Etlik Vet Mikrobiol Derg, 2025; **36 (1):** 27-37
doi: https://doi.org/10.35864/evmd.1607193

Original Article
Özgün Araştırma

The determination of enterotoxigenic virulence genes, serotypes, and antibiotic susceptibilities of *Escherichia coli* isolates from neonatal calf septicemia

Serhan Akgöz[1*], Timur Gülhan[2]

[1,2] *Ondokuz Mayıs University, Faculty of Veterinary Medicine, Microbiology Department, Samsun-Türkiye*

Geliş Tarihi / Received: 25.12.2024, **Kabul Tarihi** / Accepted: 02.05.2025

Abstract: This study aimed to isolate *Escherichia coli* from neonatal calves with septicemia and to determine antibiotic resistance, common serotypes, and virulence factors of the obtained isolates. For this purpose, important serotype, virulence, and antibiotic resistance genes related to eight different antibiotics were analyzed in confirmed isolates using PCR. According to phenotypic antibiotic resistance analysis, the isolates exhibited resistance to streptomycin (94.7%), tetracycline (93.7%), ampicillin (89.5%), trimethoprim-sulfamethoxazole (87.4%), chloramphenicol (87.4%), enrofloxacin (74.7%), gentamicin (62.1%), and cephalothin (24.2%). The detected resistance genes included *tet(B)* (88.4%), *sul3* (74.7%), *sul1* (67.4%), *cmlA* (61%), *qnr(B)* (56.8%), *tet(A)* (54.7%), *sul2* (53.7%), *CITM* (38.9%), *aadA1* (36.8%), *cat1* (28.4%), *qnr(S)* (26.3%), *aac(3)-IV* (12.6%), *tet(C)* (5.3%), and *qnr(A)* (1.05%). Serotyping revealed the prevalence of the O101, O26, and O145 serotype genes was determined to be 33.7%, 6.3%, and 2.1%, respectively. PCR analysis for virulence genes revealed that 23.2% of the isolates carried *East1*, 22.1% *F5*, 20% *STa*, 17.9% *F41*, and 3.2% *LT*. These findings highlight the presence of ETEC in calves and its high antibiotic resistance, posing a significant threat to animal health. Identifying prevalent serotypes and virulence genes also provides key insights for future vaccine development.

Keywords: Antibiotic resistance, Calf, *E. coli*, Serotype, Virulence

Yenidoğan buzağı septisemilerinden elde edilen *Escherichia coli* izolatlarının enterotoksijenik virülans genleri, serotip ve antibiyotik duyarlılıklarının belirlenmesi

Özet: Bu çalışma, yenidoğan buzağı septisemilerinden *Escherichia coli* izolasyonu, izolatlardaki antibiyotik direncinin tespiti, sık görülen serotip ve virülens faktörlerinin belirlenmesi amacıyla yürütüldü. Bu amaçla septisemili buzağılarda PCR ile doğrulanan izolatlarda önemli serotip genleri, virülens genleri ve 8 farklı antibiyotiğe ait direnç genleri araştırıldı. Fenotipik antibiyotik direnç analizine göre izolatların % 94,7'si streptomisin, % 93,7'si tetrasiklin, % 89,5'i ampisilin, % 87,4'ü trimetoprim-sülfametoksazol, % 87,4'ü kloramfenikol, % 74,7'si enrofloksasin, % 62,1'i gentamisin ve % 24,2'si sefalotine dirençli bulundu. İzolatlarda % 88,4 *tet(B)*, % 74,7 *sul3*, % 67,4 *sul1*, % 61 *cmlA*, % 56,8 *qnr(B)*, % 54,7 *tet(A)*, % 53,7 *sul2*, % 38,9 *CITM*, % 36,8 *aadA1*, % 28,4 *cat1*, % 26,3 *qnr(S)*, % 12,6 *aac[3]-IV*, % 5,3 *tet(C)* ve % 1,05 *qnr(A)* genleri belirlendi. Serotip analizi sonucunda izolatların % 33,7'sinde O101, % 6,3'ünde O26, % 2,1'inde ise O145 serotip genlerinin varlığı tespit edildi. Virülans genlerinin belirlenmesi amacıyla yapılan PCR çalışmaları sonucunda; İzolatların % 23.2'sinin *East1*, % 22,1'inin *F5*, % 20'sinin *STa*, % 17,9'unun *F41* ve % 3,2'sinin *LT* genine sahip olduğu belirlendi. Bu bulgular, buzağılarda ETEC'in varlığını ve yüksek antibiyotik direncini ortaya koymakta, bu da hayvan sağlığı için önemli bir tehdit oluşturmaktadır. Ayrıca, yaygın serotiplerin ve virülans genlerinin belirlenmesi, gelecekteki aşı geliştirme çalışmaları için önemli bilgiler sunmaktadır.

Anahtar kelimeler: Antibiyotik direnci, Buzağı, *E. coli*, Serotip, Virülens

Introduction

The genus *Escherichia* currently encompasses six species: *E. albertii*, *E. coli*, *E. fergusonii*, *E. hermannii*, *E. marmotae*, and *E. ruysiae*. Among these, *Escherichia coli* is the most well-characterized and was the first species to be described within the genus (Karakaya et al. 2022). *E. coli* is a Gram-negative, facultatively anaerobic, typically motile, rod-shaped bacterium that belongs to the family Enterobacteriaceae (Lim et al. 2010; Karakaya et al. 2022). The isolation and characterization of *Escherichia coli* were first performed by Theodor Escherich in 1885 (Lim et al. 2010). Although *E. coli* is a normal flora bacterium of the gastrointestinal tract, its pathogenic variants are recognized as one of the most prevalent human and animal pathogens, due to their association with a broad spectrum of diseases (Gülhan et al. 2009). Through the action of various virulence factors,

Yazışma adresi / Correspondence: *Serhan Akgöz, Department of Microbiology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Türkiye e-mail: akgozserhan@gmail.com*

ORCID IDs of the authors: *[1]0000-0002-5130-7120 • [2]0000-0003-4798-1427*

these variants cause both intestinal and extraintestinal infections, resulting in widespread transmission and significant mortality worldwide.

Neonatal calf septicemia is a significant cause of morbidity and mortality, leading to substantial economic losses. *E. coli* is the most frequently implicated pathogen in the etiology of septicemia; however, other Gram-negative and Gram-positive bacteria can also contribute to the condition (Çitil and Gökçe 2013; Akyüz et al. 2017). Environmental stressors and malnutrition compromise intestinal resistance, facilitating the systemic spread of pathogens via the bloodstream, which ultimately results in septicemia (Çitil and Gökçe 2013). In septicemic cases, microbial isolation from blood samples often yields negative results (Akyüz et al. 2017). The clinical manifestations of septicemia include diarrhea, pyrexia, social withdrawal, dehydration, tachycardia, increased respiratory rate, prolonged capillary refill time, anorexia, muscle weakness, and recumbency (Çitil and Gökçe 2013; Akyüz et al. 2017).

Neonatal calf diarrhea (NCD) remains one of the most significant health challenges in calves, contributing to high morbidity and mortality rates. It is characterized by secretory diarrhea and dehydration (Bartels et al. 2010). *E. coli* is one of the most common pathogens responsible for this condition (Rai et al. 2023). Diarrheagenic *Escherichia coli* (DEC) strains are classified into six pathotypes based on their virulence factors: enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Farfan et al. 2016). Among these pathotypes, ETEC is the primary pathotype most strongly associated with infectious diarrhea in calves (Algammal et al. 2020).

Neonatal calves exhibit heightened susceptibility to ETEC, which is associated with watery diarrhea. Two major virulence factors contribute to its pathogenicity: fimbriae and enterotoxins (Foster et al. 2009). ETEC produces two main types of enterotoxins in both humans and animals: heat-labile (LT) and heat-stable (STa and STb) enterotoxins (Eid et al. 2019). Adhesins involved in infections in farm animals include F4, F5, F6, F17, and F41 fimbriae all of which are encoded by fimbrial operons. Following fimbrial adhesion, ETEC colonizes the small intestine leading to severe watery diarrhea (Algammal et al. 2020).

Antimicrobial resistance (AMR) is a growing global concern, significantly impacting animal health and economic stability due to increased morbidity and mortality in livestock (Khawaskar et al. 2022). In veterinary medicine, multidrug-resistant *E. coli* presents an emerging challenge. While *E. coli* is naturally susceptible to most antimicrobial agents, it can acquire resistance genes through horizontal gene transfer, often via mobile genetic elements such as plasmids, transposons, and gene cassettes (Nagy and Fekete 2005).

The aim of this study was to isolate *E. coli* from neonatal calves with septicemia in Kars, to determine the presence of O101, O9, O26, O145 and O111 serotype genes, as well as *F17*, *F5*, *F41*, *ST*, *LT* and *EAST1* virulence genes, and to screen *E. coli* isolates for antimicrobial resistance.

Materıal and Methods

Bacterial Isolates

This study was conducted using 200 fecal samples collected from neonatal calves with septicemia younger than 10 days of age in Kars and its surrounding districts, with sample collection and laboratory work carried out between January and August 2022. All of the laboratory studies were carried out at Department of Microbiology, Faculty of Veterinary Medicine, Ondokuz Mayis University. For the isolation of *E. coli*, the fecal samples were inoculated onto MacConkey agar (Oxoid, UK), and the media were incubated for 24 hours at 37 °C in an aerobic atmosphere. Following incubation, a single lactose-positive colony was transferred to Eosin Methylene Blue (EMB) agar (Oxoid, UK) and incubated under the same conditions. At the end of incubation, a single lactose-positive colony was evaluated and subcultured on Tryptic Soy Agar (TSA) (Oxoid, UK) for various purposes (e.g. DNA extraction).

Bacterial DNA Extraction

The boiling method was employed for bacterial DNA extraction. For this procedure, a few colonies grown in pure culture on TSA were selected and suspended in 150 µl of sterile distilled water. The suspensions were then boiled at 100°C for 10 minutes. Following the boiling process, the mixture was centrifuged at 11,000 × g for 3 minutes, and the resulting supernatant was stored at -20°C for use as template DNA (González Pasayo et al. 2019).

E. coli Genotypic Confirmation

For PCR-based genotypic confirmation of *E. coli* isolates, the 16S rRNA gene was amplified using the primer pair Eco 2083 (F): 5′-GCT TGA CAC TGA ACA TTG AG-3′ and Eco 2745 (R): 5′-GCA CTT ATC TCT TCC GCA TT-3′. The presence of a 662 bp amplicon was considered positive for *E. coli* (El-Razik et al. 2010).

Antibiotic Susceptibility Test

The Kirby-Bauer standard disk diffusion method was employed to assess the resistance and susceptibility of the isolates to eight antibiotics commonly used in veterinary medicine. Antibiotic susceptibility was determined using commercial antibiotic disks (Bioanalysis, Türkiye) representing different antibiotic classes: ampicillin (AMP; 10 µg), gentamicin (CN; 10 µg), chloramphenicol (C; 30 µg), tetracycline (TE; 30 µg), cephalothin (KF; 30 µg), trimethoprim/sulfamethoxazole (SXT; 1.25/23.75 µg), streptomycin (S; 10 µg) and enrofloxacin (ENR; 5 µg). The *E. coli* ATCC 25922 strain was used as a quality control strain. Isolates exhibiting resistance to three or more antibiotic classes were classified as multidrug-resistant (MDR) (Magiorakos et al. 2012).

Antibiotic Resistance Genes Determination

To investigate the presence of antibiotic resistance genes, simplex and multiplex PCR (mPCR) assays were conducted using specific primers in parallel with the disk diffusion method. Modified and optimized PCR protocols were applied to detect genes encoding resistance to ampicillin (*CITM*) (Pérez-Pérez and Hanson 2002), gentamicin (*aac[3]-IV*) (Shahrani et al. 2014), chloramphenicol (*cat1* and *cmlA*), sulfonamides (*sul1*, *sul2*, *sul3*), tetracyclines (*tetA*, *tetB*, *tetC*) (Kozak et al. 2009), cephalothin (*blaSHV*) (Chanawong et al. 2000), streptomycin (*aadA1*), and fluoroquinolones (*qnrA*, *qnrB*, *qnrS*) (Cattoir et al. 2007) (Table 1).

Table 1. Primers used in identifying antibiotic resistance genes

Antibiotic	Target Gene	Primer	Sequence (5'–3')	Amplicon Size (bp)
Ampicillin	*CITM*	*CITM-F*	TGGCCAGAACTGACAGGCAAA	462
		CITM-R	TTTCTCCTGAACGTGGCTGGC	
Tetracycline	*tetA*	*TetA-L*	GGCGGTCTTCTTCATCATGC	502
		TetA-R	CGGCAGGCAGAGCAAGTAGA	
	tetB	*TetBGK-F2*	CGCCCAGTGCTGTTGTTGTC	173
		TetBGK-R2	CGCGTTGAGAAGCTGAGGTG	
	tetC	*TetC-L*	GCTGTAGGCATAGGCTTGGT	888
		TetC-R	GCCGGAAGCGAGAAGAATCA	
Sulfonamide	*sul1*	*sul1-F*	CGGCGTGGGCTACCTGAACG	433
		sul1-B	GCCGATCGCGTGAAGTTCCG	
	sul2	*sulII-L*	CGGCATCGTCAACATAACCT	721
		sulII-R	TGTGCGGATGAAGTCAGCTC	
	sul3	*sul3-GKa-F*	CAACGGAAGTGGGCGTTGTGGA	244
		sul3-GKa-R	GCTGCACCAATTCGCTGAACG	
Fluoroquinolone	*qnr(A)*	*QnrAm-F*	AGAGGATTTCTCACGCCAGG	580
		QnrAm-R	TGCCAGGCACAGATCTTGAC	
	qnr(B)	*QnrBm-F*	GGMATHGAAATTCGCCACTG	264
		QnrBm-R	TTTGCYGYYCGCCAGTCGAA	
	qnr(S)	*QnrSm-F*	GCAAGTTCATTGAACAGGGT	428
		QnrSm-R	TCTAAACCGTCGAGTTCGGCG	
Cephalothin	*blaSHV*	*SHV-F*	AAGATCCACTATCGCCAGCAG	768
		SHV-R	ATTCAGTTCCGTTTCCCACCGG	
Gentamicin	*aac[3]-IV*	*aac[3]-IV-F*	CTTCAGGATGGCAAGTTGGT	111
		aac[3]-IV-R	TCATCTCGTTCTCCGCTCAT	
Streptomycin	*aadA1*	*aadA1-F*	TATCCAGCTAAGCGCGAACT	447
		aadA1-R	ATTTGCCGACTACCTTGGTC	
Chloramphenicol	*cat1*	*cat1-F*	AGTTGCTCAATGTACCTATAACC	547
		cat1-R	TTGTAATTCATTAAGCATTCTGCC	
Chloramphenicol	*cmlA*	*cmlA-F*	CCGCCACGGTGTTGTTGTTATC	698
		cmlA-R	CACCTTGCCTGCCCATCATTAG	

Determination of Serotype Genes

The isolates were analyzed using multiplex PCR (mPCR) with specific primers to detect the serotype genes O101, O9, O26, O145, and O111, which are commonly associated with neonatal calf septicemia. For this purpose, PCR protocols previously established for the detection of serotype genes O101 and O9 (Liu et al. 2010) as well as O26, O145, and O111 (Shahrani et al. 2014) were modified and optimized accordingly (Table 2).

Table 2. Primers used in identifying serotype genes

Target Gene	Primer	Sequence (5'–3')	Amplicon Size (bp)
O9	*wzt-F*	TGGGTGTTAAAAGACATCAA	1142
	wzt-R	CCCAGAAATCCATGCTC	
O101	*wzm-F*	GTGTTACTTTCATATCGTCCAG	507
	wzm-R	ATGCAATGCGGTTTCTAC	
O26	*wzx-F*	CAGAATGGTTATGCTACTGT	423
	wzx-R	CTTACATTTGTTTTCGGCATC	
O145	*wzx-F*	CCATCAACAGATTTAGGAGTG	609
	wzx-R	TTTCTACCGCGAATCTATC	
O111	*wzx-F*	TAGAGAAATTATCAAGTTAGTTCC	406
	wzx-R	ATAGTTATGAACATCTTGTTTAGC	

Identification of Virulence Genes

Simplex and multiplex PCR assays were conducted to identify enterotoxigenic virulence genes associated with neonatal calf septicemia in the isolates. The *F41* (Güler et al. 2008), *F5* (K99) (Umpiérrez et al. 2016), *F17c* (Shahrani et al. 2014), *STa* (Güler et al. 2008), *LT-II* (Shahrani et al. 2014), and *EAST1* (Zajacova et al. 2012) virulence genes were detected using modified and optimized PCR protocols (Table 3).

Table 3. Primers used in identifying virulence genes

Target Gen	Primer	Sequence (5'–3')	Amplicon Size (bp)
F5	*K99-F*	TATTATCTTAGGTGGTATGG	314
	K99-R	GGTATCCTTTAGCAGCAGAGTATTTC	
F41	*F41-F*	GCATCAGCGGCAGTATCT	380
	F41-R	GTCCCTAGCTCAGTATTATCACCT	
F17c	*F17c-F*	GCAGGAACCGCTCCCTTGGC	416
	F17c-R	CAACTAACGGGATGTACAGTTTC	
STa	*STa-F*	GCTAATGTTGGCAATTTTTATTTCTGTA	190
	STa-R	AGGATTACAACAAAGTTCACAGCAGTAA	
LT-II	*LTII-F*	GCACACGGAGCTCCTCAGTC	218
	LTII-R	TCCTTCATCCTTTCAATGGCTTT	
EAST1	*east 11a*	CCATCAACACAGTATATCCGA	111
	east 11b	GGTCGCGAGTGACGGCTTTGT	

Results

Isolation and Identification

PCR verification of *E. coli* isolates was conducted on 95 (47.5%) isolates that were phenotypically characterized from 200 fecal samples collected from neonatal calves with septicemia younger than 10 days old in Kars and its surrounding districts. All isolates were confirmed as *E. coli* through the detection of the 16S rRNA *E. coli*-specific gene.

Antibiotic Susceptibility Test

The results indicated that 90 (94.7%) isolates were resistant to streptomycin, 89 (93.7%) to tetracycline, 85 (89.5%) to ampicillin, 83 (87.4%) to trimethoprim-sulfamethoxazole, 83 (87.4%) to chloramphenicol, 71 (74.7%) to enrofloxacin, 59 (62.1%) to gentamicin and 23 (24.2%) to cephalothin (Table 4).

Multiple Antibiotic Resistance

Evaluation of multiple antibiotic resistance revealed that 90 out of 95 (94.7%) *E. coli* isolates exhibited resistance to three or more antibiotic classes, classifying them as multidrug-resistant (MDR). The MDR rates of *E. coli* isolates are presented in Table 5.

Table 4. Antibiotic resistance/susceptibility rates of *E. coli* isolates

Antibiotic	R (n=95) (%)	S (n=95) (%)
AM10	85 (% 89,5)	10 (10.5)
CN10	59 (% 62,1)	36 (37.9)
KF30	23 (% 24,2)	72 (75.8)
TE30	89 (% 93,7)	6 (6.3)
ENR5	71 (% 74,7)	24 (25.3)
SXT25	83 (% 87,4)	12 (12.6)
C30	83 (% 87,4)	12 (12.6)
S10	90 (% 94,7)	5 (5.3)

Table 5. Multiple antibiotic resistance status of isolates

Antibiotic Resistance Phenotype	MDR Status	Number of Isolates (n=95) (%)
AM-CN-KF-TE-ENR-SXT-C-S	8	16 (17.8)
AM-CN-TE-ENR-SXT-C-S	7	31
AM-KF-TE-ENR-SXT-C-S		2
AM-CN-KF-ENR-SXT-C-S		2
AM-CN-KF-TE-SXT-C-S		1
AM-CN-KF-TE-ENR-C-S		1
	Total	*37 (41.1)*
AM-TE-ENR-SXT-C-S	6	11
AM-CN-ENR-SXT-C-S		2
AM-CN-SXT-C-S		2
CN-TE-ENR-SXT-C-S		2
AM-KF-TE-SXT-C-S		1
	Total	*18 (20)*
AM-TE-SXT-C-S	5	7
AM-TE-ENR-SXT-S		2
AM-TE-SXT-C-S		2
CN-TE-SXT-C-S		2
	Total	*13 (14.4)*
AM-TE-SXT-S	4	2
TE-ENR-C-S		1
	Total	*3 (3.3)*
AM-TE-S	3	2
TE-C-S		1
	Total	*3 (3.3)*

Antibiotic Resistance Genes Determination

As a result of simplex and multiplex PCR procedures conducted on a total of 95 *E. coli* isolates to identify antibiotic resistance genes, the following resistance genes were detected: *sul1* (67.4%), *sul2* (53.7%), *sul3* (74.7%), *qnrA* (1.05%), *qnrB* (56.8%), *qnrS* (26.3%), *tetA* (54.7%), *tetB* (88.4%), *tetC* (5.3%), *aac[3]-IV* (12.6%), *aadA1* (36.8%), *cmlA* (12.6%), *cat1* (28.4%), and *CITM* (38.9%). The *blaSHV* gene was not detected in any of the isolates (Table 6).

Table 6. Antibiotic resistance genes status of isolates

Antibiotic	Target Gene	Number of Isolates (n=95) (%)
Gentamicin	*aaca[3]-IV*	12 (12.6)
Streptomycin	*aadA1*	35 (36.8)
Chloramphenicol	*cat1*	27 (28.4)
	cmlA	58 (61)
Ampicillin	*CITM*	37 (38.9)
Enrofloxacin	*qnr-A*	1 (1.05)
	qnr-B	54 (56.8)
	qnr-S	25 (26.3)
Trimethoprim-sulfamethoxazole	*Sul-I*	64 (67.4)
	Sul-II	51 (53.7)
	Sul-III	71 (74.7)
Tetracycline	*tetA*	52 (54.7)
	tetB	84 (88.4)
	tetC	5 (5.3)
Cephalothin	blaSHV	0 (0)

Determination of Serotype Genes

Serotyping analysis revealed that 32 (33.7%) of the isolates belonged to the O101 serotype, 6 (6.3%) to O26, and 2 (2.1%) to O145. The O111 and O9 serotypes were not detected in any of the isolates, while 55 (57.9%) isolates tested negative for all the serotypes analyzed.

Identification of Virulence Genes

Simplex and multiplex PCR analyses identified the presence of various virulence genes among the isolates. Specifically, 22 (23.2%) isolates harbored the *EAST1* gene, 21 (22.1%) the *F5* gene, 19 (20%) the *STa* gene, 17 (17.9%) the *F41* gene, and 3 (3.2%) the *LT* gene. The *F17c* gene was not detected in any of the isolates. The distribution of virulence genes is summarized in Table 7.

Table 7. Single and multiple virulence gene profiles of *E. coli* isolates

Virulence Genes	Number of Isolates (n=95) (%)
F5,F41,STa,LT	2 (2,1)
F5, F41,STa,EAST1	1 (1)
F5,F41,STa	12 (12,6)
F5,EAST1	3 (3,1)
STa,F41	2 (2,1)
F5,STa	1 (1)
STa, EAST1	1 (1)
EAST1	17 (17,9)
F5	2 (2,1)
LT	1 (1)

Discussion and Conclusion

Enterotoxigenic *Escherichia coli* (ETEC) is a leading cause of travelers' diarrhea in humans (Al-Abri et al. 2005) and watery diarrhea in newborn farm animals, often resulting in severe dehydration, electrolyte imbalance, and potential fatality (Picco et al. 2015). ETEC infections in calves have long negatively impacted the livestock economy. Frequently observed in newborn farm animals, ETEC causes watery diarrhea, which can lead to severe dehydration, electrolyte imbalance, and potentially death (Khawaskar et al. 2022). *E. coli* infections in calves are caused by strains with different serotypes and various virulence factors, which may vary by region (Eid et al. 2019).

Infections caused by antibiotic-resistant bacteria pose a growing threat to both human and animal health (Wernli et al. 2011). Therefore, a better understanding of antibiotic resistance patterns, including the sources and vectors of resistance genes, is essential to mitigate the risks associated with increasing antibiotic resistance (Zhao et al. 2020; Skarżyńska et al. 2021).

This study aimed to isolate *E. coli* from neonatal calves with septicemia, determine antibiotic resistance patterns, and investigate common serotypes and virulence factors. The phenotypic antibiotic resistance test revealed high resistance rates to streptomycin (94.7%), tetracycline (93.7%), ampicillin (89.5%), trimethoprim-sulfamethoxazole (87.4%), chloramphenicol (87.4%), enrofloxacin (74.7%),

and gentamicin (62.1%). Additionally, 94.7% of *E. coli* isolates were found to be multidrug-resistant (MDR). A similar finding was reported by Haque et al. (2022), who observed a 100% MDR rate. Other studies conducted in Türkiye also investigated the resistance profile of *E. coli*, reporting MDR rates of 77.3% (Güler et al. 2008) and 71.4% (Cengiz and Adıgüzel 2020). However, MDR rates vary across different regions, with some studies reporting high rates ranging from 50% to 100% (Salaheen et al. 2019; Sobhy et al. 2020; Feuerstein et al. 2021), while others have documented lower MDR rates below 50% (Gharieb et al. 2019; Algammal et al. 2020). It is hypothesized that variations in multidrug resistance may be attributed to differences in animal care and feeding practices, the extent of antibiotic usage, and geographic factors.

Previous studies have reported a relatively high degree of resistance to streptomycin in *E. coli* isolates from diarrheic calves (Aydın et al. 2001; Shahrani et al. 2014; Algammal et al. 2020). In this study, a similarly high resistance rate to streptomycin (94.7%) was observed. In contrast, research conducted in different regions of the world has reported lower rates of streptomycin resistance (Khachatryan et al. 2004; de Verdier et al. 2012; Tyson et al. 2015; Sobhy et al. 2020). The *aadA1* gene, associated with streptomycin resistance, was detected in 36.8% of isolates in this study. This finding aligns with previous reports (Hakim et al. 2017; Gow et al. 2008). However, some studies have reported a higher prevalence of the *aadA1* resistance gene compared to the findings of this study (Guerra et al. 2006; Pourtaghi and Sodagari 2016).

In this study, a high level of resistance to tetracycline (93.7%) was detected. Tetracycline is widely used in veterinary medicine and plays a crucial role in the treatment of bacterial infections (OIE 2021). This finding is consistent with studies conducted in various regions on *E. coli* isolates obtained from neonatal calves with septicemia (Aydın et al. 2001; Khachatryan et al. 2004; Gow and Waldner 2009; Shahrani et al. 2014; El Ayis et al. 2015; Astorga et al. 2019; Sobhy et al. 2020). However, previous studies have also reported lower resistance rates (below 50%) (de Verdier et al. 2012; Hasan et al. 2018; Jia et al. 2022; Prieto et al. 2022). When the isolates were examined for tetracycline resistance genes, the *tet(A)*, *tet(B)*, and *tet(C)* genes were detected at rates of 54.7%, 88.4%, and 5.3%, respectively. The prevalence of *tet(B)* and *tet(C)* genes was consistent with previous studies (Gow et al. 2008; Gow and Waldner 2009); however, the prevalence of the *tet(A)* gene in this study was higher than reported in those studies.

The ampicillin resistance rate found in this study (89.5%) is comparable to a study conducted in Iran, which reported a 71.1% resistance rate to this antibiotic (de Verdier et al. 2012). Similarly, several studies have documented high ampicillin resistance rates ranging from 50% to 100% in various regions worldwide, consistent with our findings (Güler et al. 2008; Gharieb et al. 2019; Sobhy et al. 2020; Khawaskar et al. 2022; Jia et al. 2022). Conversely, other studies have reported relatively lower ampicillin resistance rates (below 50%) (Khachatryan et al. 2004; Gow and Waldner 2009; Prieto et al. 2022). In a study conducted in Kars in 2001, the resistance rate to ampicillin was found to be 20.4%, which contrasts with the findings of this study (Aydın et al. 2001). This finding demonstrates the increasing antibiotic resistance in the region over the years.

The resistance of the isolates to trimethoprim-sulfamethoxazole, another commonly used antibiotic in treatment, was examined, and the high resistance rate (87.4%) observed in this study was consistent with previous studies conducted in Türkiye (Cengiz and Adıgüzel 2020) and Iran (Shahrani et al. 2014). However, the trimethoprim-sulfamethoxazole resistance rate found in this study was relatively higher than those reported in other studies (Güler et al. 2008; Gow and Waldner 2009; Gharieb et al. 2019; Sobhy et al. 2020). In this study, the *sul1*, *sul2*, and *sul3* genes, which are associated with sulfonamide resistance, were detected in 67.4%, 53.7%, and 74.7% of the isolates, respectively. Consistent with our findings, studies conducted in different regions of the world have reported *sul1* gene detection rates ranging from 57.1% to 87% (Shahrani et al. 2014; Pourtaghi and Sodagari 2016; Hakim et al. 2017) and *sul2* gene detection rates between 67% and 81% (Gow et al. 2008; Guerra et al. 2008; Gow and Waldner 2009). In contrast to our study, other research has reported relatively lower *sul1* gene detection rates, ranging from 17.1% to 42% (Gow et al. 2008; Gow and Waldner 2009; Algammal et al. 2020). Additionally, in a previous study conducted in Kars (Aydın et al. 2001), the resistance rate to trimethoprim-sulfamethoxazole was reported to be relatively low (12.5%). However, in light of the findings of the current study, an increasing trend in resistance rates is evident.

In this study, the chloramphenicol resistance rate in isolates retrieved from neonatal calves with septicemia was determined to be 87.4%. The World Health Organization (WHO, 2019) has classified

chloramphenicol as a highly important antimicrobial for human health. The findings of this study are consistent with previous studies conducted in Iran, which reported chloramphenicol resistance rates of 73.8% (Shahrani et al. 2014) and 74% (El Ayis et al. 2015). However, studies conducted in other parts of the world have reported considerably lower chloramphenicol resistance rates than those observed in this study (Khachatryan et al. 2004; Gow and Waldner 2009; Hasan et al. 2018). When the presence of the *cmlA* and *cat1* genes, which are associated with chloramphenicol resistance, was examined using multiplex PCR (mPCR), 61% of the isolates were found to be positive for *cmlA*, while 28.4% carried the *cat1* gene. These findings are higher than those reported in a study conducted in the USA, which detected *cmlA* (2.6%) and *cat1* (3.9%) genes (Tyson et al. 2015), but lower than the results of a study from Iran, where both genes were detected in 100% of the isolates (Pourtaghi and Sodagari 2016).

The enrofloxacin resistance rate found in this study (74.7%) was relatively high compared to the resistance rates of 6.3–41.3% reported in previous studies (Güler et al. 2008; Algammal et al. 2020; Jia et al. 2022). However, other studies have documented resistance rates ranging from 52.6% to 100%, which align with the findings of this study (Shahrani et al. 2014; Cengiz and Adıgüzel 2020; Hasan et al. 2019). In the mPCR analysis of fluoroquinolone resistance determinants, the *qnr(A)* gene was detected in 1.05% of the isolates, *qnr(B)* in 56.8%, and *qnr(S)* in 26.3%. A study conducted in Iran (Pourtaghi and Sodagari 2016) investigated only the *qnr(A)* resistance gene, identifying it in 9% of isolates.

Among the antibiotics examined in this study, the lowest resistance (24.2%) was observed against cephalothin. This finding is consistent with a study conducted in Canada (Gow and Waldner 2009). However, contrary to the results of this study, other studies have reported higher cephalothin resistance, ranging from 52.1% to 96.9% (Shahrani et al. 2014; Hasan et al. 2018). In this study, the *blaSHV* gene, which is associated with cephalothin resistance, was not detected in any of the isolates. A similar result was reported in a previous study, where the *blaSHV* gene was also absent in all isolates (Gow et al. 2008). Conversely, a more recent study reported the presence of this resistance gene in 9.5% of isolates (Jia et al. 2022). It is suggested that the observed differences in antibiotic resistance rates between countries and even within different regions of the same country may be attributed to variations in calf care and feeding practices on farms, the quantities of antibiotics administered, as well as regional and seasonal factors. Furthermore, the high antibiotic resistance observed in the isolates may indicate increased exposure of calves to environments contaminated with bacteria resistant to these antibiotics.

In this study, serotyping for O101 revealed that 33.7% of the isolates carried this gene. The findings of this study are consistent with those reported in a study conducted in Iran (Yadegari et al. 2019). However, the result in a more recent study (Feuerstein et al. 2021) was relatively lower than that observed in this study. Another serotype gene examined in this study was O26, which was detected in 6.3% of the isolates. This result aligns with recent studies (Shahrani et al. 2014; Gharieb et al. 2019; Algammal et al. 2020). Conversely, some studies have reported relatively higher rates of the O26 serotype than observed in this study (Engelen et al. 2021). Regarding the O145 serotype gene, mPCR analysis revealed that 2.1% of the isolates carried this gene. While some studies report O145 serotype rates that are consistent with the results of this study (Shahrani et al. 2014; Mainga et al. 2018), there are also studies where all isolates tested negative for the O145 serotype (Taghadosi et al. 2018). In terms of O111 and O9 serotypes, PCR-based genotyping did not detect these genes in any of the isolates in this study. Similarly, studies conducted in various regions of the world also failed to detect O9 serotype (Yadegari et al. 2019; Feuerstein et al. 2021) and O111 serotype (Mainga et al. 2018; Taghadosi et al. 2018) in any isolates. However some studies (Shahrani et al. 2014; Gharieb et al. 2019; Algammal et al. 2020) reported relatively higher prevalence of the O111 serotype than observed in this study.

As a result of single and multiplex PCR assays conducted to determine virulence genes, it was found that 22 (23.2%) isolates carried the *East1* gene, 21 (22.1%) isolates carried the *F5* gene, 19 (20%) isolates carried the *STa* gene, 17 (17.9%) isolates carried the *F41* gene, and 3 (3.2%) isolates carried the *LT* gene. The *F17c* gene could not be detected in any of the isolates. Virulence genes were detected either singly or in combination. Specifically, 2.1% of the isolates carried the *F5, F41, STa* and *LT* genes, 1% carried *F5, F41, STa* and *East1*, 12.6% carried *F5, F41* and *STa*, 3.1% carried *F5* and *East1*, 2.1% carried *STa* and *F41*, 1% carried *F5* and *STa*, and 1% carried the *STa* and *East1* virulence genes together. Additionally, 17.9% of the isolates were found to carry the *East1* gene, 2.1% carried the *F5* gene, and 1% carried the *LT* gene individually. In

a comprehensive study conducted in Iran (Shahrani et al. 2014), it was reported that 1.25% of the isolates carried *LT, STa* and *F5*, 11.4% carried *F5* and *F41*, 28.4% carried *F5* and *LT*, 10.6% carried *F41* and *LT*, and 0.6% carried *F41*, *STa* and *LT* genes. A study conducted in Egypt (Algammal et al. 2020) found that 12.7% of the isolates carried *LT* and *F41*, 5% carried *LT* and *STa*, 5% carried *LT*, *F41* and *STa*, and 8.9% carried only the *STa* gene. In a study conducted in Erzurum, a province neighboring Kars, where this study was performed (Cengiz and Adıgüzel 2020), 5.7% of the isolates had the *STa* gene, 2.6% had both *STa* and *F5*, and 5.7% had *STa*, *F41* and *F5* genes. A study conducted in Egypt on diarrheal and healthy calves (Sobhy et al. 2020) reported that 3.4% of the isolates carried the *STa* gene, 12.5% carried the *F5* gene, and the isolates were negative for the *LT* gene. In a recent study conducted in Germany (Feuerstein et al. 2021), it was reported that 13.9% of the isolates carried *F17*, 2.8% carried *F5* and *STa*, and 3.7% carried *F5*, *F41* and *STa*, while all isolates were found to be negative for the *LT* gene. The differences observed in the distribution of virulence genes may be attributed to geographical diversity, serotype variations, and the different techniques employed in gene screening.

In conclusion, this study highlights *E. coli* as a major pathogen in neonatal calf septicemia, with high resistance to commonly used antibiotics. The O101 serotype and presence of virulence genes such as *East1*, *F5*, *STa*, and *F41* suggest these strains' potential pathogenicity. Given the high resistance rates, stricter antibiotic stewardship and monitoring programs are essential to curb the spread of resistant strains. Further research is needed to better understand the mechanisms of resistance and infection dynamics in livestock.

Ethics Committee Permission: Ethics committee approval is not required for this research.

Acknowledgements: This article is extracted from first author's Ph.D. dissertation entitled ''Identification Of Enterotoxigenic Virulence Genes In Calf Originated *Escherichia Coli* Isolates With Neonatal Septicemia'', supervised by Prof. Dr. Timur GÜLHAN and supported by Ondokuz Mayıs University with the project number PYO. VET.1904.21.031. Also presented as conference abstract paper at "XV. National Congress Of Veterinary Microbiology", 26-28 October 2022, Şanlıurfa, Türkiye.

Funding Support: This study was financially supported by Ondokuz Mayis University (project number: PYO.VET.1904.21.031).

Conflict of Interests: The authors declare that they have no conflicts of interest.

References

Akyüz E, Naseri A, Erkılıç EE, Makav M, Uzlu E, Kırmızıgül AE, Gökçe G. (2017) Neonatal Buzağı İshalleri ve Sepsis. *Kafkas Univ Vet Fak Derg*. 10(2), 181-191.

Al-Abri SS, Beeching NJ, Nye FJ. (2005) Traveller's diarrhoea. *Lancet Infect Dis.* 5 (6), 349-360. doi: 10.1016/s1473-3099(05)70139-0.

Algammal AM, El-Kholy AW, Riad EM, Mohamed HE, Elhaig MM, Yousef SAA, Hozzein WN, Ghobashy MOI. (2020) Genes Encoding the Virulence and the Antimicrobial Resistance in Enterotoxigenic and Shiga-Toxigenic *E. coli* Isolated from Diarrheic Calves. *Toxins (Basel).* 12 (6). doi: 10.3390/toxins12060383.

Astorga F, Navarrete-Talloni MJ, Miró MP, Bravo V, Toro M, Blondel CJ, Hervé-Claude LP. (2019) Antimicrobial resistance in *E. coli* isolated from dairy calves and bedding material. *Heliyon.* 5 (11), e02773. doi: 10.1016/j.heliyon.2019.e02773.

Aydın F, Umur S, Gökçe G, Genç O, Güler MA. (2001) The Isolation and Identification of Bacteria and Parasites from Diarrhoeic Calves in Kars District. *Kafkas Univ Vet Fak Derg*. 7(1), 7-14.

Bartels CJ, Holzhauer M, Jorritsma R, Swart WA, Lam TJ. (2010) Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves. *Prev Vet Med.* 93 (2-3), 162-169. doi: 10.1016/j.prevetmed.2009.09.020.

Cattoir V, Poirel L, Rotimi V, Soussy C-J, Nordmann P. (2007) Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. *Journal of Antimicrobial Chemotherapy*. 60 (2), 394-397. doi: 10.1093/jac/dkm204.

Cengiz S, Adıgüzel MC. (2020) Determination of virulence factors and antimicrobial resistance of *E. coli* isolated from calf diarrhea, part of eastern Turkey. *Ankara Üniv Vet Fak Derg.* 67 (4), 365-371. doi: 10.33988/auvfd.640990.

Chanawong A, M'Zali FH, Heritage J, Lulitanond A, Hawkey PM. (2000) Characterisation of extended-spectrum beta-lactamases of the SHV family using a combination of PCR-single strand conformational polymorphism (PCR-SSCP) and PCR-restriction fragment length polymorphism (PCR-RFLP). *FEMS Microbiol Lett*. 184 (1), 85-89. doi: 10.1111/j.1574-6968.2000.tb08995.x.

Çitil M, Gökçe E. (2013) Neonatal Septisemi. *Turk Klin J Vet Sci*. 4(1), 62-70.

de Verdier K, Nyman A, Greko C, Bengtsson B. (2012) Antimicrobial resistance and virulence factors in *Escherichia coli* from Swedish dairy calves. *Acta Vet Scand.* 54 (1), 2. doi: 10.1186/1751-0147-54-2.

Eid HM, Algammal AM, Elfeil WK, Youssef FM, Harb SM, Abd-Allah EM. (2019) Prevalence, molecular typing, and antimicrobial resistance of bacterial pathogens isolated from ducks. *Vet World.* 12 (5), 677-683. doi: 10.14202/vetworld.2019.677-683.

El Ayis AA, Elgaddal AA, Almofti YA. (2015) Isolation, Identification and Enterotoxin Detection of *Escherichia Coli* Isolated from Calf Diarrhea and their Virulence Characteristics. *J App Ind Sci.* 3 (4), 141-149.

El-Razik K, Abdelrahman K, Ahmed Y, Gomaa AM, Eldebaky H. (2010) Direct Identification of Major Pathogens of the Bubaline Subclinical Mastitis in Egypt using PCR. *Am J Sci*. 6(10), 652-660.

Engelen F, Thiry D, Devleesschauwer B, Heyndrickx M, Mainil J, De Zutter L, Cox E. (2021) Pathogenic potential of *Escherichia coli* O157 and O26 isolated from young Belgian dairy calves by recto-anal mucosal swab culturing. *J Appl Microbiol.* 131 (2), 964-972. doi: 10.1111/jam.14909.

Farfan AE, Ariza SC, Vargas FA, Vargas LV. (2016) Virulence mechanisms of enteropathogenic *Escherichia coli*. *Chilean J Infectol*, 33, 438–450. doi: 10.4067/S0716-10182016000400009.

Feuerstein A, Scuda N, Klose C, Hoffmann A, Melchner A, Boll K, Rettinger A, Fell S, Straubinger RK, Riehm JM. (2021) Antimicrobial Resistance, Serologic and Molecular Characterization of *E. coli* Isolated from Calves with Severe or Fatal Enteritis in Bavaria, Germany. *Antibiotics (Basel).* 11 (1), 23. doi: 10.3390/antibiotics11010023.

Foster DM, Smith GW. (2009) Pathophysiology of diarrhea in calves. *Vet Clin North Am Food Anim Pract.* 25 (1), 13-36. doi: 10.1016/j.cvfa.2008.10.013.

Gharieb R, Fawzi E, Elsohaby I. (2019) Antibiogram, virulotyping and genetic diversity of *Escherichia coli* and *Salmonella* serovars isolated from diarrheic calves and calf handlers. *Comp Immunol Microbiol Infect Dis.* 67, 101367. doi: 10.1016/j.cimid.2019.101367.

González Pasayo RA, Sanz ME, Padola NL, Moreira AR. (2019) Phenotypic and genotypic characterization of enterotoxigenic *Escherichia coli* isolated from diarrheic calves in Argentina. *Open Vet J.* 9 (1): 65-73. doi: 10.4314/ovj.v9i1.12.

Gow SP, Waldner CL, Harel J, Boerlin P. (2008) Associations between antimicrobial resistance genes in fecal generic *Escherichia coli* isolates from cow-calf herds in western Canada. *Appl Environ Microbiol.* 74 (12), 3658-3666. doi: 10.1128/aem.02505-07.

Gow SP, Waldner CL. (2009) Antimicrobial resistance and virulence factors stx1, stx2, and eae in generic *Escherichia coli* isolates from calves in western Canadian cow-calf herds. *Microb Drug Resist.* 15 (1), 61-67. doi: 10.1089/mdr.2009.0860.

Guerra B, Junker E, Schroeter A, Helmuth R, Guth BE, Beutin L. (2006) Phenotypic and genotypic characterization of antimicrobial resistance in *Escherichia coli* O111 isolates. *J Antimicrob Chemother.* 57 (6), 1210-1214. doi: 10.1093/jac/dkl127.

Güler L, Gündüz K, Ok U. (2008) Virulence factors and antimicrobial susceptibility of *Escherichia coli* isolated from calves in Turkey. *Zoonoses Public Health.* 55 (5), 249-257. doi: 10.1111/j.1863-2378.2008.01121.x.

Gülhan T, İlhan Z, Aksakal A, Solmaz H, Ekin İH. (2009) Hayvan Orijinli *Escherichia coli* Suşlarının Enterotoksin Tiplerinin (LT, ST) Belirlenmesi. *YYU Vet Fak Derg.* 20(2), 27-31.

Hakim AS, Omara ST, Syame SM, Fouad EA. (2017) Serotyping, antibiotic susceptibility, and virulence genes screening of *Escherichia coli* isolates obtained from diarrheic buffalo calves in Egyptian farms. *Vet World.* 10 (7), 769-773. doi: 10.14202/vetworld.2017.769-773.

Haque MA, Hossain MT, Islam MS, Islam MZ, Islam P, Shaha SN, Sikder MH, Rafiq K. (2022) Isolation of multidrug-resistant *Escherichia coli* and *Salmonella* spp. from sulfonamide-treated diarrheic calves. *Vet World.* 15 (12), 2870-2876. doi: 10.14202/vetworld.2022.2870-2876.

Hasan M, Hussein M, Yousif AA. (2018) Confirmatory Detection of *Escherichia coli* O157:H7 in Diarrheic and Non-Diarrheic Calves by using Real Time PCR with Studying the Antimicrobial Susceptibility of these Bacteria. *J Glob Pharma Technol.* 10, 90-96. doi: 10.5958/0974-360X.2019.00046.5.

Jia Y, Mao W, Liu B, Zhang S, Cao J, Xu X. (2022) Study on the drug resistance and pathogenicity of *Escherichia coli* isolated from calf diarrhea and the distribution of virulence genes and antimicrobial resistance genes. *Front Microbiol.* 13, 992111. doi: 10.3389/fmicb.2022.992111.

Karakaya E, Aydin F, Kayman T, Abay S. (2022) *Escherichia coli* in different animal feces: phylotypes and virulence genes. *World J Microbiol Biotechnol*, 39(1), 14. doi: 10.1007/s11274-022-03451-w.

Khachatryan AR, Hancock DD, Besser TE, Call DR. (2004) Role of calf-adapted *Escherichia coli* in maintenance of antimicrobial drug resistance in dairy calves. *Appl Environ Microbiol.* 70 (2), 752-757. doi: 10.1128/aem.70.2.752-757.200432.

Khawaskar DP, Sinha DK, Lalrinzuala MV, Athira V, Kumar M, Chhakchhuak L, Mohanapriya K, Sophia I, Abhishek, Kumar ORV, Chaudhuri P, Singh BR, Thomas P. (2022) Pathotyping and antimicrobial susceptibility testing of *Escherichia coli* isolates from neonatal calves. *Vet Res Commun.* 46 (2), 353-362. doi: 10.1007/s11259-021-09857-5.

Kozak G, Boerlin P, Janecko N, Reid-Smith R, Jardine C. (2009) Antimicrobial Resistance in *Escherichia coli* Isolates from Swine and Wild Small Mammals in the Proximity of Swine Farms and in Natural Environments in Ontario, Canada. *J Appl Environ Microbiol*. 75, 559-566. doi: 10.1128/AEM.01821-08.

Lim JY, Yoon J, Hovde CJ. (2010) A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. *J Microbiol Biotechnol.* 20(1), 5-14.

Liu B, Wu F, Li D, Beutin L, Chen M, Cao B, Wang L. (2010) Development of a serogroup-specific DNA microarray for identification of *Escherichia coli* strains associated with bovine septicemia and diarrhea. *Vet Microbiol*. 142 (3-4), 373-378. doi: 10.1016/j.vetmic.2009.10.019.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect.* 18 (3), 268-281. doi: 10.1111/j.1469-0691.2011.03570.x.

Mainga AO, Cenci-Goga BT, Malahlela MN, Tshuma T, Kalake A, Karama M. (2018) Occurrence and characterization of seven major Shiga toxin-producing *Escherichia coli* serotypes from healthy cattle on cow-calf operations in South Africa. *Zoonoses Public Health.* 65 (7), 777-789. doi: 10.1111/zph.12491.

Naderi Z, Ghanbarpour R, Sami M. (2016) Antimicrobial Resistance Characteristics and Phylogenetic Groups of *Escherichia coli* Isolated From Diarrheic Calves in Southeast of Iran. *Journal of Enteric Pathogens*. 4(4), 21-27.

Nagy B, Fekete PZ. (2005) Enterotoxigenic *Escherichia coli* in veterinary medicine. *Int J Med Microbiol.* 295 (6-7), 443-454. doi: 10.1016/j.ijmm.2005.07.003.

Pérez-Pérez FJ, Hanson ND. (2002) Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol.* 40 (6), 2153-2162. doi: 10.1128/jcm.40.6.2153-2162.2002.

Picco NY, Alustiza FE, Bellingeri RV, Grosso MC, Motta CE, Larriestra AJ, Vissio C, Tiranti KI, Terzolo HR, Moreira AR, Vivas AB. (2015) Molecular screening of pathogenic *Escherichia coli* strains isolated from dairy neonatal calves in Cordoba

province, Argentina. *Rev Argent Microbiol*. 47 (2), 95-102. doi: 10.1016/j.ram.2015.01.006.

Pourtaghi H, Sodagari H. (2016) Antimicrobial Resistance of Entrotoxigenic and Non-Entrotoxigenic *Escherichia coli* Isolated From Diarrheic Calves in Iran. *Int J Enteric Pathog*. 4(2), e34557. doi: 10.17795/ijep34557.

Prieto A, López-Novo C, Díaz P, Díaz-Cao JM, López-Lorenzo G, Antón C, Remesar S, García-Dios D, López C, Panadero R, Díez-Baños P, Morrondo P, Fernández G. (2022) Antimicrobial Susceptibility of Enterotoxigenic *Escherichia coli* from Diarrhoeic Neonatal Calves in Spain. *Animals*. 12(3), 264.

Rai S, Kumar M, Jas R, Mandal GP, Samanta I, Rajendar M, Tripura S, Das SK, Mondal M, Mandal DK. (2023) Antibacterial effect of kitchen herbs against pathogenic multidrug-resistant *E. coli* isolates from calf diarrhoea. *Trop Anim Health Prod*. 55 (3), 211. doi: 10.1007/s11250-023-03628-x.

Salaheen S, Kim SW, Cao H, Wolfgang DR, Hovingh E, Karns JS, Haley BJ, Van Kessel JAS. (2019) Antimicrobial Resistance Among *Escherichia coli* Isolated from Veal Calf Operations in Pennsylvania. *Foodborne Pathog Dis*. 16 (1), 74-80. doi: 10.1089/fpd.2018.2530.

Shahrani M, Dehkordi FS, Momtaz H. (2014) Characterization of *Escherichia coli* virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. *Biol Res*. 47 (1), 28. doi: 10.1186/0717-6287-47-28.

Skarżyńska M, Zaja CM, Bomba A, Bocian Ł, Kozdruń W, Polak M, Wia Cek J, Wasyl D. (2021) Antimicrobial Resistance Glides in the Sky-Free-Living Birds as a Reservoir of Resistant *Escherichia coli* With Zoonotic Potential. *Front Microbiol*. 12, 656223. doi: 10.3389/fmicb.2021.656223.

Sobhy NM, Yousef SGA, Aboubakr HA, Nisar M, Nagaraja KV, Mor SK, Valeris-Chacin RJ, Goyal SM. (2020) Virulence factors and antibiograms of *Escherichia coli* isolated from diarrheic calves of Egyptian cattle and water buffaloes. *PLoS One*. 15 (5), e0232890. doi: 10.1371/journal.pone.0232890.

Taghadosi R, Shakibaie MR, Alizade H, Hosseini-Nave H, Askari A, Ghanbarpour R. (2018) Serogroups, subtypes and virulence factors of shiga toxin-producing *Escherichia coli* isolated from human, calves and goats in Kerman, Iran. *Gastroenterol Hepatol Bed Bench*. 11 (1), 60-67.

Tyson GH, McDermott PF, Li C, Chen Y, Tadesse DA, Mukherjee S, Bodeis-Jones S, Kabera C, Gaines SA, Loneragan GH, Edrington TS, Torrence M, Harhay DM, Zhao S. (2015) WGS accurately predicts antimicrobial resistance in *Escherichia coli*. *J Antimicrob Chemother*. 70 (10), 2763-2769. doi: 10.1093/jac/dkv186.

Umpiérrez A, Acquistapace S, Fernández S, Oliver M, Acuña P, Reolón E, Zunino P. (2016) Prevalence of *Escherichia coli* adhesion-related genes in neonatal calf diarrhea in Uruguay. *J Infect Dev Ctries*. 10 (5), 472-477. doi: 10.3855/jidc.7102.

Wernli D, Haustein T, Conly J, Carmeli Y, Kickbusch I, Harbarth S. (2011) A call for action: the application of The International Health Regulations to the global threat of antimicrobial resistance. *PLoS Med*. 8(4),e1001022. doi: 10.1371/journal.pmed.1001022.

Yadegari Z, Nikbakht Brujeni G, Ghorbanpour R, Moosakhani F, Lotfollahzadeh S. (2019) Molecular characterization of enterotoxigenic *Escherichia coli* isolated from neonatal calves diarrhea. *Vet Res Forum*. 10 (1), 73-78. doi: 10.30466/vrf.2019.34313.

Zajacova ZS, Konstantinova L, Alexa P. (2012) Detection of virulence factors of *Escherichia coli* focused on prevalence of EAST1 toxin in stool of diarrheic and non-diarrheic piglets and presence of adhesion involving virulence factors in astA positive strains. *Vet Microbiol*. 154 (3-4), 369-375. doi: 10.1016/j.vetmic.2011.07.029.

Zhao H, Sun R, Yu P, Alvarez PJJ. (2020) High levels of antibiotic resistance genes and opportunistic pathogenic bacteria indicators in urban wild bird feces. *Environ Pollut*. 266 (Pt 2), 115200. doi: 10.1016/j.envpol.2020.115200.