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Original article

Prevalence of virulent *Escherichia coli* pathotypes in calf diarrhoea: molecular characterisation and antibiotic resistance profiles in Türkiye

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Abstract

Neonatal calf diarrhoea caused by *Escherichia coli* (*E. coli*) pathotypes remains a major health concern in livestock. This study aimed to characterise *E. coli* pathotypes isolated from diarrhoeic calves in the Küçük Menderes Basin, Türkiye, and to evaluate their antimicrobial resistance profiles. Faecal samples were collected from 100 calves across five districts. Twenty-five *E. coli* isolates were obtained and analysed by PCR for virulence genes. Antimicrobial susceptibility was tested using the disk diffusion method, following guidelines from the Clinical and Laboratory Standards Institute (CLSI). Enterotoxigenic *E. coli* (ETEC) was the most prevalent pathotype (64%). Multiple virulence gene combinations were detected, including one isolate (4%) that carried virulence genes associated with ETEC, enterohaemorrhagic *E. coli* (EHEC), and enteropathogenic *E. coli* (EPEC). Antimicrobial susceptibility testing revealed high effectiveness of florfenicol (80%) and tetracycline (76%), while resistance was highest to penicillin G (88%), streptomycin (52%), and aztreonam (64%). Multidrug resistance (MDR), defined as phenotypic resistance to three or more antimicrobial classes, was observed in 36% (9/25) of the isolates. The high prevalence of ETEC confirms its dominant role in neonatal calf diarrhoea, whereas substantial β-lactam resistance underscores the need for prudent antibiotic use and regionally adapted management strategies.

Keywords: antimicrobial resistance, calf diarrhoea, *Escherichia coli*, pathotype, polymerase chain reaction, Türkiye

Introduction

Escherichia coli (*E. coli*) is a Gram-negative member of the *Enterobacteriaceae* family and is recognised as a major cause of neonatal calf diarrhoea worldwide, including in Türkiye (Blanco et al. 2006, Nikkhah and Alimirzaei 2022). Calf diarrhoea caused by pathogenic *E. coli* is associated with increased morbidity and mortality, reduced weight gain, and higher veterinary costs (Hou et al. 2025). *E. coli* pathotypes affecting calves are primarily classified as enterotoxigenic (ETEC), enteropathogenic (EPEC), and enterohaemorrhagic (EHEC), based on their virulence factors. ETEC strains, especially those with K99 fimbriae, promote intestinal colonisation and secretory diarrhoea (Stenutz et al. 2006). EPEC is responsible for chronic diarrhoea due to attaching and effacing lesions, while EHEC produces Shiga toxins (*stx1*, *stx2*) that can cause haemorrhagic colitis (Bruyand et al. 2018). Molecular techniques, particularly PCR-based assays, are widely used to detect *E. coli* pathotypes and their virulence genes in calf diarrhoea. Although O-antigen serotyping provides additional epidemiological insights, it was not performed in this study due to resource constraints. Antibiotic resistance remains a major challenge, as the extensive utilisation of antimicrobials may promote the proliferation of resistant strains that carry genes such as *blaSHV*, *tetA/tetB*, *sul1*, and *qnr (*Aarestrup 1999, Martinez and Baquero 2002).

This study aimed to investigate the distribution of virulent Escherichia coli pathotypes and their antimicrobial resistance profiles in diarrhoeic calves from the Küçük Menderes Basin, Türkiye. These findings are expected to improve the epidemiological understanding of regional populations and support the implementation of evidence-based veterinary treatment strategies.

Materials and Methods

Ethical statement

Ethical committee permission was not required, as the samples were collected during routine examinations performed by a veterinarian, or from the environment.

Sample collection

Faecal samples were aseptically collected from 100 neonatal calves presenting with diarrhoea, which were selected consecutively during routine veterinary visits, without prior stratification by age, sex, or breed to ensure a broad representative cross-section of the regional calf population. The calves were selected from livestock farms in five districts of Izmir province: Bayındır (B), Beydağ (BY), Kiraz (K), Ödemiş (Ö), and Tire (T). The 100 sampled calves were distributed as follows: Holstein (n=78), Simmental (n=16), and Montofon (n=6). The study area, located between 38°00′–38°30′ N and 27°30′–28°30′ E, is characterised by intensive agricultural and livestock production. Samples were transported to the laboratory under cold chain conditions and stored at -20°C until bacteriological analysis was performed.

Isolation of *Escherichia coli* and DNA extraction

Faecal samples were cultured on 5% sheep blood agar and eosin methylene blue (EMB) agar (Oxoid, UK), and incubated aerobically at 37°C for 24-48 h. Typical *E. coli* colonies were identified by Gram staining, colony characteristics, and standard biochemical tests (e.g., indole, methyl red, Voges-Proskauer, and citrate test). Genomic DNA was extracted from confirmed isolates using a commercial kit (HiMedia, USA) following the manufacturer's instructions and stored at -20°C until PCR analysis.

Primers and genotypic identification of *Escherichia coli* isolates

Major virulence genes of *Escherichia coli* pathotypes were detected by PCR using previously described primers (Chandra et al. 2013). The forward and reverse primer sequences, expected amplicon sizes, and target genes used for PCR amplification are provided (Table 1). Classification of isolates was based on the presence of the respective target gene(s).

Isolates were classified as a given pathotype based on the presence of the respective target gene(s), EPEC (*bfpB*), EHEC (*stx, stx2*), ETEC (*est1b*), EIEC (*ipaH*, *virF*), EAEC (*pic, aafII*), DAEC (*daaE*). The *uidA* gene was used as a universal marker, and the *E. coli* ATCC 25922 strain was included as a positive control. PCR amplification was performed in 25 µl volumes containing Taq DNA polymerase, $MgCl_2$, dNTPs, and 100 ng of template DNA. The cycling conditions were as follows: initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 1 min, followed by a final extension at 72°C for 5 min (Vidal et al. 2005, Müller et al. 2007). PCR products were analysed by agarose gel electrophoresis and visualised with a gel documentation system. Isolates producing a 1487 bp *uidA* amplicon were genotypically confirmed as *E. coli*.

Identification of antibiotic resistance genes

The presence of antibiotic resistance genes, including *aadA1*, *tetA*, *tetB*, *dfrA1*, *qnrA*, *aac(3)-IV*, *sul1*, *blaSHV*,

Table 1. Virulence genes and corresponding pathotypes used for identification of *Escherichia coli* isolates in calves.

No	*E. coli* pathotype	Target gene(s)	Product	Primer 5′–3′	Product (bp)
1	*E. coli*	*uidA*	*β*-D-glucuronidase	F: ATGCCAGTCCAGCGGTTTTCGG R: ACCGGCTTTTATGACGATGAC	1487
2	EPEC	*bfpB*	Bundle-forming pili protein B	F: GACACACATTGCTGAAGCTG R: GGTCTTATCGGCTTATGAGTT	910
3	EPEC / EHEC	*eae*	Intimin	F: TGAACGTTACCGCCGCTTT R: GATCGTCTGCGGAGGTTCAG	482
4	EHEC	*stx1*	Shiga toxin 1	F: ATGCCACGTCACAGCTGTTG R: AACTTCAGGCTGGTGGTGAC	244
5	EHEC	*stx2*	Shiga toxin 2	F: ACGGCTGTCTGTTGCATCTG R: TTTGCCGGTAGTGTTGTTGC	324
6	ETEC	*est1b*	Heat-stable toxin	F: CTTTTCCAGAGCGTCTTCAC R: CTCGACGACTTTTAATGCTG	171
7	EIEC	*ipaH*	Invasion plasmid antigen	F: CTTTCGCAGCAGCTTGTTC R: AGCTTGCTTGATGATGAAAC	933
8	EIEC	*virF*	Transcriptional regulator VirF	F: TGGCGCTTGATGTTCAGTGC R: AAGTCGACTGAGATATAGTC	618
9	EAEC	*pic*	Serine protease autotransporter	F: AACTGTCAGTGAACGACTTGCG R: ATTCCCATGTGAAGCACTTC	1111
10	EAEC	*aafII*	Aggregative adherence fimbrial I protein AafB	F: ATTCCCATGTGAAGCACTTC R: GAAAGTTTAATGGTGCGGTAA	378
11	DAEC	*daaE*	Dr adhesin family protein	F: TCAGGCGGTGATACGTTGCG R: ATCACAGCGTTGTGACGCAA	542

blaCITM and *ereA*, was determined by PCR. Forward (F) and reverse (R) primer sequences, expected amplicon sizes, and the associated antibiotic class/resistance are listed for each gene in Table 2 (Shahrani et al. 2014). PCR reactions were performed in a final volume of 25 µl, containing 10X Taq buffer (5 µl), 2 U Taq DNA polymerase, 25 mM $MgCl_2$, 200 µM of each dNTP, 0.5 µM of each primer, and 1 µl template DNA (100 ng). Amplification was performed using a thermal cycler (Eppendorf, Germany) as follows: initial denaturation at 94°C for 8 min, 32 cycles of 95°C for 60 s, annealing at 55°C for 70 s, and extension at 72°C for 2 min with a final extension at 72°C for 8 min. The PCR products were then analysed via 1.5% agarose gel electrophoresis, after which the amplicon bands with the appropriate sizes were evaluated using a gel imaging system. All primer sequences (Table 2) and PCR conditions were adapted from (Shahrani et al. 2014).

Antibiotic susceptibility testing

Antibiotic susceptibility of confirmed *E. coli* isolates was determined by the Kirby-Bauer disc diffusion method (Bauer et al. 1959) following the Clinical and Laboratory Standards Institute (CLSI, 2022) standards. The antibiotic discs used included amikacin (30 µg), aztreonam (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), enrofloxacin (5 µg), florfenicol (30 µg), gentamicin (10 µg), penicillin G (10 IU), streptomycin (10 µg), sulfamethoxazole–trimethoprim (25 µg), and tetracycline (30 µg). The results were interpreted in accordance with the CLSI (2022) standards. The antibiotics included in the susceptibility panel were selected based on their frequent use in bovine practice in Türkiye, their relevance for the treatment of neonatal calf diarrhoea, and their representation of major antimicrobial classes with reported resistance in *Escherichia coli*.

Statistical analysis

Statistical analyses were not performed, as the study was designed to be descriptive. All results were presented as descriptive data, including the percentages and distributions of antimicrobial resistance and virulence genes. As the study aimed to characterise the isolated *E. coli* strains rather than evaluate statistical associations, no group-wise comparisons or significance testing were conducted. Multidrug resistance (MDR) was defined based on phenotypic susceptibility results as non-susceptibility to at least one agent in three or more antimicrobial categories, and not solely on the presence of resistance genes.

Table 2. Primers used for the detection of antibiotic resistance genes in *Escherichia coli* isolates in calves.

Genes	Primer (5′–3′)	Product (bp)	Antibiotic resistance
aadA1	F-TATCCAGCTAAGCGCGAACT R-ATTTGCCGACTACCTTGGTC	447	Streptomycin
tetA	F- GGTTCACTCGAACGACGTCA R- CTGTCCGACAAGTTGCATGA	577	Tetracycline
tetB	F- CCTCAGCTTCTCAACGCGTG R- GCACCTTGCTGATGACTCTT	634	Tetracycline
dfrA1	F- GGAGTGCCAAAGGTGAACAGC R- GAGGCGAAGTCTTGGGTAAAAAC	367	Trimethoprim
sul1	F- TTCGGCATTCTGAATCTCAC R- ATGATCTAACCCTCGGTCTC	822	Sulfonamide
aac(3)-IV	F- CTTCAGGATGGCAAGTTGGT R- TCATCTCGTTCTCCGCTCAT	286	Gentamicin
blaCITM	F- TGGCCAGAACTGACAGGCAAA R- TTTCTCCTGAACGTGGCTGGC	462	Ampicillin
blaSHV	F- TCGCCTGTGTATTATCTCCC R- CGCAGATAAATCACCACAATG	768	Cephalothin
ereA	F- GCCGGTGCTCATGAACTTGAG R- CGACTCTATTCGATCAGAGGC	419	Erythromycin
qnrA	F- GGGTATGGATATTATTGATAAAG R- CTAATCCGGCAGCACTATTTA	670	Fluoroquinolone

Results

A total of 25 *E. coli* isolates (25%) were obtained from rectal swabs of diarrhoeic calves. PCR analysis was used to detect the presence of regions associated with virulence genes. The pathotypes detected included enteropathogenic (*bfpB*), enteropathogenic/enterohemorrhagic (*eae*), enterohemorrhagic (*stx1*, *stx2*), enterotoxigenic (*est1b*), enteroinvasive (*ipaH*, *virF*), enteroaggregative (*pic*, *aafII*), and diffusely adherent *E. coli* (*daaE*). All isolates were positive for the *uidA* gene (100%, 1487 bp), confirming their identity as *E. coli*, consistent with the 16S rRNA PCR results (Fig. 1).

PCR-based virulence profiling revealed that the predominant pathotype was enterotoxigenic *Escherichia coli* (ETEC; *est1b*-positive), detected in 64% (16/25) of the isolates (B5, B13, B20, TR3, TR10, TR16, TR19, Ö8, Ö16, BY8, BY11, BY14, BY16, BY17, BY19, K20). These isolates were mainly from 15-day-old Holstein calves and were evenly distributed between sexes. Enterohaemorrhagic *E. coli* (EHEC) carrying *stx1* was identified in 16% of isolates (4/25; K6, K9, K15, Ö10), while *stx2* was found in 8% of isolates (2/25; K8, TR20), always in combination with either *est1b* or *eae*, indicating the absence of *stx2*-only isolates. These EHEC-positive strains were predominantly isolated from 10-20-day-old Holstein and Simmental calves and were associated with more severe clinical signs. The *eae* gene, associated with EPEC/EHEC, was detected in 12% (3/25; BY8, K8, Ö14) of isolates, and diffusely adherent *E. coli* (DAEC; *daaE*-positive) in 4% (1/25; Ö11). None of the isolates carried *bfpB* (typical EPEC), *pic/aafB* (EAEC) or *ipaH/virF* (EIEC). Several isolates exhibited virulence profiles combining multiple pathotype markers: Ö11 harboured *est1b* + *stx1* + *daaE* (ETEC + EHEC + DAEC), K8 carried *est1b* + *eae* + *stx2* (ETEC + EPEC/EHEC), Ö14 carried *stx1* + *eae* (mixed EHEC/EPEC), K8 and TR20 carried *est1b* + *stx2* (ETEC + EHEC), and BY8 carried only *eae*, consistent with an EPEC/EHEC-like profile. These combinations may increase the potential for pathogenicity by co-expressing fimbrial adhesins, enterotoxins and Shiga toxins. The distribution of pathotypes was similar between male (52%, 13/25) and female (48%, 12/25) calves, with ETEC being the most frequently detected in both sexes. Of the 25 *E. coli*-positive isolates, 21 were from Holstein calves, 3 from Simmental calves, and 1 from a Montofon calf. Among the *E. coli*-positive isolates, most originated from Holstein calves (84%, 21/25), which exhibited the broadest range of pathotypes. In contrast, Simmental (12%, 3/25) and Montofon (4%, 1/25) calves contributed relatively few isolates. In terms of age, ETEC predominated in 15-day-old calves (64%, 16/25), while EHEC (*stx1*) was more prevalent in 10-day-old calves and mixed profiles (ETEC + *eae* or ETEC + *stx2*) were found in older calves (20-25 days) (Table 3). These patterns suggest an increased susceptibility in Holstein calves and younger calves, highlighting the importance of screening for virulence genes for effective diagnostics and control.

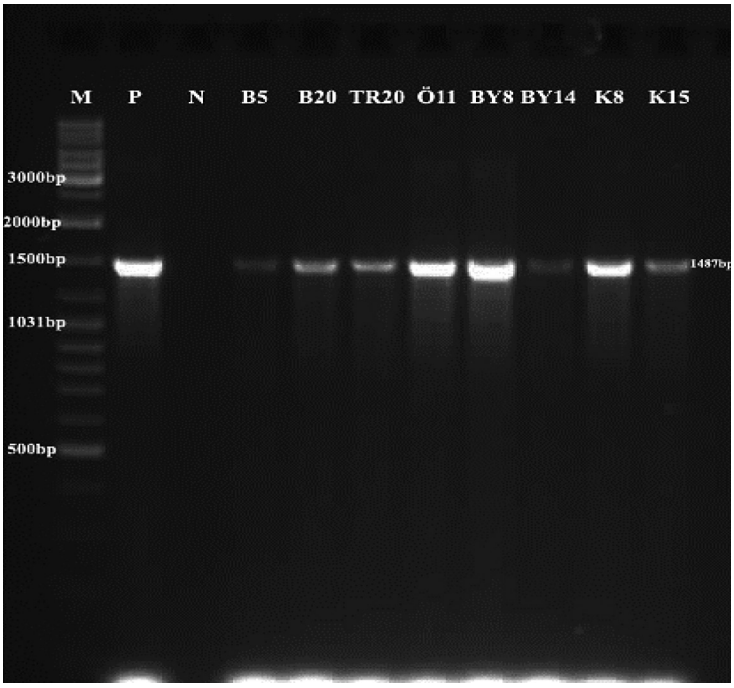

Fig. 1. 16S rRNA PCR results for *Escherichia coli* isolates M: 100bp DNA ladder; P: Positive control (*E. coli* ATCC 25922); N: Negative control (deionized water); B5, B20, TR20, Ö11, BY8, BY14, K8, K15: some PCR positive samples.

Table 3. Pathotype distribution of *Escherichia coli* isolates in calves along with virulence markers, host factors, and clinical associations.

Pathotype	Prevalence (%)	Genetic Marker(s)	Amplicon Size (bp)	Age	Breed	Clinical Significance
ETEC	64% (16/25)	*est1b*	171	15 days (d)	Holstein (dominant)	Causes diarrhoea via intestinal colonisation, the most common pathotype.
EHEC (*stx1*)	16% (4/25)	*stx1*	244	10-20 d	Holstein and Simmental	Toxin-mediated pathology, often associated with more severe symptoms.
EPEC/EHEC (*eae*) + EHEC (*stx1*)	4% (1/25)	*eae, stx1*	482, 244	15-20 d	Holstein	Adhesion and toxin production together may worsen disease severity.
ETEC + EHEC (*stx1*) + DAEC	4% (1/25)	*est1b, stx1, daaE*	171, 244, 542	15 d	Holstein	Combined adhesion and toxin genes (increased virulence).
ETEC + EHEC (*stx*2)	4% (1/25)	*est1b, stx2*	171, 324	20-25 d	Montofon	Linked to more severe intestinal lesions.
ETEC + EPEC/ EHEC (*eae*)	4% (1/25)	*est1b, eae*	171, 482	20-25 d	Holstein	Adhesion and enterotoxin synergy.
ETEC + EPEC/ EHEC (*eae*) + EHEC (*stx2*)	4% (1/25)	*est1b, eae, stx2*	171, 482, 324	20-25 d	Holstein	Multi-virulence (potentially complex infection pattern).

* ETEC, enterotoxigenic *E. coli*, EHEC, enterohemorrhagic *E. coli*, EPEC, enteropathogenic *E. coli*, DAEC, diffusely adherent *E. coli*.

Antimicrobial susceptibility testing of the 25 *E. coli* isolates (Fig. 2) revealed marked variation across antibiotic classes. Amikacin showed moderate activity, with 52% (13/25) of the isolates classified as susceptible (62.5% of the ETEC). In contrast, β-lactam antibiotics, including penicillin G, showed limited efficacy, with penicillin G exhibiting the highest resistance rate, affecting 88% (22/25) of isolates. Similarly, the monobactam aztreonam demonstrated poor activity, with only 16% (4/25) susceptibility, while resistance was observed in 64% (16/25) of strains. Third-generation cephalosporins also displayed restricted activity, with susceptibility rates of 36% (9/25) for ceftazidime and 32% (8/25) for ceftriaxone, whereas resistance to ceftri-

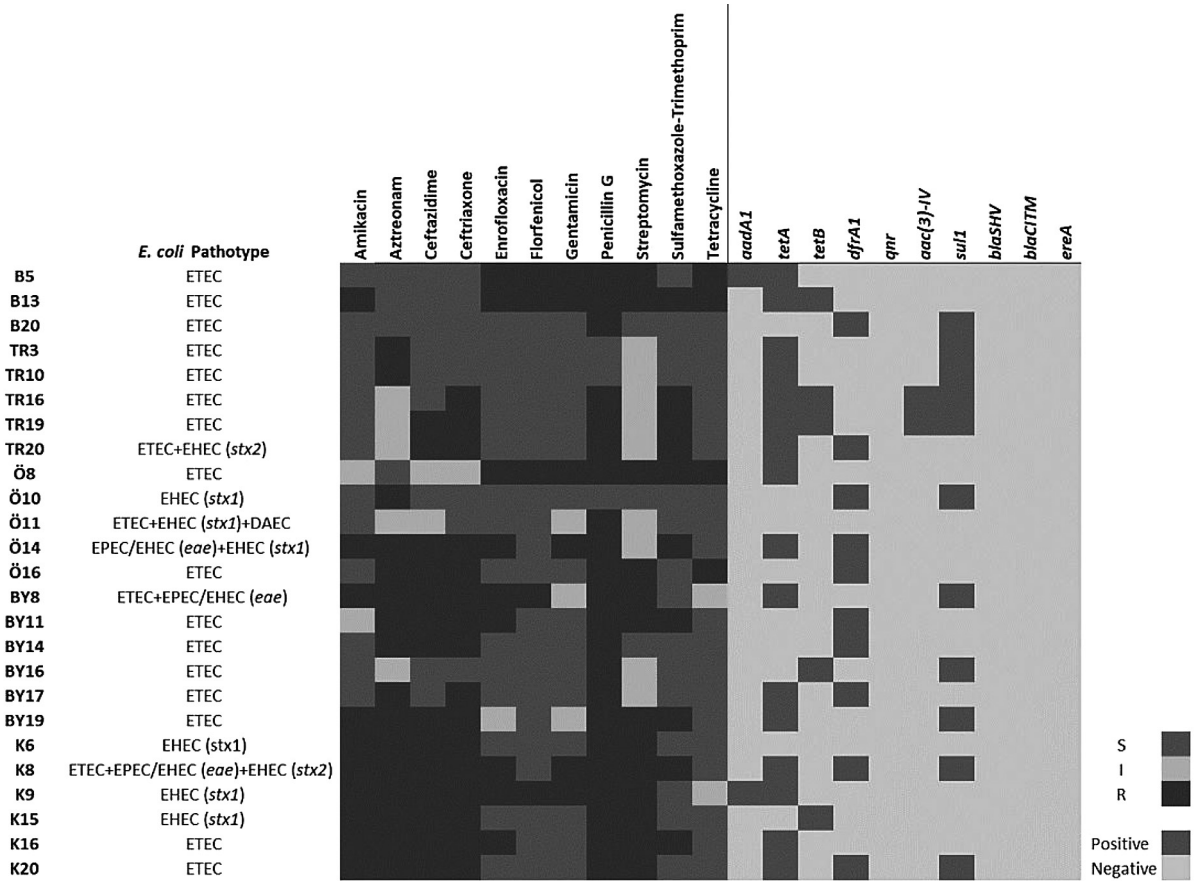

Fig. 2. Antimicrobial susceptibility profiles and distribution of resistance genes among the 25 diarrheagenic *Escherichia coli* isolates in calves. Phenotypic susceptibility results are shown for each isolate based on disk diffusion testing and classified as susceptible (S), intermediate (I), or resistant (R). ETEC, enterotoxigenic *E. coli*, EHEC, enterohemorrhagic *E. coli*, EPEC, enteropathogenic *E. coli*, DAEC, diffusely adherent *E. coli*.

axone reached 64% (16/25), intermediate susceptibility was infrequent (4%, 1/25). Among non-β-lactam agents, florfenicol exhibited the highest and most consistent in vitro activity, with 80% (20/25) of isolates classified as susceptible and no intermediate responses detected. Tetracycline also demonstrated good efficacy (76%, 19/25), followed by gentamicin (64%, 16/25) and the fluoroquinolone enrofloxacin (60%, 15/25), with intermediate susceptibility observed in only 4% (1/25) of isolates. In contrast, streptomycin showed substantial resistance (52%, 13/25), accompanied by a high proportion of intermediate responses (36%, 9/25). Sulfamethoxazole–trimethoprim exhibited moderate activity, with 60% (15/25) of isolates remaining susceptible. Overall, heterogeneous resistance phenotypes were observed among the tested isolates. The EHEC (*stx1*, Ö10) isolate demonstrated the highest level of susceptibility (90.9%), showing sensitivity to nearly all tested antibiotics, including florfenicol, amikacin, and tetracycline. In contrast, the EHEC (*stx1*, K9) isolate exhibited the highest resistance level (81.8%). The TR20 isolate, carrying an ETEC + EHEC (*stx2*) profile, displayed intermediate susceptibility (54.5%). It responded best to florfenicol and enrofloxacin, but showed resistance to β-lactams and streptomycin. In summary, penicillin G and other β-lactams were the least effective agents, while florfenicol, tetracycline and aminoglycosides exhibited the highest activity (Fig. 2).

The distribution of ten resistance genes among 25 *E. coli* isolates is shown in Fig. 2. The most prevalent tetracycline resistance genes were *tetA (*64%, 16/25) of isolates, and *tetB* (24%, 6/25). The *sul1* gene was identified in 44% of the samples (11 out of 25), followed by *dfrA1* (40%, 10 out of 25) and *aadA1* (8%, 2 out of 25). These genes have been shown to confer resistance to trimethoprim and streptomycin, respectively. The *aac(3)-IV* gene, which is associated with gentamicin resistance, was present in two isolates (8%). The *qnr* and *ereA* were not detected by PCR, suggesting a low prevalence of plasmid-mediated fluoroquinolone and macrolide resistance determinants. Similarly, *blaSHV* and *blaCITM* were PCR-negative in the tested isolates. The presence of the *tetA* and *tetB* genes, which are known to confer resistance to tetracyclines, was consistent with reduced susceptibility to tetracycline observed in several isolates. Detection of *aadA1* and *aac(3)-IV* may explain resistance to aminoglycosides such as streptomycin and gentamicin, while

resistance to trimethoprim and sulfonamides was supported by the identification of *dfrA1* and *sul1,* respectively. Multidrug resistance (MDR), defined as resistance to three or more antimicrobial classes based on phenotypic susceptibility testing, was observed in 36% (9/25) of the isolates. TR16 and TR19 carried *tetA*, *tetB*, *sul1* and *blaSHV*, and exhibited phenotypic resistance consistent with the tetracycline, sulfonamide and β-lactam classes. K8 was found to harbour *tetA*, *dfrA1* and *sul1*; B20 and K20, meanwhile, carried *tetB* + *dfrA1* + *sul1* and *tetA* + *dfrA1* + *sul1*, respectively. The remaining isolates (64%, 16/25) carried fewer than three resistance genes and thus exhibited limited profiles, though the combinations varied. For example, B5 and K9 carried *aadA1* + *tetA* (resistance to streptomycin/tetracycline), TR3, TR10, BY8 and BY19 carried *tetA* + *sul1* (resistance to tetracycline/sulfonamide), and Ö10, Ö14 and BY17 carried *dfrA1* + *sul1* (trimethoprim-sulfonamide resistance). Single-gene carriers included Ö8 (*tetA*) and BY11, BY14, Ö16 and K15 (*dfrA1*). Two isolates (Ö11, K6) lacked resistance genes entirely. The predominance of tetracycline and sulfonamide resistance genes and a 36% MDR rate indicate substantial antimicrobial pressure in the study region.

Discussion

Diarrhoea is a major cause of morbidity and mortality in neonatal calves during the early postnatal period. In this study, enterotoxigenic *E. coli* (ETEC) was the dominant pathotype detected in 64% of the isolates. This finding is consistent with earlier reports that identified ETEC as the primary agent of neonatal diarrhoea (Meganck et al. 2014, Picco et al. 2015, Tutija et al. 2022). Its high prevalence in 15-day-old calves corroborates previous findings that *E. coli* infections peak during the initial weeks of life, declining as intestinal maturation decreases fimbrial receptor expression (Awad et al. 2020, Fouad et al. 2022). The distribution of ETEC isolates was equal across sexes, with predominating recovery from Holstein calves. This distribution may reflect the composition of the sampled population rather than a direct breed-related susceptibility. Although some studies have reported elevated levels of diarrhoea in male subjects (Citil et al. 2003), no gender-related disparities were identified in the present study. The prevalence of *E. coli* was found to be 25%, consistent with the wide range reported in the literature (1-89%) (Younis et al. 2009, Izzo et al. 2011, Kumar et al. 2022). These observations are consistent with the concept that postnatal immune immaturity may facilitate early ETEC colonisation. The increased expression of fimbrial receptors in immature villus cells during the early neonatal period facilitates intestinal colonisation by ETEC (Meganck et al. 2014). As the epithelium matures, receptor density decreases, and infection rates typically decline. The increased detection of infections around 15 days of age may reflect additional pathogenic or management-related factors (Tutija et al. 2022). A higher proportion of ETEC isolates originated from Holstein calves (80% of isolates). However, no statistical analysis was performed to assess breed-related susceptibility (Aydın et al. 2001). Although infection rates appeared marginally higher in males, no statistical comparison was performed to confirm gender-related differences. The findings underscore the prominent role of ETEC in neonatal enteric disease, while host- and environment-related factors may contribute to the observed distribution. Tutija et al. (2022) reported *E. coli* prevalence rates of 96.3% in cattle faecal samples and 92.6% in dairy cows, highlighting its persistence and potential for transmission. In contrast, the prevalence in our study was 25%, consistent with the previously reported range of 16.3-25.0% for the Küçük Menderes Basin (Akane et al. 2022). This comparatively lower rate may be linked to management-related factors such as insufficient colostrum intake, poor hygiene, high stocking density, and limited biosecurity (Akyüz et al. 2017). The reported prevalence across Türkiye varies widely (6-69%) (Emre et al. 1998, Güneş et al. 2004, Coşkun and Kaya 2018), reflecting differences in sampling design, housing, and husbandry practices. The prevalence observed in this study was comparable to rates reported in Sivas (26%) and Kars (18.2%), but higher than those documented in Tokat (7.5%), Kayseri (14.3%), and Siirt (6%). These findings emphasise the importance of regional hygiene protocols and farm management practices. The observed epidemiological heterogeneity highlights the need for region-specific preventive strategies. Enterohemorrhagic *E. coli* (EHEC) was identified in 16% of isolates (*stx1*: 16%; *stx2*: 8%), indicating variation in pathogenicity associated with Shiga toxins. By contrast, Tutija et al. (2022) reported much higher rates (*stx1*: 76.7%; *stx2*: 35.2%), while Ferreira et al. (2018) found intermediate values (*stx1*: 20.4%; *stx2*: 4.5%). These discrepancies suggest the need for further investigation into the regional distribution and genetic characteristics of EHEC infections in calves. The *eae* gene, typically associated with EPEC and EHEC pathotypes, was detected in 3 isolates (12%). However, none of these were classified as pure EPEC due to the simultaneous presence of other virulence markers. This prevalence is higher than the 5.6% reported by Picco et al. (2015) but lower than the 19.9% reported by Tutija et al. (2022). These differences suggest that diagnostic methods, genetic diversity, and environmental factors may influence the distribution

of EPEC strains. Several studies conducted in Türkiye have also reported a higher prevalence of *eae* than that observed internationally (Aydin et al. 2001), supporting the notion of regional variation. Accordingly, EPEC should be considered an essential pathotype from an epidemiological perspective. Similarly, diffuse adherent *E. coli* (DAEC) was detected in 4% of isolates. The ability of these strains to adhere to intestinal epithelial cells further supports their potential role in pathogenesis. Several isolates exhibited mixed profiles (e.g., ETEC + EPEC; EHEC (*stx2*) combinations), which may exacerbate clinical outcomes. These findings support the hypothesis that neonatal diarrhoea often results from polymicrobial or multi-pathotype infections rather than single strains. Comparison with other data from Türkiye further underscores regional variation in disease presentation. For example, the prevalence of *E. coli* O157 has been reported at 18.2% in Kars (Güneş et al. 2004) and 26% in Sivas (Küliğ et al. 2019), which is broadly consistent with the 25% observed in this study. Detecting multi-pathotype isolates, including ETEC + EHEC (*stx1*) + DAEC, suggests concurrent toxin production and adhesion mechanisms may amplify clinical severity. These results highlight the importance of incorporating molecular-level characterisation into routine diagnostics, complementing conventional culture methods. The antibiotic susceptibility profiles of the isolates were largely consistent with previous reports, though regional differences were evident. Susceptibility percentages were interpreted according to CLSI guidelines; intermediate categories were reported separately and were not included in resistance calculations. The highest recorded resistance was observed against penicillin G (88%), which is in agreement with the elevated levels reported by Shahrani et al. (2014) and Yue et al. (2021). Furthermore, resistance to other β-lactams was also notable, with ceftriaxone and ceftazidime showing resistance rates of 64% and 56%, respectively. This suggests that increasing resistance within this class may substantially compromise the effectiveness of empirical therapy for calf diarrhoea. Among the remaining agents, florfenicol demonstrated the highest efficacy (80% susceptibility), thus indicating its potential as a promising alternative for treatment. Tetracycline also demonstrated its continued therapeutic efficacy, exhibiting an overall susceptibility rate of 76% (62.5% among ETEC isolates), despite documented reports of high resistance in other regions (Nepomuceno et al. 2016, De Verdier et al. 2012). The prevalence of streptomycin resistance was moderate (52%), although it approached the high levels reported in some regions (~90%) (Duse et al. 2015). Resistance rates were calculated based on resistant isolates only, in accordance with the CLSI interpretation criteria. Gentamicin demonstrated moderate activity (64% susceptibility), although regional variation was evident, with resistance as low as 7.2% in the study of Astorga et al. (2019). Overall, florfenicol and tetracycline were identified as the most effective agents, whereas penicillin G, aztreonam, and streptomycin demonstrated poor efficacy. These findings are consistent with previous reports (Nepomuceno et al. 2016, Fouad et al. 2022) and reflect the ongoing escalation of resistance to broad-spectrum antibiotics. To ensure the preservation of therapeutic options, the selection of antibiotics must be guided by regional susceptibility profiles. A particular emphasis should accompany this process on the prudent use of florfenicol and tetracycline (Caneschi et al. 2023). To ensure the continued efficacy of antimicrobial treatments, it is essential to implement regular surveillance and revise treatment protocols at regular intervals.

Conclusion

The present study corroborates that enterotoxigenic *Escherichia coli* (ETEC) is the predominant pathotype associated with neonatal calf diarrhoea. The detection of multi-pathotype strains and age- and breed-related susceptibilities highlights the complex and multifactorial nature of the disease. Antimicrobial resistance patterns exhibited regional variability, with florfenicol and tetracycline retaining the highest efficacy, whereas high resistance rates were observed to penicillin G and other β-lactam antibiotics. These findings emphasise the need to continuously monitor *E. coli* pathotypes and resistance profiles to inform regionally adapted treatment strategies. The prudent use of effective agents, particularly florfenicol and tetracycline, is critical to preserving their therapeutic value. The results also support the integration of molecular diagnostics into routine surveillance and the strengthening of antimicrobial stewardship. Future research should focus on the genetic determinants of resistance to advance the development of targeted control strategies.

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Author Declarations

Ethics approval

Ethical approval was not required, as the samples were collected during routine veterinary diagnostic procedures and no experimental interventions were performed on animals specifically for research purposes.

Use of generative artificial intelligence

The authors declare that no generative artificial intelligence tools were used in the preparation of this manuscript.

Conflict of interest

The authors declare no conflicts of interest.

Data Availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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