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Phylogenetic Analysis of *Pasteurella multocida* Strains Isolated from Saiga Antelope (Saiga Tatarica) in the West Kazakhstan Region

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ABSTRACT

The purpose of this study was to obtain and molecularly identify *Pasteurella multocida* isolates from pathological material obtained from saigas in Kazakhstan. This work was conducted in the context of recurring pasteurellosis outbreaks among migratory saiga populations, which pose a threat to both wildlife health and ecosystem stability in Central Asia. Polymerase Chain Reaction methods with universal primers specific for the 16S rRNA gene and subsequent sequencing of the amplified fragments were used for identification. Although universal primers were employed for 16S rRNA gene amplification, species-specific primers for *P. multocida* were not used, which may limit discriminatory power in cases involving closely related taxa. During the work, two isolates of *P. multocida* were obtained, for which molecular genetic identification was performed. Sequencing demonstrated the high accuracy and reliability of the data obtained, enabling a phylogenetic analysis. The degree of homology of nucleotide sequences with the reference strain *P. multocida* subsp. *gallicida* strain CCUG 17978 (GenBank: NR_041811.1) amounted to 99.86%. The results showed that both isolates had a high genetic affinity to this strain, confirming their similarity to typical *P. multocida* strains and highlighting the importance of these microorganisms in the context of epizootic processes. A phylogenetic tree constructed using the NJ method further confirmed the clustering of isolates with reference strains, supporting the robustness of the identification. The data emphasize the importance of using molecular methods to study the microbial ecology of wild animals, diagnose infectious diseases, and control the epidemiological situation in Kazakhstan. These findings contribute to improved molecular surveillance protocols and may assist in the development of early-warning systems for wildlife epizootics in Kazakhstan and beyond.

Keywords: Saiga antelopes, Pasteurellosis, *Pasteurella multocida*, Identification, Sequencing.

INTRODUCTION

In recent decades, there has been a growing interest in studying zoonotic infections that pose a threat to both animal and human health (Stufano et al. 2025; Sun et al. 2025). Pathogens with a wide geographical distribution and the ability to overcome inter-species barriers are becoming especially relevant (Sabour et al. 2022; Pronyk et al. 2023).

Pasteurella multocida is a zoonotic pathogen with a wide geographical distribution and the ability to infect a wide range of mammals, birds, and reptiles. Infections caused by this gram-negative bacterium are associated with high morbidity and mortality among humans and animals, as well as significant economic losses in animal husbandry (Mir et al. 2011; Smith et al. 2021; Gondaira et al. 2022). Along with other representatives of the *Pasteurellaceae* family,

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P. multocida is capable of causing both endemic and epizootic outbreaks of infectious diseases in domestic and wild animals, as well as birds.

The most important members of the *Pasteurellaceae* family that pose a serious threat to animals are *Mannheimia haemolytica*, *P. multocida* and *Pasteurella trehalosi* (*Biberstainia*) (Haque et al. 2024). The most virulent bacterial strains of the *Pasteurellaceae* family are capable of acting as primary pathogens in susceptible animals. However, many representatives of this family are components of the normal microflora and can exist in healthy carriers, exhibiting pathogenic properties only under certain conditions, that is, acting as secondary or opportunistic pathogens. Thus, in some cases, the recorded death of animals is likely associated with the penetration of a highly virulent strain into an immunologically naive population, while in others, internal or external factors that enhance the pathogenicity of *Pasteurellaceae* or suppress the host organism's immune defense may play a significant role. A combination of these mechanisms is also possible, in which the introduction of a new pathogen leads to a decrease in immunity and subsequent activation of opportunistic microflora (Robinson et al. 2019).

P. multocida is the most frequently reported pathogen of this family, causing mainly respiratory diseases in cattle and ungulates, including saigas. Hemorrhagic septicemia (HS) is a specific, severe, acute, and highly fatal disease common in tropical regions, primarily affecting cattle and saiga. Still, there are reports of several other affected species, most often with *P. multocida* serotype B2 (Li et al. 2023; Duan et al. 2024).

A review by Wilson and Ho (2013) demonstrated the widespread nature of HS, with potential latency in the herd, as bacteria are present in nasopharyngeal secretions periodically and persist in the amygdala crypts. The progression of the disease in animals is rapid, with infection to death in just a few hours or a few days, and with both aerosol and oral routes of infection demonstrated.

P. multocida is capable of infecting a wide range of domestic and wild mammals, birds, reptiles, and humans, causing various diseases, including economically significant ones (Piorunek et al. 2023; Wei et al. 2025). Today, it is becoming increasingly obvious that *Pasteurella* should be considered as a potential etiological agent of severe infectious diseases (Nambiar et al. 2025). In this regard, pasteurellosis poses a serious threat to animals. The arguments below relate to the *P. multocida* microorganism. However, none of the above excludes the possibility that representatives of other species of the genus *Pasteurella* may also serve as etiological agents (Taubaev et al. 2024).

Pasteurellosis is an acute infectious disease affecting a wide range of animals, including both domestic and wild animals, particularly the saiga. The disease is characterized by high mortality and can cause massive epizootics, making it a significant problem for both veterinary medicine and the protection of wild fauna. The diagnosis of pasteurellosis is complicated by the fact that it's clinical and pathoanatomical features are similar to those of other, often more severe, infections. Under these conditions, traditional bacteriological methods, despite their importance, usually fail to provide a reliable identification of the pathogen. The steady circulation of the pathogen in natural foci and outbreaks of the disease, especially in saiga populations in Kazakhstan, emphasize the need for high-precision molecular diagnostic methods. Sequencing of nucleotide sequences enables the confirmation of species affiliation, identification of strain differences, and establishment of epizootiological relationships, which is especially important when monitoring pathogens in wild fauna (Amirgazin et al. 2019).

In this context, the study of *Saiga tatarica*, a migratory ungulate native to the steppe regions of the Caspian Sea and Central Asia, is particularly important. Its natural range includes Russia, Kazakhstan, and Uzbekistan, and the subspecies *S. tatarica* mongolica is found in western Mongolia. The high mobility of these animals, which undergo seasonal migrations over considerable distances within Kazakhstan, contributes to both the spread of infectious agents and an increased risk of epizootics in populations (Ichshanova et al. 2018; Abdybekova et al. 2023; Kushaliyev et al. 2023; Kushaliyev et al. 2024).

Seasonal migrations play a crucial role in the survival of the saiga population, maintaining its numbers and the health of individual animals. However, the high mobility of animals also contributes to the widespread spread of infectious diseases, including pasteurellosis, which requires constant epizootic monitoring, including the use of molecular genetic methods (Olymon et al. 2025).

Epidemiological studies involving molecular phylogenetic analysis of bacterial populations are increasingly relying on complete genome sequencing as the preferred method for analysis. Comparative genomics approaches enable the identification of both similarities and differences between isolates or strains, contributing to a deeper understanding of their clustering, transmission pathways, and relationship to clinical manifestations and the spatiotemporal characteristics of disease occurrence (Prajapati et al. 2022).

Thus, the study aimed at the molecular identification of *P. multocida* isolated from saiga antelopes and its subsequent sequencing represents an essential step towards improving methods for the diagnosis, monitoring, and prevention of pasteurellosis. This is the first known study to genetically characterize *P. multocida* isolates from saigas using 16S rRNA sequencing and construct a phylogenetic tree to assess strain similarity to global reference sequences.

The purpose of the study was to isolate bacterial pathogens from saiga carcasses during an epizootic event, perform molecular identification of *P. multocida* using 16S rRNA gene sequencing, and assess the phylogenetic relationship of the isolates with reference strains deposited in global databases (NCBI GenBank). This allows for clarification of species identity and potential epidemiological tracing. Although based on two isolates, this study provides a molecular reference point for future diagnostic screening in saiga populations and contributes to the genetic profiling of circulating *P. multocida* strains in Kazakhstan. The phylogenetic comparison may assist in identifying potential transmission routes, evaluating the source of outbreaks, and establishing baseline data for national wildlife disease surveillance programs.

MATERIALS AND METHODS

We utilized epizootic strains of bacteria from the genus *Pasteurella*, isolated from saiga antelopes in West Kazakhstan. The samples were collected during a saiga

Fig. 1: Study region.

mortality event in 2024 in the Zhanibek district of the West Kazakhstan region (geographic coordinates: 49.4532°N, 46.7458°E, Fig. 1).

A total of 11 carcasses were examined, from which lung and liver tissues were aseptically sampled. Only animals with visible signs of hemorrhagic septicemia and no evidence of trauma were included. As a result, two *P. multocida* isolates were obtained from the pathological material.

Bacterial isolates of *P. multocida* were obtained from pathological material taken from dead animals and cultured on standard nutrient media - meat infusion agar (MIA) and meat infusion broth (MIB). The primary identification of microorganisms was carried out based on morphological, tinctorial, and cultural characteristics following generally accepted microbiological techniques (Eshmukhametov and Kamsaev 2014). Colonies appeared smooth, grayish-white, and non-hemolytic on meat infusion agar after 24–48 hours at 37°C. Microscopy revealed small, gram-negative coccobacilli with bipolar staining. Catalase and oxidase tests were positive. The isolates fermented glucose, sucrose, and mannitol but not lactose. No growth was observed on MacConkey agar. These findings were consistent with the biochemical profile of *P. multocida*. The Sanger sequencing method was used for molecular genetic identification.

DNA isolation and amplification

Genomic DNA was extracted from single-day cultures using a commercial PureLink Genomic DNA Kit (Invitrogen, USA) according to the manufacturer's protocol. The concentration of isolated DNA was quantified on a Qubit 2.1 fluorimeter using the Qubit dsDNA HS Assay Kit (Invitrogen, USA).

A fragment of the 16S ribosomal RNA gene was used as a genetic marker for identification. The reaction mixture with a volume of 25µL included 12.5µL of Q5 Hot Start High-Fidelity 2X Master Mix (New England Biolabs, USA), 1.25µL of each primer (10µm), 1.5µL of a DNA sample, and nuclease-free water to the total volume.

Polymerase chain reaction (PCR) was performed in a Mastercycler pro S thermal cycler (Eppendorf, Germany) according to the following program: pre-denaturation at 95°C for 7min; then 30 30-second cycles, including denaturation at 95°C, annealing at 55°C for 40 seconds and elongation at 72°C for 1min, followed by final elongation at 72°C for 10min. Negative controls (reaction mixture without template DNA) were included in each PCR run to detect contamination. For positive control, DNA from a known *P. multocida* reference strain (ATCC 12945) was used. All culture media were subjected to sterility controls. During sequencing, an internal sequencing control (pGEM plasmid) was run in parallel to verify instrument accuracy.

The amplified products were separated by electrophoresis in a 1.5% agarose gel, followed by visualization in an ultraviolet (UV) transilluminator after staining with ethidium bromide. 1×Tris-Borate-ethylenediamine tetraacetic acid (EDTA) (TBE) was used as an electrophoretic buffer. Purification of PCR products was carried out using the ExS-Pure Enzymatic PCR Cleanup Kit (Nimagene, the Netherlands).

Sequencing and data analysis

The sequencing of the amplified fragments of the 16S rRNA gene was performed using reagents from the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to the manufacturer's instructions. The sequenced products were purified using the BigDye XTerminator Purification Kit (Applied Biosystems, USA). Capillary electrophoresis and nucleotide sequence determination were performed on an automatic ABI 3500 DNA Analyzer sequencer (Applied Biosystems, USA).

The analysis of the obtained sequenograms was performed in the SeqA software (Applied Biosystems). The search for homologous nucleotide sequences was carried out using the BLAST algorithm (Basic Local Alignment Search Tool) in the GenBank international database of the National Center for Biotechnology Information of the USA (NCBI, http://www.ncbi.nlm.nih.gov).

Phylogenetic analysis

The nucleotide sequence alignment was performed using the ClustalW algorithm. To build phylogenetic trees, the neighbor-joining (NJ) method was used in the MEGA 6 software environment. The data obtained enabled the estimation of the genetic proximity of the studied *P. multocida* isolates to the strains presented in international databases. The robustness of the tree topology was assessed using bootstrap analysis with 1000 replicates. Bootstrap values were indicated at the nodes of the phylogenetic tree to demonstrate statistical support.

RESULTS AND DISCUSSION

For the molecular genetic identification of microorganisms from pathological material isolated from saiga, an amplification process was performed using universal primers specific for the 16S rRNA gene (Fig. 2). The resulting PCR product was then used for further analysis. The PCR amplification yielded a single sharp band in both isolates, corresponding to the expected fragment size of approximately 650bp. Electrophoresis was performed on a 1.5% agarose gel, and the band was observed in lanes 2 and 3, with lane 1 containing a 1kb DNA ladder (GeneRuler, Thermo Scientific). The observed fragment length matched the expected size for the 16S rRNA gene region. No amplification was observed in the negative control lane.

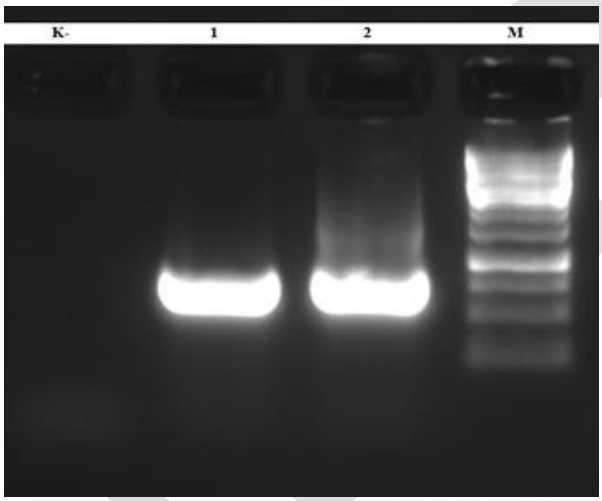


Fig. 2: The PCR product amplified using universal primers for the 16S rRNA gene; Note: M is a marker of 1,000 base pair (bp) lengths.

The image shows the resulting strip corresponding to the expected size of the amplified fragment (approximately 650bp) on a 1.5% agarose gel. The strip has a bright glow, which indicates the presence of the target PCR product.

The amplification process was successful, as confirmed by a clear and bright fragment on the gel, which was subsequently used for sequencing. During the study, two *P. multocida* isolates were obtained from the pathological material. For their molecular identification, an analysis was performed using the 16S rRNA gene sequencing method (Fig. 3).

Fig. 3 shows the sequencing sequence obtained for both isolates. Each peak on the sequenogram corresponds to one of the four nucleotides (A, T, C, G). The sequencing results demonstrated high accuracy and a distinct difference between the peaks, enabling us to obtain accurate nucleotide sequences for both isolates. Sequencing was successful for both isolates, generating high-quality reads with an average read length of 738bp. The QV (quality value) scores exceeded 30 for >95% of bases, indicating high confidence in base calling. Coverage was adequate for consensus sequence generation, and no ambiguous base calls were present in the final alignments.

GGTAGCAGGAAGAAAGCTTGCTTTCTTTGCTGACGAGTGGCGGACGGGTGAGTA
ATGCTTGGGAATCTGGCTTATGGAGGGGGATAACTGTGGGAAACTGCAGCTAAT
ACCGCGTATTCTCTGAGGAGGAAAGGGTGGGACCTTAGGGCCACCTGCCATAAG
ATGAGCCCAAGTGGGATTAGGTAGTTGGTGGGGTAAAGGCCTACCAAGCCTGCG
ATCTCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCC
AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCGCAATGGGGGGAACCCTGA
CGCAGCCATGCCGCGTGAATGAAGAAGGCCTTCGGGTTGTAAAGTTCTTTCGGTA
ATGAGGAAGGGATGTTGTTAAATAGATAGCATCATTGACGTTAATTACAGAAGA
AGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCGAGCGTT
AATCGGAATAACTGGGCGTAAAGGGCACGCAGGCGGACTTTTAAGTGAGATGTG
AAATCCCCGAGCTTAACTTGGGAACTGCATTTCAGACTGGGAGTCTAGAGTACTT
TAGGGAGGGGTAGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGA
ATACCGAAGGCGAAGGCAGCCCCTTGGGAATGTACTGACGCTCATGTGCGAAAG
CGTGGGGAGCAAACAGGATTAGATACCCTGGGTAG

Fig. 3: Sequenograms of fragments of the 16S rRNA gene for two *P. multocida* isolates obtained from pathological saiga material.

Based on the nucleotide sequences of the 16S rRNA gene obtained for two *P. multocida* isolates, a phylogenetic analysis was performed using the NJ algorithm in the MEGA 6 software (Fig. 4). The ClustalW algorithm was used to align the sequences.

Comparison with reference sequences from the NCBI database revealed that both isolates exhibited a high degree of similarity with various *P. multocida* strains previously reported and formed a distinct cluster closely related to the typical strains. A comparison of the nucleotide sequences of the 16S rRNA gene obtained from the isolates with the NCBI database showed a high degree of homology. The closest match, according to the BLAST analysis results, turned out to be *P. multocida* subsp. *gallicida* strain CCUG 17978 (GenBank: NR_041811.1, items 41-778) (Fig. 5a).

The degree of nucleotide similarity was 99.86%, which confirms the species identity of the isolates and their high genetic proximity to the specified reference strain (Fig. 5b). Comparable near-identity in the 16S region has been reported across geographically and host-diverse collections, where species-level calls are robust while strain-level diversity emerges only with MLST or WGS (Theissinger et al. 2023; Yeo et al. 2025). For example, a large 656-isolate survey spanning poultry, cattle, swine, rabbits, rodents and humans showed extensive genomic diversity despite near-identical 16S sequences, underscoring the limited subspecies resolution of this marker (Geurtsen et al. 2022). Recent genome-scale studies likewise document multiple lineages and cross-species transmission among production animals, indicating epidemiological connectivity that would not be apparent from 16S alone (Alhamami et al. 2023; Hassell et al. 2023). Regionally, HS-focused work in Eurasia and elsewhere highlights circulating epidemic sequence types, supporting the value of our 16S result as a species-level baseline while motivating higher-resolution typing in future saiga investigations (Erdélyi et al. 2025).

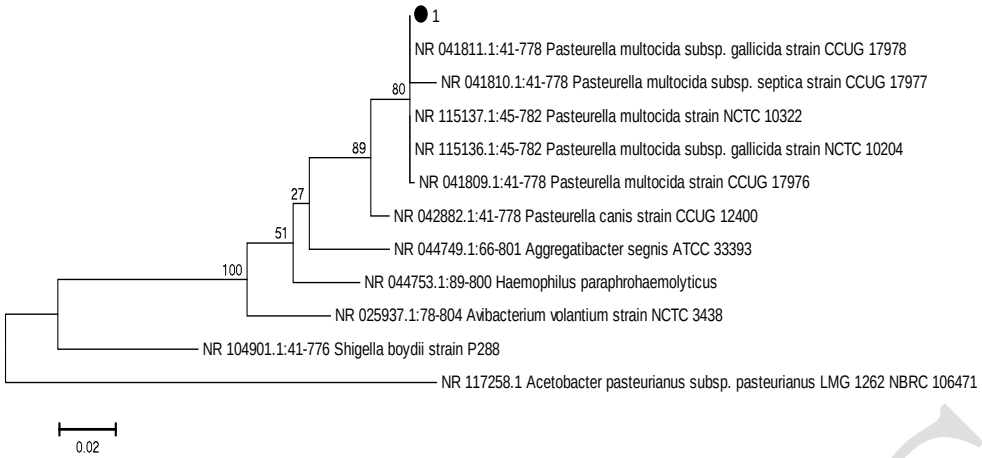
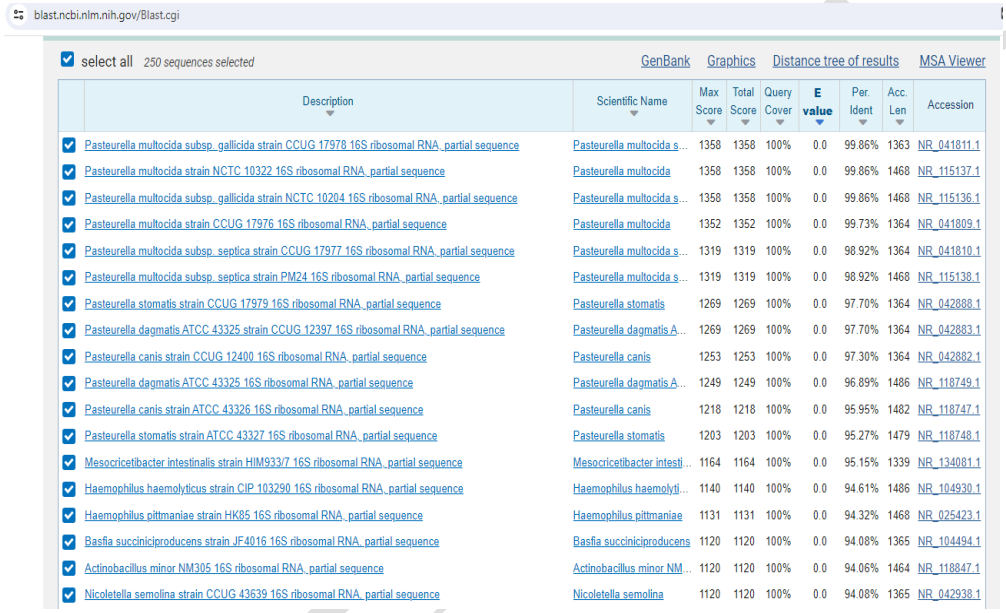


Fig. 4: A phylogenetic tree based on the sequences of the 16S rRNA gene constructed using the NJ method



blast.ncbi.nlm.nih.gov/Blast.cgi

select all 250 sequences selected — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Pasteurella multocida subsp. gallicida strain CCUG 17978 16S ribosomal RNA, partial sequence	Pasteurella multocida s...	1358	1358	100%	0.0	99.86%	1363	NR_041811.1
Pasteurella multocida strain NCTC 10322 16S ribosomal RNA, partial sequence	Pasteurella multocida	1358	1358	100%	0.0	99.86%	1468	NR_115137.1
Pasteurella multocida subsp. gallicida strain NCTC 10204 16S ribosomal RNA, partial sequence	Pasteurella multocida s...	1358	1358	100%	0.0	99.86%	1468	NR_115136.1
Pasteurella multocida strain CCUG 17976 16S ribosomal RNA, partial sequence	Pasteurella multocida	1352	1352	100%	0.0	99.73%	1364	NR_041809.1
Pasteurella multocida subsp. septica strain CCUG 17977 16S ribosomal RNA, partial sequence	Pasteurella multocida s...	1319	1319	100%	0.0	98.92%	1364	NR_041810.1
Pasteurella multocida subsp. septica strain PM24 16S ribosomal RNA, partial sequence	Pasteurella multocida s...	1319	1319	100%	0.0	98.92%	1468	NR_115138.1
Pasteurella stomatis strain CCUG 17979 16S ribosomal RNA, partial sequence	Pasteurella stomatis	1269	1269	100%	0.0	97.70%	1364	NR_042888.1
Pasteurella dagmatis ATCC 43325 strain CCUG 12397 16S ribosomal RNA, partial sequence	Pasteurella dagmatis A...	1269	1269	100%	0.0	97.70%	1364	NR_042883.1
Pasteurella canis strain CCUG 12400 16S ribosomal RNA, partial sequence	Pasteurella canis	1253	1253	100%	0.0	97.30%	1364	NR_042882.1
Pasteurella dagmatis ATCC 43325 16S ribosomal RNA, partial sequence	Pasteurella dagmatis A...	1249	1249	100%	0.0	96.89%	1486	NR_118749.1
Pasteurella canis strain ATCC 43326 16S ribosomal RNA, partial sequence	Pasteurella canis	1218	1218	100%	0.0	95.95%	1482	NR_118747.1
Pasteurella stomatis strain ATCC 43327 16S ribosomal RNA, partial sequence	Pasteurella stomatis	1203	1203	100%	0.0	95.27%	1479	NR_118748.1
Mesocricetibacter intestinalis strain HIM933/7 16S ribosomal RNA, partial sequence	Mesocricetibacter intesti...	1164	1164	100%	0.0	95.15%	1339	NR_134081.1
Haemophilus haemolyticus strain CIP 103290 16S ribosomal RNA, partial sequence	Haemophilus haemolyti...	1140	1140	100%	0.0	94.61%	1486	NR_104930.1
Haemophilus pittmaniae strain HK85 16S ribosomal RNA, partial sequence	Haemophilus pittmaniae	1131	1131	100%	0.0	94.32%	1468	NR_025423.1
Basfia succiniciproducens strain JF4016 16S ribosomal RNA, partial sequence	Basfia succiniciproducens	1120	1120	100%	0.0	94.08%	1365	NR_104494.1
Actinobacillus minor NM305 16S ribosomal RNA, partial sequence	Actinobacillus minor NM...	1120	1120	100%	0.0	94.06%	1464	NR_118847.1
Nicoletella semolina strain CCUG 43639 16S ribosomal RNA, partial sequence	Nicoletella semolina	1120	1120	100%	0.0	94.08%	1365	NR_042938.1

Fig. 5a: BLAST analysis results: the highest degree of homology (99.86%) was found with the sequence of *P. multocida* subsp. *gallicida* strain CCUG 17978 (NR_041811.1)

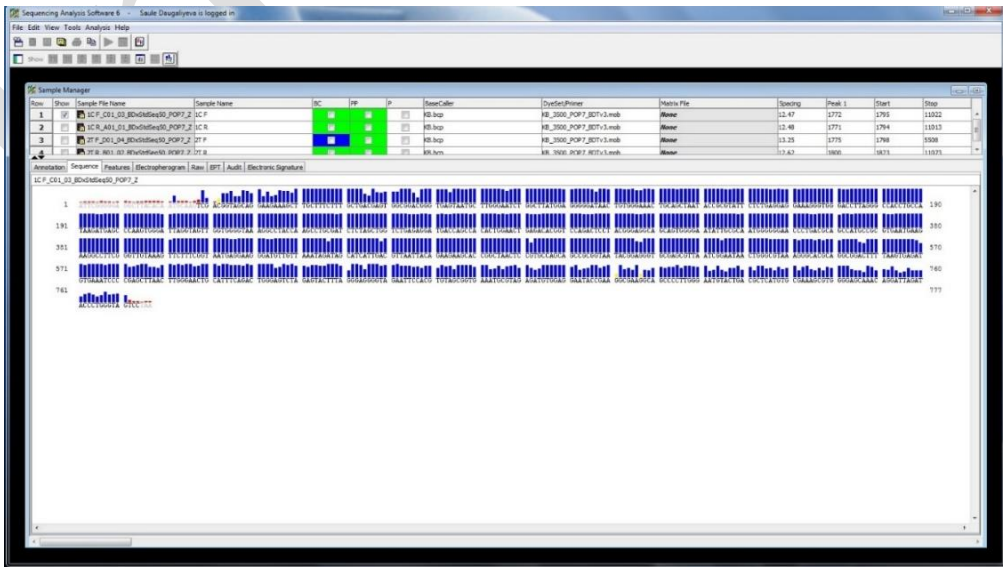


Fig. 5b: The results of BLAST alignment of the *16S rRNA* gene sequence of *P. multocida* isolate with the reference strain CCUG 17978 (*P. multocida* subsp. *gallicida*, NR_041811.1); Note to Fig. 4b: The color scale indicates the level of homology: red is a complete match, and blue is a partial match. The length of the matching fragment was 738bp, and the similarity was 99.86%.

In the current stage of biological sciences development, alongside traditional methods of microorganism identification based on cultural, morphological, and biochemical characteristics, molecular genetic approaches that analyze nucleotide sequences are of particular importance (Ichshanova et al. 2025). The results of the molecular genetic identification of the two *P. multocida* isolates in this study confirm their high degree of similarity with the well-known strain *P. multocida* subsp. *gallicida* strain CCUG 17978 (GenBank: NR_041811.1), which demonstrates the importance of molecular methods for accurate diagnosis and classification of microorganisms.

Amplification using universal primers specific for the 16S rRNA gene demonstrated the presence of a target PCR product, which confirms the successful isolation and identification of bacterial strains from the pathological saiga material. The bright stripe on the 1.5% agarose gel indicates the high specificity and effectiveness of the method. Sequencing of the PCR product yielded accurate nucleotide sequences, which enabled further phylogenetic analysis.

Phylogenetic analysis using the NJ algorithm showed that both *P. multocida* isolates formed a cluster with typical strains, indicating their high genetic similarity. Clustering with reference strains is consistent with recent reports that 16S rRNA phylogeny places wildlife and livestock isolates within broad *P. multocida* clades, while finer structure becomes evident only with multilocus or whole-genome approaches. A 2021 PLOS ONE analysis resolved host-associated genotypes across continents by genome-wide data rather than 16S alone, and subsequent studies in cattle cohorts confirmed multiple co-circulating lineages despite conserved 16S signatures (Smith et al. 2021). Pan-population genomic work in 2025 further showed repeated cross-species jumps and mobile-element flux across hosts, reinforcing the need to complement saiga 16S surveillance with MLST/WGS to map transmission routes and virulence repertoires (Allen et al. 2025). At the wildlife–livestock interface, HS surveillance and sero-epidemiological studies from 2024–2025 document active regional circulation and risk, aligning with our finding of species-level identity yet signaling the importance of strain-resolved monitoring in migratory ungulates (Shome et al. 2024). The high degree of similarity (99.86%) with the reference strain of *P. multocida* subsp. *gallicida* CCUG 17978 confirms the species identity of the isolates and their origin. This result is consistent with previous studies by the authors of (Ichshanova et al. 2023), who show that *Pasteurella* isolated from wild animals may have an identical genetic structure to pathogenic strains isolated from domestic animals.

Thus, in Shamshidin and Tleulenov (2024), the authors note a high degree of similarity between *Pasteurella* isolates obtained from saigas and other wild animals in Kazakhstan and *P. multocida* strains, which supports the results of our study. Previous studies have also confirmed that *P. multocida* plays a crucial role in the development of diseases in wild animals, including pasteurellosis. The results of the BLAST analysis, which showed a 99.86% degree of similarity with the reference strain, emphasize the importance of using genetic methods to establish accurate bacterial identification. The degree of similarity exceeding 99% indicates that the isolates are characterized by high genetic similarity with typical strains, confirming their pathogenicity and ability to cause infectious diseases in natural populations of wild animals.

Conclusion

Our results confirm the importance of using molecular methods, such as 16S rRNA gene sequencing, for the identification and analysis of bacterial strains, as well as their further application in monitoring and controlling epizootic situations in Kazakhstan's ecosystems. This is the first report to perform molecular genetic identification and phylogenetic analysis of *Pasteurella multocida* isolated from saiga antelopes in the West Kazakhstan region. The finding of 99.86% sequence similarity with the *P. multocida* subsp. *gallicida* reference strain confirms the genetic proximity of field isolates to pathogenic strains known from domestic animals. These results expand the molecular database of wildlife-associated pathogens in Central Asia and may serve as a genetic reference point for future surveillance and outbreak investigations in migratory ungulates.

DECLARATIONS

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Conflict of Interest: The authors declare no conflict of interest.

Data Availability: Data available upon request from the corresponding author.

Ethics Statement: Ethical Statement: Field sampling was conducted under approval from the Committee for Veterinary Control and Supervision, Ministry of Agriculture of the Republic of Kazakhstan (Approval No. 012, dated 12-05-2025). Although no procedures on live vertebrates were performed, our conduct followed the principles set out in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123) and aligned with the Recommendation of the Board of the Eurasian Economic Commission No. 33 of 14 November 2023 “Guidelines for work with the laboratory (experimental) animals in preclinical (non-clinical) studies”.

Author’s Contribution: AI, BY, IA: Sample collection, bacteriological work; AK, SA, NK: Molecular identification and PCR analysis; BK, AB, NK: Sequencing and phylogenetic data interpretation; BS, SB: Study design, supervision, manuscript writing and editing. All authors reviewed and approved the final version of the manuscript.

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