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Antibiotic Resistance of Enterobacterales Isolates Isolated from Livestock Products of Almaty Region, Kazakhstan

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ABSTRACT

This article presents the results of a study on antibiotic resistance in *Escherichia coli* and *Salmonella* species isolated from livestock products in the Almaty region of Kazakhstan. A total of 600 samples (beef, lamb, pork, poultry meat, and chicken eggs) were analyzed using classical microbiological methods and confirmed by 16S rRNA gene sequencing. Among these, *E. coli* was most commonly isolated from pork (24.32%) and mutton (34.23%), while *Salmonella* was mainly found in chicken meat (37.3%) and eggs (18.66%). Antimicrobial susceptibility was assessed by the disk diffusion method according to EUCAST guidelines against a panel of beta-lactams, fluoroquinolones, aminoglycosides, and other agents. Resistance to fluoroquinolones, beta-lactams, and aminoglycosides was observed in 43% of *E. coli* and 18% of *Salmonella* isolates. The highest resistance rates were to norfloxacin (29.25% and 15.15%) and ciprofloxacin (15.09% and 25.76%), with the lowest for meropenem (0.94% and 0%). Notably, 38% of all isolates were sensitive to all tested antibiotics. No resistance was recorded to cefazolin, tetracycline, azithromycin, erythromycin, amoxiclav, clindamycin, or doxycycline. Of 172 Enterobacteriaceae isolates, 38% were sensitive to all tested antibiotics. Species identification was confirmed via 16S rRNA gene sequencing, which showed 100% similarity with *E. coli* and *Salmonella enterica* sequences from the NCBI database. In addition, a culture medium excipient, recently synthesized and imbued with defibrinated horse blood, was appraised and deemed appropriate to bacterial cultivation plus susceptibility assays, although further comparative validation utilizing standard media remains indicated. Collectively, the results stress that antimicrobial-resistant microbes exist in foodstuffs. They additionally stress that continuing observation as well as suitable utilization of antimicrobials remain important, instead of proposing prompt alterations within veterinary practice.

Keywords: Antibiotic resistance, *Salmonella enterica*, *Escherichia coli*, Animal products.

INTRODUCTION

One of the major global public health concerns is the increasing antimicrobial resistance (AMR) of microorganisms, which continues to intensify each year (Economou and Gousia 2015; European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC, 2017).

The excessive use of antibiotics in livestock production and the food industry – including for disease prevention and growth promotion in addition to treatment – contributes to the spread of resistant bacteria and resistance genes, which can be transmitted to humans (Prestinaci et al. 2015). Antibiotic-resistant bacteria cause hard-to-treat infections and increase the risk of mortality in various countries worldwide (Shulyak et al. 2018). Irrespective of the cultural acceptance of horse blood medium in Europe, it also offers significant advantages when compared to sheep or goat blood medium. Studies and technical analysis has shown that horse blood medium generally provides X- (hemin) and V factors (NAD) required for the growth of fastidious microorganisms.

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It also contains essential nutrients and enzymes needed to supply macro and micronutrients that support cell growth (Sakur et al. 2024). A major advantage is that horses tolerate frequent blood collection of huge amounts up to 8L (16mL/kg bodyweight) extremely well, and there are no deleterious consequences in the hematological variables (Hagen et al. 2021). The availability and standardization of horse blood, especially in central Asian regions like Kazakhstan, also make it a rational option (Ombelet et al. 2022).

The World Health Organization (WHO) has developed a priority list of pathogenic microorganisms exhibiting multiple and increasing drug resistance, which require urgent development of new antibiotics. Antimicrobial resistance (AMR) is a looming global problem that requires urgent action and innovation in new drug discovery and treatment strategies. WHO Bacterial Priority Pathogens List (WBPPL) for 2024 grouped pathogens into 3 priority classes: critical, high, and medium, and utilized a methodology of Multi-Criteria Decision Analysis (MCDA) with eight criteria, such as Mortality, Incidence, Non-fatal health burden (YLDs), Trend of resistance, Transmissibility, Preventability, Treatability, Pipeline (R&D status). From this documentation, gram-negative bacteria maintained the highest priority, RR-TB (rifampicin-resistant tuberculosis), and community-acquired pathogens were also indicated as increased priorities together with MRSA (Methicillin-resistant Staphylococcus aureus) (WBPP 2024).

According to the joint report by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) for 2021–2022, published in 2024, antimicrobial resistance (AMR) continues to be monitored in bacteria isolated from humans, animals and food products. Of particular concern is the high level of resistance found in *Salmonella* spp. and *Escherichia coli* isolated from poultry meat (chicken and turkey) to ampicillin, fluoroquinolones, tetracyclines, and sulfonamides (European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC, 2024). Between 2019 and 2022, WHO member states implemented national action plans to combat AMR. Kazakhstan actively participates in these global efforts by enacting measures outlined in its 2023–2027 National Roadmap, approved by Order No. 1104 of the Chief State Sanitary Doctor of the Republic of Kazakhstan, dated 8 December 2022 (Vice-Minister of Health of the Republic of Kazakhstan 2022).

According to the study by Xu et al. (2022), 50 to 80% of all antibiotics are used in animal agriculture, primarily for the prevention of bacterial infections and to promote animal health across various countries. The misuse and overuse of antibiotics in livestock production has contributed to the emergence and spread of numerous antimicrobial-resistant bacterial strains (Martin et al. 2015). Studies conducted in CIS countries have also shown that AMR in livestock is a pressing issue, especially in border regions, where the risk of resistant microorganism transfer is high (Maistrenko et al. 2023). In Russia, studies have reported resistance to ampicillin, fluoroquinolones, and tetracyclines in *Escherichia coli* and *Salmonella* strains isolated from animals and meat products (Zaiko et al. 2019). In Tajikistan, foodborne bacterial isolates were predominantly *E. coli* and Staphylococcus spp., with high resistance rates observed in Staphylococcus spp. to kanamycin (66.7%), rifampicin (66.7%), ceftriaxone (54.6%) and lincomycin (45.5%) (Kayumova et al. 2024).

A study conducted in Northern Kazakhstan from 2018 to 2020 identified *Salmonella* spp. and *Staphylococcus aureus* among isolates from livestock, poultry and animal-derived products. *Salmonella* spp. showed high resistance to tetracyclines and nitrofurans, while *Staphylococcus aureus* isolates demonstrated significant resistance to β-lactam antibiotics and macrolides (Mendybayeva et al. 2022). In 2024, a study in the Almaty region examined the prevalence and resistance profile of *Escherichia coli* isolated from calf feces with clinical signs of colibacillosis. A multidrug-resistant strain was identified, along with the detection of 55 antibiotic resistance genes (Alexyuk et al. 2025).

Standard and reliable cultivation methods are used to isolate fastidious microorganisms. Traditionally, nutrient agar supplemented with 5% animal blood (from horses, sheep, or goats) is used, providing optimal growth conditions (Shulyak et al. 2018). However, in some countries, human donor blood is still used in laboratories, which can distort the phenotypic characteristics of bacteria, complicating their identification and antibiotic susceptibility testing. Many microorganisms exhibit atypical growth patterns – or fail to grow altogether – on media containing human blood, unlike media enriched with animal blood (AMRcloud 2024).

In addition, the use of human blood in laboratory practice carries biological risks, including the potential transmission of bloodborne pathogens such as hepatitis viruses and HIV. Accidental contact with human blood, especially through needle sticks or other sharp injuries, may result in infection (U.S. Centers for Disease Control and Prevention (CDC) n.d.). This issue is particularly critical when working with fastidious microorganisms such as Haemophilus spp., Neisseria spp., and anaerobes (AMRcloud 2024). Therefore, standardizing laboratory diagnostic methods and utilizing optimal culture media are essential for accurate research outcomes.

Given the relevance and importance of diagnostic investigations for both medical and veterinary services, a novel technology for producing a culture media component was developed for the first time in the Republic of Kazakhstan. This innovation enables the production of media enriched with micro- and macro elements, allowing for reliable and high-quality laboratory analyses.

It is worth noting that *Salmonella enterica* and *Escherichia coli* are the most frequently encountered bacteria, present in the majority of tested samples, and have demonstrated virulence in laboratory animals (Course et al. 2021). In recent years, the incidence of acute intestinal infections of bacterial etiology caused by conditionally pathogenic and pathogenic Enterobacteriaceae has been increasing in Kazakhstan. Their proportion in various regions of the country ranges from 10% to 30% (Orysbayeva et al. 2022).

In this context, the present study aimed to determine the antimicrobial resistance levels of *Salmonella* and *Escherichia coli* isolates obtained from livestock products, using culture media containing a component derived from defibrinated horse blood.

MATERIALS AND METHODS

Study objects

The study included 600 samples of five types of livestock products: beef (n=123), lamb (n=119), pork (n=122), poultry meat (n=114), and chicken eggs (n=122). The sample size was not determined through a formal power calculation; instead, it was based on feasibility, availability across retail outlets in the Almaty region, and the aim of ensuring balanced representation of the main categories of livestock products consumed locally. This approach allowed coverage of diverse product types while providing sufficient isolates for meaningful descriptive analysis. The owners of the retail outlets were not informed about the purpose or nature of the study, which helped to exclude any potential intentional influence on product quality. Meat samples were taken from whole pieces weighing between 500 and 600g, temporarily stored at ambient market temperature (20–22°C), and immediately placed in insulated containers with cooling elements for transport. The time from sampling to laboratory analysis did not exceed 2 hours, which minimized the risk of bacterial proliferation. Although WHO/ISO standards typically recommend cold-chain preservation (4±2°C), the short transport duration and controlled conditions ensured that sample integrity was maintained. All egg samples were refrigerated at the time of collection. Each sample was placed in an individual disposable polyethylene bag and transported in a thermal container with cooling elements to the bacteriology laboratory. The time from sampling to the start of laboratory analysis did not exceed 2 hours (State Committee of the USSR for Standards 1985a).

Bacteriological analysis

Two bacteriological analysis methods were used for meat samples: surface swabbing and the classical sample homogenization method. Before performing surface swabs, the entire piece of meat was swabbed for 1 minute using a sterile isotonic sodium chloride solution and a sterile swab. The obtained swabs were inoculated in multiple 0.1mL aliquots into test tubes and onto the surfaces of culture media plates (State Committee for Standards of the Council of Ministers of the USSR, 1973, 1975; State Committee of the USSR for Standards, 1985b; State Standard of the Russian Federation 1993; Deputy Chief State Sanitary Doctor of the Russian Federation 1996).

Additionally, the classical microbial isolation method was applied: sterile scissors were used to cut 1.5–2.0cm tissue pieces from different parts of each sample, which were then minced and placed into sterile homogenizer cups. To prepare a suspension, 15cm³ of sterile physiological saline (in a 1:1 ratio to the sample mass) was added to the minced tissue and homogenized. The resulting suspension was used for inoculation: 1cm³ of supernatant, containing 0.5g of product, was transferred into tubes with meat-peptone broth and onto meat-peptone agar plates, evenly spreading over the surface of the medium (State Committee for Standards of the Council of Ministers of the USSR, 1973, 1975; State Committee of the USSR for Standards, 1985b; State Standard of the Russian Federation 1993; Deputy Chief State Sanitary Doctor of the Russian Federation 1996).

Both universal and differential diagnostic culture media were used in the study. Nutrient agar (TM media, India) was employed for the indication of *E. coli* and *Salmonella*. Plates inoculated with *E. coli* and *S. enterica* were incubated in a thermostat (Memmert, Germany) at 37.5°C. Results were recorded after 18 hours of incubation. In cases where no visible growth was observed, incubation was extended to 48 hours with daily checks for bacterial growth. Upon detection of colonies morphologically characteristic of *S. enterica* and *E. coli*, identification was performed, including Gram staining microscopy and subculturing on differential diagnostic media.

For species identification of *Salmonella*, including lactose-positive strains, the selective chromogenic medium CHROMagar™ *Salmonella* Plus (CHROMagar, France) was used. For *Escherichia coli* identification, the chromogenic medium CHROMagar™ *E. coli* (CHROMagar, France) was applied. According to the manufacturer, the sensitivity of these selective chromogenic media is 99%. Isolated cultures were stored throughout the study period in tubes containing semi-solid agar based on meat-peptone broth (MPB, medium No. 3) with the addition of 2.5–3.0g standard infusion agar (pH 7.2–7.4) at +4°C (Eppendorf, Germany). Isolates were kept at +4°C as their storage duration did not exceed 30 days, which ensured stable conditions for maintaining culture viability while minimizing the risk of cell damage.

Blood collection and preparation of culture media component

Defibrinated horse blood was obtained following a careful selection of donor animals exclusively from epizootically safe farms in the Almaty region. Consideration was given to humane housing conditions, balanced feeding, and implementation of preventive and anti-epizootic measures, including all necessary vaccinations and regular veterinary check-ups.

Before blood collection, the animals' body temperature was measured, and general and biochemical blood analyses were performed. A mandatory criterion for selection was the absence of antibiotic administration for at least 30 days before the procedure.

After satisfactory laboratory control results, blood was collected aseptically from the jugular vein of the selected animals in volumes not exceeding 15% of the total circulating blood volume, following all aseptic protocols. Blood was collected into sterile tubes containing glass beads, which were manually rotated to separate fibrin strands (Rotkina and Pronina 2015). The collected blood was transported to the laboratory, where it was aseptically filtered to remove fibrin and subsequently aliquoted into sterile 50.0mL bottles (Federal Agency for Technical Regulation and Metrology 2012).

After aliquoting, the collected blood was tested for sterility by inoculating samples onto meat-peptone agar and into meat-peptone broth, followed by incubation for 14 days. The absence of microbial growth confirmed sterility. Only after obtaining negative microbiological culture results was the defibrinated horse blood used for the preparation of culture media (Federal Agency for Technical Regulation and Metrology 2012). Subsequently, plates with nutrient agar (TM Media, India) and Mueller–Hinton agar (Oxoid, UK) supplemented with 5% defibrinated horse blood were prepared, along with control plates of the same media without blood supplementation.

Hemolytic activity assessment

Hemolytic activity of the isolated cultures was evaluated on meat-peptone agar (MPA) supplemented with 5% defibrinated horse blood. Daily cultures of control strains *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), and *Streptococcus pyogenes* (ATCC 19615) were used as references. These strains were provided by the branch of the Scientific and Practical Center for Sanitary and Epidemiological Expertise and Monitoring of the National Center for Public Health, Ministry of Health of the Republic of Kazakhstan. Hemolytic activity was assessed based on the diameter of the hemolysis zone, degree of clearing within the hemolytic zone, and colony morphology for each tested culture. Observations were recorded twice: at 24 and 72 hours of incubation at 37°C. Identification of Gram-positive strains was performed by Gram-stained smear microscopy, with additional confirmation through phenotypic and biochemical testing.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of *E. coli* and *Salmonella* isolates was determined according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2024) using the disk diffusion method on Mueller–Hinton agar (Oxoid, UK) with and without 5% supplementation of defibrinated horse blood. The inoculum was prepared from 24-hour cultures of isolated *E. coli* and *Salmonella*, diluted in 3–5mL of sterile isotonic sodium chloride solution to a turbidity equivalent to 0.5 McFarland standard (DEN-1 McFarland Densitometer, BIOSAN, Latvia). Plates were inoculated directly with sterile cotton swabs and incubated for 16–20 hours at 37.5°C. Susceptibility was tested against cefotaxime (30µg), cefazolin (30µg), ampicillin-sulbactam (20µg), meropenem (10µg), ciprofloxacin (5µg), norfloxacin (10µg), levofloxacin (5µg), tetracycline (30µg), trimethoprim (5µg), gentamicin (10µg), azithromycin (15µg), erythromycin (15µg), amoxiclav (20/10µg), clindamycin (10µg), and doxycycline (30µg). Quality control of media and verification of growth properties were performed using *E. coli* ATCC 25922 RK/5 and *Salmonella choleraesuis serotype Typhimurium ATCC 14028* control strains.

Molecular genetic studies

DNA extraction

Genomic DNA was extracted from 24-hour microbial cultures using the PureLink Genomic DNA Kit following the manufacturer's protocol (Invitrogen, Carlsbad, USA). DNA concentration in the samples was measured using a Qubit® 2.0 fluorometer with the Qubit™ dsDNA HS Assay Kit (Life Technologies, Oregon, USA).

16S rRNA gene amplification

A fragment of the 16S rRNA gene was used as the genetic marker. The PCR reaction mixture (25µL total volume) contained: 12.5µL of Q5® Hot Start High-Fidelity 2X Master Mix (New England Biolabs, USA); 1.2µL each of universal primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 806R (5'-GGACTACCAGGGTATCTAAT-3') at 10 µM concentration; template DNA and nuclease-free water to a final volume of 25µL (Vegas et al. 2006). The amplification protocol was as follows: initial denaturation at 95°C for 5min; 30 cycles of 95°C for 30s, 55°C for 40s, and 72°C for 50s; final elongation at 72°C for 10min. PCR products were purified using CleanSweep™ PCR Purification reagent (Life Technologies, Carlsbad, CA).

16S rRNA gene sequencing

Sequencing of bacterial 16S rRNA gene fragments was performed using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to the manufacturer's instructions. The sequencing fragments were separated on an automated genetic analyzer, the 3500 DNA Genetic Analyzer (Applied Biosystems, Hitachi, Tokyo, Japan). Sequence data were processed with the SeqA software (Applied Biosystems). Homologous nucleotide sequences were identified using BLAST (Basic Local Alignment Search Tool) in the International GenBank database of the National Center for Biotechnology Information (NCBI), USA (World Health Organization (WHO, 2015). Phylogenetic analysis was performed using MEGA 6 software (Talukder et al. 2023). Nucleotide sequences were aligned using the ClustalW algorithm. Phylogenetic neighbors were identified with BLASTN (NJ) (Buienbayeva et al. 2023).

Data Analysis

All collected data were entered into Microsoft Excel (Microsoft Corp., USA) and subsequently analyzed using IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to calculate absolute and relative frequencies (percentages) of *E. coli* and Salmonella isolates in different livestock products. The prevalence of isolates was presented with 95% confidence intervals (CI) calculated using the Wilson method. Differences in prevalence between product categories (beef, lamb, pork, poultry, and eggs) were assessed using the chi-square (χ^2) test. A $P<0.05$ was considered statistically significant. Resistance profiles were visualized as bar graphs using GraphPad Prism 10 (GraphPad Software, USA). The proportions of antibiotic-resistant isolates were compared between *E. coli* and Salmonella using two-proportion z-tests. No imputation was performed for missing data, and only viable isolates were included in the final analysis.

RESULTS

A total of 600 samples of livestock products were analyzed using classical microbiological methods. From these samples, 111 strains of *Escherichia coli* (18.3%) and 75 strains of *Salmonella* (12.5%) were isolated. Significant differences ($\chi^2 = 12.84$, df = 4, P = 0.012) were observed in the distribution of E. coli among product types, with the highest occurrence in pork (31.15%) and lamb (22.69%). Similarly, for Salmonella, prevalence varied significantly between product categories ($\chi^2 = 10.97$, df = 4, P = 0.027), primarily due to the high contamination rate in poultry meat (24.56%) and eggs (11.48%). Prevalence by product type, with 95% confidence intervals, is presented in Table 1. Fourteen strains lost viability during storage.

Thus, the monitoring of livestock products showed that *E. coli* contamination was highest in pork and lamb meat samples, at 34.23 and 24.32%, respectively. The presence of *Salmonella* was notably frequent in poultry

Table 1: Criteria for assessing the sensitivity of *E. coli and Salmonella* strains to antimicrobials (Vegas et al. 2006)

The antimicrobial agent	Limit values of diameters of growth suppression zones (mm)	
	sensitive strain, ≥	resistant strain, <
Cefotaxime 30mcg - 3rd generation cephalosporins (betta-lactams)	20	17
Ampicillin sulbactam 20mcg - penicillins (beta-lactams)	14	14
Meropenem 10mcg - carbopenems (beta-lactams)	22	16
Ciprofloxacin 5mcg is a 1st generation fluoroquinolones.	25	22
Norfloxacin 10mcg- 2nd generation fluoroquinolones	24	24
Levofloxacin 5mcg - 3rd generation fluoroquinolones	23	19
Trimethoprim 5mcg – diaminopyrimidines	15	15
Gentamicin 10mcg – aminoglycosides	17	17

Interpretation criteria for the sensitivity of *Enterobacterales* to antimicrobials according to EUCAST (version 14.0 2024)

Table 2: Prevalence of *E. coli* and *Salmonella* by product type (with 95% CI)

Product type	Sample size (n)	*E. coli*		*Salmonella* prevalence (%)	
		No.	%	No.	%
Poultry meat	114	25	21.93	28	24.56
Lamb meat	119	27	22.69	11	9.24
Beef	123	11	8.94	11	8.94
Pork	122	38	31.15	11	9.02
Eggs	122	10	8.20	14	11.48
	χ^2 Value	12.84		10.97	
	df	4		4	
	P Value	0.012		0.027	

Data analysis by Chi-Square. For *E. coli*, significant variation among product types was detected ($\chi^2 = 12.84$, df = 4, P = 0.012). For Salmonella, differences were also significant ($\chi^2 = 10.97$, df = 4, P = 0.027).

meat and eggs, with contamination rates of 37.3 and 18.66%, respectively. In total, 186 isolates were obtained, including 111 *E. coli* and 75 *Salmonella* strains, which were further used for subsequent studies. Antimicrobial susceptibility testing by the disk diffusion method was performed on 172 isolates against eight antibiotics. To compare resistance proportions between E. coli and Salmonella isolates, two-proportion z-tests were applied for each antibiotic. Statistically significant differences were detected for ciprofloxacin (z = 2.44, P = 0.015) and gentamicin (z = 2.11, P = 0.035), indicating higher resistance among *E. coli* isolates. No significant differences were found for the remaining antibiotics (all P>0.05). These data are presented in Fig. 1 and 2. In summary, Table 2 includes χ^2 for prevalence comparisons, while Fig. 1 and 2 include z-test results comparing antibiotic resistance proportions. All statistical tests used a significance level of P<0.05.

It was found that all isolates exhibited varying degrees of sensitivity and resistance to the tested antibiotics (Fig. 1 and 2). Interpretation of susceptibility results was carried out according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (version 14.0) (European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2024). The results are presented in Fig. 1 and 2. The majority of isolates exhibited resistance to at least one of the tested antibacterial agents. Some strains demonstrated resistance to antibiotics from multiple pharmacological classes, indicating the presence of multidrug resistance.

A total of 74 isolates (43%) of *E. coli* demonstrated resistance to fluoroquinolone antibiotics (1st, 2nd, and 3rd generation), beta-lactams (1st and 2nd generation), and aminoglycosides. According to the data obtained, the resistance frequency to cefotaxime in *E. coli* was 2.83% (n=3), 95% CI 0.97–7.99; to ampicillin-sulbactam – 2.83% (n=3), 95% CI 0.97–7.99; to norfloxacin – 29.25% (n=31), 95% CI 21.43–38.51; to ciprofloxacin – 15.09% (n=16), 95% CI 9.51–23.12; to meropenem – 0.94% (n=1), 95% CI 0.17–5.15; to levofloxacin – 2.83% (n=3), 95% CI 0.97–7.99; and to gentamicin – 16.04% (n=17), 95% CI 10.26–24.19. Isolates of *Salmonella* (n=32, 18%) showed resistance only to fluoroquinolone antibiotics (1st, 2nd, and 3rd generation). Resistance to norfloxacin was 15.15% (n=10), 95% CI 8.44–25.69; to ciprofloxacin – 25.76% (n=17), 95% CI 16.75–37.43; and to levofloxacin – 7.58% (n=5), 95% CI 3.28–16.54. The tested *Salmonella* isolates were sensitive to beta-lactams and aminoglycosides. No resistant strains of *E. coli* or *Salmonella* to cefazolin, tetracycline, azithromycin, erythromycin, amoxiclav, clindamycin, or doxycycline were detected. Overall, the highest resistance rates among *E. coli* and *Salmonella* isolates were observed for norfloxacin (29.25 and 15.15%, respectively) and ciprofloxacin (15.09 and 25.76%, respectively), while the lowest resistance was observed for meropenem (0.94 and 0.00%, respectively). It should be noted that out of 172 enterobacterial isolates tested, 66 (38%) were sensitive to all groups of tested antibiotics.

During the study of antibiotic-resistant and -sensitive enterobacteria, the developed nutrient medium component, obtained from defibrinated horse blood, was tested for microbial cultivation. Hemolytic activity determination confirmed that all tested cultures exhibited the necessary phenotypic properties. Cultivation of *Enterococcus faecalis* strain on solid nutrient medium resulted in small, round, convex, shiny colonies sized 0.5 to 1mm without hemolysis zones. Growth of *Staphylococcus aureus* was characterized by colonies 0.5 to 1.5mm in diameter, surrounded by hemolysis zones not exceeding 2.0mm. *Streptococcus pyogenes* grew gray colonies approximately 1mm in size with clearly defined hemolysis zones ranging from 3 to 4mm. Notably, on blood-enriched meat-peptone agar, the described hemolysis zone sizes were observed after 24 hours of incubation, with significant enlargement after 72 hours. Moreover, cultivation of *Staphylococcus aureus* and *Streptococcus pyogenes* on nutrient media containing the proposed component from defibrinated horse blood produced clearer and more extensive hemolysis zones, indicating enhanced lysis.

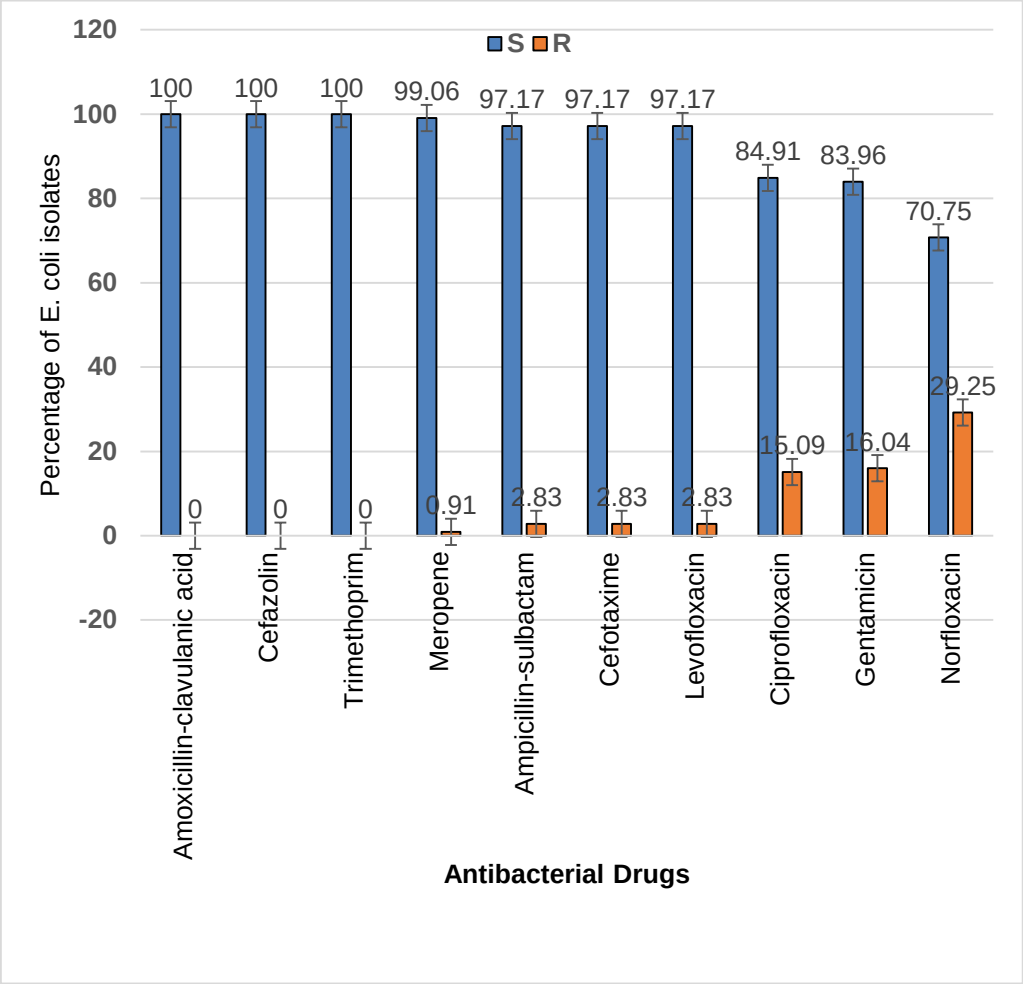

Fig. 1: Sensitivity and resistance of isolated *E. coli* isolates to antibacterial drugs (S - sensitivity; R - resistance): Note: For ciprofloxacin (z = 2.44, P = 0.015) and gentamicin (z = 2.11, P = 0.035), E. coli showed significantly higher resistance. No significant differences were found for other antibiotics (P>0.05). Significance threshold: P<0.05.

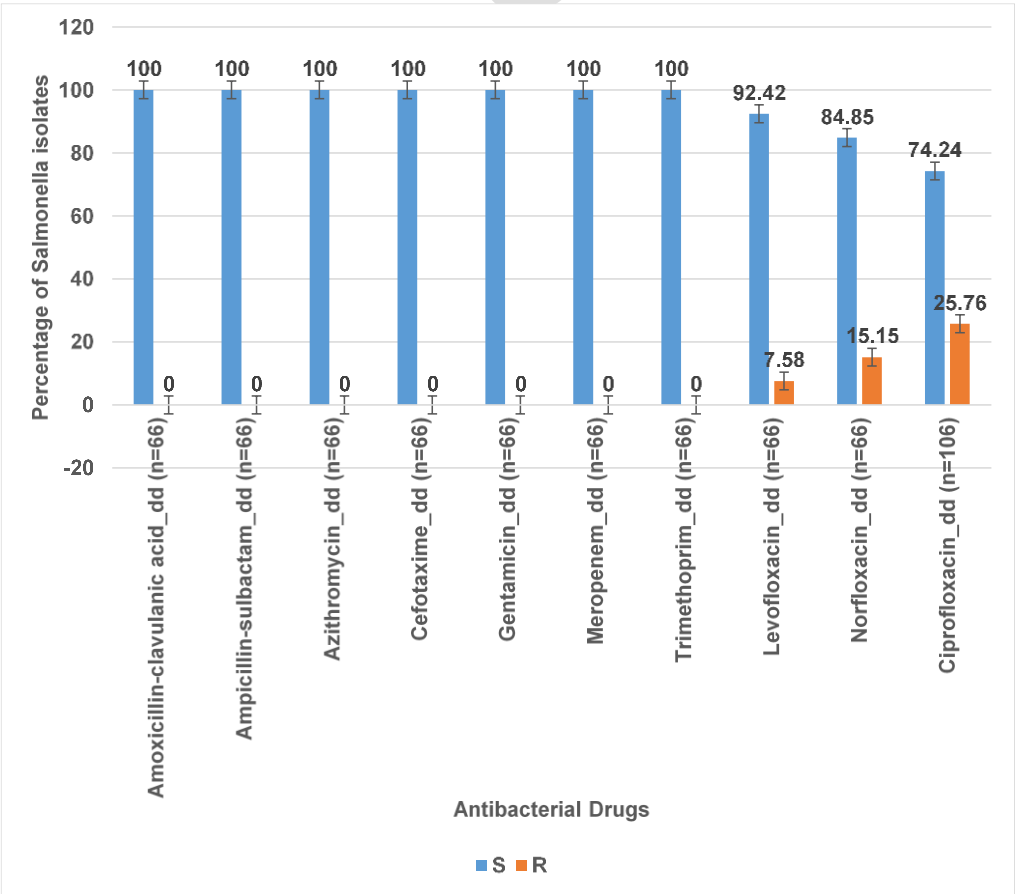

Fig. 2: Sensitivity and resistance of isolated *Salmonella* isolates to antibacterial drugs (S - sensitivity; R - resistance): Note: Two-proportion z-tests confirmed significant differences between E. coli and Salmonella resistance profiles for ciprofloxacin (P = 0.015) and gentamicin (P = 0.035): Other antibiotics showed no significant inter-species difference (P>0.05); Significance threshold: P<0.05.

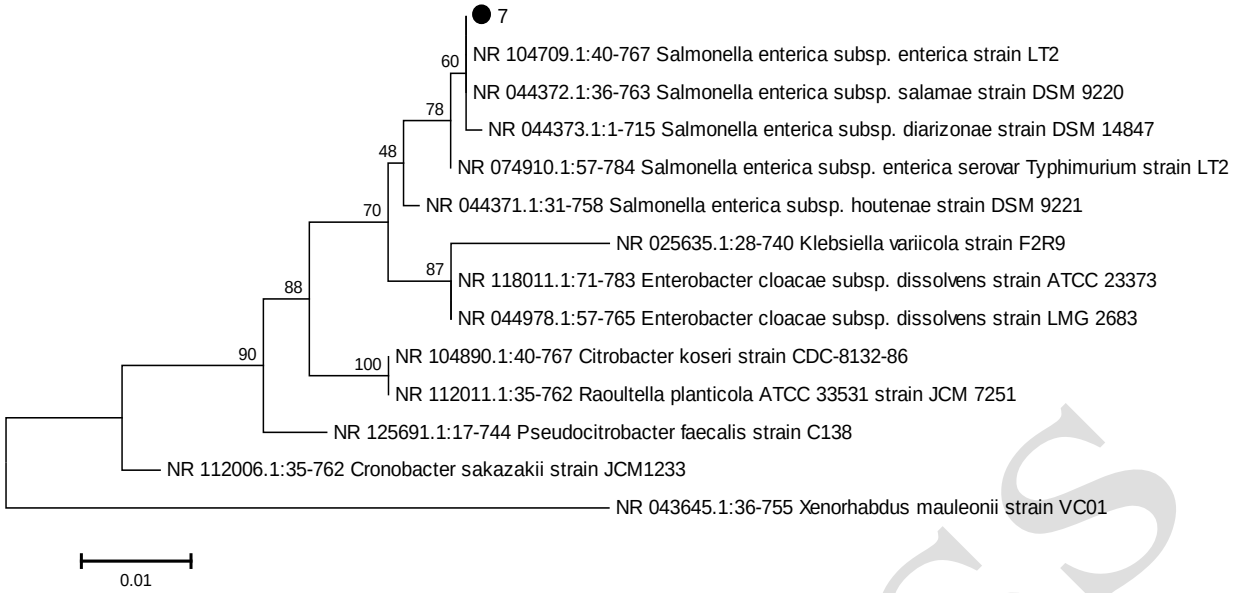

Fig. 3: Phylogenetic tree based on comparison of the nucleotide sequence of the 16S rRNA gene of the studied *S. enterica* sample with reference sequences from the NCBI database. The *S. enterica* sample under study is listed as 'Isolate 7 gene for 16S rRNA'.

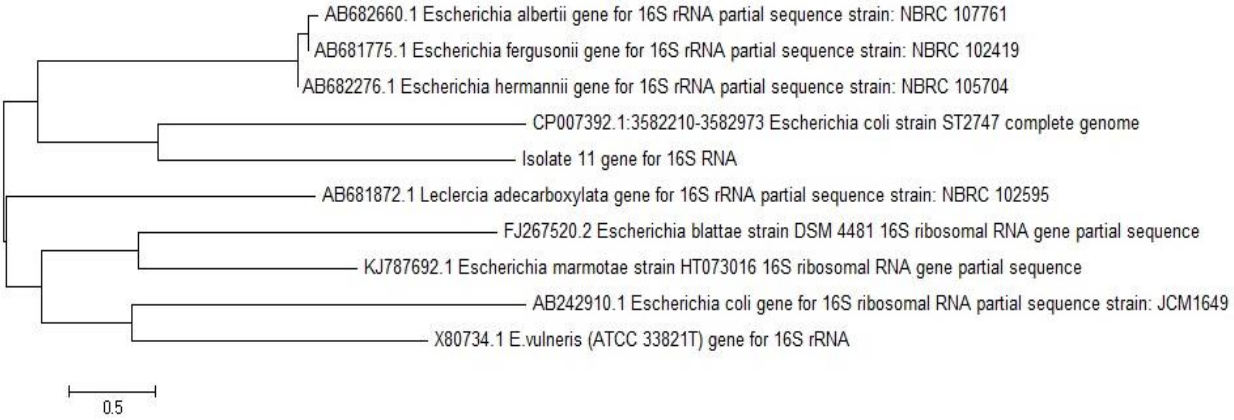

Fig. 4: Phylogenetic tree based on comparison of the nucleotide sequence of the 16S rRNA gene of the studied *E. coli* sample with reference sequences from the NCBI database. The investigated *E. coli* sample is indicated as 'Isolate 11 gene for 16S rRNA'.

To confirm the species identity of the isolated enterobacteria from meat, 16S rRNA gene sequencing was performed on two isolates of *E. coli* and *Salmonella*, and results were compared with reference sequences from the NCBI database. The findings are presented in Fig. 3 and 4. In Fig. 3, according to the constructed phylogenetic tree, the sample "Isolate 7 gene for 16S rRNA" clusters within the *Salmonella enterica* group, forming a monophyletic clade with a single common ancestor. Fig. 4 shows a phylogenetic tree representing the evolutionary relationships among various *E. coli* strains based on nucleotide sequence comparisons. The *E. coli* isolate "Isolate 11 gene for 16S rRNA" is 100% homologous to other *Escherichia* species, while possessing distinct genetic features that differentiate it from other *Escherichia* species and strains.

DISCUSSION

The study revealed a high level of resistance of *E. coli* to norfloxacin (29.25%), ciprofloxacin (15.09%), and gentamicin (16.04%), as well as *Salmonella* resistance to norfloxacin (15.15%) and ciprofloxacin (25.76%). In some cases, *E. coli* isolates exhibited combined resistance to norfloxacin and gentamicin. The observed prevalence of *E. coli* and *Salmonella* resistant to norfloxacin and gentamicin exceeded the global average resistance thresholds for these antibiotics. A comparatively high level of resistance to levofloxacin (7.58%) was also noted in *Salmonella* isolates. Low resistance rates were observed in *E. coli* to cefotaxime (2.83%), ampicillin-sulbactam (2.83%), levofloxacin (2.83%), and meropenem (0.94%). The results align with the studies of Böger et al. (2021) who highlighted that in a Brazilian study, analyzed water samples showed that *E. coli* was resistant to amoxicillin, norfloxacin, ciprofloxacin, doxycycline and sulfamethoxazole. The study also aligned with Hryhoriv et al. (2021) whose approach focused on the chemical modification of Ciprofloxacin and Norfloxacin to counteract E coli resistance. The results on *Salmonella* resistance aligns strongly with the study of El-Saeed et al. (2024), who extracted Colistin-, cefepime-, and levofloxacin-resistant *Salmonella* from Egyptian chicken carcass. From the results, a trend of selective resistance is

observed and this could encourage future research by helping scientist identify antibiotics that can be easily tweaked to treat pathogenic infections due to their low resistance profile.

Similar results were reported in other regions of Kazakhstan. In a study by Mendybayeva et al. (2023) conducted in Northern Kazakhstan, 46 *Salmonella* strains were isolated from 398 poultry product samples, with over 90% showing resistance to at least one antimicrobial agent, and 64.3% classified as multidrug-resistant. Resistance genes including tetA, blaTEM, aadA, sul3, catII, as well as class 1 and 2 integrons were identified in the isolated cultures, indicating a high potential for horizontal transfer of resistance. The increase in *Salmonella* infection poses a huge threat to the chicken poultry industry and global public health as a whole (Tariq et al. 2022). Subsistence and commercial farmers are advised to direct more attention to harnessing the methods of preventing *Salmonella* infection. These methods contribute to a cost expenditure reduction, a reduction in chemical inputs, increase in meat quality and an increase in general global health (Wessels et al. 2021). These methods includes and are not limited to pest control, ensuring farm hygiene and ensuring access to *Salmonella* free food. Early life protection using probiotics, prebiotics, and bacteriophages can also help develop favorable gut flora (Ruvalcaba-Gómez et al. 2022).

The authenticity of the cultivated *E. coli* and *Salmonella* strains was confirmed through phylogenetic analysis of 16S rRNA genes. No nucleotide differences or substitutions were found in the 16S rRNA gene sequences of *E. coli* isolate "Isolate 11 gene for 16S rRNA," showing 100% homology with Escherichia genus sequences. Similarly, the gene "Isolate 7 gene for 16S rRNA" showed 100% identity with reference sequences of *Salmonella enterica*, with no nucleotide substitutions detected.

A meta-analysis covering 10 years of research in South Asian countries highlights the widespread prevalence of resistant *Salmonella* strains, especially in animal-derived products. The authors identified resistance genes tetA, blaTEM, aadA, sul3, catII, and class 1 and 2 integrons, confirming the global scale of the antibiotic resistance problem (Talukder et al. 2023). These findings emphasize the need for systematic epidemiological surveillance and stricter antimicrobial stewardship in livestock and food industries to contain the spread of antibiotic resistance.

During the evaluation of the developed culture medium component derived from defibrinated horse blood, its suitability for the detection and susceptibility testing of the studied microorganisms was confirmed. It is imperative to note that a primary limitation of this study lies in its cross-sectional design, which encompasses antibiotic resistance prevalence at a specific point in time. This experimental design discourages the tracking of evolution and trends of resistance patterns over longer periods (Christaki et al. 2019; Larsson and Flach 2021). Future research should focus on long term monitoring of antimicrobial resistance so as to obtain comprehensive data and to understand the roles of underlying factors like seasonal changes in antimicrobial resistance (Palmer and Buckley 2021; Sajjad et al. 2024). Furthermore, the geographical scope is restricted to the Almaty region, which, while valuable for local surveillance, limits the generalizability of the results to other regions in Kazakhstan or to countries with different agricultural and antibiotic use practices (Patel et al. 2023). It is imperative that the locally produced culture medium component made from defibrinated horse blood needs to be thoroughly assessed in comparative studies with commercially available and globally recognized media. This would impartially verify its efficacy, affordability, and dependability for standard diagnostic and antimicrobial susceptibility testing in Kazakhstani labs (Giancola and Hart 2022).

The culture medium component utilizing defibrinated horse blood offers specific biochemical and practical advantages that enhance diagnostic reliability over suboptimal alternatives, particularly human blood, such as the absence of clot formation due to the absence of fibrin, a longer shelf life, and the presence of homogeneity, which is essential for microbial culture (Hardy et al. 2024a, b). A key improvement lies in the consistent composition of horse blood, which is naturally deficient in certain antibodies and complement proteins that can inhibit the growth of fastidious microorganisms. A more permissive environment for bacterial cultivation is created by the lack of growth-inhibiting factors, which results in more robust and distinctive colonial morphology and hemolytic patterns.

By providing a richer and more uniform nutrient profile, the medium ensures optimal expression of bacterial resistance mechanisms, leading to more accurate and unambiguous zone edge definition. This minimizes the risk of falsely sensitive or falsely resistant antimicrobial sensitivity test results that could occur from poor growth on inferior media. Consequently, this locally developed reagent provides a safer, more standardized, and diagnostically superior option for both isolation and AST, directly addressing a critical gap in laboratory infrastructure and improving the fidelity of AMR surveillance data (Latypova et al. 2024).

Conclusion

Almaty region can be classified as an area with low resistance of *E. coli* to cefotaxime, ampicillin-sulbactam, levofloxacin, and meropenem, as well as low resistance of *Salmonella* to levofloxacin. Conversely, high resistance levels were observed in *E. coli* to norfloxacin, ciprofloxacin, and gentamicin, and in *Salmonella* to norfloxacin and ciprofloxacin. The authenticity of two Enterobacteriaceae isolates was confirmed by phylogenetic analysis. Based on these findings, it is advisable to reconsider the use of the aforementioned antibiotics in veterinary practice. The developed domestic reagent can be recommended for widespread application in laboratory practice across Kazakhstan.

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Ethics Statement: All procedures involving animals (blood collection from donor horses for culture media preparation) were conducted in accordance with national and international animal welfare standards.

Author's Contribution: ZL, ShS, and MY contributed to the conception and design of the study. MK, AB, VS, and ZhA carried out bacteriological analyses. GK, ZhT, AM, and SM were responsible for antimicrobial susceptibility testing. ESh, AL and LA performed molecular analyses. AT supervised the project, analyzed data, and prepared first version of the manuscript. All authors reviewed and approved the final version of the paper.

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