

Identification of a *Salmonella* Abortusequi isolate based on morphological and serological characteristics and assessment of its antimicrobial resistance profile

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Abstract

Background and Aim. Equine salmonellosis-associated abortion remains an important infectious disease of horses, with significant implications for reproductive health and horse breeding. *Salmonella enterica* subsp. *enterica* serovar Abortusequi is recognized as one of the etiological agents associated with abortion in mares and may persist within affected horse populations. This study is aimed to characterize and identify an *S. Abortusequi* isolate recovered from pathological material obtained from an aborted equine fetus in the Aiyrtau District of the North Kazakhstan Region through comprehensive assessment of its morphological, cultural, biochemical, and serological characteristics, and to determine its antimicrobial susceptibility profile.

Materials and Methods. A pure bacterial culture was obtained through sequential subculturing followed by the selection of colonies exhibiting characteristic morphological features. Identification of the isolate was performed using an integrated approach based on morphological, cultural, biochemical, and antigenic characteristics. Cellular morphology and staining properties were examined by light microscopy, while cultural characteristics were evaluated based on growth patterns and colony morphology on selective and differential diagnostic media. Biochemical activity was assessed using standard diagnostic tests. The antigenic profile of the isolate was determined by slide agglutination with polyvalent and monoreceptor diagnostic antisera specific for *Salmonella* O- and H-antigens. Species-level identification was additionally confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Antimicrobial susceptibility was assessed against 12 antimicrobial agents using the disk diffusion method, with results evaluated based on the diameters of bacterial growth inhibition zones.

Results. The recovered isolate exhibited morphological, cultural, and biochemical characteristics consistent with the genus *Salmonella*, including Gram-negative, motile, rod-shaped cells, facultative anaerobic growth, characteristic colony morphology on selective and differential media, and hydrogen sulfide (H₂S) production. Biochemical testing demonstrated glucose fermentation with acid and gas production, absence of lactose and sucrose fermentation, negative indole and urease reactions, and positive citrate utilization. Serological analysis revealed specific agglutination with O:4 (O-IV) and O:12 (O-XII) diagnostic antisera, assigning the isolate to *Salmonella* serogroup B; the antigenic formula was determined as 4,12:-: n,x, consistent with *Salmonella enterica* subsp. *enterica* serovar Abortusequi. Identification was additionally supported by MALDI-TOF MS analysis. Antimicrobial susceptibility

testing showed inhibition zone diameters ranging from 26 to 41 mm, with the largest zones observed for imipenem (41 mm), ofloxacin (40 mm), and ceftriaxone (38 mm), followed by amoxicillin/clavulanic acid and norfloxacin (35 mm each), cefoxitin (34 mm), co-trimoxazole and tobramycin (32mm each), ampicillin (31 mm), gentamicin (30 mm), azithromycin (27 mm), and tetracycline (26 mm).

Conclusion. Comprehensive assessment of the morphological, cultural, biochemical, serological, and mass spectrometric characteristics enabled identification of the isolate recovered from pathological material of an aborted equine fetus as *S. Abortusequi*. Disk diffusion testing demonstrated growth inhibition zone diameters ranging from 26 to 41 mm across the panel of 12 antimicrobial agents tested. These findings provide a phenotypic characterization of the *S. Abortusequi* isolate and describe its antimicrobial susceptibility profile, contributing to the laboratory characterization of this pathogen associated with equine abortion.

Keywords: antimicrobial resistance; equine salmonellosis-induced abortion; epizootic strain; *Salmonella Abortusequi*; serological identification.

Introduction

Identifying the biological properties represents a critical step in the laboratory diagnosis of equine salmonellosis-induced abortion and serves as the basis for taxonomic verification of isolates [1, 2]. Contemporary studies emphasize the need to comprehensively assess both the phenotypic and molecular-genetic characteristics of strains [3, 4, 5].

Morphologically, *S. Abortusequi* is a Gram-negative, motile rod, facultative anaerobe that does not form spores. These characteristics have been confirmed both through classical microbiological identification and in the characterization of strains isolated in Kazakhstan and China [1, 2, 3].

Its cultural properties include forming smooth colonies on solid nutrient media and producing hydrogen sulfide (H₂S) on differential diagnostic media, which are utilized in primary laboratory diagnostics [2, 3]. Its biochemical profile is characterized by glucose fermentation with acid and gas production, absence of lactose and sucrose fermentation, negative indole and urease reactions, and the ability to utilize citrate [1, 4]. These characteristics retain diagnostic significance for differentiation from other *Salmonella* serovars.

Antigenic structure plays a decisive role in identification. Serotyping is based on the detection of somatic (O) and flagellar (H) antigens. *S. Abortusequi* is characterized by the antigenic formula O: 4,12: - : e,n,x, corresponding to serological group B [1, 3, 5]. Genomic studies have confirmed the stability of this antigenic formula and its conformity with the international nomenclature of serovars [3, 5].

Modern molecular methods, including whole-genome sequencing, have significantly enhanced identification capabilities by enabling precise determination of strains' phylogenetic affiliation and the detection of virulence genes and antimicrobial resistance markers [3, 5, 6]. Genomic analyses of Kazakhstani isolates indicate phylogenetic relatedness among strains while maintaining typical phenotypic characteristics [3].

Serological diagnostic methods, particularly the development of domestic enzyme-linked immunosorbent assays and the use of recombinant antigens, aim to enhance the sensitivity and specificity of confirming a pathogen's antigenic structure [4, 7, 8, 9, 10]. Their application is especially important for epizootic monitoring and for detecting latent carriers [11, 12, 13, 14].

The scientific novelty of this study is represented by the comprehensive characterization of an epizootic isolate of *S. Abortusequi* recovered from pathological material of an aborted equine fetus in the North Kazakhstan Region. The isolate was characterized using an integrated approach combining cultural, morphological, biochemical, serological, and mass spectrometric methods, together with phenotypic assessment of its antimicrobial susceptibility. The findings provide additional data on the phenotypic and serological characteristics of *S. Abortusequi* associated with equine abortion and contribute to improving its comprehensive laboratory identification. The established antimicrobial susceptibility profile provides a basis for further comparative studies of independent *S. Abortusequi* isolates and for assessing the variability of antimicrobial susceptibility within this serovar.

Materials and Methods

Ethical approval

This study was approved by the local Bioethics Committee of the Institute of Animal Science and Veterinary Medicine at S. Seifullin Kazakh Agrotechnical Research University (protocol number: 3, approval date: January 12, 2026) and conducted in accordance with bioethical principles.

Isolate Preparation

The study material consisted of a single aborted equine fetus at 5-6 months of gestation, obtained in February 2026 from a horse-breeding farm in the Aiyrtau District of the North Kazakhstan Region. The fetus was transported to the laboratory in accordance with established veterinary and sanitary requirements for the packaging and transportation of pathological material.

The packaging and transportation of pathological material were carried out in accordance with the requirements of the Rules for Sampling of Transported Objects and Biological Material, approved by Order No. 7-1/393 of the Minister of Agriculture of the Republic of Kazakhstan dated April 30, 2015, as well as applicable guidelines for the collection of biological material for the laboratory diagnosis of infectious diseases in farm animals. Necropsy of the aborted fetus and collection of internal organ samples for bacteriological examination were performed under laboratory conditions at S. Seifullin Kazakh Agrotechnical Research University in accordance with aseptic procedures and precautions aimed at preventing secondary contamination of the collected material.

For bacteriological examination, samples of the liver, spleen, kidneys, and regional lymph nodes were aseptically collected using sterile instruments. From each tissue sample, 1.0 g was homogenized in 9 mL of sterile 0.85% sodium chloride solution to obtain a 1:10 suspension. The resulting tissue suspensions were subsequently used for bacterial isolation and identification.

Primary isolation was performed by inoculating the prepared tissue suspensions onto differential and selective media, including Endo agar and *Salmonella*-Shigella (SS) agar. The inoculated plates were incubated aerobically at 37 °C for 18-24 h. Following incubation, bacterial growth and colony morphology were evaluated, and colonies exhibiting cultural characteristics consistent with the genus *Salmonella* were selected for further purification. A pure culture was obtained by sequential subculturing using the Drygalski plating method and was subsequently used for identification and antimicrobial susceptibility testing.

Isolate Characterization

The morphological characteristics of the isolated culture were examined by light microscopy of Gram-stained smears. Cell shape, size, arrangement, and Gram reaction were evaluated. Cultural characteristics were assessed based on bacterial growth and colony morphology on Endo agar and *Salmonella*-Shigella (SS) agar following incubation at 37 °C for 18-24 h. Biochemical characterization included assessment of glucose, lactose, and sucrose fermentation, hydrogen sulfide (H₂S) and indole production, urease activity, and citrate utilization. The combined morphological, cultural, and biochemical characteristics were used for preliminary identification of the isolate as a member of the genus *Salmonella*.

Serological identification of the pure culture was carried out by slide agglutination using *Salmonella* diagnostic antisera (SSI Diagnostica A/S, Hillerød, Denmark). Initial serogroup determination was performed using the *Salmonella* Sero-Quick Group Kit, containing diagnostic antisera for the major *Salmonella* serogroups. Assignment of the isolate to serogroup B was based on a positive reaction with the corresponding group-specific antiserum detecting the somatic O:4 antigen. Further characterization of the somatic antigenic profile was performed using *Salmonella* Factor O:12 antiserum, while the flagellar antigenic profile was assessed using *Salmonella* Phase H,n,x antiserum (SSI Diagnostica A/S, Hillerød, Denmark). Slide agglutination tests were conducted according to the manufacturer's instructions, with the formation of visible specific agglutination interpreted as a positive reaction. The resulting profile of somatic O- and flagellar H-antigens was interpreted according to the White-Kauffmann scheme for *Salmonella* serovar determination.

Species-level verification was performed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) at the National Center for Biotechnology (Astana, Republic of Kazakhstan). Spectra were recorded using a Microflex LT MS and Biotyper software (Bruker Daltonics, Bremen, Germany); identification was considered reliable when the score was ≥ 2.0 .

Antimicrobial susceptibility of the isolated culture was assessed using the disk diffusion method. The isolate was tested against 12 antimicrobial agents: co-trimoxazole (sulfamethoxazole/trimethoprim), 25 µg (23,75/1,25 µg); amoxicillin/clavulanic acid, 30 µg (20/10 µg); ampicillin, 10 µg; cefoxitin, 30 µg; ceftriaxone, 30 µg; gentamicin, 10 µg; tobramycin, 10 µg; ofloxacin, 5 µg; imipenem, 10 µg; norfloxacin, 10 µg; azithromycin, 15 µg; and tetracycline, 30 µg.

Following incubation, the diameters of the growth inhibition zones around the antimicrobial discs were measured in millimeters. The obtained values were used to characterize the phenotypic antimicrobial susceptibility profile of the isolate. Results were interpreted according to the applicable EUCAST criteria. When no validated clinical breakpoint was available for a specific organism-

antimicrobial combination, the measured inhibition zone diameter was reported without categorical interpretation.

The study was limited to the characterization of a single bacterial isolate recovered from a single case of equine abortion. Samples collected from different organs of the same fetus were used exclusively for pathogen isolation and identification and were not treated as independent biological or epidemiological observations.

Results

The sampling and subsequent bacteriological analysis of pathological material obtained from an aborted equine fetus (5-6 months of gestation) from the Aiyrtau District, North Kazakhstan Region, resulted in the isolation of a microorganism taxonomically assigned to the genus *Salmonella* (Figure 1).



Figure 1. The aborted equine fetus (Aiyrtau District, North Kazakhstan Region)

During postmortem examination of the aborted equine fetus, samples of the liver, spleen, kidneys, thyroid lymph nodes, and mesenteric lymph nodes were collected for bacteriological examination. Analysis of the collected pathological material resulted in the isolation of a bacterial culture, which was subsequently subjected to phenotypic, serological, and mass spectrometric identification (Figure 2).

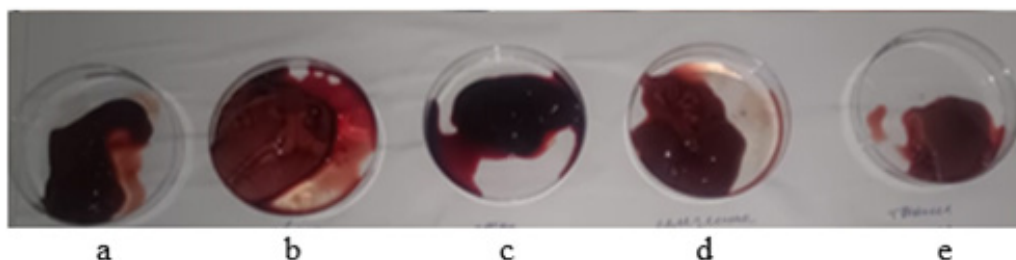


Figure 2. Pathological material collected from the internal organs and regional lymph nodes of an aborted equine fetus for bacteriological examination

Note: a-kidney; b-spleen; c-liver; d- thyroid lymph node; e- mesenteric lymph node.

Following sample homogenization and initial cultivation on selective and differential diagnostic media (Endo and *Salmonella-Shigella* agar) at 37 °C for 18-24 hours, colonies exhibiting morphology typical of the *Salmonella* genus were observed. On *Salmonella-Shigella* agar, the colonies appeared colorless with a characteristic black center, indicating H₂S production. Subsequent subculturing yielded a pure culture that maintained stable morphological characteristics.

Microscopic examination of Gram-stained smears revealed Gram-negative rods 3-5 µm long and 0.5 µm wide, occurring singly or in pairs (Figure 3). The observed morphology was consistent with bacteria belonging to the genus *Salmonella*.

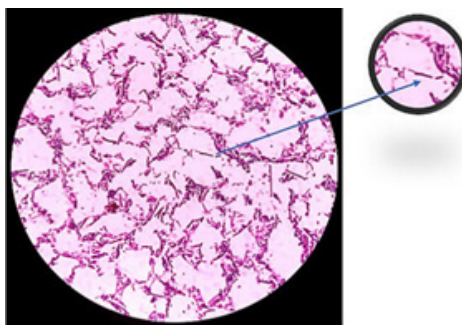


Figure 3. Morphological characteristics of the *Salmonella* Abortus equi isolate revealed by Gram staining and light microscopy

In meat-peptone broth, the culture exhibited uniform turbidity and moderate sedimentation. The isolate was characterized biochemically by glucose fermentation with acid and gas production, absence of lactose and sucrose fermentation, negative indole and urease reactions, the ability to utilize citrate, and H₂S production. This combination of biochemical characteristics corresponds to the diagnostic criteria for the genus *Salmonella*.

The phenotypic antimicrobial susceptibility profile of the *S. Abortusequi* isolate was evaluated against 12 antimicrobial agents from different pharmacological groups using the disk diffusion method. The growth inhibition zone diameters varied depending on the antimicrobial agent tested and ranged from 26 to 41 mm. The results of antimicrobial susceptibility testing are presented in Table 1.

Table 1. Antimicrobial susceptibility profile of the epizootic *S. Abortus equi* isolate determined by the disk-diffusion method

No	Antimicrobial agents and their concentrations	S ≥	R <	ATU	Inhibition zone diameter, mm	Result
1	Co-trimoxazole (sulfamethoxazole trimethoprim) – 25 µg (23.75/1.25 µg)	15	15	-	32	S
2	Amoxiclav (amoxicillin/clavulanic acid) – 30 µg (20/10 µg)	19	19	19–20	35	S
3	Ampicillin – 10 µg	14	14	-	31	S
4	Cefoxitin – 30 µg (screen only)	19	19	-	34	S
5	Ceftriaxone – 30 µg	27	24	-	38	S
6	Gentamicin – 10 µg	17	17	-	30	S
7	Tobramycin – 10 µg	16	16	-	32	S
8	Ofloxacin (5 µg)	24	22	-	40	S
9	Imipenem (10 µg)	22	19	-	41	S
10	Norfloxacin (10 µg)	24	24	-	35	S
11	Azithromycin (15 µg)	12	12	-	27	S
12	Tetracycline (30 µg)	30	30	-	26	S

Abbreviations: ATU = area of technical uncertainty, R = resistant, S = susceptible

Analysis of the antimicrobial susceptibility results showed that the largest growth inhibition zone was observed for imipenem (41 mm), followed by ofloxacin (40 mm) and ceftriaxone (38 mm). Inhibition zone diameters of 35 mm were recorded for both amoxicillin/clavulanic acid and norfloxacin, followed by cefoxitin (34 mm), co-trimoxazole and tobramycin (32 mm each), ampicillin (31 mm), and gentamicin (30 mm). Smaller inhibition zones were observed for azithromycin (27 mm) and tetracycline (26 mm), with tetracycline showing the smallest zone among the 12 antimicrobial agents tested.

Comparison of the measured inhibition zone diameters with the EUCAST interpretive criteria presented in Table 1 showed that the results for co-trimoxazole, amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftriaxone, gentamicin, tobramycin, ofloxacin, imipenem, norfloxacin, and azithromycin met the corresponding susceptibility criteria. For amoxicillin/clavulanic acid, the observed inhibition zone diameter of 35 mm was also outside the Area of Technical Uncertainty (ATU; 19-20 mm) specified by EUCAST.

For tetracycline, the inhibition zone diameter was 26 mm, which was below the susceptibility breakpoint ($S \geq 30$ mm) applied in this study; therefore, this result did not meet the specified susceptibility criterion. Overall, the *S. Abortusequi* isolate met the applied susceptibility criteria for most antimicrobial agents included in the tested panel, whereas tetracycline produced the smallest growth inhibition zone among the antimicrobial agents examined.

The obtained data indicate that the studied *S. Abortusequi* isolate exhibited in vitro susceptibility to most of the antimicrobial agents tested, based on the interpretive criteria applied in this study. These findings characterize the phenotypic antimicrobial susceptibility profile of the isolate and provide baseline information on its response to antimicrobial agents. However, given that the present study was limited to a single isolate, further investigation involving a larger collection of *S. Abortusequi* isolates from different geographical regions and epidemiological settings is required to assess the distribution and potential emergence of antimicrobial resistance in equine salmonellosis.

Species identity was confirmed by MALDI-TOF MS. The obtained mass spectra, analyzed with the Bruker Biotyper software, yielded a score ≥ 2.0 , providing reliable species-level identification as *S. Abortusequi* (Figure 4).

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
<u>A1</u> (+++)(A)	A1	Salmonella sp	2.353	Salmonella sp	2.258
<u>B1</u> (++)(A)	B1	Salmonella sp	2.151	Salmonella sp	2.086
<u>C1</u> (+)(B)	C1	Salmonella sp	1.742	not reliable identification	1.626
<u>D1</u> (-)(C)	D1	not reliable identification	1.604	not reliable identification	1.053
<u>E1</u> (++)(A)	E1	Salmonella sp	2.162	Salmonella sp	2.158
<u>F1</u> (++)(A)	F1	Salmonella sp	2.009	Salmonella sp	1.974
<u>G1</u> (-)(C)	G1	not reliable identification	1.502	not reliable identification	1.353
<u>H1</u> (++)(A)	H1	Salmonella sp	2.215	Salmonella sp	2.152
<u>A2</u> (++)(A)	A2	Salmonella sp	2.161	Salmonella sp	2.086
<u>B2</u> (++)(A)	B2	Salmonella sp	2.189	Salmonella sp	2.146

Figure 4. Mixed-sample analysis demonstrating the detection of protein profiles characteristic of members of the genus *Salmonella*. The labeling of the analyzed samples corresponds to the following anatomical sites: A1 = kidneys; B1 = spleen; C1 = liver; E1, F1 = thyroid (cervical) lymph nodes; H1, A2, B2 = mesenteric lymph nodes

During the pathological examination of the aborted equine fetus (5-6 months of gestation), pronounced morphological changes were observed in the internal organs, indicative of a generalized infection process. The kidneys (A1) exhibited signs of parenchymal dystrophy, moderate cortical hyperemia, and vascular congestion, reflecting the toxico-septic impact of the infectious agent. The spleen (B1) was enlarged, with pronounced blood engorgement and a tense capsule, characteristic

of reactive lymphoid hyperplasia in septicemia. The liver (C1) showed congestive hyperemia, focal granular hepatocyte dystrophy, and a dull cut surface, consistent with intrauterine bacterial infection. The thyroid lymph nodes (E1 and F1) were enlarged and firm, with pronounced vascular injection, reflecting immune system activation in response to antigenic stimulation. The mesenteric lymph nodes (H1, A2, and B2) exhibited signs of serous inflammation, vascular injection, and tissue edema, indicating systemic dissemination of an infectious agent. This combination of pathological changes corresponds to intrauterine septicemia, confirming the development of a generalized infection-inflammatory process typical of equine salmonellosis-induced abortion caused by members of the genus *Salmonella*.

Serological characterization of the isolate was performed by slide agglutination using diagnostic O- and H-antisera. The isolate was assigned to serogroup B based on a positive agglutination reaction with antiserum specific for the somatic O:4 antigen. Further serotyping demonstrated a positive reaction with O:12 antiserum, confirming the presence of the corresponding somatic antigen. Analysis of the flagellar antigenic profile revealed specific agglutination with H-antiserum e,n,x. The results of serological characterization are presented in Table 2.

Table 2. Serotyping results of the *S. Abortus equi* isolate according to the Kaufmann-White scheme

No	Diagnostic sera used	Detected antigens	Agglutination result	Interpretation
1	Salmonella Sero-Quick Group Kit, Group B	O:4	++++	Belonging to serogroup B
2	Salmonella Factor O:12 serum	O:12	+++	Positive reaction; presence of somatic antigen O:12 confirmed
3	Salmonella Phase H:e,n,x serum	H:e,n,x	+++	Positive reaction; flagellar antigens e,n,x detected
4	Antigenic formula	O:4,12:-:e,n,x	-	Consistent with <i>S. Abortus equi</i>

The combined results of serological typing established the antigenic profile of the isolate as O:4,12:-:e,n,x. The detection of somatic antigens O:4 and O:12 in combination with flagellar antigens e,n,x was consistent with the antigenic characteristics of *S. Abortusequi* according to the White-Kauffmann scheme. These findings were consistent with the cultural, morphological, and biochemical characteristics of the isolate, while additional identification by MALDI-TOF MS confirmed its taxonomic assignment to *S. Abortusequi*.

Thus, serological typing constituted an important component of the comprehensive identification of the isolate and enabled characterization of its somatic and flagellar antigenic profile. In combination with the morphological, cultural, biochemical, and mass spectrometric findings, the serological results provided consistent evidence supporting the identification of the isolate as *S. Abortusequi*.

Discussion

The obtained results demonstrate the diagnostic value of an integrated approach combining cultural-morphological, biochemical, serological, and mass spectrometric methods for the identification of *S. Abortusequi*. These findings are consistent with previous studies demonstrating the association of *S. Abortusequi* with reproductive disorders, particularly abortion, in horses and other equids [8, 15, 16]. Notably, an epidemiological study conducted in China between 2017 and 2022 reported the isolation of 243 *S. Abortusequi* strains from 377 examined samples, all of which were characterized by the antigenic formula 4,12:-:e,n,x [17]. The consistency of this antigenic profile among a large collection of isolates highlights the diagnostic relevance of serotyping for the identification and characterization of *S. Abortusequi*, particularly when used in combination with complementary phenotypic and instrumental identification methods.

Current research emphasizes the importance of combining conventional bacteriological methods with molecular approaches for the reliable detection and characterization of *S. Abortusequi*. Wang et al.

[8] developed a specific real-time PCR assay for the detection of *S. Abortusequi* and demonstrated high analytical sensitivity and specificity, supporting its potential application for the examination of clinical samples. Whole-genome sequencing provides additional opportunities for detailed characterization of isolates, including assessment of their phylogenetic relationships, virulence-associated factors, and genetic determinants of antimicrobial resistance [8, 10]. Whole-genome analysis of three *S. Abortusequi* strains isolated from cases of equine abortion in Kazakhstan revealed a high degree of genomic similarity, 12 *Salmonella* pathogenicity islands, three prophages, and the antimicrobial resistance-associated gene *aac* (6')-*laa* [10]. These findings demonstrate the complementary value of molecular methods for the confirmation and further characterization of *S. Abortusequi* isolates initially recovered by conventional bacteriological methods.

The antimicrobial susceptibility testing performed in the present study provides a phenotypic characterization of the *S. Abortusequi* isolate examined. Growth inhibition zone diameters ranged from 26 to 41 mm, with the isolate meeting the applied susceptibility criteria for most of the antimicrobial agents tested. However, published studies indicate variability in antimicrobial susceptibility among *S. Abortusequi* isolates. Manikandan et al. [5] reported resistance to 8 of 26 antimicrobial agents in an isolate from India and observed only 32% concordance between phenotypic resistance and the presence of corresponding genetic resistance determinants. This discrepancy demonstrates that the detection of antimicrobial resistance genes does not necessarily predict phenotypic resistance and highlights the importance of integrating phenotypic susceptibility testing with molecular characterization of resistance determinants when evaluating antimicrobial resistance in *S. Abortusequi*.

Thus, the present findings provide phenotypic and serological characterization of a single *S. Abortusequi* isolate recovered from a case of equine abortion and contribute additional data on the characteristics of this pathogen. In light of recent studies, further investigations should include larger collections of epidemiologically independent *S. Abortusequi* isolates from different geographical regions and combine standardized antimicrobial susceptibility testing with molecular genetic approaches. Such studies will provide a more comprehensive understanding of the phenotypic and genomic diversity of *S. Abortusequi* and facilitate the assessment of antimicrobial resistance patterns among circulating isolates.

This study provided a comprehensive characterization of a single *S. Abortusequi* isolate recovered from pathological material obtained from an aborted equine fetus. The findings established its morphological, cultural, biochemical, and serological characteristics and defined its phenotypic antimicrobial susceptibility profile. Given that the study was limited to a single isolate, further investigations should include a larger collection of epidemiologically independent *S. Abortusequi* isolates from different farms and geographical regions. Comparative phenotypic and molecular genetic analyses of such isolates would provide a more comprehensive understanding of the diversity, epidemiological characteristics, and antimicrobial resistance patterns of this pathogen.

Conclusion

This study provided a comprehensive characterization of an epizootic isolate of *S. Abortusequi* recovered from pathological material obtained from an aborted equine fetus. The combined assessment of morphological, cultural, biochemical, serological, and mass spectrometric characteristics enabled reliable identification of the isolate, while antimicrobial susceptibility testing defined its phenotypic susceptibility profile *in vitro*. The findings contribute additional data on the phenotypic and antigenic characteristics of *S. Abortusequi* and demonstrate the value of an integrated laboratory approach for its identification. However, the antimicrobial susceptibility results obtained in this study are specific to the examined isolate and should not be extrapolated to *S. Abortusequi* populations as a whole or interpreted as evidence of the clinical efficacy of the antimicrobial agents tested. Further studies involving epidemiologically independent isolates from different farms and geographical regions, together with standardized phenotypic susceptibility testing and molecular genetic analyses, are required to better characterize the diversity of *S. Abortusequi* and the distribution and genetic basis of antimicrobial resistance.

Author Contributions

The authors jointly developed the concept and study design; TB, ZhB: collected pathological material and performed bacteriological, morphological, cultural, biochemical, and serological analyses. ZhB: conducted; MS: verification of the isolate using MALDI-TOF MS, determined its antimicrobial susceptibility profile according to EUCAST standards. ZhB, GE, AK, IA, MB: analyzed and interpreted the data, prepared the illustrative material and the manuscript. SA and ZhB: approved the final version of the manuscript for publication.

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Conflict of Interest

The authors declare that they have no known financial interests or personal relationships that could have influenced the results presented in this work.

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