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Species (biological, biochemical) features of the causative agent of bovine streptococcosis isolated on the territory of Kazakhstan

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Abstract. *Ilimbayeva A, Yegorova N, Bakiyeva F, Kydyrova G, Nussupova S. 2025. Species (biological, biochemical) features of the causative agent of bovine streptococcosis isolated on the territory of Kazakhstan. Biodiversitas 26: 1114-1123.* The research was focused on identifying and characterizing streptococci responsible for mastitis in cattle, highlighting their pathogenic characteristics and antibiotic resistance. The goal was to develop more effective diagnostic, preventive, and treatment methods to reduce the impact of streptococcal infections on animal husbandry. Molecular techniques like Polymerase Chain Reaction (PCR) and DNA sequencing were used for precise species identification, while microbiological and biochemical analyses provided insights into their pathogenicity and resistance profiles. The identified streptococci included *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus bovis* complex, *Streptococcus pyogenes*, *Streptococcus zooepidemicus*, *Streptococcus canis*, and *Streptococcus suis*, and each species exhibited unique characteristics. For example, *S. agalactiae* showed beta-hemolytic activity, making it highly pathogenic to the mammary gland, while *S. dysgalactiae* demonstrated both alpha- and beta-hemolytic activity. *S. uberis* could thrive in high NaCl concentrations and showed notable enzymatic activity, and *S. bovis* complex exhibited gamma-hemolysis with the ability to grow in saline conditions. Additionally, beta-hemolytic species like *S. pyogenes*, *S. zooepidemicus*, *S. canis*, and *S. suis* highlighted their roles in respiratory diseases and interspecies transmission. The study underscores the importance of species-specific differentiation in streptococcal infections, as it is crucial for tailoring effective treatment and prevention strategies. This contributes to improved approaches in diagnosing, preventing, and treating mastitis in cattle, particularly in the context of increasing antibiotic resistance.

Keywords: Antibiotic, antibiotic resistance, hemolytic activity, mastitis, streptococci

INTRODUCTION

Streptococcosis is an infectious disease caused by bacteria of the genus *Streptococcus* (Kukeyeva et al. 2023), it is a pressing area of study for veterinary researchers, animal health professionals, and agricultural scientists. The investigation of bovine streptococcosis, caused by *Streptococcus* species in Kazakhstan, is essential due to its impact on animal health and agricultural efficiency. This infectious disease affects various livestock, presenting acutely or chronically, especially in young animals. The genus includes over 20 species, with pathogenic hemolytic strains causing purulent infections. Modern classification relies on antigenic structure, particularly group polysaccharides identified through precipitation reactions (Ospanov et al. 2024). In animal pathology, serogroups A, B, C, D, and E are most pathogenic. Streptococci exhibit significant serological, pathogenic, and genetic diversity, leading to varying infection forms across species and often complicating viral infections. They are categorized as specific disease agents or non-specific suppurative pathogens and show low environmental resistance. Reservoirs include infected young animals and adult carriers, maintaining the epizootic cycle. Variations in infection forms may result from immunological changes and the emergence of highly virulent strains (Kukeyeva et al. 2023).

Mastitis-causing streptococci produce various toxins that enhance their pathogenicity, leading to a rapid progression of infection. Erythrogenic toxin induces local inflammation, while hemolytic toxin lyses erythrocytes, indicating strain virulence, observable on blood agar. The necrotic toxin causes tissue necrosis, and leukocidin destroys leukocytes (Umitzhanov et al. 2014). Virulent strains also secrete fibrinolysin and hyaluronidase, enzymes that amplify their pathogenic effects. The most virulent streptococci are found in purulent exudates from cows with acute mastitis (Zhumanov et al. 2015). Intraperitoneal injection of 0.1-0.2 cm^3 of such exudate into mice leads to death within 24 hours, with hemolytic streptococci isolated from the heart blood of deceased animals. In cows, mastitis pathogenesis is closely linked to postpartum endometritis, where streptococci from the inflamed uterus spread hematogenous to the udder, triggering infection (Eleodoro et al. 2023).

Mendybayeva et al. (2022) and Mukhamadieva et al. (2022) consider mastitis a major economic burden for Kazakhstan's dairy industry, leading to increased antibiotic use, reduced milk production, and higher operational costs. Kirimbayeva et al. (2023) report that financial losses from mastitis go beyond reduced milk yield and milk loss, including treatment costs and mortality. About 78% of economic losses result from decreased milk production, 8% from treatment, and 14% from culling. Contaminated animal

products also pose a public health risk, potentially causing severe toxic infections in humans.

Global estimates indicate a cattle population of approximately 1.489 billion, with over 40% affected by various forms of mastitis (Livestock Primary 2022). In Kazakhstan, prevalence ranges from 20% to 40%, with subclinical mastitis occurring 2-4 times more frequently than clinical cases, often going undetected without specialized testing. The primary pathogens responsible for bovine mastitis are *Streptococcus agalactiae* (GBS, Group B *Streptococcus*), *Streptococcus dysgalactiae* subsp. *dysgalactiae* (GCS, Group C *Streptococcus*), and *Streptococcus uberis*. While *S. uberis* and *S. dysgalactiae* primarily infect animals, *S. agalactiae* also poses significant human health risks, causing severe infections in newborns, pregnant women, the elderly, and immunocompromised adults, sometimes with fatal outcomes. *S. uberis*, a major cause of mastitis in cattle, is notable for its ability to proliferate within and outside the host. According to Zhylkaidar et al. (2021), the primary sources of infection in mammary tissues include the skin and udder surface. Additionally, *S. uberis* is commonly isolated from environmental sources such as straw bedding in winter and pastures grazed by infected cattle, highlighting its adaptability and ability to survive in diverse conditions.

Various approaches and medications are used to treat mastitis, but their effectiveness varies significantly. Zhumakayeva et al. (2023) emphasize a major drawback of antibiotic treatment: the risk of milk contamination with drug residues, which poses health risks to consumers, lowers dairy product quality, and disrupts fermentation in dairy production. Furthermore, prolonged antibiotic use promotes microbial resistance, diminishing long-term treatment efficacy.

Research in Kazakhstan reveals significant diversity among *Streptococcus* species affecting cattle, presenting a major challenge to the agricultural sector. However, existing studies lack comprehensive analysis and a systematic classification of these species based on their key characteristics. This study aimed to thoroughly examine the morphological, genetic, and enzymatic traits of various *Streptococcus* strains to understand better their specificity, variability, and adaptive mechanisms to both hosts and environmental conditions.

MATERIALS AND METHODS

Procedures

The research was conducted in 2024 at the Kazakh Scientific Research Veterinary Institute LLP, Almaty, Kazakhstan. Milk samples were taken from cows with clinical and subclinical forms of mastitis. The identification of the isolated cultures was carried out based on the study of cultural and morphological, tinctorial, biochemical, and genetic properties by Bergey's Classification of Bacteria. The pathogenicity of streptococci was determined by conducting a bioassay on laboratory animals (white mice). Microorganisms were isolated from selected milk samples from animals with mastitis. Next, the cows were administered a polyvalent inactivated vaccine aimed at preventing mastitis in cattle. Inoculations from the mean milk sample of cows taken from different locations and different depths of containers were done on Meat Peptone Broth (MPB) and Meat Peptone Agar (MPA). The samples were inoculated in a thermostat for 20 hours at 37°C. Abundant growth of coccal microflora was observed in the culture. Uniform turbidity without a ring and mucous sediment was observed on the MPB. Streptococci on MPA grew in the form of small, round, opaque convex colonies with smooth edges of white or yellowish color in the S-shape. In smears prepared from daily agar cultures and Gram-stained, large Gram-positive cocci of spherical shape arranged in chains, clusters, and groups were observed.

Then, to investigate the biological properties and pathogenicity of various types of streptococci that cause mastitis in cattle, an integrated approach was applied, including not only molecular biological and microbiological methods but also epidemiological studies. As part of this approach, special attention was paid to the isolation and cultivation of streptococci from clinical samples, such as milk from infected cows. The use of MPA with blood addition for the cultivation of streptococci, the preparation of smears from daily agar cultures, and Gram staining allowed isolating and identifying Gram-positive cocci characteristic of the genus *Streptococcus*, determining the type of hemolysis on blood agar, which was the first step towards further diagnosis. Molecular diagnostic techniques such as Polymerase Chain Reaction (PCR) and DNA sequencing have been used to identify streptococcal species accurately. These methods not only accurately determined the type of pathogen but also identified specific genetic markers associated with the pathogenicity and resistance of streptococci to antibiotics. This was made possible by analyzing genes encoding virulence factors and molecules involved in resistance mechanisms.

Data analysis

The study of virulence mechanisms has been expanded by using quantitative PCR to analyze the expression of relevant genes and physiological tests on models of living organisms. Such experiments included studying the interaction between bacterial virulence factors and the host immune system and evaluating the ability of streptococci to form protective biofilms, which play a significant role in their survival and the development of antibiotic resistance. Serological identification using specific antiserums was complemented by subtler methods of assessing the immune response, allowing not only the classification of isolated strains into serotypes but also the assessment of the level and specificity of the antibodies produced in response to infection. This provided additional information for the development of vaccines and immunoprophylactic strategies. In vitro tests, such as the determination of the biochemical profile of bacteria and their ability to form biofilms, were supplemented by a comprehensive analysis of the interaction between the pathogen and the host. The study included an assessment of the effect of various environmental factors on the pathogenesis and development of mastitis and an analysis of the molecular mechanisms underlying the

interaction of the microbe with the host immune system.

The analysis of the genetic sequence of DNA of bacteria of the genus *Streptococcus* was carried out by the Restriction Fragment Length Polymorphic (RFLP) and Random Amplified Polymorphic DNA (RAPD) methods, showing a profile characteristic of streptococcal DNA. DNA isolation of streptococci was carried out using the column technique. RAPD/RFLP analysis using a primer of 15 bp, from the 3' end of the restriction site of msel (TTAA) endonuclease, often found in the streptococcal gene, with the addition of 3' end of the selective nucleotide. Serological identification of streptococci was conducted using specific antiserums to classify the isolated strains into serological groups. The streptococci were categorized into groups A, B, C, and G based on their antigenic properties. Group A streptococci were identified using specific antisera that react with the group-specific carbohydrate present on the bacterial cell wall. Similarly, groups B, C, and G were identified using their respective antisera, which recognize unique antigenic determinants on the bacterial surface. The PCR reaction was performed with universal primers 16SF 190 ATTAGCTAGTAGGTGGGGTAA and 16SR 1100 TTACTAGCGATTCCGACTTCA in a total volume of 25 µL. The PCR mixture contained 15 ng. DNA, 2.5 of the mixture (Synthol), 10 µL of deionized water, and 10 pmol of each primer. The PCR amplification program included denaturation of 94°C for 3 minutes; 27 cycles: 94°C - 30 seconds, 60°C - 30 seconds, 72°C - 30 seconds; final elongation of 7 minutes at 72°C.

Purification of PCR products from unbound primers was carried out by the enzymatic method using Exonuclease I (Fermentas) and alkaline phosphatase (Shrimp Alkaline Phosphatase, SibEnzyme). The sequencing reaction was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions, followed by fragment separation on an automatic genetic analyzer 3500 DNA Analyzer (Applied Biosystems).

Flow cytometry was actively used for the detailed analysis of streptococci associated with mastitis in cattle. This process began with the preparation of bacterial cultures grown from milk samples of infected animals. After reaching the required concentration of cells, they were treated in a standardized manner using specific labeled antibodies that bound to target antigens on the bacterial surface. This procedure helped not only to identify and quantify the presence of certain antigens but also to investigate various aspects of cellular physiology, such as viability, stages of the cell cycle, and apoptosis. Next, using flow cytometry, a large number of cells were quickly analyzed, obtaining data on the distribution and intensity of fluorescence, which reflected the expression of antigens. Thus, this method provided an opportunity for a deep understanding of the mechanisms of virulence and adaptation of streptococci to host conditions.

An important part of the process was also assessing the viability of bacterial cells. Special fluorescent dyes were used to distinguish between living, dead, and apoptotic cells. This provided valuable information for developing infection control strategies, including choosing the most effective antibiotics and evaluating their effects on the pathogen.

RESULTS AND DISCUSSION

Streptococcus spp. is a genus of Gram-positive bacteria, some species of which are significant pathogens for cattle in Kazakhstan. These species include *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Streptococcus bovis* complex, *Streptococcus pyogenes, Streptococcus zooepidemicus, Streptococcus canis,* and *Streptococcus suis.* These bacteria can cause a variety of infectious diseases, including mastitis, pneumonia, septicemia, and meningitis, which highlights their pathogenic potential for cattle (Mendybayeva et al. 2022).

S. agalactiae, or Group B *Streptococcus*, is the main causative agent of contagious mastitis, one of the most economically significant cattle diseases. This microorganism is especially dangerous for dairy herds, as it can significantly reduce milk yield and quality, leading to an increase in the content of somatic cells. Control and prevention measures include regular veterinary examinations, sanitation of milking equipment, and vaccination (Table 1). Figure 1 shows columns of mastitis streptococcus surrounded by a hemolysis zone.

Table 1. Species features and pathogenic potential of the causative agent of bovine streptococcosis isolated in the territory of the Republic of Kazakhstan

Variant	Habitat/commonly found in	Caused by the disease(s)
Streptococcus agalactiae	Milk ducts	Persistent infection, recurrent acute mastitis
Streptococcus dysgalactiae	Oral cavity, skin, genitals	Acute mastitis
Streptococcus uberis	Skin, tonsils, vagina	Clinical mastitis without systemic signs
Streptococcus bovis complex	Digestive system, feces	Calf septicemia, neonatal septicemia, meningitis
Streptococcus pyogenes	Udder	Mastitis
Streptococcus zooepidemicus	Udder	Mastitis
Streptococcus canis	-	Mastitis of dairy cattle
Streptococcus suis	Udder	Mastitis

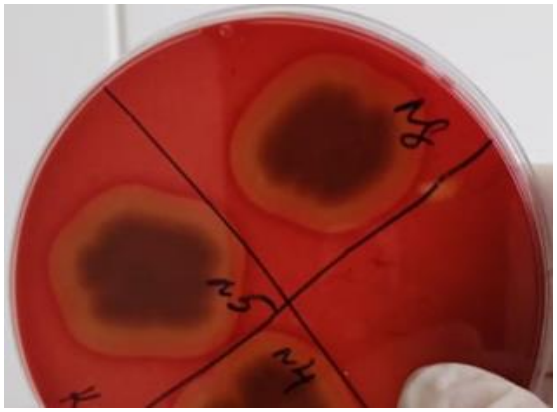

Figure 1. Colonies of *S. agalactiae* surrounded by a hemolysis zone

Figure 1 shows hemolysis on blood agar, which indicates the hemolytic activity of streptococci. *S. dysgalactiae* causes both contagious and non-contagious mastitis, and infections can be both acute and chronic. Especially dangerous is the fact that this species can survive in various environmental conditions, which complicates the fight against the spread of infection. To prevent the disease, it is critically important to maintain a high level of hygiene during milking and ensure adequate nutrition and animal care.

Streptococcus uberis, responsible for a significant proportion of cases of non-contagious mastitis, is highly adaptable to various habitats. This pathogen can be found in soil, manure, and even on animal skin, making its control particularly difficult. Strategies to control *S. uberis* include improving animal welfare and the use of targeted antibiotics after accurate diagnosis (Zhylkaidar et al. 2021).

Streptococcus bovis complex (now often called *S. equinus* complex) is important in terms of possible systemic infections such as bacterial endocarditis and septicemia. In the context of bovine cattle, this complex may be associated with digestive disorders and an imbalance of the rumen microflora. Maintaining optimal nutrition and monitoring rumen health is key to minimizing the risk of diseases associated with these bacteria (Mussayeva et al. 2023).

Although *S. pyogenes* is better known as a human pathogen that causes sore throat, scarlet fever, and a number of other infections, it can also be reported in cattle, causing mastitis and dermatitis. The interaction between animals and humans requires strict biosafety measures to prevent cross-infections. The management of *S. pyogenes* includes the use of antimicrobials to treat infected animals and the introduction of control measures to reduce the risk of infection (Li et al. 2023).

Streptococcus zooepidemicus most often causes upper respiratory tract infections, mastitis, septicemia, and arthritis in cattle. Infections caused by this species can spread both directly from animal to animal and through the environment in which they are kept. Important control measures are to maintain good ventilation in the rooms where the animals are kept and to ensure their good nutrition to maintain immunity (Smistad et al. 2022).

Streptococcus canis is relatively rare in cattle, but it can cause serious diseases, including mastitis and wound infections. Although *S. canis* is more typical for dogs, where it can cause a variety of infections, including respiratory and skin infections, it is also capable of cross-infection between species, which underscores the importance of biosecurity on farms where different animal species are present (Wataradee et al. 2023).

Streptococcus suis is one of the most important pathogens affecting pigs, but it can also cause diseases in bovine cattle. However, cases of infection among cattle in Kazakhstan are quite rare. In case of cross-infection, *S. suis* can cause septicemia, meningitis, and other serious conditions. Control and prevention include strict biosecurity measures and the isolation of sick animals (Srithanasuwan et al. 2023).

When studying the biological characteristics of various *Streptococcus* species associated with infections in cattle, both common characteristics and unique features of each species were identified. All *Streptococcus* spp. are Gram-positive cocci, which can be arranged in pairs or form chains. They are facultative anaerobes, preferring microaerophilic conditions for growth with an optimal temperature of about 35-37°C (Tables 2 and 3). Continuing to consider the biological characteristics of various *Streptococcus* species, it is important to note that their adaptations and morphological characteristics play a key role in their ability to cause infectious processes in cattle and other hosts. By examining microorganisms in a deeper context, one can better understand their interactions with the environment and the host and develop more effective methods to combat the infections they cause.

Table 2 shows that the isolated cultures had typical biological properties for bacteria of the genus *Streptococcus*. *S. agalactiae* forms cocci that tend to cluster into chains. One of the key factors of its virulence is the presence of a polysaccharide capsule, which helps to evade the host's immune response. The capsule protects against phagocytosis, which is critical for the survival and spread of bacteria in host tissues. The lack of a polysaccharide capsule in species (*S. dysgalactiae*, *S. uberis*, and *S. bovis* complex) suggests they may rely on other mechanisms for survival and virulence. The capsule typically aids in evading the host's immune response, so these species might have alternative strategies for infection and persistence. These species employ diverse mechanisms for survival and virulence, including the production of specific proteins and toxins, varying abilities to form biofilms, and distinct antibiotic resistance profiles. The genome of *S. agalactiae* contains many genes responsible for its adaptation to various hosts and living conditions. It includes genes encoding virulence factors, such as surface proteins involved in the adhesion and invasion of host cells. Virulence mechanisms include the production of cytolysins, which destroy the cell membranes of the host, and enzymes that promote the spread of infection. *S. agalactiae* can form biofilms on medical devices and internal surfaces of the host, which increases its resistance to antibiotics and contributes to chronic infection.

Table 2. Biological characteristics of *Streptococcus* bacteria isolated on the territory of the Republic of Kazakhstan

Species	Morphology	Growth conditions	Optimal temperature	Presence of a capsule	Genetic features
S. agalactiae	Gram-positive cocci	Facultative anaerobic	37°C	Yes	Polysaccharide capsule
S. dysgalactiae	Gram-positive cocci	Facultative anaerobic	37°C	No	M-protein
S. uberis	Gram-positive cocci	Facultative anaerobic	37°C	No	Unique plasmids
S. bovis complex	Gram-positive cocci	Strictly anaerobic	37°C	No	Genetic polymorphism
S. pyogenes	Gram-positive cocci	Facultative anaerobic	37°C	Yes	M-protein, exotoxins
S. zooepidemicus	Gram-positive cocci	Facultative anaerobic	37°C	Yes	Variable genes of surface proteins
S. canis	Gram-positive cocci	Facultative anaerobic	37°C	Yes	FBP54 gene
S. suis	Gram-positive cocci	Facultative anaerobic	37°C	Yes	Capsule polysaccharides

Table 3. Virulence mechanisms and serological identification of *Streptococci*

Species	Antibiotic resistance	Biofilm formation	Virulence mechanisms	Serological identification
S. agalactiae	Penicillin-G	High	Capsule, C5a peptidase	Group B
S. dysgalactiae	Erythromycin	Average	M-protein, hyaluronidase	Group C or G
S. uberis	Varies	High	Uberolysin, a fibrinogen-binding protein	Not applicable
S. bovis complex	High resistance to macrolides	Low	Enterotoxins	Not applicable
S. pyogenes	Beta-lactams	Average	Streptolysin O, DNA bases	Group A
S. zooepidemicus	Varies	High	Hyaluronic acid	Not applicable
S. canis	Varies	Low	Fibronectin-binding proteins	Group G
S. suis	High resistance to tetracyclines	Average	Suilisin, a polysaccharide capsule	Serotyping

In *S. dysgalactiae*, the morphology is also characterized by a coccoid shape formed into chains, which is typical for streptococci. The specific feature of *S. dysgalactiae* is its ability to adapt to different hosts and environments. The genome of *S. dysgalactiae* contains numerous genes responsible for its adaptation to the host and virulence, including genes encoding surface proteins, which promote its attachment to host tissues. Antibiotic resistance in this species may vary, but there is a tendency to increase resistance to traditionally used drugs, which requires constant monitoring and adaptation of treatment protocols (Zhumakayeva et al. 2023).

Streptococcus uberis is characterized by a high degree of genetic variability, which allows it to adapt to a wide range of ecological niches and hosts. Morphologically, it is similar to other streptococci, forming cocci arranged in pairs or chains. A feature of *S. uberis* is its ability to form biofilm on various surfaces, including the milk ducts, which makes it difficult to eradicate and requires an integrated approach to the treatment of mastitis. Regarding antibiotic resistance, *S. uberis* also demonstrates resistance to certain antibiotics, which is the result of both natural evolution and the pressure created by antimicrobial therapy.

Streptococcus bovis complex, which includes several species, is characterized by its role in the development of diseases of the gastrointestinal tract in ruminants. Morphologically, these bacteria are cocci capable of forming long chains. They are adapted to live in the anaerobic conditions of the gastrointestinal tract, where the optimal temperature for their growth corresponds to the body temperature of the host. An important characteristic of the *S. bovis* complex is its ability to alter the metabolism of the host, which can lead to undesirable consequences such as acidosis. The genetic characteristics of these species allow them to efficiently absorb nutrients from the ruminant diet, contributing to their rapid growth and reproduction. Despite the relatively low antibiotic resistance compared to other streptococci, the emergence of resistant strains requires careful use of antimicrobials in veterinary practice.

Morphologically, *S. pyogenes* forms cocci, which can be arranged in pairs or short chains. An important factor in its virulence is the presence of a capsule of acidic hyaluronic polysaccharide, which not only protects bacteria from phagocytosis but also promotes their penetration into host tissues. Antibiotic resistance in *S. pyogenes* may vary, but the presence of macrolide resistance genes requires special attention when choosing therapy.

A feature of *S. zooepidemicus* is its ability to spread rapidly among animal populations, which makes it an important pathogen in veterinary medicine. It has a variety of virulence genes that encode various pathogenicity factors, such as surface proteins that promote adhesion to host cells and invasion and enzymes that destroy tissues, which allows bacteria to penetrate deep tissues and spread throughout the body. In general, *S. zooepidemicus* remains sensitive to many antibiotics, such as macrolides and tetracyclines used in veterinary practice. Like many other pathogenic bacteria, *S. zooepidemicus* can form biofilms on surfaces, which can contribute to its survival in the external environment. One of the key mechanisms of *S. zooepidemicus* virulence is its ability to induce a strong immune response, which can lead to damage to host tissues. In addition, the production of streptolysins and other toxins contributes to

the destruction of cell membranes and tissues, increasing the severity of the infectious process (Bonsaglia et al. 2023).

Streptococcus canis and *S. suis* are examples of streptococci, which, although primarily associated with dogs and pigs, can pose a threat to cattle only under certain conditions. The presence of the capsule plays an important role in the virulence of *S. suis*, providing protection against phagocytosis and contributing to the evasion of the host's immune response. *S. canis* may also have a capsule that affects its pathogenic properties, but to a lesser extent compared to *S. suis*. The genetic features of both species contain many genes encoding virulence factors, such as adhesives that promote attachment to host tissues and toxins that cause damage to cells and tissues. Antibiotic resistance in *S. suis* and *S. canis* varies, but some strains show resistance to standard antibacterial drugs, which makes treatment difficult.

Next, to better understand the differences between key streptococcal species, their biochemical characteristics were considered based on a wide range of indicators. These indicators include not only catalase, coagulase, and reaction to hemolysis but also fermentation of various substrates, the ability to grow in an environment with a high concentration of NaCl, and other important factors determining their biological and pathogenic properties (Table 4).

It follows from Table 4 that the isolated cultures possessed biochemical properties characteristic of bacteria of the genus *Streptococcus*, which allowed them to be identified. The beta-hemolytic activity of *S. agalactiae* on blood agar is one of its most noticeable features, which makes it easy to identify this species due to the clean destruction zone of red blood cells around the colonies. This catalase-negative bacterium is like most other streptococci, which means there is no activity of catalase, an enzyme that breaks down hydrogen peroxide. *S. agalactiae* is unable to produce coagulase, unlike some pathogenic staphylococci, which use this enzyme to coagulate host plasma. This species also does not show growth in an environment with 6.5% NaCl, which emphasizes its sensitivity to high salt concentrations and can be used as another diagnostic marker. Esculin hydrolysis, a process in which esculin decomposes into glucose and esculetin in the presence of bile salts, is not typical for *S. agalactiae*, which also serves as an additional differentiating criterion. The ability to ferment various carbohydrates, including lactose, highlights the metabolic activity of *S. agalactiae* and its ability to adapt to diverse habitats. Carbohydrate fermentation not only plays an important role in pathogenesis but also provides another way to identify this species in the laboratory.

S. dysgalactiae demonstrates both alpha- and beta-hemolytic activity, which indicates its ability to cause partial or complete hemolysis of erythrocytes on blood agar. This diversity in the hemolysis reaction provides the key to its identification, as many other streptococci exhibit a strictly defined type of hemolysis. Like most streptococci, *S. dysgalactiae* is catalase negative, which means there is no activity of the catalase enzyme that breaks down hydrogen peroxide. Unlike some other streptococci, *S. dysgalactiae* does not show the ability to grow in an environment with 6.5% NaCl, which indicates its sensitivity to high salinity. This property helps to distinguish *S. dysgalactiae* from some species capable of surviving in more extreme conditions. Another important diagnostic feature is the ability of *S. dysgalactiae* to hydrolyze esculin. An important biochemical characteristic of *S. dysgalactiae* is its ability to ferment a variety of carbohydrates, including mannitol, which distinguishes it from some other streptococci that are not capable of fermenting this substrate. However, unlike *S. agalactiae*, *S. dysgalactiae* may not ferment lactose, which is a key difference between the two species.

Streptococcus uberis is a primary etiological agent of mastitis in cattle and is classified as an alpha-hemolytic streptococcus. Like most streptococci, *S. uberis* is catalase negative, indicating the absence of catalase, an enzyme that decomposes hydrogen peroxide. This is a key diagnostic feature that helps distinguish streptococci from staphylococci, which are catalase-positive.

Streptococcus uberis does not exhibit coagulase activity, which is another important difference from some pathogenic staphylococci that use coagulase to form protective blood clots around colonies. This feature highlights the differences in pathogenicity mechanisms between different groups of Gram-positive bacteria.

Unlike some other streptococci, *S. uberis* can hydrolyze esculin, which is a significant diagnostic feature. Regarding enzymatic activity, *S. uberis* demonstrates the ability to ferment several carbohydrates, including lactose and sucrose. This metabolic flexibility plays an important role in its ability to colonize mammary glands and cause mastitis. Fermentation of various carbohydrates also serves as an additional method for identification and classification in the laboratory.

Table 4. Biological characteristics of *Streptococcus* bacteria isolated on the territory of the Republic of Kazakhstan

Species	Catalase	Coagulase	Reaction to hemolysis	Hydrolysis of esculin	Growth in 6.5% NaCl	Fermentation
S. agalactiae	Negative	Negative	Beta-hemolysis	Negative	Negative	Lactose, maltose
S. dysgalactiae	Negative	Negative	Alpha- or beta- hemolysis	Positively	Negative	Sucrose, lactose
S. uberis	Negative	Negative	Alpha-hemolysis	Positively	Positively	Lactose, sucrose
S. bovis	Negative	Negative	Gamma-hemolysis	Negative	Positively	Does not ferment
S. pyogenes	Negative	Negative	Beta-hemolysis	Negative	Negative	Lactose, maltose
S. zooepidemicus	Negative	Negative	Beta-hemolysis	Positively	Negative	Sucrose, lactose
S. canis	Negative	Negative	Beta-hemolysis	Positively	Negative	Sucrose, maltose
S. suis	Negative	Negative	Alpha- or beta- hemolysis	Positively	Negative	Glucose, sucrose

Streptococcus bovis, currently classified within the *S. bovis* complex and often specified as *S. gallolyticus*, is a significant microorganism in clinical microbiology and veterinary medicine. This type of *Streptococcus* exhibits a unique biochemical profile that contributes to its identification and distinction from other streptococci. The hemolytic activity of *S. bovis* can vary, but most often, it demonstrates alpha-hemolysis or gamma-hemolysis (absence of hemolysis), which makes it a distinctive feature on blood agar. This ability to alpha-hemolysis is associated with the production of partial destruction of red blood cells, which leads to the formation of greenish staining on agar. In addition, *S. bovis* can grow in an environment with 6.5% NaCl, which distinguishes it from many other streptococci that are unable to survive in similar conditions. Regarding enzymatic activity, *S. bovis* is characterized by the ability to ferment glucose, but unlike some other streptococci, it may not ferment lactose. This specificity in carbohydrate metabolism is another factor that helps in its identification.

A key feature of *S. pyogenes* is its beta-hemolytic activity on blood agar, which means the bacterium's ability to destroy red blood cells, forming clear, transparent zones around colonies. This characteristic makes *S. pyogenes* easily distinguishable on a beta-hemolytic background and is an important diagnostic feature. The lack of coagulase activity in *S. pyogenes* is also an important differential indicator since coagulase is usually associated with some types of staphylococci and promotes the formation of blood clots around bacterial colonies, which protects them from the host immune system. Regarding the ability to hydrolyze esculin, *S. pyogenesis*, as well as *S. bovis* and *S. agalactiae*, usually do not demonstrate this activity, which distinguishes it from some other streptococcal species capable of hydrolyzing esculin to form a dark pigment in the medium. The growth of *S. pyogenes* in an environment with 6.5% NaCl is usually suppressed, which reflects its sensitivity to high salinity and helps to differentiate it from some salt-resistant microorganisms. In terms of fermentation, *S. pyogenes* can ferment a variety of carbohydrates, which emphasizes its metabolic flexibility and ability to adapt to various environmental conditions. This fermentation ability helps in the diagnosis and classification of the bacterium.

Streptococcus zooepidemicus exhibits beta-hemolytic activity on blood agar, destroying red blood cells and leaving a transparent halo around the colonies. A feature of *S. zooepidemicus* is its ability to hydrolyze esculin, demonstrating activity in the presence of bile salts. *Streptococcus zooepidemicus* is usually unable to grow in an environment with a high concentration of NaCl, for example, 6.5% NaCl. Regarding carbohydrate metabolism, *S. zooepidemicus* demonstrates the ability to ferment various carbohydrates, including lactose, which is an important factor for its energy metabolism and can be used for biochemical identification.

Streptococcus canis and *S. suis* demonstrate the characteristic features of streptococci, including catalase negativity and hemolysis. However, they also have specific characteristics that determine their interaction with the host and the diseases they cause. Beta-hemolytic activity is found in both species on blood agar, which indicates their ability to destroy red blood cells. Another common feature is complete hydrolysis of esculin and lack of growth in the presence of 6.5% NaCl. The enzymatic activity of *S. suis* includes the ability to ferment various carbohydrates, including glucose and maltose, which emphasizes its metabolic activity and can serve as an additional criterion for its identification. However, the ability to ferment other substrates, such as lactose, may vary, which highlights the need for a comprehensive approach to the diagnosis of this species. While *S. canis* demonstrates the ability to ferment several sugars, including maltose and trehalose, but may not ferment other sugars, which serves as an important differential criterion.

Streptococcal infections, particularly those caused by *S. agalactiae* (Group B *Streptococcus*, GBS), pose a significant zoonotic risk, meaning they can be transmitted from animals to humans. GBS is known to cause severe infections in newborns, pregnant women, the elderly, and immunocompromised individuals. The transmission can occur through contact with infected animals or consumption of contaminated dairy products. In humans, GBS can lead to serious health implications such as sepsis, meningitis, and pneumonia, which can be life-threatening if not treated promptly. The health implications extend to increased medical costs, prolonged hospital stays, and potential long-term health complications for survivors. Effective prevention strategies include stringent hygiene practices in animal husbandry, pasteurization of dairy products, and regular veterinary check-ups to monitor and control streptococcal infections in livestock (Li et al. 2023).

Several factors, including housing conditions, feeding practices, and the presence of concurrent infections, influence the transmission of streptococcal infections in cattle. Overcrowded and poorly ventilated housing conditions can exacerbate the spread of infection due to increased contact between animals and higher concentrations of airborne pathogens. Inadequate feeding practices, such as poor-quality feed or insufficient nutrition, can weaken the immune system of cattle, making them more susceptible to infections. Concurrent infections, such as viral or other bacterial infections, can further compromise the immune response, facilitating the establishment and spread of streptococcal infections. Additionally, improper hygiene and sanitation practices, including inadequate cleaning of milking equipment and living areas, can contribute to the persistence and transmission of streptococci. Effective management strategies include ensuring adequate space and ventilation, providing high-quality feed, maintaining strict hygiene protocols, and implementing regular veterinary check-ups and vaccination programs (Maslov et al. 2023).

Streptococcal infections, particularly mastitis, have a substantial financial impact on livestock production. Direct losses include reduced milk yield and quality, increased veterinary costs, and higher mortality rates among infected animals. Indirect losses encompass the costs associated with treatment, including antibiotics and supportive care, as well as the economic burden of culling affected animals. The contamination of dairy products with antibiotic residues can further lead to lower product quality and potential market rejection, affecting revenue. Additionally, the need for enhanced biosecurity measures and improved housing conditions to prevent the spread of infection adds to

operational costs. The cumulative effect of these direct and indirect losses can significantly strain the financial viability of livestock operations, underscoring the importance of effective prevention and control strategies to mitigate the economic impact of streptococcal infections (Putnam et al. 2023).

Discussion

The results obtained provided a fairly wide range of species features to differentiate them from each other and other bacteria. Nevertheless, for a single study, it is necessary to compare it with other papers on similar topics to confirm their objectivity and relevance mutually. As in the above study, the streptococci strains considered by Chen et al. (2022) and Han et al. (2022) were Gram-positive cocci, immobile, and did not form spores, being arranged in pairs or the form of short chains. A negative reaction to catalase was typical for all strains. Under inoculation conditions in a broth with glucose addition and in a heart-brain broth, the strains showed uniform growth, while no gas formation was observed in the MRS broth. Under aerobic conditions on sheep blood agar, the hemolysis reaction was α-hemolytic or absent altogether. The tellurite test showed a negative result. Except for the reference strain *Enterococcus saccharolyticus*, none of the strains showed growth on bile-esculin agar and in broth with 6.5% NaCl. This indicates that the basic characteristics of the causative agent of streptococcosis do have certain trends characteristic of all types and can be used for the initial stage of differentiation of diseases in the Republic of Kazakhstan.

The nomenclature and classification of the genus *Streptococcus* have undergone numerous and diverse changes. There are 11 species and 4 subspecies of beta-hemolytic streptococci that can be identified by classification of Hassan et al. (2022) and Maćešić et al. (2022), who used various phenotypic tests, and almost all of them were identified in the study of animal infections. In the category of non-beta-hemolytic streptococci, there are 26 species of *Streptococcus viridans*, 5 species belonging to the *S. bovis* group, 5 species characterized by a lack of nutrients, 9 species of other streptococci, and 3 new genera of Gram-positive cocci forming chains. Most of the changes in the classification of non-beta-hemolytic streptococci consisted of expanding the list of species, which greatly complicated the process of identifying each species. Thus, according to the established biochemical and biological characteristics, it is possible to determine the resulting species in the category of S. *viridans* and non-beta-hemolytic streptococci, which is much more important because the latter category has been studied rather insufficiently.

Despite the successful production of isolates not typical for cattle in the above study, Dong et al. (2023) and Smistad et al. (2022) indicate that there is practically no information on the detection and molecular analysis of *S. suis* from clinical samples of cattle, which may be due to difficulties in identifying alpha-hemolytic streptococci at the species level in many laboratories due to the complexity of this procedure. As a result, *S. suis* isolates often remain undetected, and, accordingly, infections caused by this species may not receive proper diagnosis. An additional difficulty is the variability of the biochemical characteristics of *S. suis*, which further complicates the process of their identification. However, due to a comprehensive analysis focusing on the biochemical and biological characteristics of the causative agent of bovine streptococcosis isolated in the territory of the Republic of Kazakhstan, it was possible to accurately identify *S. suis*, which makes it possible to prescribe effective treatment more precisely.

To expand information about the most widespread type of *Streptococcus*, *S. uberis*, the studies by Barsi et al. (2022) and Doan et al. (2022) were considered. A study of 27 *S. uberis* isolates for their ability to form biofilms on a polystyrene surface and produce extracellular polysaccharides on Congo agar showed that the addition of milk significantly enhances the formation of biofilms by these isolates. These data indicate the possible influence of milk or its components on the development of mastitis through stimulation of biofilm formation. The study of the role of individual milk components in this process, conducted by Bonsaglia et al. (2023), found that casein and β-lactoglobulin in concentrations typical for in vivo conditions do not affect the formation of biofilms. Similarly, the fat content of milk does not affect biofilms since both skimmed and whole milk showed similar results. Lactoferrin also did not play a significant role in the process for most *S. uberis* isolates. However, the use of fructose, glucose, or sucrose stimulated the formation of biofilms, while lactose reduced it. This may explain the observed decrease in the attachment of *S. uberis* biofilms in the presence of raw milk, probably due to exposure to lactose. Iron restriction led to a decrease in the formation of biofilms by isolates without reducing the number of cells. In addition, it was found that the flora naturally present in raw milk contributes to the formation of *S. uberis* biofilms since their number decreased after the removal of this flora from milk. These papers more accurately describe the biofilm formation process of *S. uberis*, which confirms the observations in the above study and adds important components to this process.

The reference strain of *S. bovis* was isolated from a 3-year-old Jersey cow in the study by Maslov et al. (2023) and Zouharova et al. (2023). The bacteria described are Gram-positive, immobile cocci, forming chains with a diameter of approximately 0.5 μm, lacking catalase and coagulase activity. On Colombian agar with 5% addition of defibrinated sheep blood or on agar with 5% defibrinated horse blood, the strain forms translucent colonies with a diameter of about 1 mm after 48 hours of inoculation at 37°C in an atmosphere with 5% CO_2, demonstrating gamma-hemolytic activity. Biochemically, strain NZ1587 T showed positive results for arginine dihydrolase, β-glucosidase, α-galactosidase, β-glucuronidase, β-mannosidase, Voges-Proskauer on acetone production, hippurate hydrolysis, alanyl-phenylalanyl-proline-arylamidase, glycol-tryptophanarylamidase; negative results were obtained for alkaline phosphatase and ureases. This strain is also capable of producing acid from carbohydrate sources such as ribose, mannitol, sorbitol, lactose, trehalose, raffinose, sucrose, pullulan, maltose, melibiose, methyl-β-d-

glucopyranoside, and tagatose, but not from l-arabinose, d-arabitol, cyclodextrin, and glycogen. Agglutination with Lancefield sera AD and FG did not occur. There are noticeable similarities when drawing parallels between the above and these studies, which confirm the reliability of the data obtained and instill confidence in the research.

According to the standards for antimicrobial sensitivity testing developed by Li et al. (2023), Ostler and Jones (2023), and Woudstra et al. (2023), streptococcal strains were evaluated as sensitive, resistant, or having an intermediate degree of sensitivity to various antibiotics. Sensitivity analysis of the isolates revealed that up to 100% of them showed sensitivity to aminoglycosides, including kanamycin, gentamicin, neomycin, and tobramycin, and 70.5% were sensitive to streptomycin. However, all 105 tested isolates showed resistance to beta-lactam antibiotics such as penicillin, amoxicillin, ceftazidime, and ceftriaxone, with a resistance level of up to 98.1%. Piperacillin resistance was observed in 29.5% of cases. In the study of streptococcal species isolated in Kazakhstan, the situation had an almost similar appearance. This indicates rapidly developing antibiotic resistance, which can have severe consequences.

Considering streptococci not only as pathogens but also as laboratory models, Ancuelo and Perez (2023), Ma et al. (2023), and Wataradee et al. (2023) found that hyaluronate lyase obtained from the strain *S. agalactiae* 4755 by a biotechnological method, which provides it with a higher level of purity in comparison with extracts from testicles, demonstrates the preservation of enzymatic activity after mild proteolytic cleavage by trypsin. In the context of potential therapeutic use, enzymatically active fragments with low molecular weight attract special attention due to the supposed advantages of their pharmacokinetic characteristics compared to the original protein. These important studies show a different opinion on a familiar pathogen and contribute to the development of its study and use for practical purposes.

Exploring the possibilities of *S. uberis* survival in the external environment, Eleodoro et al. (2023), Putnam et al. (2023) and Srithanasuwan et al. (2023) focused their attention on evaluating the ability of 5 *S. uberis* strains of the mastitis pathogen with natural reservoirs in the environment to survive and reproduce on 3 different types of bedding (sand, wheat straw, and pine sawdust dried in an oven). The bedding, sterilized in advance, was seeded with *S. uberis* and kept at room temperature. Monitoring of the number of bacteria over time revealed an increase in the number of *S. uberis* on all types of bedding, indicating that the presence of feces and urine may contribute to their reproduction. *S. uberis* remained viable for at least 35 days on straw and sand, while bacteria could not be detected on pine sawdust, both new and used, for more than 7 days. The results emphasize the importance of litter selection and management for controlling the survival of *S. uberis* in agricultural conditions.

In addition, as one of the possible omissions of the above study, Keyvan (2023) and Woo et al. (2023) presented a description of a new species of *Streptococcus pseudo-agalactiae* isolated from the udder of a bull. In biochemical reactions, this organism practically does not differ from *S. agalactiae*, except that in most cases, milk does not curdle, although sometimes clotting can occur after incubation for 7 days. There was also a slight decrease in the concentration of methylene blue, which is usually not typical for typical *S. agalactiae* cultures. According to Plastridge and Hartsell, although *S. pseudo-agalactiae* cultures often showed less hemolytic activity compared to *S. agalactiae* and the reactions of these 2 species in litmus and methylene milk sometimes differed slightly, none of the biochemical tests performed provided clear results sufficient for differentiation purposes. Based on this, it can be assumed that this species is also present in the Republic of Kazakhstan, but due to the complexity of differentiation with the main species and their similarity, *S. pseudo-agalactiae* has not been identified.

The results show that all species of bacteria of the genus *Streptococcus* are represented by Gram-positive cocci that tend to form pairs or chains. These organisms, being facultative anaerobes, prefer conditions with a low oxygen content and an optimal temperature in the range of 35-37°C for their growth. Based on the analysis of biological and morphological characteristics, it can be argued that these features play an important role in the ability of streptococci to cause infectious diseases in livestock and other animals. These characteristics contribute significantly to their pathogenic potential in livestock and other animals. This study identified several species, including *S. agalactiae*, *S. dysgalactiae*, *S. uberis*, *S. bovis* complex, *S. pyogenes*, *S. zooepidemicus*, *S. canis*, and *S. suis*, each with distinct biochemical and biological properties influencing their role in diseases like mastitis. For instance, *S. agalactiae* exhibits beta-hemolysis and high pathogenicity toward mammary glands, while *S. uberis* shows alpha-hemolysis and salt tolerance. Similarly, *S. pyogenes* and *S. zooepidemicus* are linked to respiratory infections, and *S. bovis* complex to gastrointestinal diseases. Research limitations include restricted access to diverse livestock populations and ethical challenges in animal experimentation. Future research should focus on molecular genetics, host-pathogen interactions, and microbiome studies to enhance understanding and develop targeted therapies.

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