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Identification of *Pasteurella multocida* Isolates using Different Methods

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ABSTRACT

The study aims to identify the species and serotypes of pasteurellosis pathogen isolates in cattle and saigas and to distinguish different species within this genus by analyzing their cultural, biological, and biochemical features. A commercial PCR kit was used to identify and confirm the pathogen *Pasteurella multocida*. Pasteurellosis pathogen isolates were fully adapted on nutrient agar and broth with 10% cattle blood serum. The isolates showed no motility in the experiments, which is characteristic of the pasteurellosis pathogen. The biochemical features of microorganisms were studied using Hiss's medium. Cultures were grown according to the generally accepted methodology. After 16 and 24 hours of incubation in the thermostat, the results of carbohydrate fermentation were recorded based on changes in the color of the nutrient medium and the formation of gaseous substances. After examining the cultural and biochemical properties of the two isolates, PCR was conducted to re-confirm the results. The studies demonstrated that both pasteurellosis pathogen isolates belonged to *P. multocida*.

Key words: Saiga, Cattle, Pasteurellosis, Identification, Differentiation, Biochemical features

INTRODUCTION

The development of contemporary animal husbandry is hindered by several unresolved veterinary issues associated with ensuring animal health and the epizootic welfare of farms (Anokhina et al. 2020). The conditions of industrial animal husbandry create prerequisites for the emergence and intensification of the epizootic process of diseases with alimentary and airborne modes of transmission (Mussynov et al. 2019). One such disease is animal pasteurellosis (Absatirov and Ishchanova 2018; Ishchanova et al. 2018a). Pasteurellosis is a highly contagious disease of many species of agricultural, synanthropic, wild animals, fur-bearing animals, and birds with high lethality and a tendency to stationarity. The annual incidence of pasteurellosis in animals testifies to the tense epizootic and epidemic associated with this disease (Ichshanova et al. 2018a; Kirkimbaeva et al. 2018).

Pasteurellosis continues to be an urgent issue in animal husbandry. It is an infectious disease of agricultural, domestic, and wild animals, birds, and humans that differs in its localization and course. Pasteurellosis is caused by bacteria of the genus *Pasteurella*, including six species: *P. multocida*, *P. haemolytica*, *P. ureae*, *P. pneumotropica*, *P. aerogenes*, and *P. gallinarum*. *P. multocida* and *P. haemolytica* are distinguished by major etiological significance in the infectious pathology of animals and birds.

P. multocida is the pathogen of hemorrhagic septicemia in animals, avian cholera, and pulmonary pasteurellosis, which complicate respiratory infections of viral and mycoplasma etiology. *P. haemolytica* causes pneumonia in cattle and sheep of all ages and septicemia in newborn lambs (Mussayeva et al. 2021; Suchshikh et al. 2023).

Of crucial importance for eliminating and preventing pasteurellosis is its timely and accurate diagnosis, which largely determines the effectiveness of treatment and veterinary and sanitary measures (Madenova et al. 2019). Any diagnostic method can only be used with other

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diagnostic methods, considering the results of pathologic-morphologic and laboratory tests (Mussayeva et al. 2021). The totality of information on epizootiology and the clinical and pathologic-anatomical picture allows for a preliminary diagnosis. In contrast, the results of bacteriological and molecular-biological studies allow for a definitive diagnosis. The progress made in studying this disease enables one to reduce the incidence of pasteurellosis in animals, but its level is still relatively high. Pasteurellosis of farm and wild animals occupies a leading position in infectious pathology and causes significant economic damage (Taubaev 2004; Kirkimbayeva et al. 2014; Taubaev et al. 2024).

In recent years, methods based on the study of morphological, cultural, tinctorial, biochemical, antigenic, and biological properties have been supplemented by modern techniques of molecular biology (Kirkimbaeva and Oryntaev 2011; Ishchanova 2012; Taubaev 2012). PCR diagnostics allow one to identify the pathogen directly. A significant distinctive feature of the PCR method is determined by the fact that it detects an original DNA region typical only of a given pathogen in the tested material. The significant sensitivity of this method allows one to detect even a small number of microorganisms. Another advantage of this method is that the results are obtained quickly, which allows one to detect and diagnose bacteria carriers (Ichshanova and Radojicic 2018; Ichshanova et al. 2018b).

It is important to study the distribution and incidence of diseases involving Pasteurellaceae bacteria in cattle and saigas in this context. This study aims to identify the species and serovar of pasteurellosis pathogen isolates in cattle and saigas and differentiate the species of this genus based on cultural, biological, and biochemical features.

MATERIALS AND METHODS

The authors confirm compliance with the regulatory and legal framework of the Republic of Kazakhstan, precisely the "Guidelines for Conducting Work with Laboratory (Experimental) Animals in Preclinical (Non-Clinical) Studies," developed by the Recommendations of the Eurasian Economic Commission Board No. 33, dated November 14, 2023.

The research was carried out based on Contract No. 131 of November 17, 2023, as part of the project AR15473404, "Intensity of pasteurellosis during the seasonal migration of saigas and its association with the occurrence of the disease in farm animals". Within the framework of this Contract, biomaterials (parenchymatous organs with regional lymph nodes, 20 samples) obtained from fallen and diseased animals (biomaterials from three cattle and two saigas) were delivered from the West Kazakhstan Agrarian-Technical University named after Zhangir Khan (Uralsk, Kazakhstan) to the Research Institute of Biological Safety Problems for further study.

We cultured 20 organ samples from dead saigas and cattle on Hottinger's nutrient agar. In addition, the 20 samples were tested for pasteurellosis pathogen DNA with PCR. DNA was isolated from the samples using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Electrophoresis was performed in a polyacrylamide gel.

Pasteurellosis pathogen culturing

The pasteurellosis pathogen was cultured on nutrient agar with 10% cattle blood serum with incubation for 24-48 hours at 37°C. We conducted experiments on their differentiation to determine the species and serovar of pasteurellosis pathogen isolates (Table 1).

Hemolytic activity assay procedure

Broth cultures of the tested samples were cultured on blood agar (nutrient agar with 5% defibrinated sheep blood). Here and in subsequent cases, the cultures were incubated at 37±1°C. We recorded the results daily (after 24 and 48 hours) by measuring the width of the hemolysis zone (a clearly limited, completely transparent zone due to the destruction of erythrocytes).

Indole detection

Indole was detected using indicator papers. The indicator papers were prepared from 10-12cm long strips of filter paper soaked in hot oxalic acid, dried in a thermostat, and stored in a jar with a lapped stopper.

Determination of urease

Two to three drops of 24h broth culture were poured into a tube containing urea medium. The cultures were cultured for 20-24 hours at 37ºC.

Biological testing

The pathogenicity of cultures was determined on white mice weighing 16-18g. Two white mice were given a hypodermic injection of 0.2cm^3 of 18-24-hour broth culture.

Biochemical properties of museum strains of the pasteurellosis pathogen

The experiments were conducted using ready-made media containing different carbohydrates (lactose, glucose, maltose, sucrose, mannitol) (Table 1). The growth of pasteurellosis strains on different nutrient media is described in Table 2.

Sensitivity of pasteurellosis isolates to benzylpenicillin sodium salt

The experiments to study the isolates' sensitivity to this antibiotic used preparations with antibiotic content of 1,000, 100, 10, 1, 0.1, and 0.01IU/mL. The strains were grown on nutrient agar. The cup with the lid ajar was dried for 30min, and then a sterile filter paper (round shape) soaked with the respective antibiotic solutions was placed in the center of the dried drop.

Sensitivity of pasteurellosis isolates to gentamicin sulfate

The strains were tested using the following concentrations of gentamicin: 50, 5, 0.5, 0.05, 0.005, and 0.0005mg/mL. After sowing the strains, filter paper soaked with the doses of gentamicin was applied to nutrient agar. The results were recorded after 24 hours of incubation at 37°C.

Sensitivity of pasteurellosis isolates to ampicillin

The experiments were carried out using 100, 10, 1, 0.1, 0.01, and 0.001mg/mL of ampicillin. After these doses of the antibiotic were applied, the strain cultures were incubated at 37°C for 24 hours.

Table 1: Variations of Hiss's serum water medium

Hiss's serum water medium	Pancreatic hydrolysate of fish meal (g/L)	Sodium chloride (g/L)	Disodium phosphate (g/L)	Primary carbohydrate (g/L)	Bromothymol blue (g/L)	Agar (g/L)
GRM with lactose	6.0	3.5	0.2	Lactose 3.5	0.04	3.5±0.2
GRM with glucose	6.0	3.5	0.2	Glucose 3.5	0.04	3.5±0.2
GRM with maltose	6.0	3.5	0.2	Maltose 3.5	0.04	3.5±0.2
GRM with sucrose	6.0	3.5	0.2	Sucrose 3.5	0.04	3.5±0.2
GRM with mannitol	6.0	3.5	0.2	Mannitol 3.5	0.04	3.5±0.2

GRM - The nutrient medium is intended to identify enterobacteria by testing the fermentation of one of the carbohydrates (lactose, glucose, sucrose, maltose) or polyhydric alcohol (mannitol). It is a fine, hygroscopic powder of a light-yellow color.

Table 2: The growth of Pasteurella strains on different nutrient media

Growth medium for pasteurellosis strains	Primary components (g/L)	Incubation
Endo-GRM	Pancreatic hydrolysate of fish meal – 12.0; Yeast extract – 1.0; Lactose – 10.0; Basic fuchsin – 0.2; Sodium chloride – 3.4; Agar – (10.0±3.0); pH 7.4±0.2	18-20 hours at 37°C
Kligler-GRM	Pancreatic hydrolysate of fish meal – 20.5; Yeast extract – 3.0; Lactose – 20.0; Glucose – 1.0; Ferrous sulfate – 0.2; pH 7.4±0.2	18-20 hours at 37°C
Simmons' citrate agar	Ammonium phosphate – 1.0; Magnesium sulfate – 0.4; Trisodium citrate – 2.0; Sodium chloride – 5.0; Bromothymol blue – 0.06; pH 6.7±0.15	24 hours a day for 5 days at 37 and 22°C
Polymyxin medium	Polymyxin – result after 24 hours of incubation	24 hours at 37°C
Nutrient agar (GRM-agar)	Pancreatic hydrolysate of fish meal – 12.0; Fermentative peptone – 12.0; Sodium chloride – 6.0; Agar – (10.0±2.0); pH 7.1-7.5	Standard incubation at 37°C

Sensitivity of pasteurellosis isolates to streptomycin

The experiments used 100, 10, 1, 0.1, 0.01, and 0.001mg/mL of streptomycin. After applying these antibiotic doses, the strain cultures were incubated at 37°C for 24 hours.

Sensitivity of pasteurellosis isolates to tetracycline

The experiments used 100, 10, 1, 0.1, 0.01, and 0.001mg/mL of tetracycline. After applying these antibiotic doses, the strain cultures were incubated at 37°C for 24 hours.

Research stages

Laboratory diagnosis of infectious diseases consists of obtaining a pure culture of the pathogen from clinical or pathological material and then typifying it. Molecular genetic methods can provide the most reliable results, but the requirements for their application often need to be revised for low-income laboratories. Our study identified the bacteria using the recommended traditional methods of determining the pathogen's cultural, biological, and biochemical properties (Taubaev et al. 2024).

We used a commercial PCR kit to identify and confirm the pathogen *Pasteurella multocida*. PCR was carried out according to the instructions attached to the kit. This method produced a PCR product 144bp in two samples: No. 4 (liver from saiga-1) and No. 13 (liver from cattle-2). The culture and biochemical properties of the isolates were further studied. The colonies formed were assessed visually (Fig. 1 and 2).

Next, ten consecutive inoculations were performed to adapt the isolates to nutrient media (nutrient agar and broth produced by Himedia). At the end of each experiment, we examined colony growth and virulence morphology in white mice and performed Gram staining with methylene blue.

The antibiotics used in the experiment included sodium salt, lincomycin, gentamicin, ampicillin, streptomycin, and tetracycline. Different concentrations of antibiotics were tested to study the sensitivity of pasteurellosis pathogen isolates to antibiotics.

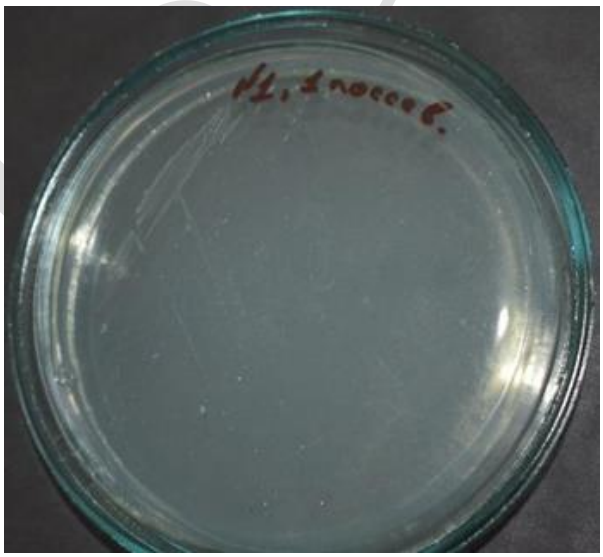

Fig. 1: The colony of isolate No. 1 on nutrient agar with 10% cattle blood serum.

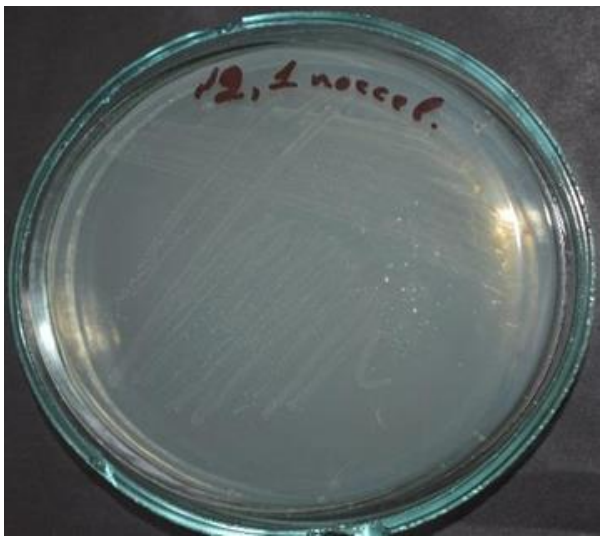

Fig. 2: The colony of isolate No. 2 on nutrient agar with 10% cattle blood serum.

RESULTS

Fig. 3 shows the PCR results. During the studies, we fully adapted the pasteurellosis pathogen isolates on nutrient agar and broth with 10% cattle blood serum. These isolates and strains were cultured for 20-48 hours at 37°C. In differentiating the samples, we relied on the standard operating procedure for laboratory diagnostics of pasteurellosis by the bacteriological method (Table 3).

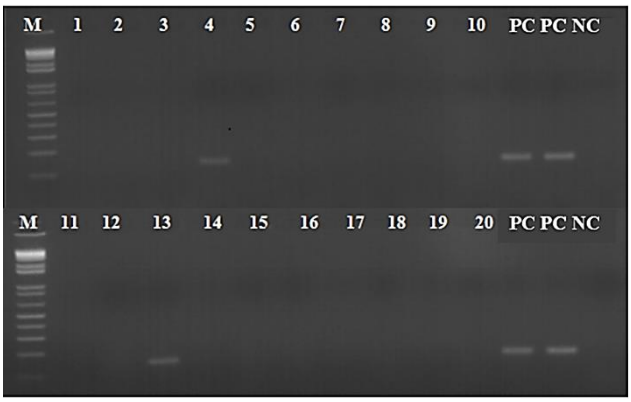

Fig. 3: PCR results to detect the pathogen *P. multocida*: M – marker; samples 1-20; PC – positive control; NC – negative control.

In the experiments, the isolates were immotile, characteristic of the pasteurellosis pathogen. The hemolytic activity test showed that the pasteurellosis pathogens did not cause hemolysis of ram erythrocytes. The tested samples did not hemolyze ram erythrocytes after 48h of cultivation at 37°C, characteristic of the pasteurellosis pathogen.

To detect indole, a strip of indicator paper was placed under a cotton plug in a tube with meat peptone broth after sowing the culture so that the lower end of the paper did not touch the medium. Then it was incubated at 37°C for 24-72 hours. When indole was isolated, the lower part of the paper turned pink. The next step was to determine the presence of urease. The medium was reddened in the presence of the urease enzyme, but the pasteurellosis pathogen did not cause it to turn red.

The virulent strains of *P. multocida*, belonging mainly to serovar B and the pathogens of hemorrhagic septicemia, kill infected white mice within 24-72 hours. The weakly virulent strains of serovars A and E, involved in the development of pneumonia, cause death after a more extended period (up to 7 days).

P. haemolytica can cause death in white mice only through intraperitoneal infection. Other *Pasteurella* species are generally non-pathogenic to laboratory animals.

After infection with the tested samples, mice died after 36 and 42 hours, while control mice remained alive for 10 days. Based on the results, we compiled the differential Table 4.

The *biochemical features* of the isolates were studied using different media with carbohydrates. Microorganisms that do not ferment carbohydrates do not change the medium's color. If carbohydrates or polyatomic alcohol are fermented with the formation of acidic products, the medium changes color. When isolates are sown on Hiss's medium-GRM with lactose, carbohydrates are not fermented.

When the isolates were grown on Hiss's medium-GRM with glucose, the carbohydrate was fermented (the medium is stained yellow) (Fig. 4), while the carbohydrate did not ferment when isolates were grown with maltose on Hiss's medium-GRM. Carbohydrate fermentation was observed when the isolates were grown on Hiss's medium-GRM with sucrose (the medium is stained yellow) (Fig. 5). When the isolates were sown on Hiss's medium-GRM with mannitol, carbohydrate fermentation occurred (the medium is stained yellow) (Fig. 6).

Table 3: Key differentiating features of *Pasteurella* species

Indicators	*P. multocida*	*P. pneumotropica*	*P. haemolytica*	*P. ureae*	*P. aerogenes*	*P. gallinarum*
Motility	-	-	-	-	-	-
Hemolysis on blood agar	-	-	+	-	-	-
Formation of indole	+	+	-	-	-	-
Presence of urease	-	+	-	+	+	-
Bioassay on mice, death after	under 7 days	-	-	-	-	-

Note; 1 – "+" – positive result; 2 – "-" – negative result

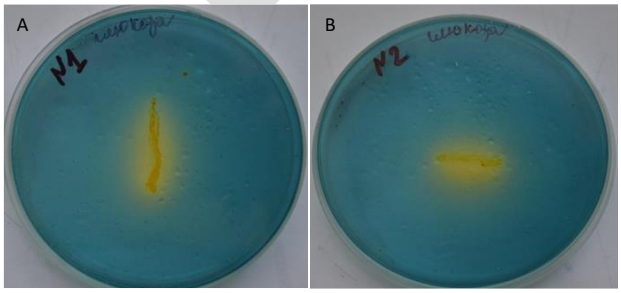

Fig. 4: The growth of pasteurellosis isolates on Hiss's medium – GRM with glucose; A – Hiss's medium – GRM with glucose, contaminated with isolate No. 1; B – Hiss's medium – GRM with glucose, contaminated with isolate No. 2.

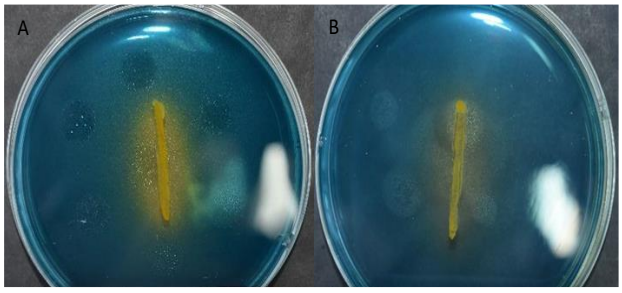

Fig. 5: The growth of pasteurellosis isolates on Hiss's medium – GRM with sucrose; A – Hiss's medium – GRM with sucrose, contaminated with isolate No. 1; B – Hiss's medium – GRM with sucrose, contaminated with isolate No. 2.

Table 4: Differentiation of the tested pasteurellosis pathogen strains

No.	Indicators	Isolate No. 1	Isolate No. 2
1	Motility	-	-
2	Hemolysis on blood agar	-	-
3	Formation of indole	+	+
4	Presence of urease	-	-
5	Bioassay on mice, death after	36h 35min	42h 06min

Note: 1 – "+" – positive result; 2 – "-" – negative result

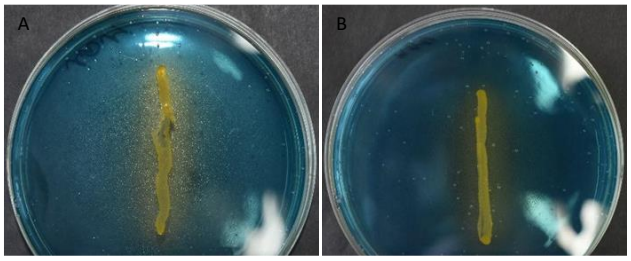

Fig. 6: The growth of pasteurellosis isolates on Hiss's medium – GRM with mannitol. A – Hiss's medium – GRM with mannitol, contaminated with isolate No. 1; B – Hiss's medium – GRM with mannitol, contaminated with isolate No. 2.

Once the bacteria were sown, the results were recorded after 18-20 hours of incubation at 37°C based on the presence of bacteria. Lactose-negative bacteria on the Endo medium form transparent (translucent) colorless colonies and may also form pale pink colonies. Lactose-positive bacteria form red colonies with or without a metallic sheen. The growth of Gram-positive microflora on the medium is completely inhibited.

The experimental data suggested that all pasteurellosis pathogen isolates cannot grow on Endo-GRM agar. The fermentation of carbohydrates on Kligler-GRM agar was indicated by the yellow coloration, which is the same as the fermentation of glucose. The formation of gas was determined by bubbles and ruptures in the agar or its detachment from the walls of the test tube. The growth of microorganisms that did not ferment lactose and glucose does not change the color of the medium. The experimental data showed that the studied pasteurellosis isolates did cause the fermentation of carbohydrates (Fig. 7).

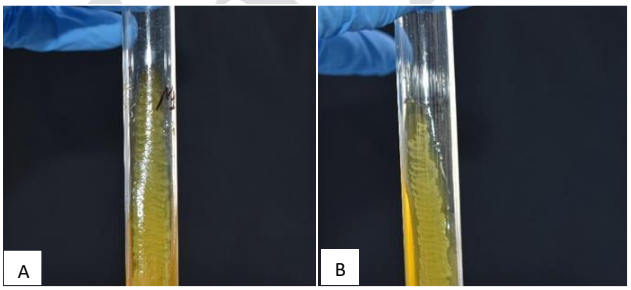

Fig. 7: The growth of pasteurellosis isolates on Kligler agar-GRM
A – Isolate No. 1 sown on Kligler agar-GRM
B – Isolate No. 2 sown on Kligler agar-GRM

Microorganisms that can metabolize sodium citrate grow on the medium and change its color from green to blue (positive reaction). Microorganisms unable to metabolize sodium citrate do not grow on the medium and the color does not change (negative reaction).

Considering the growth of pasteurellosis strains on polymyxin medium, the data showed that all pasteurellosis pathogen isolates did not grow on the agar. Finally, the results demonstrated that the pasteurellosis pathogen isolates did grow on nutrient agar (Fig. 8). The results of the growth of the isolated strains on the tested media are summarized in Table 5.

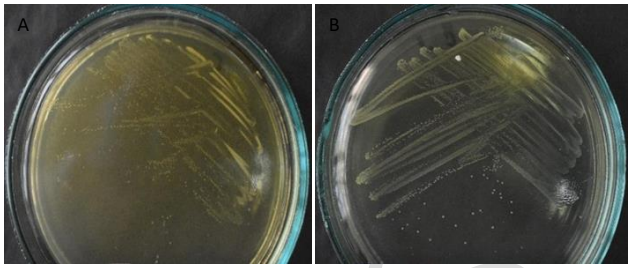

Fig. 8: The growth of pasteurellosis strains on nutrient agar (GRM-agar). A – Isolate No.1 sown on nutrient agar GRM; B – Isolate No. 2 sown on nutrient agar GRM.

Table 5: Results of the experiment on carbohydrate fermentation by pasteurellosis isolates on different media and agar.

No.	Medium and agar	Isolates		
		Reference	Isolate No. 1	Isolate No. 2
1	Hiss's – GRM with lactose	NF	NF	NF
2	Hiss's – GRM with glucose	FC	FC	FC
3	Hiss's – GRM with maltose	NF	NF	NF
4	Hiss's – GRM with sucrose	FC	FC	FC
5	Hiss's – GRM with mannitol	FC	FC	FC
6	Endo-GRM	NG	NG	NG
7	Kligler	WCF	WCF	WCF
8	polymyxin medium	NG	NG	NG
9	GRM-agar	NF	NF	NF
10	No. 14 GRM (Simmons' citrate agar)	NG	NG	NG

Note: 1 "NG" – no growth on agar; 2 "FC" – cause carbohydrate fermentation; 3 "NF" – colony growth, do not induce carbohydrate fermentation; 4 "WCF" – cause weak fermentation of carbohydrates.

Sensitivity of pasteurellosis isolates to different antibiotics

The results of experiments on the sensitivity of the isolates to benzylpenicillin sodium salt are presented in Fig. 9 and 10 show that both pasteurellosis pathogen isolates were sensitive to 10IU/mL of benzylpenicillin sodium salt. The tests on the sensitivity of pasteurellosis isolates to gentamicin sulfate showed that the strains were sensitive to 0.5mg/mL of gentamicin (Fig. 11 and 12). Fig. 11 and 12 demonstrate that isolates No. 1 and 2 were sensitive to 0.5mg of gentamicin sulfate. The sensitivity of pasteurellosis isolates to ampicillin was determined following the described technique. The results of this experiment are provided in Fig. 13 and 14. Isolate No. 1 was sensitive to ampicillin at 0.1mg/mL, while isolate No. 2 was sensitive to 1mg/mL ampicillin.

Experiments on the sensitivity of pasteurellosis isolates to different doses of streptomycin and tetracycline yielded the following results (Fig. 15 and 16). Isolate No. 1 showed sensitivity to streptomycin at a dose of 1mg/mL, and isolate No. 2 was sensitive to streptomycin at a concentration of 10mg/mL. The sensitivity of the isolates to different doses of tetracycline is shown in Fig. 17 and 18. Both isolates were sensitive to 1mg/mL tetracycline.

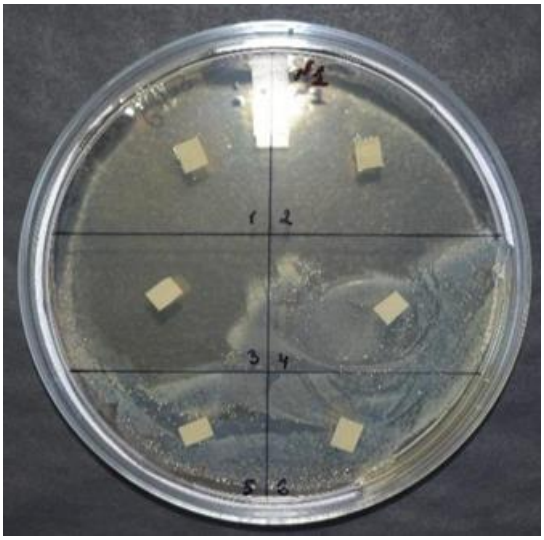

Fig. 9: Sensitivity of pasteurellosis pathogen isolate No. 1 to benzylpenicillin sodium salt; 1 – 1,000IU/mL; 2 – 100IU/mL; 3 – 10IU/mL; 4 – 1IU/mL; 5 – 0.1IU/mL; 6 – 0.01IU/mL.

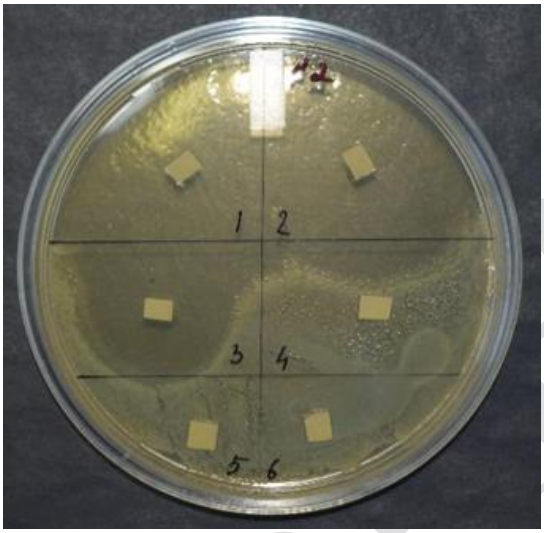

Fig. 10: Sensitivity of pasteurellosis pathogen isolate No. 2 to benzylpenicillin sodium salt; 1 – 1,000IU/mL; 2 – 100IU/mL; 3 – 10IU/mL; 4 – 1IU/mL; 5 – 0.1IU/mL; 6 – 0.01IU/mL.

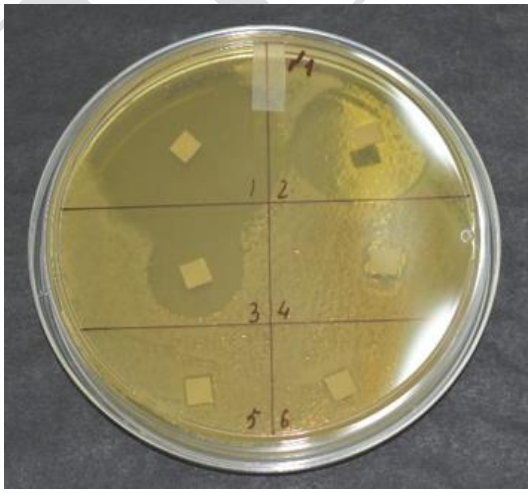

Fig. 11: Sensitivity of pasteurellosis pathogen isolate No. 1 to gentamicin; 1–50mg; 2 – 5mg; 3 – 0.5mg; 4 – 0.05mg; 5 – 0.005mg; 6 – 0.0005mg.

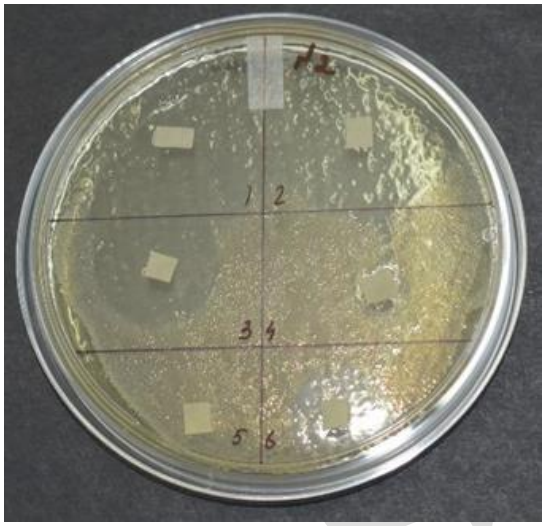

Fig. 12: Sensitivity of pasteurellosis pathogen isolate No. 2 to gentamicin; 1 – 50mg; 2 – 5mg; 3 – 0.5mg; 4 – 0.05mg; 5 – 0.005mg; 6 – 0.0005mg.

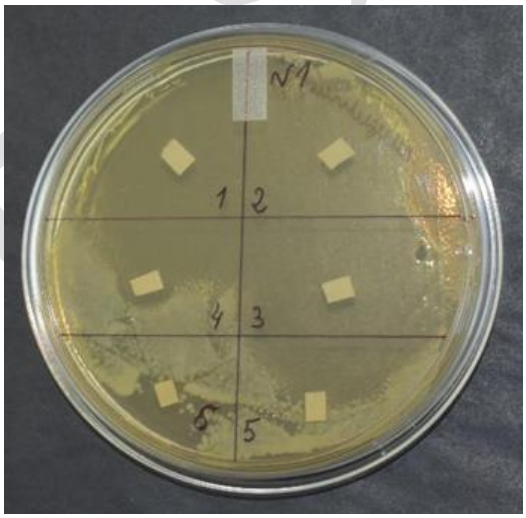

Fig. 13: Sensitivity of pasteurellosis pathogen isolate No. 1 to ampicillin; 1 – 100mg/mL; 2 – 10mg/mL; 3 – 1mg/mL; 4 – 0.1mg/mL;5 – 0.01mg/mL; 6 – 0.001mg/mL.

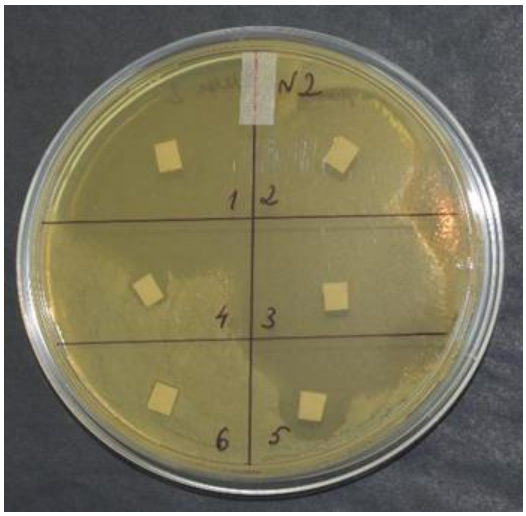

Fig. 14: Sensitivity of pasteurellosis pathogen isolate No. 2 to ampicillin; 1 – 100mg/mL; 2 – 10mg/mL; 3 – 1mg/mL; 4 – 0.1mg/mL; 5 – 0.01mg/mL; 6 – 0.001mg/mL.

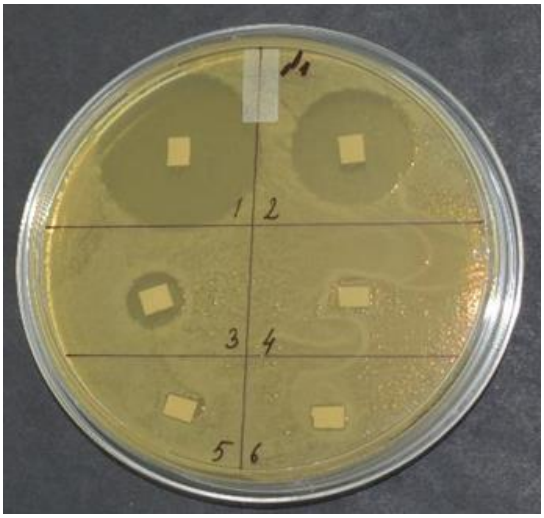

Fig. 15: Sensitivity of pasteurellosis pathogen isolate No. 1 to streptomycin; 1 – 100mg/mL; 2 – 10mg/mL; 3 – 1mg/mL; 4 – 0.1mg/mL;5 – 0.01mg/mL; 6 – 0.001mg/mL.

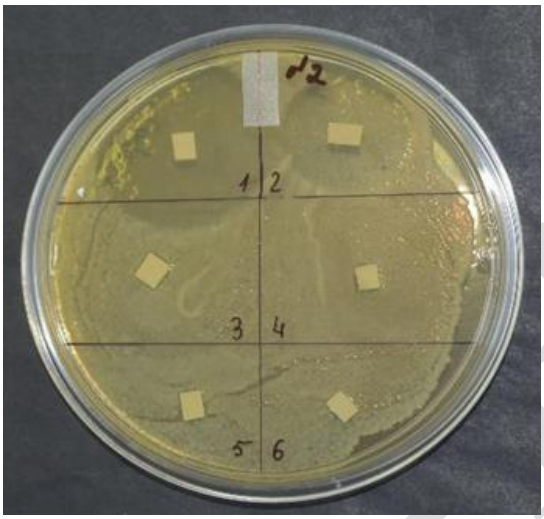

Fig. 16: Sensitivity of pasteurellosis pathogen isolate No. 2 to streptomycin; 1 – 100mg/mL; 2 – 10mg/mL; 3 – 1mg/mL; 4 – 0.1mg/mL; 5 – 0.01mg/mL; 6 – 0.001mg/mL.

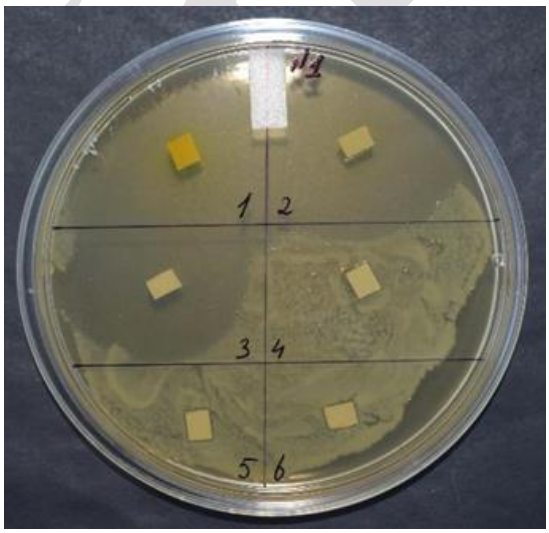

Fig. 17: Sensitivity of pasteurellosis pathogen isolate No. 1 to tetracycline; 1 – 100mg/mL; 2 – 10mg/mL; 3 – 1mg/mL; 4 – 0.1mg/mL; 5 – 0.01mg/mL; 6 – 0.001mg/mL.

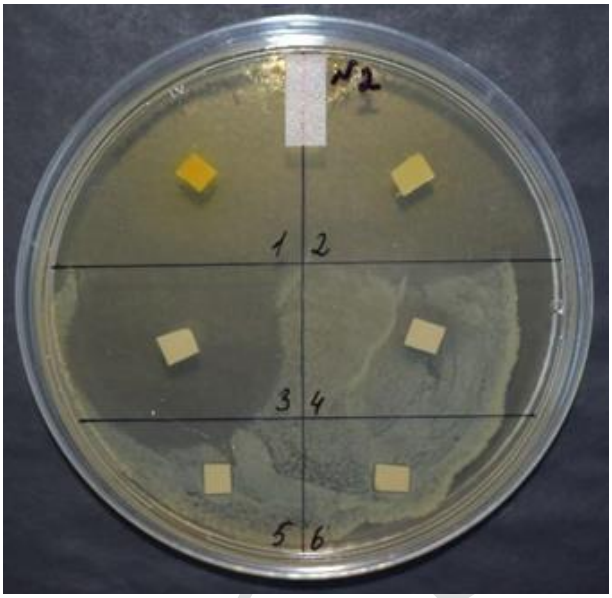

Fig. 18: Sensitivity of pasteurellosis pathogen isolate No. 2 to tetracycline; 1 – 100mg/mL; 2 – 10mg/mL; 3 – 1mg/mL; 4 – 0.1mg/mL; 5 – 0.01mg/mL; 6 – 0.001mg/mL.

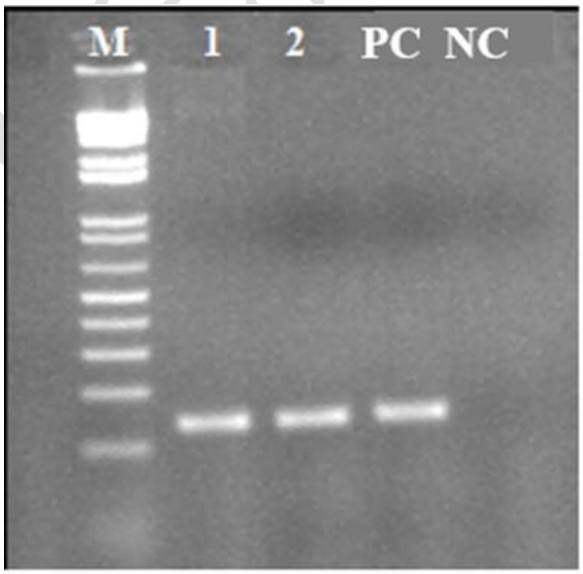

Fig. 19: Results of PCR to detect the *P. multocida* pathogen: M – marker; 1 – isolate No. 1 (sample 4); 2 – isolate No. 2 (sample 13); PC – positive control; NC – negative control.

Table 6: Results on isolates' sensitivity to antibiotics

No.	Antibiotic sensitivity level	Pasteurellosis isolates	
		No. 1	No. 2
1	Benzylpenicillin, I.U./μL	10<	10<
2	Gentamicin, mg/μL	0.5<	0.5<
3	Ampicillin, mg/μL	0.1<	1<
4	Streptomycin, mg/μL (Shymkent)	1<	10<
5	Tetracycline, mg/μL	1<	1<
6	Benzylpenicillin, I.U./μL	10<	10<

Note – "<" – above

The results on the sensitivity of the isolates to antibiotics are summarized in Table 6. After examining the cultural and biochemical properties of the two isolates, PCR was performed to re-confirm the results (Fig. 19). The results confirm that both pasteurellosis pathogen isolates belong to *P. multocida*.

DISCUSSION

The application of molecular and biochemical diagnostic methods for identifying *Pasteurella multocida* isolates from cattle and saiga populations aligns with recent research trends such as Taubaev et al. (2024) and Elsayed et al. (2021) that emphasize precision in bacterial detection and the development of antimicrobial resistance among the microbial strain. The Application of PCR and biochemical testing to classify the *Pasteurella multocida strain* aligns with the works of El-Demerdash et al. (2023) and Malik et al. (2023), who similarly used PCR to classify and amplify *P. multocida* strains and achieved accurate results, thereby reinforcing the robustness of PCR in precise pathogen identification.

The immotility and non-hemolytic trait of *P. multocida* observed in our study aligns with the works of Desem et al. (2023) and Dablool (2023), who reported similar characteristics in the *P. multocida* strains isolated from cattle, thereby suggesting preliminary criteria in identifying *P. multocida*. Our result showing that *P. multocida* caused the death of white mice aligns with the work of Yuan et al. (2024), who also exposed that aside from being a pathogen of hemorrhagic septicemia, *P. multocida* was also responsible for a host of diseases, including fowl cholera.

Our study also involved the biochemical differentiation of *P. multocida* isolates through carbohydrate fermentation using the Hiss medium, which aligned with the works of Taubaev et al. (2024) and Buienbayeva et al. (2024). Our results concluded that the isolates can ferment glucose and sucrose but not lactose and mannitol. These results partially agree with the work of Desem et al. (2023) and Taubaev et al. (2024), who provided information that there were rare cases where *P. multocida* could ferment lactose. Our results also partially agreed with the work of Ahmed et al. (2024), who concluded that lactose could not be fermented but claimed that *P. multocida* can ferment mannitol.

In our study, isolates displayed sensitivity to antibiotics such as gentamicin, ampicillin and tetracycline. Our results agree with Bahr et al. (2020), who highlighted that among the three tested antibiotics, gentamicin showed the highest susceptibility and the least percentage of antibiotic resistance when used on *P. multocida* obtained from cattle, buffalo, sheep, and goats. Our result did not align with another recent study (Kandimalla et al. 2022), which concluded that gentamicin had a higher resistance percentage than tetracycline and ampicillin when used on *P. multocida* obtained from sheep and goats. This difference shows the presence of underlying factors such as dosage and environmental factors, creating a gap for more research on how these factors play roles in antibiotic resistance. Our data suggest gentamicin could be a viable treatment option for pasteurellosis outbreaks, particularly in mixed animal environments where resistance development must be managed effectively. This conclusion agrees with the work of Abood et al. (2021).

Additionally, adopting molecular diagnostics and surveillance on a wider scale could transform pasteurellosis control. We agree with Manessis et al. (2022) and Goecke et al. (2021), who demonstrated the potential of lab-on-a-chip devices and nPCR to facilitate rapid field testing, which could be especially useful in remote areas and for migrational animals like saigas. Future studies should also focus on expanding the antibiotic susceptibility database for *P. multocida*, as regional variability in resistance profiles remains a significant challenge.

Conclusion

We successfully identified and confirmed the isolates of *P. multocida* using biochemical and molecular methods, including PCR. The isolates were tested on culture media, including Endo-GRM, Kligler-GRM, and citrate agar, allowing for differentiation based on their biochemical properties. The results demonstrated that both isolates belong to *P. multocida* serovar B, further confirmed by PCR analysis. The isolates' biochemical characteristics, including their inability to ferment lactose and their ability to ferment glucose and maltose, align with the expected properties of P. multocida. The isolates showed typical growth patterns on media, providing valuable insights into their metabolic capacities. The findings highlight the effectiveness of combining traditional biochemical methods with modern molecular diagnostics, ensuring precise identification and differentiation of bacterial pathogens. These results are crucial for improving diagnostic practices, disease management, and control strategies in livestock and wildlife populations during seasonal migration and disease outbreaks in saigas and cattle.

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