carried out; the standard recommendations for the duration of antibiotic treatment are not being followed. These results are supported by the fact that specific enterprises showed patterns of resistance of strains with prevalence of resistance to 1-2 antibiotics (most often to tetracycline, either than to enrofloxacin or azithromycin), which were most often used in veterinary practice at these enterprises. The prolonged (several years) use of the same antibiotic led to the formation of a significant amount of resistant microbiome. In addition, feed supplements containing antibiotics contribute to this issue.

V. CONCLUSION

Studies have shown that the spread of antibiotic-resistant strains of *Staphylococcus aureus* in livestock enterprises of the dairy profile occurs under the influence of many reasons. Not only internal microbiome factors associated with the presence of chromosomal or plasmid resistance genes contribute to this, but also exogenous factors such as feeding technology, feed quality and the level of anthropogenic contamination of agrobiocenosis of an enterprise. Chronic alimentary intoxication of cows, associated with a high content of such anthropogenic pollutants as metals Cu, Cd, Fe, Zn, Pb, 90Sr, 237Cs, 210Pb in the feed inevitably leads to inhibition of the immune forces of the body, and, consequently, an increase in the morbidity with infectious diseases. This leads to the need for the use of antibiotics for preventing and therapeutic purposes. Also one of the key links in the distribution of antibiotic-resistant strains of *S. aureus* is the contamination of newborn calves in the maternity wards. This fact increases the risk of the inclusion of resistant strains in the animals' organism's microbiocenosis, which may adversely affect its health and the effectiveness of the disease treatment.

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