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Antimicrobial susceptibility of mastitis pathogens of dairy cows in Ukraine

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ABSTRACT
Mastitis is one of the most common diseases on dairy farms. It causes significant economic damage associated with the cost of treating sick cows, reduced milk yield and quality indicators of dairy products, and the risk of premature culling of animals. Treatment of cows with mastitis on dairy farms is carried out mainly with antimicrobial drugs, which are usually used without a preliminary test to identify the causative agent of the disease and determine its sensitivity to antimicrobial substances, which is an important part of the effectiveness of therapy. Increasing the resistance of bacteria to antimicrobial substances poses a threat not only to the animal but also to humans, as a consumer of dairy products. The availability of data on the sensitivity of mastitis pathogens to antimicrobial drugs makes it possible for veterinary doctors to choose the most effective antibiotic for treating animals with the shortest duration of treatment. The presented results of studies of breast secret samples taken from cows indicate that in 57.5% of cases, contagious pathogens of mastitis were identified. In particular, *Streptococcus agalactiae* made 24.1%, *Staphylococcus aureus* – 18.4%, *Corynebacterium* spp. – 7.2%, *Streptococcus dysgalactiae*– 5.6%, *Streptococcus uberis* – 2.2%. Environmental pathogens accounted for 42.5% of the total number of isolated isolates, among which *Streptococci* represented gram-positive microflora at 11.5 *Streptococcuscus* spp. (6.2% *Streptococcuscus parauberis* (4.4% *Streptococcuscus Bovis* (0.9%) and *Staphylococcus* spp. – 10.3%. Gram-negative microflora is 20.6%, among which the largest percentage belongs to *E. coli* – 8.4% and *Klebsiella pneumonia* – 1.9%. Mastitis caused by yeast accounted for 1.4% of all diagnosed pathogens. Antimicrobial sensitivity was evaluated using the disk diffusion method (Kirby-Bauer). According to the results of determining the sensitivity of mastitis pathogens to antimicrobial substances, it was found that the highest sensitivity of the isolated isolates was to Ceftiofur, Amoxicillin/clavulanic acid, Rifampicin, Amoxicillin, Gentamicin, Ampicillin, Bacitracin, Cephalexin, Cloxacillin, Enrofloxacin, Trimethoprim/sulfamethoxazole, Oxytetracycline, Lincomycin. The least sensitive – to Spiramycin, Tylosin, streptomycin, neomycin, Marbofloxacin, Tilmicosin, and Danofloxacin.

Keywords: mastitis, antimicrobial substances, contagious, environmental, the causative agent of mastitis

INTRODUCTION
Farm owners and producers of dairy products suffer significant economic losses due to various infectious and non-infectious diseases, among which one of the main one is inflammation of the mammary gland. Mastitis, by its nature, is a complex, reasonably common, and expensive disease of cows on dairy farms **[1]**. Economic losses are associated with treatment costs, reduced milk production, and the quality of milk obtained, as well as the risks of premature culling of highly productive animals **[3]**, **[5]**, **[6]**. According to data **[3]**, the total cost of expenses caused by bovine mastitis is estimated at an average of USD 147 per cow per year. Bovine mastitis therapy is the most common reason for using antimicrobials on dairy farms **[7]**, **[11]**. In addition, it is known that broad-spectrum antimicrobials affect the development of resistance to a greater extent than narrow-spectrum antimicrobials **[20]**, **[47]**. Antimicrobial drugs for the treatment of animals with mastitis have been used for about sixty years and are often prescribed without a preliminary test to identify the pathogen and determine its sensitivity, which is a fairly important part of therapy **[2]**. Pathogens of mastitis are divided into two groups, the so-called contagious and environmental. Contagious pathogens are transmitted mainly from one cow to another, especially through milking equipment. In contrast, environmental pathogens enter the mammary gland from the external environment (through bedding, flies, or even cow skin) **[8]**, **[13]**. Contagious pathogens

include such types as *Staphylococcus aureus* and *Streptococcus agalactiae* and less common ones – such as *Mycoplasma bovis* and *Corynebacterium*, which are localized on the udder and skin. Environmental pathogens such as *Escherichia coli* or *Streptococcus uberis* penetrate and reproduce in the udder of cows, induce an immune response, and are rapidly eliminated **[9]**. Monitoring the resistance of mastitis pathogens to antimicrobials over time becomes extremely important to ensure the long-term effectiveness of antibacterial drugs. Access to antimicrobial sensitivity data helps veterinary doctors choose the most effective drug for treating animals with mastitis, especially given that therapy for this pathology usually begins before testing the sensitivity of the pathogen **[10]**, **[12]**. Increasing the resistance of bacteria to antimicrobial substances poses a threat to both animals and humans, as consumers of dairy products. Therefore, the World Organization for Animal Health (WOAH) recommends monitoring the resistance of pathogens and commensal bacteria if necessary. Such monitoring provides significant information for therapeutic measures and, at the same time, shows trends in the development of bacterial resistance, which can be taken into account when using individual antimicrobial drugs in practice **[11]**, **[14]**, **[15]**.

This study aimed to identify pathogens of excretion from samples of cow mammary glands secretions and determine the sensitivity of the main pathogens of mastitis to commonly used antimicrobial substances.

Scientific Hypothesis

We expect that isolated isolates of pathogens from the secretion of cows with mastitis will show different sensitivity to a wide range of antimicrobial substances, which will make it possible to isolate those with the highest antibacterial activity and recommend them for animal therapy. Testing the secretion of cows suffering from mastitis for antimicrobial substances is an effective tool in increasing the indicators of obtaining high-quality and safe dairy products.

MATERIAL AND METHODOLOGY

Samples

Samples of cow mammary glands secretions were submitted for research to the laboratory of bacteriology and path anatomy of LLC "Center for veterinary diagnostics" from different regions of Ukraine in sterile test tubes.

Chemicals

Blood agar (Oxoid, UK), MacConkey Agar (Oxoid, UK), Muller-Hinton Agar (Oxoid, UK), Condalab antimicrobial discs (Spain), Erba lachema indole test (Czech Republic), oxidase test HiMedia Laboratories (India), catalase test of Technopharm LLC (Ukraine), Química Clínica Aplicada S. A. Gram dye. (Spain).

Animals and Biological Material

The animals were of different breeds (Holstein, Ukrainian black, and piebald), age, and had different lactation duration and productivity. There was no information about the size of livestock, diet, maintenance, watering, milking system, or milk supply. The secret of the udder was taken from cows with mastitis.

Instruments

Petri dishes, microbiological loop.

Laboratory Methods

Udder secretion samples were examined microbiologically using standard laboratory methods **[16]**. Mammary gland secretions (approximately 0.1 mL) were applied in a loop to the surface of blood agar (agar-based medium enriched with 5% sterile sheep's blood) (Biocorp, Poland). Bacterial dishes were incubated at 37 °C for 24 – 48 hours under aerobic conditions. After that, the morphology of the colony was evaluated and described. Samples that produced more than three types of microorganisms were identified as contaminated. Individual bacterial colonies were subcultivation to produce pure isolates by repeated bacteriologic culture technique. Pure isolates were identified using phenotyping tests, including Química Clínica Aplicada S. A. (Spain) gram staining, HiMedia Laboratories oxidase test (India), indole tester Lachema (Czech Republic), and Technopharm LLC (Ukraine) catalase. Bacterial species were identified based on biochemical profiles using the API 20E BioMerieux system (France) and Streptotest 16 erga Lachema (Czech Republic). Gram-negative bacteria were identified based on growth on MacConkey Agar (Oxoid, UK), indole, and oxidase tests. Blood agar (Oxoid, UK) was used to cultivate yeast and mold. Determination of the sensitivity of isolated isolates to antimicrobial substances was performed using the Kirby Bauer Disk Diffusion method **[17]**, **[18]**, **[19]**, **[21]** in vitro on Muller-Hinton Agar (Oxoid, UK), using commercial Condalab disks (Spain).

Description of the Experiment

Sample preparation: According to the bacteriological study of 346 samples of udder secretions selected from cows with clinical and subclinical forms of mastitis, 264 samples were found to be positive. 21 samples with a negative result- no growth of microorganisms. Contamination was found in 61 samples of udder secretions (Figure 1).

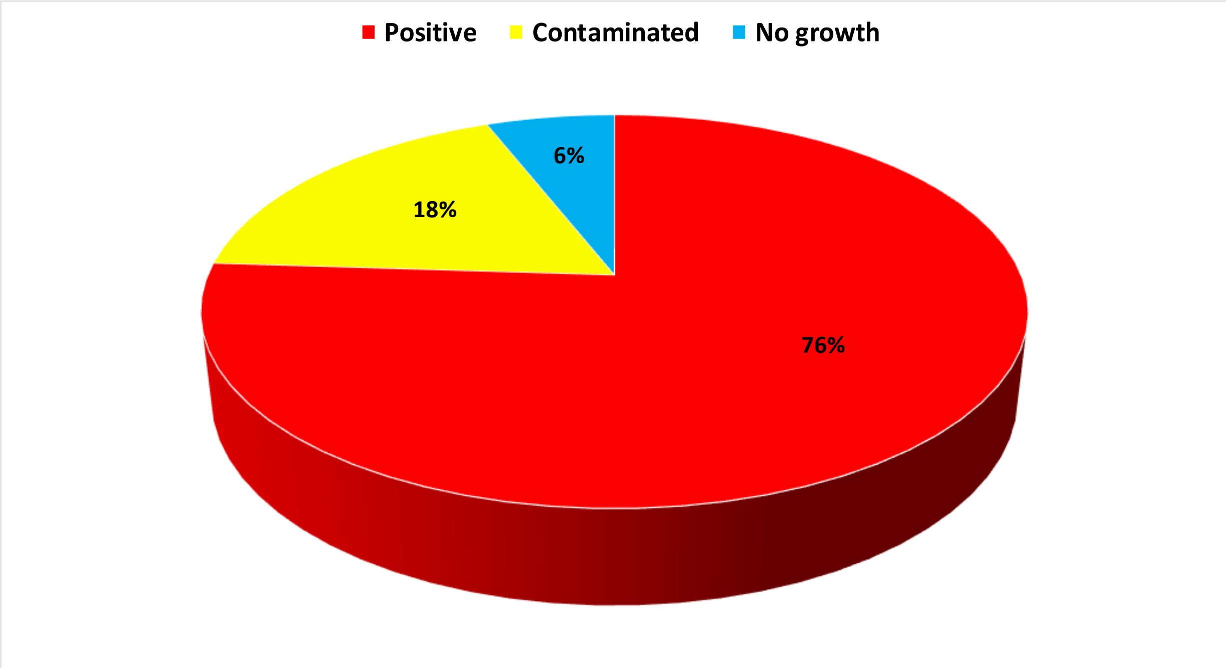

Figure 1 Results of the study of individual samples of udder secretions.

Number of samples analyzed: 320 samples were analyzed.

Number of repeated analyses: Each study was carried out five times, with the number of samples being four, which amounted to twenty repeated analyses.

Number of experiment replication: The number of repetitions of each experiment to determine one value was 5 times.

Design of the experiment: The study was conducted on 3 dairy farms, in separate research units of the National University of Life and Environmental Sciences of Ukraine, "Velikosnityn educational and research farm named after O. V. Muzychenka", "Agronomic Research Station", "Educational and Research Farm "Vorzel" of Kyiv Region, Ukraine.

All research in research farms was conducted by a group of researchers consisting of 5 people in the period from July 2021 to October 2022. Management practices, housing conditions and milking procedures were assessed and documented in a standardized data collection form. Milking patterns were recorded by observing regular milking during one milking period. Observations during the visit were recorded during the keeping of cows with mastitis.

After conducting a clinical examination of the udder of cows and a laboratory study of its secretion, using the California mastitis test, samples of secretion from animals with mastitis were collected in sterile test tubes.

Then the samples were cooled to a temperature of +2 to +4 °C and immediately transported to the laboratory. Selected samples of udder secretions were subjected to bacteriological examination, followed by testing of selected isolates for antimicrobial substances.

Statistical Analysis

Simple descriptive statistics were used. The results of bacteriological cultures were expressed as a percentage of individual microbial species isolated. Sensitivity results were expressed as a percentage – as the percentage of sensitive isolates to each type of antimicrobial substance.

RESULTS AND DISCUSSION

Studies conducted in Slovakia showed that 21 yeast strains and 500 bacterial strains of 25 types were isolated from 633 samples of mammary gland secretions. The most common pathogens were coagulase-negative staphylococci, which made up 35.9% of positive results; the second most common was *E. coli* – 14.8%, followed by *S. aureus* (12.5%), *Str. uberis* (10.9%) and *Streptococcus agalactiae* (5.8%). We found that contagious pathogens of mastitis in cows accounted for 184 (57.5%) of isolated isolates: *Streptococcus agalactiae* – in 77 (24.1%), *Staphylococcus aureus* – in 59 (18.4%), *Corynebacterium* spp. – in 23 (7.2%), *Streptococcus dysgalactiae* – in 18 (5.6%), *Streptococcus uberis* – in 7 (2.2%) isolates, and environmental (non-infectious) mastitis pathogens – in 136 (42.5%) isolates. Most of the bacteria belonged to Gram-positive microflora, in particular to staphylococci in 33 samples (*Staphylococcus* spp. – 10.3%) and streptococci in 37

(11.5%) samples (*Streptococcus spp.* – 20 (6.2%), *Streptoccocus parauberis* – 14 (4.4%) samples, *Streptoccocus bovis* – 3 (0.9%) isolates. Gram-negative bacteria accounted for 66 (20.6%) isolates, among which the largest percentage was accounted for by *E. coli* 27 (8.4%) samples and *Klebsiella pneumonia* 6 (1.9%) samples.

The results of the bacteriological study of individual samples of udder secretions (from the affected udder lobes) showed (Table 1, Figure 2), which was most often isolated from the studied samples *Streptococcus agalactiae* (Figure 3 and 4), *Staphylococcus aureus* (Figure 5 and 6), *Staphylococcus spp.* (Figure 7 and 8) and *E. coli* (Figure 9 and 11).

Table 1 Isolated microflora from samples of cow udder secretions for mastitis.

It. no.	Microflora	RESULT	
		Total	Total
1	*Streptococcus agalactiae*	77	24.1
2	*Staphylococcus aureus*	59	18.4
3	*Staphylococcus* spp.	33	10.3
4	*E. coli*	27	8.4
5	*Corynebacterium* spp.	23	7.2
6	*Streptococcus* spp.	20	6.2
7	*Streptococcus dysgalactiae*	18	5.6
8	*Streptococcus parauberis*	14	4.4
9	*Trueperella pyogenes*	10	3.1
10	*Bacillus* spp.	10	3.1
11	*Streptococcus uberis*	7	2.2
12	*Klebsiella pneumoniae*	6	1.9
13	*Yeast*	5	1.6
14	*Enterobacteriaceae*	4	1.3
15	*Klebsiella terrigenous*	4	1.3
16	*Streptococcus Bovis*	3	0.9
	Total	**320**	**100**

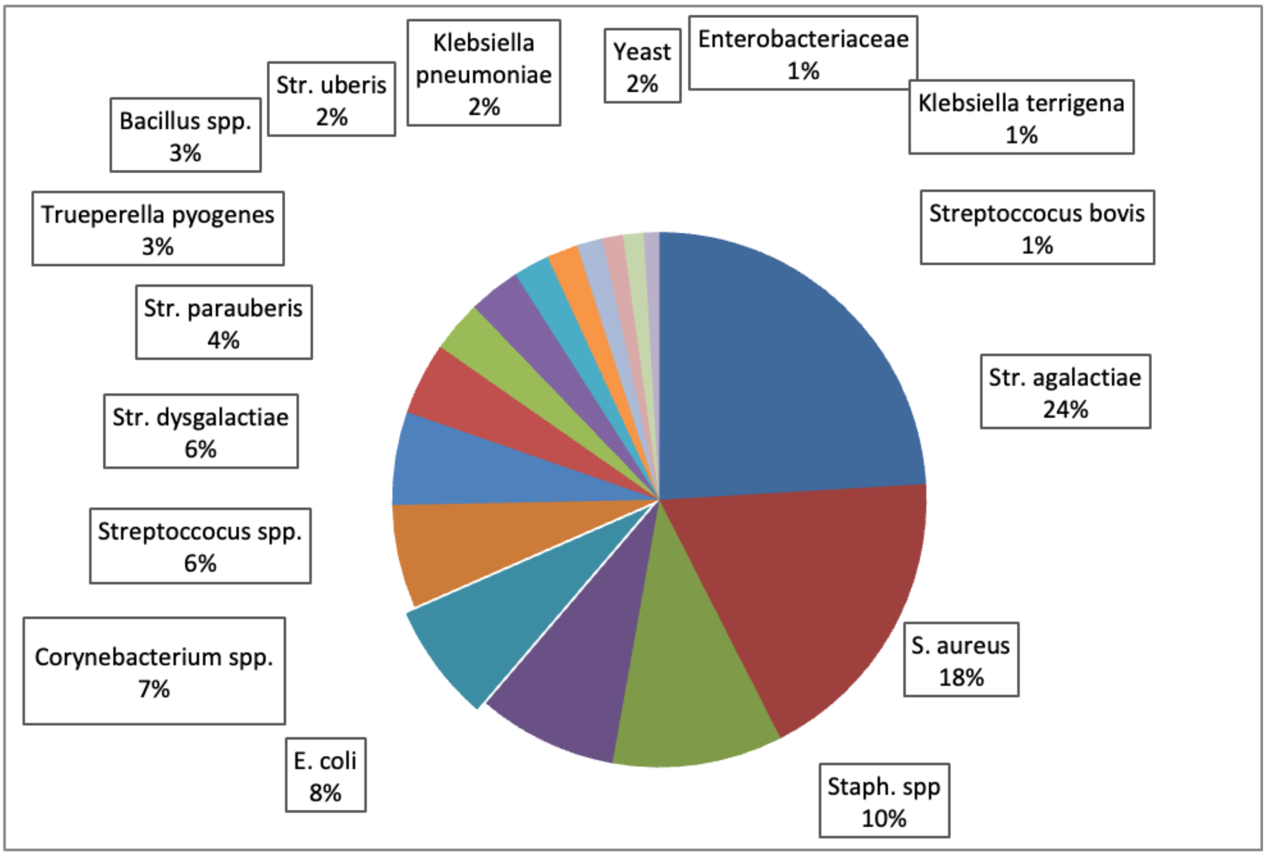

Figure 2 Total number of isolated isolates from milk samples from cows with mastitis.

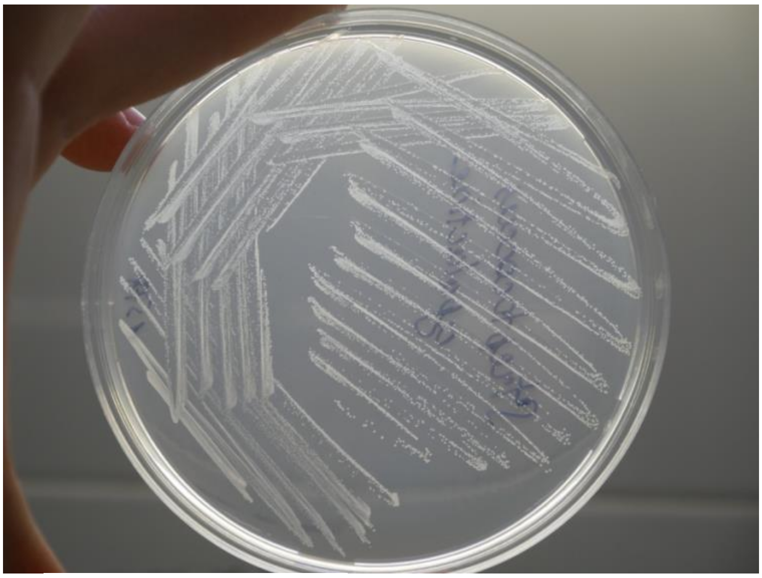

Figure 3 Bacterial colonies *Streptococcus agalactiae* on Muller-Hinton agar.

Figure 4 Bacterial colonies *Streptococcus agalactiae* on blood agar.

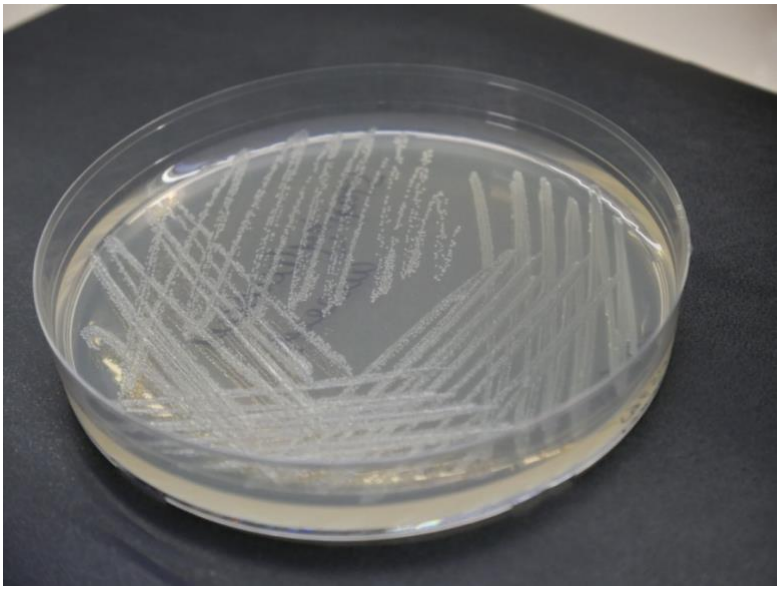

Figure 5 Bacterial colonies *Staphylococcus aureus* Muller-Hinton agar.

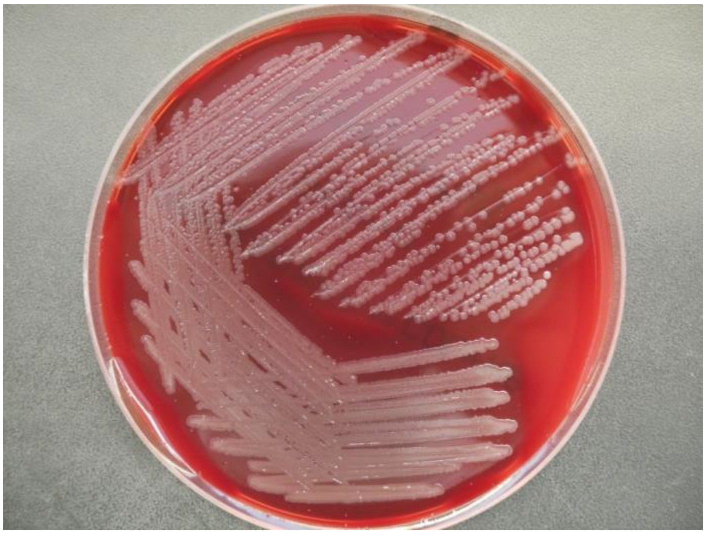

Figure 6 Bacterial colonies *Staphylococcus aureus on* Blood agar.

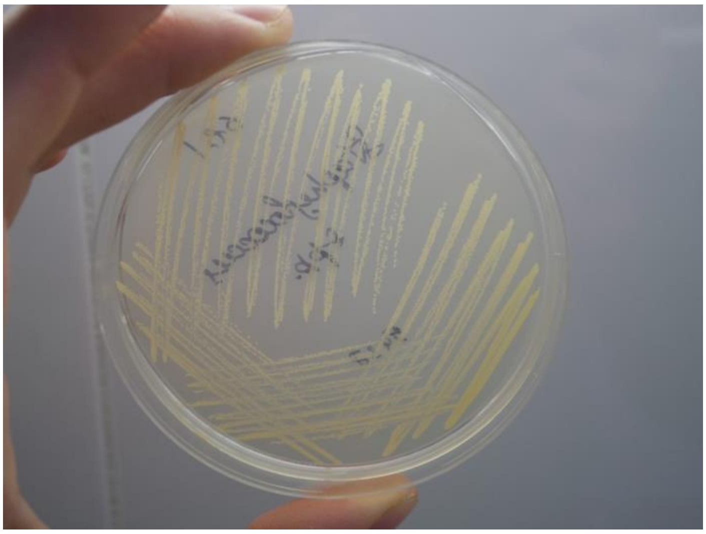

Figure 7 Bacterial colonies *Staphylococcus spp.* on Muller-Hinton agar.

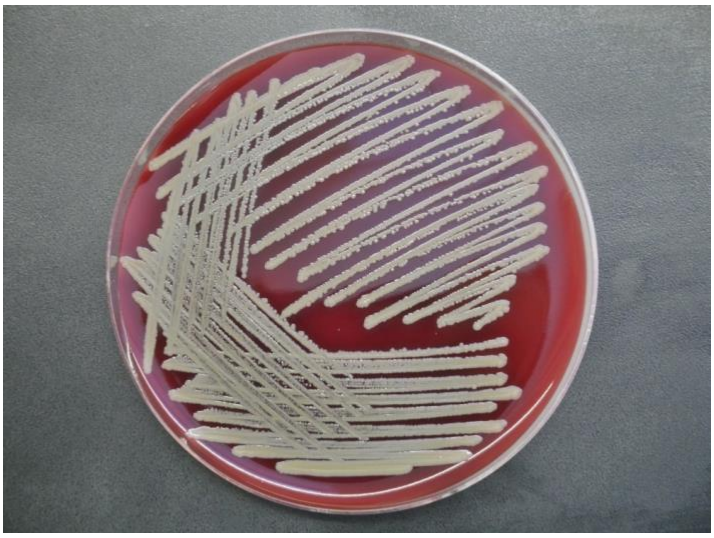

Figure 8 Bacterial colonies *Staphylococcus spp.* on Blood agar.

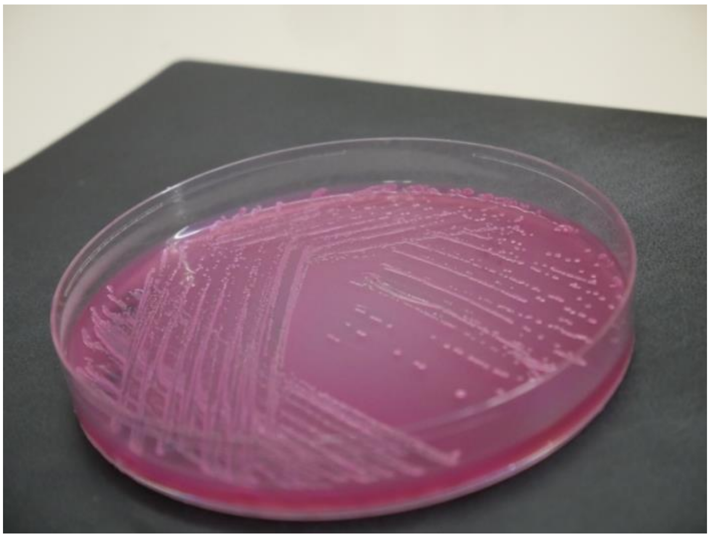

Figure 9 Bacterial colonies *E.coli* on McConkey agar.

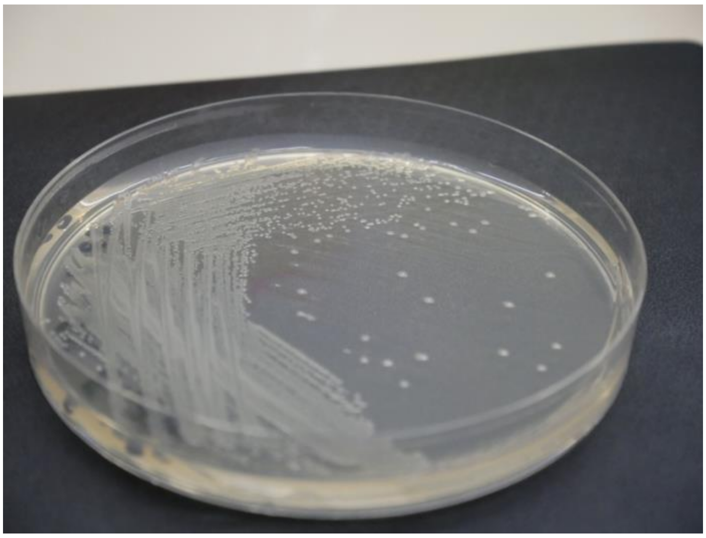

Figure 10 Bacterial colonies *E.coli* on Muller-Hinton agar.

Figure 11 Bacterial colonies *of E. coli* on Blood agar.

Studies conducted in Germany on dairy farms out of 751 clinical cases of cow mastitis indicate the spread of bacterial pathogens of mastitis *Staphylococcus aureus* – 10.0%, *Streptococcus uberis* – 8.5% and coliforms, mainly Escherichia coli, were isolated in 10.2% **[22]**. Studies in France have shown that 707 positive isolates of mammary gland secretions taken from cows with clinical mastitis *S. aureus* occurred in 15.8% of cases, *S.*

uberis in 22.1%, and *E. coli* 16.0% **[23]**. Studies conducted in Sweden **[24]** out of 743 isolates from 669 cows with a clinical mastitis showed that *S. aureus*, *S. uberis*, and *E. coli* made were 28.4%, 15.2%, and 21.9%, respectively. In the Netherlands out of 438 mammary gland secretion samples from cows with subclinical mammary isolates, *S. aureus* was detected in 18.0%, and *S. uberis* in 9.6% of cases **[25]**, **[44]**.

Studies conducted in Mexico showed that 20 different types of yeast were identified in 282 (25.75%) secret samples **[26]**, **[36]**.

According to the results of our studies, bovine mastitis caused by yeast was detected in 5 (1.4%) isolates of the total number of diagnosed pathogens. The data obtained by us are consistent with the results of research by other authors **[4]**, **[37]**.

Based on the results of the obtained bacteriological studies, the sensitivity of isolated mastitis pathogens to antimicrobial substances was determined (Table 2 – 4).

Studies conducted in Brazil show that out of 89 isolates of *Str. agalactiae*high sensitivity was to Ceftiofur, enrofloxacin, ampicillin, gentamicin, and lincomycin, and the isolates were resistant to neomycin and tetracycline **[27]**, **[38]**. In Germany, studies of milk from cows with mastitis show that this isolate was resistant to Sulfatrimethoprim 50.5%, tetracycline 46.2%, and erythromycin 15.4% **[28]**, **[39]**. As the results of our study show, the isolation of isolated *Str. agalactiae* showed a high level of sensitivity to amoxicillin in 73 (94.8%) isolates, Amoxicillin/claulanic acid in 71 (92.2%) samples. Moderately sensitive to Rifampicin in 65 (84.4%), Ampicillin in 64 (83.1%), Ceftiofur in 61 (77.2%), lincomycin, Cloxacillin, Bacitracin in 61 (77.2%) isolates, to Cephalexin in 55 (71.4%) and Oxytetracycline in 39 (50.6%) isolates. Weakly sensitive to Trimethoprim/sulfamethoxazole, Gentamicin in 36 (46.7%) isolates, Enrofloxacin in 22 (28.5%), Tylosin in 17 (22%), Tilmicosin in 21 (27.3%), to Danofloxacin in 14 (18.2%), to Marbofloxacin in 13 (16.9%), to Spiramycin in 12 (15.5%) isolates, Neomycin and Streptomycin in 6 (7.8%) isolates.

Studies conducted on farms in Ukraine show that isolate *S. aureus* was sensitive to Gentamicin in 77.97% **[29]**, and in 70% of isolates, *S. aureus* – was resistant to Ampicillin, Oxacillin, and Tetracycline **[32]**. Our study shows that *staphylococcus aureus* was highly sensitive to Gentamicin in 59 (100%) isolates, Ceftiofur in 58 (98.3%), Rifampicin in 57 (96.6%), to Cloxacillin in 56 (94.9%), to Cephalexin in 54 (91.5%) isolates. Moderately sensitive to Bacitracin in 54 (86.4%) isolates, to Trimethoprim/sulfamethoxazole in 49 (83%), to Amoxicillin/claulanic acidenrofloxacin in 48 (81.3%) isolates, to Amoxicillin in 40 (67.8%), to Oxytetracycline in 39 (66.1%), to Neomycin in 38 (64.4%), to Lincomycin in 33 (55.95%) isolates. The isolates were weakly sensitive to ampicillin in 28 (47.4%) isolates, Danofloxacin in 25 (42.4%), Tilmicosin in 24 (40.7%), Streptomycin in 22 (37.3%), Marbofloxacin in 18 (30.5%), Tylosin in 10 (16.9%) and Spiramycin in 4 (6.8%) isolates.

Studies of secretions from sick cows with mastitis in Algeria demonstrate the sensitivity of isolate*d staphylococcus spp* to gentamicin and Neomycin **[30]**, **[40]**, **[41]**. which coincides with the results of our studies, which showed that isolate*d staphylococcus spp*, which showed high sensitivity to Rifampicin in 30 (90.9%) isolates, to Amoxicillin/Claulanic acid, to Enrofloxacin, Ceftiofur, Gentamicin – in 29 (87.9%) isolates, to Cloxacillin, Bacitracin, Cephalexin – in 27 (81.8%) isolates, to Neomycin in 24 (72.7%), to Ampicillin in 23 (69.7%), to Amoxicillin in 22 (66.7%), to Oxytetracycline in 21 (63.6%), to Trimethoprim in 19 (57.6%). Moderately sensitive and weakly sensitive were to Streptomycin in 16 (48.5%) isolates, Lincomycin and Tilmicosin in 14 (42.4%) isolates, Marbofloxacin in 12 (36.3%), Danofloxacin in 10 (30.3%), to Tylosin in 6 (18.2%), to Spiramycin in 4 (12.1%) isolates.

Studies of milk from sick cows for the clinical form of mastitis on farms in Bangladesh have shown high resistance of Escherichia coli to Amoxicillin, Ampicillin, and Tetracycline **[31]**, **[42]**. Studies conducted in Canada indicate that this isolate was insensitive to Streptomycin, Tetracycline, Ampicillin, and Colistin, but showed sensitivity to Ciprofloxacin and Gentamicin **[33]** According to the authors **[32]**, more than 60% of isolates *of E. coli* showed resistance to Oxacillin and Sulfamethoxazole-trimethoprim.

Our research has shown that *E. coli* showed high sensitivity to Ceftiofur, which is consistent with the results of the researchers **[34]**, **[45]** and Gentamicin – in 27 (100%) isolates, to Enrofloxacin and Oxytetracycline – in 25 (92.5%) isolates. The medium-sensitive was isolating to Amoxicillin/Claulanic acid and Ampicillin – in 24 (88.8%) isolates, to Danofloxacin in 20 (74%), to Trimethoprim/sulfamethoxazole in 19 (70.3%), to Amoxicillin and Marbofloxacin – in 18 (66.6%), to Streptomycin in 6 (22.2%), to Cephalexin in 5 (18.5%)and Neomycin in 4 (14.8%) isolates. Highly resistant isolate *E. coli* was to Lincomycin, Cloxacillin, Tylosin, Bacitracin, Spiramycin, Tilmicosin, and Rifampicin.

Isolates*Corynebacterium spp* were highly sensitive to Gentamicin and Rifampicin in 23 (100%) isolates, to Ampicillin in 22 (95.6%) isolates, to Ceftiofur, Amoxicillin, and Bacitracin in 21 (91.3%) samples. Medium-sensitive isolates turned out to be Amoxicillin/claulanic acid, Lincomycin. Cephalexin – in 20 (86.9%) isolates, Enrofloxacin in 18 (78.3%), Oxytetracycline in 17 (73.9%) isolates, to Streptomycin, Marbofloxacin and

Tilmicosin – in 14 (60.9%) isolates, to Danofloxacin and Tylosin – in 13 (56.5%) isolates. Low sensitivity of the isolates was shown to Cloxacillin and Neomycin – in 11 (47.8%), Spiramycin in 9 (39.1%), and Trimethoprim/sulfamethoxazole in 4 (17.3%) isolates.

Table 2 Sensitivity of isolated mastitis pathogens to antimicrobial substances.

it. no.	Antibiotic	*Streptoccocus agalactiae*		*Staphylococcus aureus*		*Staphylococcus* spp.		*E. coli*		*Corynebacterium* spp.	
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
1	Amoxicillin (25 µg/disc)	73	94.8	40	67.8	22	66.7	18	66.6	21	91.3
2	Amoxicillin+Cl.acid (30µg/disc)	71	92.2	48	81.3	29	87.9	24	88.8	20	86.9
3	Enrofloxacine (10 µg/disc)	22	28.5	48	81.3	29	87.9	25	92.5	18	78.3
4	Streptomycin (10 µg/disc)	6	7.8	22	37.3	16	48.5	6	22.2	14	60.9
5	Trimethoprim/ Sulfamethoxazole (25µg/disc)	36	46.7	49	83	19	57.6	19	70.3	4	17.3
6	Oxytetracycline (30 µg/disc)	39	50.6	39	66.1	21	63.6	25	92.5	17	73.9
7	Ceftiofur (30 mcg)	61	77.2	58	98.3	29	87.9	27	100	21	91.3
8	Ampicillin (10 µg/disc)	64	83.1	28	47.4	23	69.7	24	88.8	22	95.6
9	Gentamicin (10 µg/disc)	36	46.7	59	100	29	87.9	27	100	23	100
10	Neomycin (30 µg/disc)	6	7.8	38	64.4	24	72.7	4	14.8	11	47.8
11	Lincomycin (15 µg/disc)	61	77.2	33	55.9	14	42.4	0	0	20	86.9
12	Cloxacillin (5 µg/disc)	61	77.2	56	94.9	27	81.8	0	0	11	47.8
13	Tylosin (30µg/disc)	17	22	10	16.9	6	18.2	0	0	13	56.5
14	Bacitracin (0.04 µg/disc)	61	77.2	51	86.4	27	81.8	0	0	21	91.3
15	Cephalexin (30 µg/disc)	55	71.4	54	91.5	27	81.8	5	18.5	20	86.9
16	Danofloxacin (5 µg/disc)	14	18.2	25	42.4	10	30.3	20	74	13	56.5
17	Spiramycin (100 µg/disc)	12	15.5	4	6.8	4	12.1	0	0	9	39.1
18	Marbofloxacin (5 µg/disc)	13	16.9	18	30.5	12	36.3	18	66.6	14	60.9
19	Tilmicosin (15 µg/disc)	21	27.3	24	40.7	14	42.4	0	0	14	60.9
20	Rifampicin (5 µg/disc)	65	84.4	57	96.6	30	90.9	0	0	23	100

The results presented in Table 3 showed that *Streptococcus spp* showed high sensitivity to the following antimicrobial substances: Ceftiofur in 18 (90%) isolates, Ampicillin, and Bacitracin – 17 (85%) isolates. Average sensitivity was to Amoxicillin in 16 (80%) isolates, Rifampicin in 15 (75%) isolates, Amoxicillin/claulanic acid, Gentamicin – in 14 (70%) isolates, Cephalexin in 13 (65%), Cloxacillin in 12 (60%) isolates. Low sensitivity was to Enrofloxacin, and trimethoprim/sulfamethoxazole – 9 (45%) isolates, lincomycin in 8 (40%), Tilmicosin in 7 (35%), Danofloxacin in 6 (30%), to oxytetracycline in 4 (20%), to Marbofloxacin in 3 (15%) isolates, to Streptomycin, Spiramycin, Neomycin, and Tylosin-only in 2 (10%) isolates. However, previous studies conducted in Poland show that the highest resistance of the bacterium of the genus *Streptococcus spp* was to Gentamicin, Kanamycin, and Tetracycline. In contrast, the highest sensitivity was observed to Penicillin, Enrofloxacin, and Marbofloxacin **[35]**, **[43]**.

The high sensitivity of isolate*d streptococcus dysgalactiae* was to Ceftiofur and Bacitracin – 18 (100%) isolate, to Cloxacillin – 17 (94.4%) isolates. Medium-sensitive of isolates were Cephalexin in 16 (88.9%) isolates, Amoxicillin/claulanic acid in 5 (83.3%), Ampicillin in 14 (77.8%) isolates, Rifampicin and Lincomycin -13 (72.2%) isolates, to Trimethoprim/sulfamethoxazole and Enrofloxacin – 12 (66.6%) isolates, to Gentamicin-

9 (50%) isolates. Low sensitivity was shown to Amoxicillin in 8 (44.4%), Tilmicosin 6 (33.3%), Marbofloxacin 5 (27.8%), Danofloxacin 4 (22.2%), Spiramycin 3 (16.6%), to Neomycin 2 (11.1%) isolates and was almost resistant to Tylosin, Streptomycin, and Oxytetracycline – only 1 (5.5%) isolate.

Streptococcus parauberis was insensitive to Spiramycin, Marbofloxacin, and Tilmicosin but was sensitive to Bacitracin 12 (85.7%), Amoxicillin 11 (78.6%) isolates, Ampicillin and Rifampicin 10 (71.4%) isolates, to Amoxicillin/claulanic acid, Ceftiofur, Cloxacillin and Cephalexin 9 (64.3%) isolate, to Trimethoprim/sulfamethoxazole 7 (50%) isolates and weakly sensitive – to Enrofloxacin 6 (42.8%), Gentamicin 5 (35.7%), Lincomycin 3 (21.4%) isolates, Streptomycin, Oxytetracycline, and neomycin – 2 (14.2%) isolates, to Tylosin and Danofloxacin only 1 (7.1%) isolate.

Bacteria *Trueperella pyogenes* showed high sensitivity to Amoxicillin, Ceftiofur, rifampicin-10 (100%) isolates, Amoxicillin/claulanic acid, Cephalexin, Ampicillin – 9 (90%) isolates, medium sensitivity to Enrofloxacin, Lincomycin – showed 8 (80%) isolates, to Gentamicin, Bacitracin, Marbofloxacin, Cloxacillin – showed 7 (70%) isolates, to Tilmicosin 6 (60%), Oxytetracycline 5 (50%) isolates. Low sensitivity to the following antibiotics: Trimethoprim/sulfamethoxazole 4 (40%) isolates, Tylosin, Danofloxacin, Spiramycin – 3 (30%) isolates, Streptomycin 2 (20%) isolates, insensitive to Neomycin. Recent studies show that most isolates *T. pyogenes*, were highly sensitive to Amoxicillin, Ampicillin, Gentamicin, and Ceftiofur. At the same time, a high level of resistance was observed to Trimethoprim/sulfamethoxazole and Tylosin, which coincides with our research results.

Isolates*Bacillus spp* were highly sensitive to Enrofloxacin 10 (100%) isolates, Rifampicin, Ceftiofur, and Ampicillin 9 (90%) isolates, and were moderately sensitive to Amoxicillin, Trimethoprim/sulfamethoxazole, Gentamicin, Cephalexin, Tilmicosin 8 (80%) isolates, Amoxicillin/claulanic acid and Oxytetracycline 7 (70%) isolates, Neomycin, Streptomycin, and Cloxacillin – 6 (60%) isolates. They were weakly sensitive to Lincomycin, and Bacitracin 4 (40%) isolate, to Danofloxacin 3 (30%), Tylosin 2 (20%) isolate, and Spiramycin 1 (10%) isolates and generally not sensitive *to Bacillus spp.* was to Marbofloxacin.

The study presented in table 4 shows that the highest sensitivity of *Streptococcus uberis* showed (100%) isolates to Ampicillin Ceftiofuria 7. It was moderately sensitive to Amoxicillin, Cloxacillin, Bacitracin, Cephalexin, and Rifampicin – 6 (85.7%) isolates and Oxytetracycline 4 (57.1%) isolates. Hypersensitive was to Amoxicillin / claulanic acid, and Danofloxacin 3 (42.2%) isolates, Gentamicin and Marbofloxacin only 2 (28.6%) isolates. Once isolate*d streptococcus uberis* was sensitive to Enrofloxacin, Trimethoprim/sulfamethoxazole, Lincomycin, Tylosin, and Spiramycin, which is 14.2%, respectively, and resistant to Streptomycin, Neomycin, and Tylmycosin.

All isolates *of Klebsiella pneumoniae* we have selected demonstrated high sensitivity to only one antimicrobial substance– Gentamicin 6 (100%) isolates. The average sensitivity was up to Amoxicillin/claulanic acid 5 (83.3%) isolates, weakly sensitive to Ceftiofur 2 (33.3%) isolates, once the isolate showed sensitivity to Enrofloxacin, Trimethoprim/sulfamethoxazole, Oxytetracycline, Danofloxacin, Marbofloxacin, which is 16.7%, and showed high resistance to Amoxicillin, Streptomycin, Ampicillin, Neomycin, Lincomycin, Cloxacillin, Tylosin, Bacitracin, Cephalexin, Spiramycin, Tilmicosin, and Rifampicin.

Isolates *Klebsiella terrigen*ous was highly sensitive to Trimethoprim/sulfamethoxazole 4 (100%), Oxytetracycline 4 (100%), Gentamicin 4 (100%), Ceftiofur 4 (100%), medium-sensitive to Amoxicillin/claulanic acid 3 (75%), Enrofloxacin 3 (75%), to Danofloxacin 3 (75%), to Marbofloxacin 3 (75%), to Cephalexin 2 (50%) and Streptomycin 1 (25%) and insensitive to Amoxicillin, Ampicillin, Neomycin, Lincomycin, Cloxacillin, Tylosin, Bacitracin, Spiramycin, Tilmicosin, and Rifampicin.

Table 3 Sensitivity of isolated mastitis pathogens to antimicrobial substances.

It. no.	Antibiotic	*Streptococcus spp.*		*Streptococcus dysgalactiae*		*Streptococcus parauberis*		*Trueperella pyogenes*		*Bacillus spp.*	
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
1	**Amoxicillin (25 µg/disc)**	16	80	8	44.4	11	78.6	10	100	8	80
2	**Amoxicillin+Cl.acid (30 µg/disc)**	14	70	15	83.3	9	64.3	9	90	7	70
3	**Enrofloxacine (10 µg/disc)**	9	45	12	66.6	6	42.8	8	80	10	100
4	**Streptomycin (10 µg/disc)**	2	10	1	5.5	2	14.2	2	20	6	60
5	**Trimethoprim/ Sulfamethoxazole (25µg/disc)**	9	45	12	66.6	7	50	4	40	8	80
6	**Oxytetracycline (30 µg/disc)**	4	20	1	5.5	2	14.2	5	50	7	70
7	**Ceftiofur (30 mcg)**	18	90	18	100	9	64.3	10	100	9	90
8	**Ampicillin (10 µg/disc)**	17	85	14	77.8	10	71.4	9	90	9	90

Table 3 Cont.

It. no.	Antibiotic	*Streptococcus spp.*		*Streptococcus dysgalactiae*		*Streptococcus parauberis*		*Trueperella pyogenes*		*Bacillus spp.*	
		n	***%***	***n***	***%***	***n***	***%***	***n***	***%***	***n***	***%***
9	**Gentamicin (10 µg/disc)**	14	70	9	50	5	35.7	7	70	8	80
10	**Neomycin (30 µg/disc)**	2	10	2	11.1	2	14.2	0	0	6	60
11	**Lincomycin (15 µg/disc)**	8	40	13	72.2	3	21.4	8	80	4	40
12	**Cloxacillin (5 µg/disc)**	12	60	17	94.4	9	64.3	7	70	5	50
13	**Tylosin (30 µg/disc)**	2	10	1	5.5	1	7.1	3	30	2	20
14	**Bacitracin (0.04 µg/disc)**	17	85	18	100	12	85.7	7	70	4	40
15	**Cephalexin (30 µg/disc)**	13	65	16	88.9	9	64.3	9	90	8	80
16	**Danofloxacin (5 µg/disc)**	6	30	4	22.2	1	7.1	3	30	3	30
17	**Spiramycin (100 µg/disc)**	2	10	3	16.6	0	0	3	30	1	10
18	**Marbofloxacin (5 µg/disc)**	3	15	5	27.8	0	0	7	70	0	0
19	**Tilmicosin (15 µg/disc)**	7	35	6	33.3	0	0	6	60	8	80
20	**Rifampicin (5 µg/disc)**	15	75	13	72.2	10	71.4	10	100	9	90

Enterobacteriaceae bacteria family showed a high sensitivity to Enrofloxacin, Trimethoprim/sulfamethoxazole, and Gentamicin-4 (100%) isolates. Average sensitivity was shown to Danofloxacin and Marbofloxacin – 3 (75%) isolates, Bacitracin and Oxytetracycline – 2 (50%) isolates. Once, they were sensitive to Amoxicillin, Ampicillin, Neomycin, Cloxacillin, Cephalexin, Tilmicosin, and rifampicin, which is 25%. Amoxicillin / claulanic acid, Streptomycin, Ceftiofur, Lincomycin, Tylosin, and Spiramycin were highly resistant.

Only 3 isolates *of Streptococcus Bovis* were isolated during the study of mammary glands secretions, which showed 100% sensitivity to Amoxicillin, Amoxicillin / claulanic acid, Ceftiofur, Ampicillin, Gentamicin, Tylosin, Cephalexin, and Rifampicin. The average sensitivity was to Trimethoprim/sulfamethoxazole, Cloxacillin, Bacitracin, Danofloxacin, and Tilmicosin – 2 (66.7%) isolates. Once, they were sensitive to Oxytetracycline, and Lincomycin, 33.3 %, respectively, and were highly resistant to Enrofloxacin, Streptomycin, Neomycin, Spiramycin, and Marbofloxacin.

Table 4 Sensitivity of isolated mastitis pathogens to antimicrobial substances.

It. no.	Antibiotic	*Streptococcus uberis*		*Klebsiella pneumoniae*		*Enterobacteriaceae*		*Klebsiella terrigenous*		*Streptoccocus bovis*	
		n	***%***	***n***	***%***	***n***	***%***	***n***	***%***	***n***	***%***
1	**Amoxicillin (25 µg/disc)**	6	85.7	0	0	1	25	0	0	3	100
2	**Amoxicillin+Cl.acid (30 µg/disc)**	3	42.8	5	83.3	0	0	3	75	3	100
3	**Enrofloxacine (10 µg/disc)**	1	14.2	1	16.7	4	100	3	75	0	0
4	**Streptomycin (10 µg/disc)**	0	0	0	0	0	0	1	25	0	0
5	**Trimethoprim/ Sulfamethoxazole (25µg/disc)**	1	14.2	1	16.7	4	100	4	100	2	66.7
6	**Oxytetracycline (30 µg/disc)**	4	57.1	1	16.7	2	50	4	100	1	33.3
7	**Ceftiofur (30 mcg)**	7	100	2	33.3	0	0	4	100	3	100
8	**Ampicillin (10 µg/disc)**	7	100	0	0	1	25	0	0	3	100
9	**Gentamicin (10 µg/disc)**	2	28.6	6	100	4	100	4	100	3	100
10	**Neomycin (30 µg/disc)**	0	0	0	0	1	25	0	0	0	0
11	**Lincomycin (15 µg/disc)**	1	14.2	0	0	0	0	0	0	1	33.3
12	**Cloxacillin (5 µg/disc)**	6	85.7	0	0	1	25	0	0	2	66.7
13	**Tylosin (30µg/disc)**	1	14.2	0	0	0	0	0	0	3	100
14	**Bacitracin (0,04 µg/disc)**	6	85.7	0	0	2	50	0	0	2	66.7
15	**Cephalexin (30 µg/disc)**	6	85.7	0	0	1	25	2	50	3	100
16	**Danofloxacin (5 µg/disc)**	3	42.8	1	16.7	3	75	3	75	2	66.7
17	**Spiramycin (100 µg/disc)**	1	14.2	0	0	0	0	0	0	0	0
18	**Marbofloxacin (5 µg/disc)**	2	28.6	1	16.7	3	75	3	75	0	0
19	**Tilmicosin (15 µg/disc)**	0	0	0	0	1	25	0	0	2	66.7
20	**Rifampicin (5 µg/disc)**	6	85.7	0	0	1	25	0	0	3	100

As can be seen from the results of the study shown in Table 5, most of the isolated isolates were sensitive to Ceftiofur – 86.3%, Amoxicillin/claulanic acid – 76.6%, Rifampicin – 75.6%.

Table 5 Distribution of the total number of isolated mastitis pathogens by sensitivity to various antimicrobial substances.

It. no.	Antibiotic	The total number of isolates obtained	Number of sensitive isolates	% sensitive isolates
1	**Amoxicillin (25 µg/disc)**	320	237	74.1
2	**Amoxicillin+Cl.acid (30 µg/disc)**		245	76.6
3	**Enrofloxacine (10 µg/disc)**		196	61.3
4	**Streptomycin (10 µg/disc)**		78	24.4
5	**Trimethoprim/ Sulfamethoxazole (25 µg/disc)**		179	55.9
6	**Oxytetracycline (30 µg/disc)**		172	53.8
7	**Ceftiofur (30 mcg)**		276	86.3
8	**Ampicillin (10 µg/disc)**		231	72.2
9	**Gentamicin (10 µg/disc)**		236	73.6
10	**Neomycin (30 µg/disc)**		96	30.0
11	**Lincomycin (15 µg/disc)**		166	51.9
12	**Cloxacillin (5 µg/disc)**		214	66.9
13	**Tylosin (30 µg/disc)**		59	18.4
14	**Bacitracin (0,04 µg/disc)**		228	71.3
15	**Cephalexin (30 µg/disc)**		228	71.3
16	**Danofloxacin (5 µg/disc)**		111	34.7
17	**Spiramycin (100 µg/disc)**		39	12.2
18	**Marbofloxacin (5 µg/disc)**		99	30.9
19	**Tilmicosin (15 µg/disc)**		103	32.2
20	**Rifampicin(5µg/disc)**		242	75.6

Weak sensitivity of 320 isolated isolates was shown to Spiramycin (Spiramycin) – 12.2%, Tylosin – 18.4%, Streptomycin – 24.4%, neomycin – 30%, Marbofloxacin – 30.9%, Tilmicosin – 32.2%, Danofloxacin – 34.7%.

A significant percentage (74-51.9%) of the obtained isolates were sensitive (in descending order) to Amoxicillin – 74%, Gentamicin – 73.6%, Ampicillin – 72.2% and Bacitracin – 71.3% and Cephalexin – 71.3%, Cloxacillin – 66.9%, Enrofloxacin – 61.3%, Trimethoprim/sulfamethoxazole – 55.9%, Oxytetracycline – 53.8% and Lincomycin – 51.9%.

CONCLUSION

Contagious pathogens of cow mastitis are diagnosed in 57.5% of isolates from the total number of 24.1%, *Staphylococcus aureus* 18.4%, *Corynebacterium spp*. 7.2%, *Streptococcus dysgalactiae* 5.6%, *and Streptococcus uberis* 2.2% were most often detected.

Environmental pathogens make up 42.5% of all isolated isolates, most bacteria are Gram-positive microflora. In particular, streptococci 11.5% (*Streptococcus spp*. 6.2%, *Str. parauberis* 4.4%, *Str. bovis* 0.9%), *Staphylococcus spp*. 10.3 %. The landscape of Gram-negative microflora is 20.6%, among which the most significant percentage belongs to *E. coli* 8.4% and *Klebsiella pneumoniae* 1.9%.

Mastitis caused by fungi (yeast) accounts for more than 1.4% of the total number of diagnosed mastitis pathogens.

A significant percentage of the obtained isolates showed sensitivity (in descending order) to Ceftiofur, Amoxicillin/claulanic acid, Rifampicin, Amoxicillin, Gentamicin, Ampicillin, Bacitracin, Cephalexin, Cloxacillin, Enrofloxacin, Trimethoprim/sulfamethoxazole, Oxytetracycline, Lincomycin. The least sensitive isolates were Spiramycin, Tylosin, Streptomycin, Neomycin, Marbofloxacin, Tilmicosin, and Danofloxacin.

The prospect of further research will be to improve the analysis of the sensitivity of pathogens to antimicrobial substances and establish the terms of care and flow to the quality indicators of milk.

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