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Research article

Phenotypic antimicrobial susceptibility of *Escherichia coli* isolated from bovine superficial cervical (prescapular) lymph nodes from market-derived bovine products in Semey, Kazakhstan

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Abstract

Background and Aim. Bovine lymph nodes are relevant to veterinary diagnostics and beef safety because bacteria within lymphatic tissue may evade carcass surface decontamination and enter the food chain. The aim of this study was to describe the phenotypic antimicrobial susceptibility profile of *E. coli* isolated from bovine superficial cervical (prescapular) lymph nodes collected from market-derived bovine products in Semey, Kazakhstan.

Materials and Methods. Thirty-two *E. coli* isolates recovered in autumn from bovine prescapular lymph nodes during pre-sale veterinary-sanitary inspection were tested by disk diffusion on Mueller-Hinton agar. Eighteen antimicrobial agents were evaluated. Final susceptible/intermediate/resistant (S/I/R) categories were determined using CLSI breakpoints where applicable, and Wilson 95% confidence intervals were calculated for susceptible proportions. Isolate-level MDR profiles were summarized from the available phenotypic susceptibility profiles.

Results. The highest susceptibility was observed for gentamicin and amikacin (32/32 each; 100%), followed by enrofloxacin and ceftriaxone (31/32 each; 97%), and ciprofloxacin, cefotaxime, and amoxicillin/clavulanate (30/32 each; 94%). High susceptibility was also observed for azithromycin and tetracycline (29/32 each; 91%), streptomycin (28/32; 88%), and kanamycin and neomycin (27/32 each; 84%). Lower susceptibility was observed for imipenem (18/32; 56%), amoxicillin (14/32; 44%), and doxycycline (4/32; 12%). The highest resistance rates were detected for benzylpenicillin (32/32; 100% R), tylosin (30/32; 94% R), doxycycline (15/32; 47% R), and amoxicillin (10/32; 31% R). Isolate-level analysis classified 17/32 isolates (53.1%) as MDR; the dominant resistant classes in MDR isolates were penicillins/beta-lactams, macrolides, and tetracyclines.

Conclusion. *E. coli* isolated from bovine prescapular lymph nodes showed a heterogeneous susceptibility profile. Gentamicin and amikacin showed complete in vitro susceptibility, and fluoroquinolones and third-generation cephalosporins retained high activity against most isolates. In contrast, benzylpenicillin and tylosin showed poor activity based on descriptive phenotypic observations, whereas doxycycline and aminopenicillin-related agents showed mixed or reduced activity. MDR was detected in 17/32 isolates (53.1%), with isolate-level profiles dominated by combinations involving penicillins/beta-lactams, macrolides, and tetracyclines. These data support continued phenotypic surveillance of carcass-associated tissues and should be interpreted together with approved indications, food-animal regulations, and antimicrobial stewardship principles rather than as empirical treatment recommendations.

Keywords: antimicrobial susceptibility; beef safety; *Escherichia coli*; market-derived bovine product; prescapular lymph node.

Introduction

Antimicrobial resistance (AMR) in cattle-associated *E. coli* remains important for both veterinary medicine and food safety. *E. coli* is commonly used as an indicator organism in AMR surveillance because it is widespread in food-producing animals, clinically relevant, and able to acquire and disseminate resistance determinants [1].

Peripheral lymph nodes have attracted increasing attention in beef safety because bacteria located inside these tissues may escape surface decontamination and later enter trimmings used for ground products. While subiliac and popliteal lymph nodes are studied more often, data on superficial cervical (prescapular) lymph nodes remain relatively limited [2, 3].

Published studies from cattle lymph nodes, slaughterhouse environments, and dairy systems indicate heterogeneous susceptibility patterns. Resistance commonly persists against tetracyclines, aminopenicillins, and some older agents, whereas selected aminoglycosides, fluoroquinolones, and third-generation cephalosporins may retain *in vitro* activity depending on region and production context [4–9].

Such variability makes local phenotypic monitoring essential, particularly when isolates originate from products intended for human consumption. In the present case, the isolates were obtained during pre-sale inspection at markets in Semey, Kazakhstan, giving the results direct relevance for veterinary-sanitary control and food-chain surveillance.

The aim of this study was to describe the phenotypic antimicrobial susceptibility profile of *E. coli* isolated from bovine superficial cervical (prescapular) lymph nodes collected from market-derived bovine products in Semey, Kazakhstan. This manuscript is intentionally limited to conventional phenotypic susceptibility data and does not include PCR, sequencing, virulence profiling, or phylogenetic characterization. Therefore, the results should be interpreted as a preliminary local descriptive dataset rather than as a comprehensive molecular epidemiological investigation.

Materials and Methods

Ethical approval

The study used products sampled during routine pre-sale veterinary-sanitary inspection and did not involve experimental procedures on live animals; therefore, specific animal ethics approval was not required according to local practice. No personal data or farm identifiers were used in the analytical dataset.

Study design and sample origin

This work is a descriptive laboratory study based on the antimicrobial susceptibility component of an existing diagnostic dataset. Thirty-two *E. coli* isolates recovered from bovine superficial cervical (prescapular) lymph nodes were included. The lymph nodes obtained from cattle products presented for sale at markets in Semey, Kazakhstan, and were sampled in autumn during routine pre-sale veterinary-sanitary inspection. The study did not involve experimental infection or manipulation of live animals. Because the samples were obtained from market-derived products rather than a prospective farm-level sampling design, animal age, production type (beef or dairy), farm of origin, geographic origin before marketing, and previous antimicrobial exposure history were not available in the analytical dataset.

Phenotypic confirmation by Gram staining

Before antimicrobial susceptibility testing, presumptive *E. coli* isolates were examined by Gram staining as a phenotypic confirmation method. Smears were prepared from fresh pure cultures, stained using the conventional Gram-staining procedure, and examined microscopically. Isolates showing Gram-negative, rod-shaped morphology consistent with *E. coli* were included in the antimicrobial susceptibility analysis.

Disk diffusion testing

The disk-diffusion workflow was described based on the available laboratory records and routine laboratory procedures for antimicrobial susceptibility testing. For each isolate, bacterial material from a fresh pure culture was used to prepare a homogeneous suspension following routine laboratory practice. Mueller-Hinton agar was prepared according to the manufacturer's instructions and poured into Petri dishes to an approximate depth of 4 mm. The bacterial suspension was applied to the agar surface with a sterile cotton swab by evenly streaking the plate. After the surface had dried briefly, antibiotic disks were placed at appropriate intervals on the inoculated agar surface. Plates were incubated aerobically

under routine laboratory conditions, after which inhibition zone diameters were measured in millimeters using a ruler or caliper. Representative stages of sample handling, lymph-node separation, and disk-diffusion testing are shown in Figure 1.



Figure 1. Main laboratory stages: A – pathological material; B – lymph-node separation; C – agar plate with antibiotic disks; D – incubated plates

Antimicrobial panel and interpretation

The antimicrobial panel included doxycycline, tylosin, kanamycin, streptomycin, tetracycline, neomycin, ampicillin, azithromycin, ciprofloxacin, enrofloxacin, benzylpenicillin, amoxicillin/clavulanate, amoxicillin, ceftriaxone, cefotaxime, imipenem, gentamicin, and amikacin. Final susceptible/intermediate/resistant (S/I/R) categories were determined using CLSI breakpoints where applicable. Azithromycin was interpreted using the following criteria: S $\geq$ 13.5 mm, I = 12.5-13 mm, and R $\leq$ 12 mm. The present study focuses on inhibition-zone diameters and overall phenotypic susceptibility patterns. Benzylpenicillin and tylosin were retained because they were included in the original local laboratory testing panel; however, these agents are not intended to serve as preferred clinical indicators for Enterobacterales. Agent-organism combinations with limited or non-harmonized interpretive criteria are discussed cautiously and presented as phenotypic monitoring data rather than direct therapeutic recommendations [10]. For isolate-level MDR evaluation, multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antimicrobial classes [11]. Intermediate results were not considered resistant in this classification. Resistant classes were determined from the available phenotypic susceptibility profiles, and the resistant agents contributing to each MDR profile were summarized at isolate level.

Statistical analysis

Descriptive statistics were used. For each antimicrobial agent, the mean inhibition-zone diameter and the percentage of isolates categorized as S, I, and R were recorded. Wilson 95% confidence intervals (CIs) were calculated for susceptible proportions to reflect the uncertainty associated with the limited sample size. In addition to agent-level descriptive analysis, isolate-level MDR profiles were summarized by listing resistant antimicrobial agents and antimicrobial classes for each MDR-positive isolate. The MDR proportion was calculated by dividing the number of MDR isolates by the total number of examined isolates. Inferential statistical testing and animal- or farm-level risk-factor analysis were not performed because the available metadata were limited.

Results

All isolates included in the susceptibility analysis showed Gram-negative, rod-shaped morphology on Gram staining, supporting their phenotypic consistency with *E. coli*. Representative stages of sample

handling and disk-diffusion testing are shown in Figure 1. Susceptibility results and isolate-level MDR profiles are summarized in Table 1, Figure 2, and Table 2.

Table 1. Phenotypic antimicrobial susceptibility profile of *E. coli* isolates according to CLSI breakpoints (n = 32)

Antimicrobial	Mean zone, mm	S, n (%)	I, n (%)	R, n (%)	S 95% CI, %
Doxycycline	12.9	4 (12)	13 (41)	15 (47)	5.0-28.1
Tylosin	9.1	0 (0)	2 (6)	30 (94)	0.0-10.7
Kanamycin	19.4	27 (84)	5 (16)	0 (0)	68.2-93.1
Streptomycin	19.1	28 (88)	3 (9)	1 (3)	71.9-95.0
Tetracycline	17.7	29 (91)	2 (6)	1 (3)	75.8-96.8
Neomycin	18.7	27 (84)	3 (9)	2 (6)	68.2-93.1
Ampicillin	18.3	22 (69)	4 (12)	6 (19)	51.4-82.0
Azithromycin	24.0	29 (91)	0 (0)	3 (9)	75.8-96.8
Ciprofloxacin	32.4	30 (94)	1 (3)	1 (3)	79.9-98.3
Enrofloxacin	29.7	31 (97)	0 (0)	1 (3)	84.3-99.4
Benzylpenicillin	6.5	0 (0)	0 (0)	32 (100)	0.0-10.7
Amoxicillin/clavulanate	21.3	30 (94)	0 (0)	2 (6)	79.9-98.3
Amoxicillin	15.6	14 (44)	8 (25)	10 (31)	28.2-60.7
Ceftriaxone	32.1	31 (97)	0 (0)	1 (3)	84.3-99.4
Cefotaxime	31.7	30 (94)	1 (3)	1 (3)	79.9-98.3
Imipenem	23.2	18 (56)	9 (28)	5 (16)	39.3-71.8
Gentamicin	23.4	32 (100)	0 (0)	0 (0)	89.3-100.0
Amikacin	22.6	32 (100)	0 (0)	0 (0)	89.3-100.0

Note: S/I/R, susceptible/intermediate/resistant; CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute. S/I/R categories were assigned using the provided CLSI breakpoints where applicable. Azithromycin was interpreted as S ≥ 13.5 mm, I = 12.5–13 mm, and R ≤ 12 mm. “–” entries were treated as 0 mm/no inhibition zone so that n = 32 for every antimicrobial. Wilson 95% CI is shown for the susceptible proportion. Selected agent–organism combinations with limited interpretive relevance for Enterobacterales should be regarded as descriptive phenotypic observations rather than treatment recommendations.

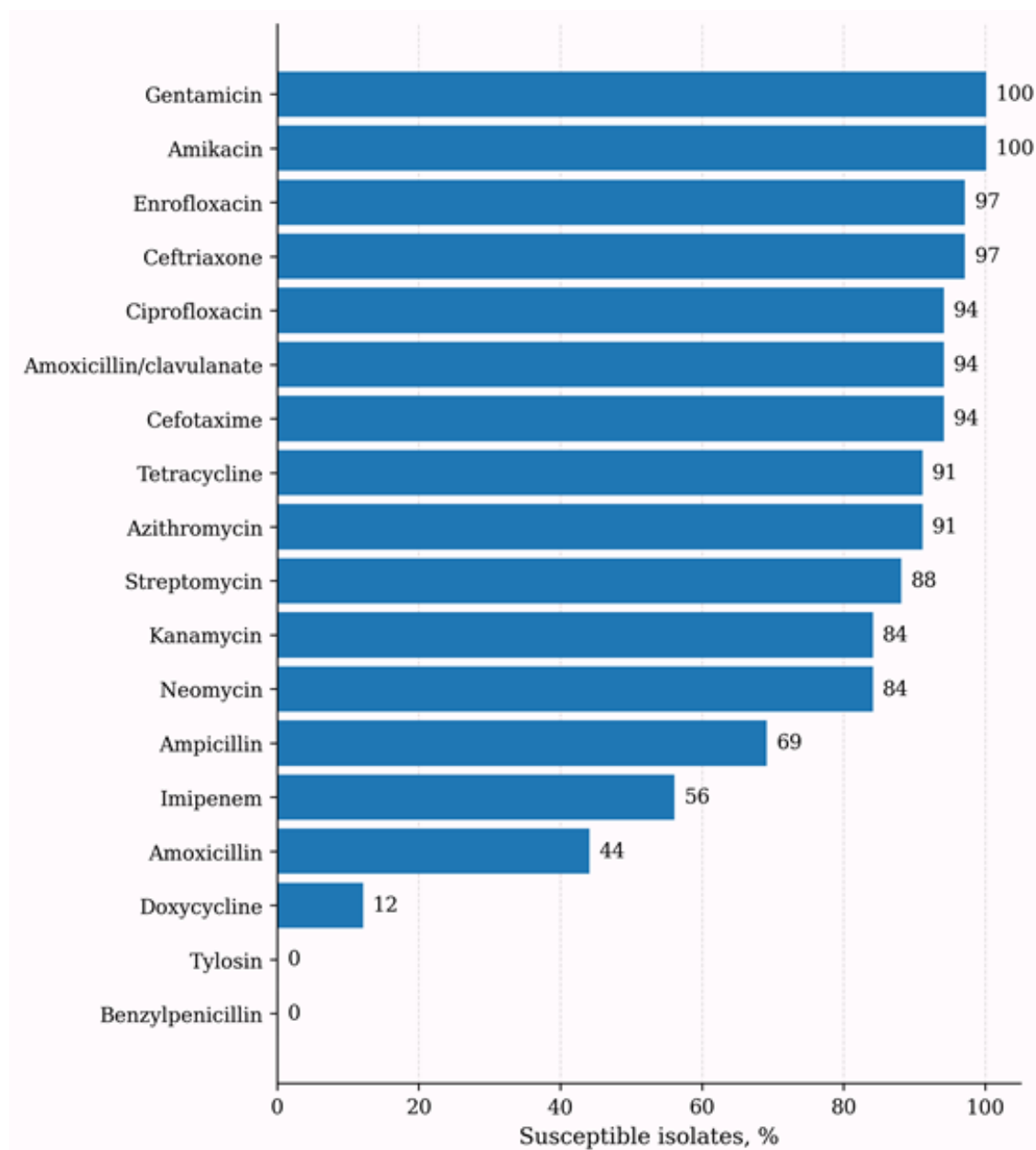


Figure 2. Percentage of susceptible isolates for the tested antimicrobials (n = 32; ordered by susceptible proportion)

Based on the updated CLSI interpretation, the highest susceptible proportions were observed for gentamicin and amikacin (32/32 each; 100%; Wilson 95% CI 89.3-100.0%). Very high susceptibility was also observed for enrofloxacin and ceftriaxone (31/32 each; 97%), followed by ciprofloxacin, cefotaxime, and amoxicillin/clavulanate (30/32 each; 94%), azithromycin and tetracycline (29/32 each; 91%), streptomycin (28/32; 88%), and kanamycin and neomycin (27/32 each; 84%).

Lower or variable susceptibility was observed for ampicillin (22/32; 69%), imipenem (18/32; 56%), and amoxicillin (14/32; 44%). Excluding benzylpenicillin and tylosin, which were retained as descriptive phenotypic observations, doxycycline showed the lowest susceptible proportion among the remaining agents (4/32; 12%) and also showed a large intermediate fraction (13/32; 41%) and high resistance (15/32; 47%).

The highest resistance rates were recorded for benzylpenicillin (32/32; 100%), tylosin (30/32; 94%), doxycycline (15/32; 47%), amoxicillin (10/32; 31%), ampicillin (6/32; 19%), and imipenem (5/32; 16%). Resistance to aminoglycosides, fluoroquinolones, and third-generation cephalosporins was

rare or absent, with no gentamicin or amikacin resistance and only one resistant isolate for ciprofloxacin, enrofloxacin, ceftriaxone, and cefotaxime.

Seventeen isolates were classified as MDR (17/32; 53.1%). Isolate-level MDR profiles are summarized in Table 2. Among MDR isolates, resistance most frequently involved penicillins/beta-lactams (17/17), macrolides (16/17), and tetracyclines (14/17), whereas fewer MDR isolates showed resistance involving carbapenems (5/17), aminoglycosides (2/17), and third-generation cephalosporins (1/17).

Table 2. Isolate-level MDR profiles of *E. coli* isolates

Isolate No.	No. of resistant classes	Resistant antimicrobial classes	Resistant antimicrobials
2	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin)
4	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (AMX)	DOX (Doxycycline), TYL (Tylosin), AMX (Amoxicillin)
6	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin)
7	4	Tetracyclines (DOX); Macrolides (TYL, AZM); Aminoglycosides (NEO); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), NEO (Neomycin), AZM (Azithromycin), PEN (Benzylpenicillin)
8	3	Tetracyclines (DOX); Macrolides (TYL, AZM); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), AZM (Azithromycin), PEN (Benzylpenicillin)
10	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin)
14	4	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN); Carbapenems (IPM)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin), IPM (Imipenem)
16	4	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN, AMX); Carbapenems (IPM)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin), AMX (Amoxicillin), IPM (Imipenem)
17	3	Tetracyclines (DOX, TET); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), TET (Tetracycline), PEN (Benzylpenicillin)
21	3	Macrolides (TYL); Aminoglycosides (STR); Penicillins/beta-lactams (PEN)	TYL (Tylosin), STR (Streptomycin), PEN (Benzylpenicillin)
23	3	Macrolides (TYL); Penicillins/beta-lactams (PEN, AMX); Third-generation cephalosporins (CRO, CTX)	TYL (Tylosin), PEN (Benzylpenicillin), AMX (Amoxicillin), CRO (Ceftriaxone), CTX (Cefotaxime)

Continuation of Table 2

25	4	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (AMP, PEN); Carbapenems (IPM)	DOX (Doxycycline), TYL (Tylosin), AMP (Ampicillin), PEN (Benzylpenicillin), IPM (Imipenem)
26	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin)
27	3	Tetracyclines (DOX); Penicillins/beta-lactams (PEN); Carbapenems (IPM)	DOX (Doxycycline), PEN (Benzylpenicillin), IPM (Imipenem)
28	3	Macrolides (TYL); Penicillins/beta-lactams (PEN, AMX); Carbapenems (IPM)	TYL (Tylosin), PEN (Benzylpenicillin), AMX (Amoxicillin), IPM (Imipenem)
31	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin)
32	3	Tetracyclines (DOX); Macrolides (TYL); Penicillins/beta-lactams (PEN)	DOX (Doxycycline), TYL (Tylosin), PEN (Benzylpenicillin)

Note: Antimicrobial abbreviations: DOX, doxycycline; TYL, tylosin; STR, streptomycin; TET, tetracycline; NEO, neomycin; AMP, ampicillin; AZM, azithromycin; PEN, benzylpenicillin; AMX, amoxicillin; CRO, ceftriaxone; CTX, cefotaxime; IPM, imipenem. Only resistant antimicrobials recorded in MDR isolates are listed.

Discussion

This dataset from prescapular lymph nodes associated with market-derived bovine products showed a heterogeneous phenotype rather than generalized resistance. Gentamicin and amikacin showed complete *in vitro* susceptibility, and susceptibility to fluoroquinolones and third-generation cephalosporins remained high, although not absolute. In contrast, benzylpenicillin and tylosin showed the lowest *in vitro* activity, while doxycycline, amoxicillin, ampicillin, and imipenem showed mixed or reduced susceptibility. The isolate-level MDR table further showed that MDR was present in more than half of the examined isolates and was mainly driven by combinations involving penicillins/beta-lactams, macrolides, and tetracyclines.

The generally high susceptibility to ceftriaxone, cefotaxime, ciprofloxacin, and gentamicin was broadly consistent with previous cattle-associated studies. Grispoldi et al. reported high susceptibility of bovine lymph-node *E. coli* isolates to ceftriaxone, ciprofloxacin, cefotaxime, and gentamicin [2]. Similarly, slaughterhouse- and red-meat-production studies by Vázquez-Villanueva et al. and Guragain et al. showed that *E. coli* resistance patterns can vary by processing environment and production context, with some higher-importance agents retaining activity in specific datasets [5, 9].

The reduced or variable susceptibility to doxycycline, amoxicillin, and ampicillin agree with broader evidence that tetracycline and aminopenicillin-related resistance remains common in cattle-associated *E. coli*. Comparable patterns have been described in Canadian dairy herds, Bavarian dairy farms, Australian pasture-based dairy systems, and cattle isolates carrying diverse resistance genes [4, 6-8]. In the present dataset, doxycycline resistance was more prominent than tetracycline resistance, which may reflect differences in local breakpoint interpretation, disk performance, or isolate-level phenotype rather than a uniform class-wide pattern.

The added MDR table provides epidemiological detail beyond the agent-level susceptibility percentages. The most frequent resistant components among MDR isolates were benzylpenicillin and tylosin (16/17 MDR isolates each) and doxycycline (14/17), followed by imipenem (5/17) and amoxicillin

(4/17). Less frequent MDR components included azithromycin, neomycin, tetracycline, streptomycin, ceftriaxone, cefotaxime, and ampicillin. This pattern suggests that MDR in the present dataset was driven mainly by specific older or locally tested agents, whereas resistance to most fluoroquinolones and aminoglycosides remained uncommon.

Direct comparison with surveillance data from Central Asia remains limited because available studies differ in animal category, sample source, and laboratory methods. Nevertheless, recent data from Kazakhstan demonstrate that bovine *E. coli* can carry clinically relevant resistance determinants; Alexyuk et al. reported a multidrug-resistant bovine *E. coli* isolate from Kazakhstan with multiple antimicrobial-resistance genes and phenotypic resistance to several drug classes [13]. A global geospatial analysis of food-animal AMR identified parts of Central Asia as predicted AMR hotspots; it also reported high average resistance prevalence to tetracycline in *E. coli* across low- and middle-income countries and found that many Asian locations were associated with penicillins or sulphonamides crossing critical resistance levels in future projections [14]. At the national level, Kazakhstan is developing AMR containment and veterinary-sector surveillance activities under a One Health framework [15].

The poor activity of benzylpenicillin and tylosin should not be interpreted as a direct therapeutic ranking because selected agent-organism combinations in the local panel have limited clinical relevance for Enterobacterales. Therefore, the MDR combinations shown in Table 2 are best understood as descriptive phenotypic patterns based on the available panel. They should not be used as empirical treatment recommendations, especially for critically important agents or combinations lacking harmonized interpretive criteria. This interpretation is consistent with antimicrobial stewardship principles and with guidance emphasizing responsible and prudent antimicrobial use in veterinary medicine [10, 12].

This study has several limitations. First, this study included 32 *E. coli* isolates collected from market-derived bovine products in Semey during one season; therefore, the findings should be interpreted as preliminary local phenotypic data rather than as regional prevalence estimates. Second, although isolate-level MDR classification and Table 2 were added, complete animal- and farm-level metadata, including cattle age, production type, geographic origin before marketing, and previous antimicrobial exposure history, were not available. Third, complete disk lot metadata and quality-control records were not fully available in the laboratory documentation, and selected agent-organism combinations have limited interpretive relevance for *E. coli*. Fourth, PCR, sequencing, virulence-factor detection, and phylogenetic characterization were outside the scope of this work. Future studies should include larger numbers of samples, include prospective multi-season and multi-site sampling, retain isolate-level raw measurements and quality-control records, and combine phenotypic testing with molecular characterization of resistance determinants.

From a food-safety standpoint, monitoring peripheral lymph nodes may complement routine carcass-surface hygiene assessment because microorganisms located within lymphatic tissue are less accessible to external decontamination and may persist until trimming or further processing. For veterinary-sanitary control in Semey, continued seasonal sampling of such tissues could improve local risk assessment and support evidence-based surveillance of beef products offered for sale.

Conclusion

E. coli isolated from bovine superficial cervical (prescapular) lymph nodes sampled from market-derived bovine products in Semey showed a heterogeneous phenotypic susceptibility profile. Gentamicin and amikacin showed complete in vitro susceptibility, and fluoroquinolones and third-generation cephalosporins retained high activity against most isolates. By contrast, benzylpenicillin and tylosin showed poor activity based on descriptive phenotypic observations, and doxycycline, amoxicillin, and imipenem showed reduced or mixed susceptibility. Seventeen *E. coli* isolates were classified as MDR, corresponding to an MDR frequency of 17/32 isolates (53.1%). The MDR profiles mainly involved resistance to penicillins/beta-lactams, macrolides, and tetracyclines, as shown in the added isolate-level table. Given the limited sample size, incomplete animal-level information, incomplete quality-control metadata, and absence of molecular characterization, these findings should be interpreted as preliminary local phenotypic surveillance data. These findings support continued monitoring of carcass-associated tissues and should be considered in conjunction with approved indications, food-animal regulations, and antimicrobial stewardship principles.

Authors' Contributions

ST: contributed to the study conception and design, sample processing, laboratory testing, data organization, descriptive analysis, visualization, and preparation of the original manuscript draft. BB: supervised the study, contributed to methodology validation and interpretation of results, and critically revised the manuscript. Both authors read and approved the final version of the manuscript.

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Statement on Competing Interests

The authors declare that they have no competing interests.

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