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Investigation of the Antibiotic Resistance and Biofilm-Forming Ability of Staphylococcus species from Bovine Mastitis cases in the Almaty Region, Kazakhstan

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ABSTRACT

Cow mastitis is a multifactorial disease caused by the interaction of various factors, including the host, specific pathogens, the environment, the season, and keeping conditions. The purpose of this study is to determine the prevalence of mastitis in farms of the Almaty region (Kazakhstan), identify its pathogens, and study their properties. The authors conducted a study at the Biological Safety Laboratory of the Kazakh National Agrarian Research University using samples from several farms. 430 lactating cows of the Holstein and Alatau breeds were selected, with a total of 468 samples over two years. The ability to form biofilm in the isolated strains, as well as antibiotic sensitivity, was studied. Most of the isolated strains were *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*. The cultures were isolated using microbiological methods, and DNA was analyzed using the CAMOMILE-NKMag-PCR kit. The results showed that the prevalence of mastitis in the Almaty region was 27.2%, mainly in the form of a subclinical manifestation. The authors also found a link between the degree of morbidity and the age of cows, as well as the number of calvings. Among the investigated strains, only 4.3% did not form biofilm. A high prevalence of antibiotic resistance was identified, and methicillin-resistant strains were isolated among *S. aureus* and *S. epidermidis*.

Key words: *Staphylococcus aureus*; *S. epidermidis*; *S. haemolyticus*; Biofilm; Sample analysis; Antibiotic resistance

INTRODUCTION

Cow mastitis is a multifactorial disease that is developed as a result of the interaction of various factors related to the host, specific pathogens, the environment, the season, and keeping conditions (Klibi et al. 2019). The mastitis pathogens can be classified into infectious, environmental microorganisms, and opportunistic microorganisms, depending on their main mode of transmission within the herd (Karabassova et al. 2022; Baymenov et al. 2023). *Staphylococcus aureus* pathogens are infectious microorganisms that adapt to the mammary gland environment and can potentially spread from cow to cow during milking. Similarly, *Escherichia coli* and coagulase-negative *Staphylococci* are considered environmental pathogens and are opportunistic mammary gland bacteria that can be transferred from a contaminated environment to the mammary gland of a cow during milking (Klibi et al. 2019; Jafarova and Abasova 2023; Reshetnikova and Krylova 2023).

Previously, we studied coagulase-positive strains of *Staphylococcus*, which were isolated from milk from both healthy cows and cows with clinical and subclinical forms of mastitis. *S. aureus* strains were detected more than other typified strains (132 out of 171 strains; 77.2%) (Zhylkaidar et al. 2019).

Several research publications focus on representatives of the *Staphylococcus* genus which do not have coagulase activity but cause mastitis in cows (Mounam et al. 2023). Researchers in many countries more often isolated coagulase-negative *Staphylococci* from cow's milk obtained from cattle with mastitis, and they were most significant in the pathology of the cow mammary gland (Fessler et al. 2010; Frey et al. 2013; Goetz et al. 2017).

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In most cases, coagulase-negative *Staphylococci* causes' subclinical mastitis, but they can also cause clinical mastitis characterized by mild clinical symptoms that are associated with an increase in the somatic cell number (Bochniarz et al. 2013).

Biofilm formation is the most important mechanism of attachment and colonization of microorganisms in nature and represents structured communities of microorganisms organized into a complex structure that adheres to the abiotic and biotic surfaces (Silva et al. 2014).

The biofilm matrix, consisting of proteins, polysaccharides, lipids, and nucleic acids, most often occupies up to 85% of its volume, due to which microbial cells are almost 100% protected from any external influence as long as they are inside the film (Akhazhanov et al. 2023; Beishova et al. 2023). This can lead to chronic or recurrent infections in the herd. As a result of biofilm formation, it becomes increasingly difficult to cure and eradicate the disease, which makes this problem more urgent (Rudenko et al. 2021; Buienbayeva et al. 2023; Babiker et al. 2024). The purpose of this study was to determine the level of mastitis spread in farms of the Almaty region (Kazakhstan), as well as to identify the causative agents of mastitis and study their phenotypic and genotypic properties.

MATERIALS AND METHODS

This work was performed at the Kazakh National Agrarian Research University Biological Safety Laboratory, and samples were collected from several farms in the Almaty region. For the study, 430 lactating cows of dairy herds of Holstein (350 heads) and Alatau (80 heads) breeds were selected, of which 280 heads were older than 6 years and 150 heads were younger from several farms in the Almaty region. In 2 years (from 2021 to 2023), 468 milk samples were collected.

Milk samples and identification of isolated cultures

A physical examination of the animals was performed to assess the presence of signs of udder inflammation, like redness, swelling, local temperature, and soreness. Milk was also tested for such changes as clots, blood, or discoloration. Milk samples were taken under aseptic conditions. For this purpose, the udder teats were wiped with a cotton swab soaked in 70% ethyl alcohol, and 5-7 ml of milk were taken into sterile test tubes. Test tubes with milk were delivered to the laboratory for analysis within 4 hours.

Culture isolation was carried out using microbiological methods. The samples were well mixed, and two or three loops of milk were streaked onto meat-peptone agar and incubated at 37°C for 24-48 hours until a characteristic growth appeared on the agar. The cultures were then subjected to bacteriological analysis. Gram staining was performed, and only gram-positive cocci collected in clusters were considered. These individual colonies from petri dishes were transplanted into meat-peptone broth (MPB) and incubated for 12 hours (at night) at 37°C to obtain good bacterial growth. 24-hour cultures were used for identification by Sanger sequencing the *16S rRNA* gene.

16S metagenomic sequencing was performed on a MiSeq new generation genome-wide sequencer (Illumina, USA).

The reaction mixture for sequencing consisted of: 2.5µL of the DNA matrix; 5µL of each primer in 1µM concentration; 12.5 µL KAPA HiFi HotStart ReadyMix in 2X concentration (KAPA Biosystems, Cape Town, South Africa).

Determination of biofilm formation

Biofilm formation was determined using a Stat FAX 2000 spectrophotometer at a wavelength of 570nm (Gogoi-Tiwari et al. 2017). The isolated cultures were inoculated in 200µL tryptone soy broth (TSB) with the addition of 1% glucose in polystyrene 96-well plates and left at 37°C for 48 hours. After that, the contents of the wells were carefully removed and washed three times with a phosphate-salt buffer solution, fixed, and stained with 2% crystalline violet. The optical density was read using a reader at a wavelength of 570nm. The threshold values for the generators to varying degrees were as follows: weak (outer diameter (OD)=0.350-0.550), medium (OD=0.600-0.800), and strong (OD=0.850-2.00).

Determination of antibiotic sensitivity

To determine antibiotic sensitivity, antibiotics such as ampicillin (10µg), chloramphenicol (30µg), erythromycin (15µg), furazolidone, gentamicin, penicillin (10IU), tetracycline (30µg), gentamicin (10µg), rifampicin (5µg), cefoxitin (30µg), vancomycin (30µg), norfloxacin (30µg) and cefoperazone/sulbactam (75/35µg) were used. The resistance profile of the isolated strains was determined by the disk diffusion method. The strains were classified as susceptible or resistant based on species-specific epidemiological thresholds issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (n.d.).

Determination of antibiotic resistance genes and genes associated with biofilm formation

DNA was isolated using the CAMOMILE-NKMag-PCR kit according to the instructions for use from the manufacturer Diamed Asia Test LLP.

The following antibiotic resistance genes were found: *blaZ, tetM, tetK,* and *mecA.* The genes that encode biofilm formation in spp. *Staphylococcus* were also studied: *aap, bap, icaA,* and *icaD.* The primers used in this study are shown in Table 1. Amplification was carried out under the following conditions: preliminary denaturation: 94°C, 6 minutes, main cycling: 30 cycles; denaturation: 94°C, 30 seconds, primer annealing: 30 seconds (annealing temperature for each primer is indicated in Table 1), elongation: 30 seconds at 72°C, final elongation: 72°C, 10 min.

After the horizontal electrophoresis was completed, the gel-documenting Infinity VX2 3026 system, WL/LC/26M XPress, Vilber Lourmat (USA) was used to view the resulting electrophoregram.

RESULTS

The spread of cow mastitis in the Almaty region

According to the results of our study, mastitis in farms of the Almaty region occurs in two forms: clinical and subclinical, which, by the nature of the inflammatory process, are represented by serous, catarrhal, and purulent-catarrhal character.

Table 1: The sequence of primers used in this study

Name	Sequence	Size	Temperature (°C)	References
blaZ	ACT TCA ACA CCT GCT GCT TTC GA CCA CTT TTA TCA GCA ACC	173	55	Srednik et al.(2017)
tetM	AGT GGA GCG ATT ACA GAA CAT ATG TCC TGG CGT GTC TA	158	55	Yang et al.(2016)
tetK	GTA GCG ACA ATA GGT AAT AGT GTA GTG ACA ATA AAC CTC CTA	360	55	Yang et al.(2016)
mecA	GTG AAG ATA TAC CAA GTG ATT ATG CGC TAT AGA TTG AAA GGA T	147	57	Srednik et al.(2017)
icaA	CTG TTT CAT GGA AAC TCC TCG ATG CGA TTT GTT CAA ACA T	200	57	Frey et al.(2013)
aap	GAAGCACCGAATGTTCCAACTATC AGTTGGCGGTATATCTATTGTA-	289	50	Frey et al.(2013)
icaD	AAA CGT AAG AGA GGT GG GGC AAT ATG ATC AAG ATA C	199	55.5	Manandhar et al.(2018)
bap	ACT TAY TRC CHT ATA TCG AAR TAG GCT GTT GAA GTT AAT ACT GTA CCT GC	900	47	Tremblay et al.(2013)

Table 2: Distribution of cow mastitis in farms of the Almaty region

Risk factors		Cows		Clinical mastitis n(%)	Subclinical mastitis n(%)
		Total number	Sick		
Age	>6 years	280	71(25.4)	9(3.2)	53(18.9)
	<6 years	150	46(30.7)	18(12)	37(24.7)
Breed	Holstein	350	98(28.0)	19(6.8)	73(20.9)
	Alatau	80	19(23.8)	8(2.9)	17(21.3)
Lactation stage	Early phase	270	76(65.0)	14(12.0)	49(41.1)
	Phase 1	125	19(16.2)	6(5.1)	30(25.6)
	Phase 2	35	22(18.8)	7(6.0)	11(9.4)
Milking method	Manual	50	12(24.4)	3(1.1)	9(18.0)
	Automatic	380	105(27.6)	24(8.6)	81(21.3)

Out of a total of 430 dairy cows, 117 were diagnosed with mastitis, which is a percentage of 27.2% (Table 2). Subclinical mastitis was found in animals that showed no signs of udder inflammation or changes in milk and demonstrated positive results in the test performed with the Milkotest express diagnostic kit.

Serous mastitis was usually recorded more often in cows after 1-3 calvings and had the following characteristics: the body temperature of the animal increased to 40.5-41°C, the heart rate to 95 beats per minute, and the respiratory rate up to 35 respiratory movements per minute. On clinical examination, the affected lobes were enlarged, and the teats of the affected cows were slightly enlarged and flaccid. Supramental lymph nodes were enlarged 1.5-2 times, with pain irradiation during palpation. Depression and loss of appetite were present in three cows with mastitis. At the beginning of inflammation, milk from the affected teats had a white color. When the disease passed to a more severe stage, it became watery and yellowish (serous) or showed the presence of blood, which was recorded in the study.

Catarrhal mastitis was detected in animals after the 4th calving. The general condition of the animals was depressed, they had no appetite, their body temperature reached 42.5°C, and pulse and respiration increased in most cases. The affected udder halves were enlarged and slightly painful to the touch, the skin was hyperemic, and the discharge from the affected udder had a serum-like consistency with an admixture of small flakes and clots. Supramammary lymph nodes were enlarged and painful to the touch.

Purulent and catarrhal mastitis was registered only in cows after 5-7 calvings. In this form, in clinical cases of mastitis, general depression and fever (elevated body temperature equaling 41.5°C) were noted, and increased breathing and heart rate were also recorded. The affected areas were swollen, hard, painful on palpation, and hot to the touch, with noticeably enlarged teats, as well as bluish-reddish spotty hemorrhages on the skin. Supramental lymph nodes were enlarged and painful on palpation. The discharge from the affected udder was thick yellow-green with an unpleasant odor.

We also found that the incidence of mastitis increases with increasing age, the number of calvings, as well as with inadequate diet and poor compliance with sanitary and hygienic rules. Thus, the incidence of both subclinical (24.7%) and clinical mastitis (6.4%) was higher in cows older than 6 years compared to younger cows. The prevalence of subclinical and clinical mastitis was highest at the early stage of lactation: 14 cows (55.6%) and 49 cows (41.1%), respectively. In this study, no effect of the milk yield method on the incidence of mastitis was found.

Identification of bacteria and their role in mastitis

Many microorganisms are actively involved in the development of the inflammatory process in the mammary gland. Conditionally pathogenic and pathogenic microorganisms are isolated from mastitis-affected udder quarters at farms.

Microbiological studies have established the participation in the etiology and pathogenesis of bacteria of the species *S. aureus, methicillin-resistant S. aureus (*MRSA), *S. epidermidis*, and *S. haemolyticus* (Table 3). It has been shown that *S. aureus* and coagulase-negative *Staphylococcus* are the main pathogens that are isolated in clinical and subclinical mastitis (Wang et al. 2018; Francisco et al. 2021). In total, 94 isolates were isolated in this study (Fig. 1).

Table 3: Distribution of isolates in clinical and subclinical mastitis

Mastitis form	*S. aureus, %*	*MRSA, %*	*S. epidermidis, %*	*S. haemolyticus, %*
Clinical mastitis	72.3(68/94)	83.3(25/30)	19.4(7/36)	30.8(8/26)
Subclinical mastitis	27.7(26/94)	16.7(5/30)	80.6(29/36)	65.4(17/26)

Table 4: Results of the biofilm formation study

Isolates	*S. aureus* n, %	*MRSA* n, %	*S. epidermidis* n, %	*S. haemolyticus* n, %	*Total* n, %
Total number	94	30	36	26	186(100)
Formation of well-expressed biofilms*	67(71.3)	20(66.7)	22(50)	16(61.5)	125(62.4)
Formation of average-expressed biofilm**	19(20.2)	6(20)	8(27.8)	2(7.7)	35(21.5)
Formation of weakly expressed biofilm***	6(6.6)	2(6.7)	6(22.2)	2(7.7)	16(8.6)
No biofilm formation	2(2.1)	2(6.7)	-	4(15.4)	8(4.3)

*strong(OD=0.850-2.00); **average(OD=0.600-0.800); ***weak(OD=0.350-0.550).

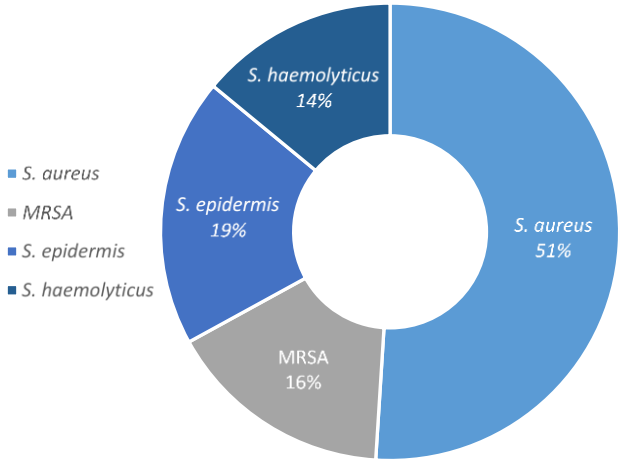

Fig. 1: The spread of pathogens that cause mastitis.

S. aureus and *MRSA* were isolated in clinical mastitis and coagulase-negative *Staphylococci* were isolated in subclinical mastitis. In clinical mastitis, 83 strains of *S. aureus* were isolated, of which 25 strains were identified as MRSA (Table 3).

The ability to form biofilms in isolated strains of Staphylococci

The ability of *Staphylococci* to form biofilms is one of the virulence factors facilitating the adhesion and colonization of these pathogens on the mammary gland epithelium, contributing to the evasion of immunological protection and recurrent or persistent infections (Pérez et al. 2020).

Among the isolated cultures, *S. aureus* and MRSA formed a well-expressed biofilm, and *S. epidermidis* formed a less expressed biofilm (Table 4). Most of the studied isolates could form a biofilm (62.4% formed a well-expressed biofilm and only 4.3% were unable to form a biofilm). It was found that *S. aureus* formed a biofilm more than other *Staphylococci*.

Investigation of antibiotic resistance of isolated cultures

The antibiotic sensitivity of the microflora isolated from the mammary gland secretions of cows with mastitis is shown in Table 5. Most of the tested isolates have multiple antibiotic resistance. Of all the isolated strains, they were more resistant to penicillin (93.5%), followed by ampicillin (87.1%) and tetracycline (75.3%).

No resistance was found in *S. epidermidis* and *S. haemolyticus* strains to such antibiotics as norfloxacin, vancomycin, and rifampicin. However, they were most sensitive to vancomycin, norfloxacin, rifampicin, and chloramphenicol. Among the isolated strains, a high degree of antibiotic resistance was observed, and resistance to three antibiotics was found with high frequency (ampicillin, penicillin, and tetracycline); strains with resistance to four antibiotics such as penicillin, ampicillin, tetracycline, and gentamicin were also noted.

The results of the study showed a high level of resistance of microorganisms isolated from sick cows to antibacterial agents of various pharmacological groups, which proves the need to monitor the antibiotic sensitivity of infectious agents isolated in the farm for the appointment of rational antimicrobial therapy, as well as the search for alternative treatment methods.

Identification of antibiotic resistance genes

Four genes responsible for antibiotic resistance of *S. aureus* strains were studied: *blaZ, mesA, tetK,* and *tetM*. Based on Table 6, most of the isolated *S. aureus* strains have the *blaZ* gene in their genome, which is responsible for the mechanism of resistance to beta-lactam antibiotics. The *blaZ* gene was detected in 120 studied *S. aureus* samples, which amounted to 96.8%. Tetracycline resistance genes *tetK* and *tetM* were found to a greater extent in *S. epidermidis* (69.4 and 80.6%, respectively). The detection of the *tetM* gene was recorded more often among all strains compared to *tetK*.

Of 124 strains of *S. aureus*, 30 MRSA strains were identified, of 36 strains of *S. epidermidis*, four MRSA strains were identified, and the presence of the *mesA* gene was also confirmed.

Identification of genes associated with biofilm formation

In this study, four genes associated with biofilm formation, *icaA, icaD, aap*, and *bap*, were studied (Table 7). The results of the study showed that the *icaA* gene was detected with high frequency in *S. aureus* and *S. epidermidis*. To a lesser extent, the *icaD* gene was detected in *S. aureus* and *S. epidermidis*. The *aap* gene was found in MRSA strains (80%) and only in 21.3% of *S. aureus*, while the *bap* gene was not found in any strain.

DISCUSSION

The incidence of mastitis in the studied farms was 27.2%, and it was found that subclinical mastitis prevailed in most cases compared with clinical mastitis. In comparison with other international studies, this study showed a relatively lower prevalence of bovine mastitis.

Table 5: Results of the antibiotic sensitivity study

Antibiotics	*S. aureus* (94) n, %	*MRSA* (30) n, %	*S. epidermidis* (36) n, %	*S. haemolyticus* (26) n, %	Total: 186
Penicillin	90(95.7)	30(100)	32(86.7)	24(86.7)	166
Tetracycline	72(76.6)	18(60)	30(83.3)	18(60)	79
Ampicillin	82(87.2)	22(73.3)	32(91.7)	22(73.3)	81
Erythromycin	20(21.3)	16(53.3)	20(55.6)	8(26.7)	64
Gentamicin	38(40.4)	14(46.7)	18(50)	6(20)	76
Vancomycin	4(4.3)	10(33.3)	0	0	14
Norfloxacin	16(17.0)	8(26.7)	0	0	26
Rifampicin	30(31.9)	12(40)	0	0	42
Cefoxitin	0	30(100)	10(31.25)	0	40
Chloramphenicol	20(21.3)	8(26.7)	6(16.7)	4(13.3)	38
Furazolidone	44(46.8)	10(33.3)	24(66.7)	6(20)	84
cefoperazone/sulbactam	20(42.6)	14(46.7)	16(44.4)	8(26.7)	58

Table 6: Results of the study on the presence of antibiotic resistance genes

Gene	*S. aureus* (124) n, %	*S. epidermidis* (36) n, %	*S. haemolyticus* (26) n, %
blaZ	120(96.8)	33(91.7)	20(76.9)
mecA	30(24.2)	4(11.1)	0
tetK	41(33.1)	25(69.4)	16(61.5)
tetM	78(62.9)	29(80.6)	19(73.1)

Table 7: Presence of genes associated with biofilm formation

Gene of interest	*S. aureus* (94) n, %	MRSA (30) n, %	*S. epidermidis* (36) n, %	*S. haemolyticus* (26) n, %
icaA	88(93.62)	13(43.3)	33(91.6)	17(65.4)
icaD	84(89.36)	16(53.3)	28(77.8)	12(46.2)
Aap	20(21.3)	24(80)	23(63.9)	15(57.7)
Bap	0	0	0	0

In our study, the incidence of subclinical mastitis was determined as 20.9%, while in Egypt the incidence of cow mastitis was 52.1% (Algammal et al. 2020), and in Kenya, the Embu and Kajido regions, 73.2% (Mbindyo et al. 2020), but in Ethiopia, the prevalence of cow mastitis was estimated at 51.2% (Ibrahim et al. 2023). The incidence of clinical mastitis in Japan was recorded at 21.9% (Fukushima et al. 2020), while 3.9% was found in Ethiopia (Almaw et al. 2008). In the studied farms of the Almaty region, the prevalence of clinical mastitis was determined at 6.3%. Our research group established a direct relationship between the incidence of mastitis with age and the lactation period of cattle. In cows older than 6 years, mastitis in both forms was diagnosed to a greater extent than in cows at a younger age. The prevalence of clinical and subclinical mastitis in cows over the age of 6 years was 12 and 24.7%, respectively. This is explained by the fact that the teat canal in older animals is more expanded or the canal constantly remains partially open due to repeated milking, which allows microorganisms to penetrate the body of cows and promotes the entrance of microorganisms associated with the environment and skin into the teat canal, which leads to mastitis and loss of milk production (Shittu et al. 2012). It was shown that significantly more cases of mastitis in two forms were registered in the early stage of lactation; the same results were obtained by Tezera and Aman Ali (2021). However, in this study there was no difference in the prevalence of mastitis between different ages and the number of cows' calvings (Tezera and Aman Ali 2021). The influence of the early stage of lactation on the prevalence of mastitis may be because the diapedesis of neutrophils into the mammary gland in recently calved cows takes longer, in addition to increased oxidative stress and a decrease in the mechanisms of antioxidant protection during early lactation (Abebe et al. 2016).

As shown in Fig. 1, *S. aureus* was identified as the predominant microorganism isolated from mastitis (51%), and similar results were obtained by many authors (Pérez et al. 2020; Maalaoui et al. 2021; Ranasinghe et al. 2021). *MRSA* was found among the isolated *S. aureus*. Recently, a lot of attention has been paid to coagulase-negative *Staphylococci*, which also play an important role in the etiology of cattle mastitis (Li et al. 2015; Sender et al. 2017). Many studies have been conducted where coagulase-negative *Staphylococci* were the predominant pathogens in subclinical and clinical mastitis of cattle (Persson Waller et al. 2011; Hosseinzadeh and Dastmalchi Saei 2014). In a study conducted in Korea, 14 types of *Staphylococcus* were identified, with the three most common ones being *S. chromogenes*, *S. simulans*, and *S. epidermidis* (Kim et al. 2019). In Argentina, *S. chromogenes* and *S. haemolyticus* were identified as the predominant *Staphylococci*. Among coagulase-negative *Staphylococci*, *S. epidermidis* and *S. saprophyticus* are more frequently isolated in clinical mastitis, and *S. hyicus* is much more common among clinical cases (Persson Waller et al. 2011). Bochniarz et al. (2013) found that *S. xylosus* caused clinical mastitis, while *S. chromogenes*, *S. warneri*, *S. hominis*, and *S. saprophyticus* were detected in subclinical mastitis. In this study, *S. haemolyticus* and *S. epidermidis* were isolated in 19 and 14%, respectively. Based on the results obtained, *S. aureus* and MRSA play an important role in the pathogenesis of clinical mastitis, and in subclinical mastitis, coagulase-negative *Staphylococci* are more common.

The results of the study of biofilm formation by microorganisms showed a high degree of biofilm formation in almost all obtained isolates of microorganism strains. *S. epidermidis* (100%), *S. aureus* (97.9%), MRSA (93.3%), and *S. haemolyticus* (84.6%) formed a biofilm to varying degrees. In a study conducted in Argentina, a colossal

degree of biofilm formation was found; all isolated *S. aureus* strains and coagulase-negative *Staphylococci* formed a biofilm (Srednik et al. 2017). In addition, other researchers noted a high degree of biofilm formation in *S. aureus* and *MRSA* strains in their studies (Filipello et al. 2019; Shah et al. 2019; Torres et al. 2023). Among all obtained isolates, only four strains of *S. haemolyticus*, two strains of *S. aureus*, and two strains of *MRSA* did not form a biofilm. Coagulase-negative *Staphylococci* were studied for their ability to form biofilms, and 18% of the isolated ones were biofilm-forming (Phophi et al. 2019). In Brazil, the majority (65.7%) formed a biofilm (Francisco et al. 2021) and in Canada, 85.1% of biofilm-forming strains were obtained (Tremblay et al. 2014). In a study where only 18% of biofilm-forming strains were obtained, the TSB nutrient medium was used, and in other studies where a high degree of biofilm-forming strains was detected, a heart-brain broth with added glucose was used, which explains the higher results, since the addition of glucose has a positive effect on biofilm formation. In this study, the biofilm was formed to a greater extent by *S. aureus* and *MRSA*. Among the strains of *S. epidermidis* non-biofilm-forming strains were not found.

The results of the study of antibiotic resistance suggest that it is necessary to select antibiotic therapy carefully and intelligently. Most of the studied strains had multiple antibiotic resistance, where the most common combination of resistance was penicillin, ampicillin, and tetracycline. However, other studies conducted in Turkey (45.5, 39.3, and 33%) (Aslantaş and Demir 2016) and Kosovo (91, 61, and 51%) (Mehmeti et al. 2016), found high resistance to penicillin, ampicillin, and tetracycline. High resistance to gentamicin and erythromycin were found in *S. epidermidis* and *MRSA* strains. Antibiotics such as vancomycin, rifampicin, norfloxacin, and chloramphenicol have shown good efficacy.

Since most strains are resistant to beta-lactam antibiotics and tetracyclines, the presence of the *blaZ*, *tetK*, and *tetM* genes and methicillin-resistant strains was investigated. Among *Staphylococci*, the blaZ gene was detected in 96.8% of *S. aureus*. *tetM* was detected to a greater extent than *tetK* in all strains that were studied. *TetM* 80.6% and *tetK* 69.4% were found most in *S. epidermidis* isolates compared to other isolates. 30 *S. aureus* isolates and four *S. epidermidis* with the *mecA* gene isolates were found; these isolates were also resistant to cefoxitin.

Other studies have shown that the blaZ antibiotic resistance gene has been found to a greater extent in *S. aureus* strains (Zhang et al. 2022; Yang et al. 2023). Ahmed et al. (2020) found a high prevalence of the blaZ b gene in coagula-negative Staphylococci of 80%, which was similar to our results in *S. epidermidis* (91.7%) and to a lesser extent in *S. haemolyticus* (76.9%). In contrast, a study conducted in China showed a lower detection rate of the blaZ gene of 69.4% (Yang et al. 2023). A very high prevalence of the tetK and tetM gene was found in Jordan (100 and 97%) in *S. aureus* (Gharaibeh et al. 2023), while in our study a lower prevalence of *tetK (*33.1%) and *tetM (*62.9%) was noted.

Among coagulase-negative *Staphylococci*, high resistance of the tetK (79%) and *tetM (*96%) genes was found in China (Qu et al. 2019), and in Egypt, the prevalence of the *tetK* gene was detected (73.3%) (Ahmed et al. 2020). In Kazakhstan, the *mecA* gene was first detected among coagulase-negative *Staphylococci*, in 11.1% of isolates. The mecA gene was detected in 24.2% of *S. aureus* isolates, and the degree of prevalence was diverse in international articles. In the study by Taponen et al. (2023), the *mecA* gene was not detected in *S. aureus* isolates but was detected in 12% of *S. epidermidis* isolates. In a study conducted in Nepal, the *mecA* gene was detected in only 6.9% of *S. aureus* isolates (Shrestha et al. 2021), and in India only in 9.6% of *S. aureus* isolates (Mahanti et al. 2020).

In this study, the confirmed presence of the *mecA* gene was observed to a greater extent in *S. aureus* (24.2%). In coagulase-negative *Staphylococci*, the *mecA* gene was found in *S. epidermidis* (11.1%) and was not detected in *S. haemolyticus* strains.

The presence of genes responsible for biofilm formation did not correlate with the results of biofilm formation. *S. aureus* isolates (97.9%) were phenotypically positive, the *aap* gene was detected in only 21.3% of *S. aureus* isolates, and the *bap* gene was not detected in any isolate. Similar results were obtained in the studies of Notcovich et al. (2018) who also suggested that following other studies, *ica* and *bap* represented alternative ways of biofilm formation. Among all the studied genes, *icaA and icaD* were identified the most and *S. haemolyticus* had the least genes responsible for biofilm formation. Other authors noted the same pattern, that the *icaA* and *icaD* genes were most identified among biofilm-forming *Staphylococci* (Khoramrooz et al. 2016; Notcovich et al. 2018; Munive Nuñez et al. 2023).

Conclusion

Our research group found that the prevalence of mastitis in the Almaty region was 27.2%, and the subclinical form of mastitis was diagnosed more often. The study demonstrated a relationship between the degree of morbidity and the age of cows, as well as the number of calvings. *S. aureus* and some coagulase-negative *Staphylococci* played an important role in the etiology of mastitis. Methicillin-resistant strains of *S. aureus* and *S. epidermidis* were isolated and most strains had multi-resistance to antibiotics. In this regard, we noted that antibiotic resistance is very high among the isolated strains. During the study of biofilm formation, many isolates had properties to form biofilm to varying degrees, which was also confirmed by the presence of the *icaA* and *icaD* genes responsible for biofilm formation, except the *bap* gene.

Authors' contributions

Lyailya Bessembayeva: Conceptualization, Methodology, Writing - Original Draft. Zhumagul Kirkimbayeva: Data curation, Investigation, Visualization. Birzhan Biyashev: Supervision, Writing - Review & Editing, Project administration. Assel Zholdasbekova: Resources, Validation, Funding acquisition. Gulnur Kuzembekova: Formal analysis, Software, Methodology. Dinara Sarybayeva: Investigation, Writing - Review & Editing, Visualization. Arman Zhylkaydar: Conceptualization, Writing - Original Draft, Supervision. Kairat Oryntaev: Validation, Resources, Writing - Review

& Editing. Flyura Bakiyeva: Data curation, Methodology, Writing - Review & Editing.

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