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The main biological features and resistance to antibacterial drugs of *Streptococcus agalactiae* isolated from the cow's milk

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Abstract

Background and Aim. The main goal of our research, as stated in the article, was to study the phenotypic characteristics of strains of *S. agalactiae* isolated from milk, identify and evaluate the effectiveness of the main groups of antibiotics recommended for use in veterinary medicine, including those provided by EUCAST.

Materials and Methods. The study of the phenotypic characteristics of *S. agalactiae* and the research on antibiotic resistance were carried out at the base of the Microbiology Laboratory of the Biotechnology Research Institute of Kostanay State University named after A. Baitursynov in 2023, using mastitis milk samples brought to the microbiology laboratory.

The biological characteristics of streptococci in the milk samples were identified using classical microbiological methods. The antibiotic susceptibility testing was performed using the disk-diffusion method in Mueller-Hinton agar.

Results. The results of the study indicate that out of 631 milk samples from animals in the region's dairy farms, 35 of them were found to be *S. agalactiae* isolates.

The text you provided seems to report the results of a study that investigated 631 samples of raw milk for mastitis, identifying 35 *S. agalactiae isolates.* It details the antibiotic susceptibility of these isolates, indicating a high susceptibility to benzylpenicillin (90.4%), amoxicillin (80.9%), kanamycin (76.1%), and moderate susceptibility to ampicillin and neomycin. However, the isolates demonstrated lower susceptibility to doxycycline (47.6%), tylosin (42.8%), gentamicin, and erythromycin (33.3%).

Conclusion. Thus, various mechanisms of antibiotic resistance and ways of their acquisition by bacteria significantly complicate the process of selecting effective antibiotic therapy both in agro-industrial organizations and in medical institutions. The mechanisms of acquired and natural antibiotic resistance are inherently complex and vary from species to species, from strain to strain of microorganisms. Basically, intraspecific and interspecific acquisition of antibiotic resistance genes is carried out through horizontal transfer - conjugation, transformation, and transduction.

Key words: antibiotic; microbiology; sensitive; strain; streptococcus; resistance.

Introduction

When cows are sick with mastitis, streptococci, staphylococci, enterococci are most often isolated. Of etiological importance are contagious, highly pathogenic *Str. agalactiae* and *S. aureus*, which cause severe mastitis and provoke chronic inflammation with a high index of somatic cells, which makes milk unusable [1, 2].

Due to the concerns of the potential impact of *S. agalactiae* on the safety of milk and the issues of antibiotic use in animals, many researchers are worried about the adverse effects on public health. Uncontrolled use of antibiotics against pathogenic microorganisms can lead to various forms of food infections, which are very difficult to treat [3].

Many researchers note that Staphylococcus aureus and agalactylus streptococcus, which affects the mammary gland of cows, pose a threat to public health due to problems with the safety of dairy products and the use of antibiotics. When infected with antibiotic-resistant forms of pathogenic microorganisms, severe forms of food infections are observed, which are very difficult to treat [4, 5].

Streptococci are one of the widely studied pathogens in clinical microbiology. The morphological structure of streptococci is described in detail, their virulence factors and dominant clinical manifestations of diseases are studied [6].

Among the infectious agents involved in mixed infections of domestic animals, streptococci occupy a special position for certain reasons [7].

Uncontrolled use of antibiotics leads to the accumulation of low sub-inhibitory concentrations in the tissues and intestines of treated animals and in the environment, which contributes to the selection of antibiotic-resistant bacteria and enhances their growth. In addition, the presence of antibiotics can stimulate biofilm formation and horizontal gene transfer in some bacteria.

There are a number of mechanisms that contribute to the development of resistance of a bacterial cell to one or more antimicrobial drugs: a decrease in the accumulation of an antimicrobial drug inside the cell through a decrease in the permeability of the antimicrobial drug wall from the bacterial cell; enzymatic modification or degradation of an antimicrobial agent.

One of the main objectives is to reduce the use of antibiotics in animal husbandry by improving the quality of life and conditions of animals. In this regard, it is recommended to apply good animal husbandry and animal handling practices in livestock enterprises and during animal transportation; improving animal welfare (for example, ensuring an optimal microclimate, high-quality water, appropriate ventilation and distribution of areas) at all stages, including production, transportation and slaughter; the use of locally adapted breeds that are more resistant to diseases and stress, or animals selected for disease resistance (resistant animals will require fewer antimicrobial treatments); compliance with veterinary and sanitary, sanitary and hygienic rules, biosafety measures at enterprises to prevent the use of medicines; compliance with strict measures to combat diseases (for example, vaccinations); the use of feed ingredients/additives that increase the efficiency of feed conversion to exclude the use of antibiotics as growth stimulants; the rejection of feed ingredients with anti-nutritional properties; the use of modern waste disposal methods. In addition, it is necessary to organize and conduct monitoring and supervision of the spread of antibiotic-resistant bacteria, including the assessment and identification of trends and sources of antimicrobial resistance in bacteria; the discovery of new mechanisms of antimicrobial resistance; providing data necessary for the analysis of risks to animal and human health; providing a basis for practical recommendations for the protection of animal and human health; providing information for monitoring antimicrobial prescriptions in agricultural organizations and the reasonable use of recommendations; evaluating and determining the effectiveness of measures to combat antibiotic resistance.

The development of modern microbiology is closely related to the improvement of methods for identifying pathogenic microorganisms. Significant progress has been made in this field, primarily through coordinated research in chemistry, immunology, and genetics. Special methods have been developed that allow detailed study of the genetic apparatus of microorganisms, as well as the identification of specific antibodies and antigens using serological techniques.

As for the novelty of the work done, in order to solve such an urgent problem of modern microbiology, *S. agalactiae* strains were isolated from cow's milk in the Kostanay region. We studied and evaluated the level of resistance of isolates to the main groups of antibiotics. The obtained research results are recommended for use in dairy farms of Kostanay region.

Materials and methods

The study of the phenotypic characteristics of *S. agalactiae* and the research on antibiotic resistance were carried out at the base of the Microbiology Laboratory of the Biotechnology Research Institute of

Kostanay State University named after A. Baitursynov in 2023, using mastitis milk samples brought to the microbiology laboratory.

The biological characteristics of streptococci in the milk samples were identified using classical microbiological methods. The antibiotic susceptibility testing was performed using the disk-diffusion method in Mueller-Hinton agar.

The susceptibility of the bacteria to antimicrobial agents was tested using the disk-diffusion method. Interpretation was carried out according to the EUCAST guidelines, version 11.0 [8], MIC 4.2.1890–04 MIC. Determination of the susceptibility of microorganisms to antimicrobial agents [9].

The tests were conducted and prepared in accordance with the requirements of the State Standard of the AIS 26809-86 "Milk and Milk Products. General Requirements for Preparation, Sampling, and Trials". Adherence criteria, research methods, and preparation for testing" [10].

Microbiological study of *S. agalactiae.*

Morphology and bacterial characteristics of colonies of the isolated S. agalactiae strains were identified microscopically and through biochemical properties, after proper cultivation.

Selective incubation of milk samples for preliminary streptococcal isolation was performed at a temperature of 35-37 °C for 18-24 hours, promoting the growth of streptococci. Further, the selective streptococcal agar was used, followed by CHROMagar Mastitis GP, CHROMagar Step B S1, and S2 agars for the isolation of different types of streptococci to maximize their growth. In blood agar, the majority of *S. agalactiae* strains form β-hemolytic colonies which are smooth, shiny, and yellow, while α-hemolytic or non-hemolytic colonies manifest differently. On CHROMagar Step B, colonies appeared as greenish.

The differences in the morphology of the colonies of *S. agalactiae* were identified through biochemical methods, comparing them with colonies of other types of streptococci and bacteria.

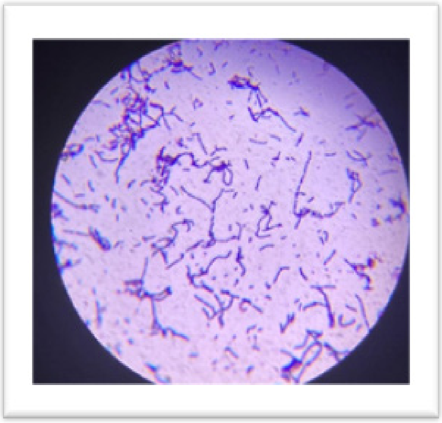
A CAMP test was performed, in which a streak of beta-toxin-producing *S. aureus* was placed at the center of blood agar. Then, perpendicular streaks of the tested beta-hemolytic streptococcal strains were made in such a way that they did not intersect the streaks of the hemolytic staphylococcal strain. The cultures were incubated at 35 ºC in a 5% CO_2 atmosphere. After incubation, the hemolytic area, indicative of the projection of the lysis zone formed by both streptococci and staphylococci, was observed where the erythrocytes had lysed.

Results

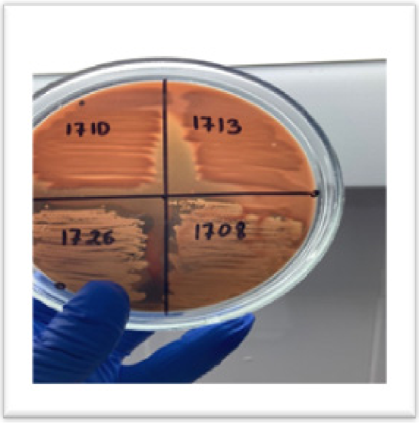
The results of the study indicate that out of 631 milk samples from animals in the region's dairy farms, 35 of them were found to be *S. agalactiae* isolates.

The characteristics of the *S. agalactiae* isolates were examined, showing that they possess the necessary properties for the growth and development of streptococci, including the presence of blood, lactose, and glucose in suitable environments.

Furthermore, the morphology and microscopic examination of the colonies of the *S. agalactiae* isolates led to the identification of their biochemical traits (figure 1).



Colored by Gram method streptococci



Hemolysis of red blood cells in blood agar

Growth of mastitis pathogens CHROMagar Mastitis GP

CHROMagar Step B *S.agalactiae* purple

Figure 1 – Morphology and biochemical properties of growths of *S. agalactiae* strains in nutrient media

S. agalactiae ferments glucose, lactose, sucrose, and maltose, but does not ferment mannitol, inulin, sorbitol, or gelatin. It also shows hyaluronidase activity.

In addition, for the differentiation of *S. agalactiae,* the CAMP test was used, with some strains of *S. aureus* producing beta-toxin, which acts synergistically with a substance (CAMP factor) produced by *S. agalactiae*. According to the literature, not all S. agalactiae strains show a positive result in the CAMP test. In our studies, 29 out of 35 strains showed a positive result in the CAMP test (82.8%).

The EUCAST recommends the use of benzylpenicillin, norfloxacin, vancomycin, tetracycline, erythromycin, and levomycetin to treat *S. agalactiae*. However, due to the wide range of antibiotics used for treating mastitis in goats, we have tested 16 different antibiotics.

The bacterial susceptibility of the 35 divided *S. agalactiae* strains, as well as at least a few to one bacterial agent, has been tested. To assess the susceptibility of bacteria to antibacterial agents, a disc-diffusion method was employed using 5% defibrinated sheep blood Mueller-Hinton agar (figure 2).

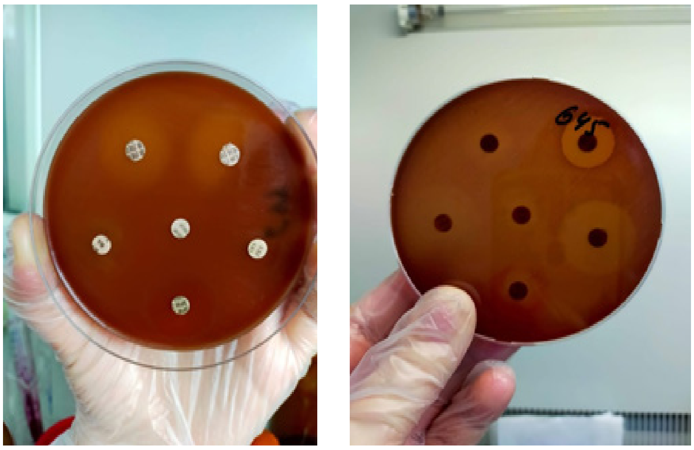


Figure – 2 Disco-diffuse method

The greatest susceptibility was demonstrated towards β-lactam agents (benzylpenicillin, amoxicillins, ampicillins), aminoglycosides (kanamycin, neomycin, gentamicin), tetracyclines (doxycycline), and the macrolide erythromycin within the tetracycline group (table 1).

Table 1– Antibioticogram of *S. agalactiae* strain

Isolates	Name of antibacterial drugs	Number of resistant strains	%
S. agalactiae	ampicillin	13	61,9
S. agalactiae	amoxicillin	17	80,9
S. agalactiae	benzylpenicillin	19	90,4
S. agalactiae	norfloxacin	6	28,5
S. agalactiae	vancomycin	5	23,8
S. agalactiae	streptomycin	9	42,8
S. agalactiae	kanamycin	16	76,1
S. agalactiae	neomycin	13	61,9
S. agalactiae	gentamicin	7	33,3
S. agalactiae	tetracycline	5	23,8
S. agalactiae	doxycycline	10	47,6
S. agalactiae	erythromycin	7	33,3
S. agalactiae	tylosin	9	42,8
S. agalactiae	sulfamethoxazole / trimethoprim	6	28,5
S. agalactiae	ciprofloxacin	5	23,8
S. agalactiae	chloramphenicol	4	19

As you can see from the table, the majority of *S. agalactiae* isolates were susceptible to benzylpenicillin (90.4%), amoxicillin (80.9%), kanamycin (76.1%), ampicillin and neomycin (61.9%), doxycycline (47.6%), tylosin (42.8%), gentamicin, and erythromycin (33.3%). The susceptibility profile of clinical and resistant strains of streptococci is shown in the figure 3.

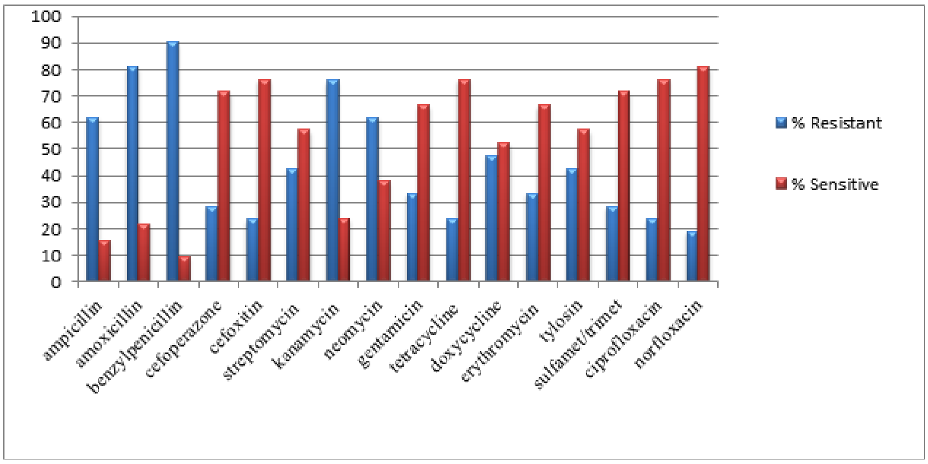


Figure 3 – Susceptibility (%) of sensitive and resistant strains of *S. agalactiae*

Discussion

According to our observations, the drugs of the natural penicillin group had the greatest activity, while resistance to tetracycline and erythromycin was quite high among all the studied groups. Fluorinated quinolones remain an effective treatment for streptococcal infections, however, an increase in the number of insensitive isolates to drugs of this group among *S. agalactiae* has been found [11].

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The majority of the isolates showed high susceptibility to β-lactam antibiotics (penicillins and cephalosporins), while demonstrating lower susceptibility to aminoglycosides, tetracyclines, and macrolides. The study highlights the varying degrees of susceptibility, ranging from mono-resistance to multidrug resistance, among the isolates.

Conclusion

Thus, various mechanisms of antibiotic resistance and ways of their acquisition by bacteria significantly complicate the process of selecting effective antibiotic therapy both in agro-industrial organizations and in medical institutions. The mechanisms of acquired and natural antibiotic resistance are inherently complex and vary from species to species, from strain to strain of microorganisms. Basically, intraspecific and interspecific acquisition of antibiotic resistance genes is carried out through horizontal transfer - conjugation, transformation, and transduction. The main measures to combat antibiotic resistance include reducing the use of antibiotics by improving the quality of life and conditions of animals; organizing and conducting monitoring and surveillance of the spread of antibiotic-resistant bacteria; developing new antibiotics and test systems for the diagnosis of antibiotic resistance of bacteria.

Authors' Contributions

GKA and MZhA: Concept development, design and planning of the study, data collection and analysis, critical review of the article and final approval, research, statistical analysis. MZhA and SKD: Sampling and delivery of samples and conducting research. All the authors have read, reviewed and approved the final version of the manuscript.

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