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Detection of virulence genes, antibiotic resistance genes and antibiotic resistance of *Escherichia coli* isolated from diarrheic lambs in Anhui Province, China[1]

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ABSTRACT.- Deng Y.F., Wang K., Zhang L.J., Yan K. & He S.J. 2024. **Detection of virulence genes, antibiotic resistance genes and antibiotic resistance of *Escherichia coli* isolated from diarrheic lambs in Anhui Province, China**. *Pesquisa Veterinária Brasileira 44:e07346, 2024*. College of Animal Science, Anhui Science and Technology University, Fengyang, Anhui 233100, China. E-mail: shaojunhe2011@126.com

Lamb diarrhea seriously restricts the development of the sheep industry. Infectious pathogens often cause diarrhea, and *E. coli* isolates are highly distributed in infectious diarrhea. The study aimed to investigate the prevalence of pathogenic *E. coli* in diarrhea lambs and determine the distribution of virulence genes, antibiotic resistance genes, and antibiotic resistance. One hundred seventy-eight *E. coli* isolates were isolated from the feces of 204 diarrhea lambs. The virulence genes *mdh, hlyF, iss, ompA, fimC, iucD, st, lt, stx1, stx2,* and antibiotic resistance genes including *tetA, tetB, aac (6')-II, blaCMY-2, qnr, aadA1, sul1, blaTEM, blaCTX-M* were detected by polymerase chain reaction (PCR). Antibiotic resistance was determined by Kirby-Bauer Disc Diffusion method. There were 109 (61.24%, 109/178) enterotoxigenic *E. coli* (ETEC), 119 (66.85%, 119/178) Shiga toxin-producing *E. coli* (STEC), and 95 (53.37%, 95/178) hybrid STEC/ETEC isolates. The highest prevalent virulence genes were *ompA* (80.90%, 144/178) and *fimC* (67.98%, 121/178), and the lowest was *iucD* (8.99%, 16/178). The most commonly detected antibiotic resistance genes were *tetA/tetB* (92.70%, 165/178), *qnr* (75.84%, 135/178), and *sul1* (62.92%, 112/178), no *blaTEM* or *blaCTX-M* genes were detected. All isolates had high antibiotic resistance to lincomycin (96.63%, 172/178), tetracycline (88.76%, 158/178), and co-trimoxazole (80.34%, 143/178), and the multidrug resistant (MDR) rate reached 93.82% (167/178). The high prevalence of ETEC and STEC indicates that *E. coli* is one of the critical pathogenic agents leading to diarrhea in lambs in the region, and its high antibiotic resistance, especially MDR, should be brought to our attention.

INDEX TERMS: *Escherichia coli*, antibiotic resistance, antibiotic resistance genes, virulence genes, diarrheic lambs.

INTRODUCTION

Escherichia coli is a ubiquitous, primarily harmless gastrointestinal bacteria (Glowacki et al. 2019). This is partly due to the acquisition of gene function, allowing commensal *E. coli* to receive different virulence factors that make them pathogenic (Bihannic et al. 2014). Pathogenic *E. coli* is one of the diarrhea pathogens and is known as diarrheagenic *E. coli* (DEC). It is divided into seven types: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffuse adhesive *E. coli* (DAEC) and adhesive invasive *E. coli* (AIEC) (Sharma et al. 2006). Furthermore, EPEC isolates usually have multiple virulence and antibiotic resistance factors (Beutin & Fach 2014), making clinical treatment extraordinarily challenging. Comprehensive antibiotic resistance analysis is essential in treating and controlling diarrheal diseases. In addition, both antibiotic-resistant bacteria and their antibiotic-resistance genes increase when exposed to sufficiently high antibiotic selection pressures (González-Plaza et al. 2018), and the development

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of *E. coli* resistance can be promoted by horizontal transfer of antibiotic-resistance genes between different strains and species (Jutkina et al. 2018). For the reasons mentioned above, antibiotic resistance of *E. coli* remains a significant public health problem worldwide. Diarrhea caused by infectious microorganisms causes most neonatal morbidities and deaths in ruminants, and it can be caused by many pathogens, including viruses, parasites, and bacteria (Smith-Palmer et al. 2003). Pathogenic *E. coli* is the most common and essential agent in variable breeding models of large farms, smallholders, and individually owned animals (Muktar et al. 2015). Lamb diarrhea is a common disease among sheep farmers, with a very high mortality rate, seriously affecting the development of the agro-economy. In contrast, sheep farming is a lifeline for the agro-economy in many parts of the world. Therefore, the study of the etiology and treatment of the disease has important economic significance. The previous study showed that the characteristics of *E. coli* have substantial regional differences (Pires et al. 2022). Hence, the current research on pathogenic *E. coli* causing lamb diarrhea still needs to be supplemented to treat and prevent it effectively.

Diarrhea and dysentery in young animals are more commonly caused by ETEC and STEC strains (Muktar et al. 2015). ETEC can cause severe diarrhea by releasing heat-labile enterotoxins (*lt*) and heat-stable enterotoxins (*sta*/*stb*). Also, the Shiga toxins 1 (*stx1*) and 2 (*stx2*) are associated with STEC strain pathogenesis (Shahrani et al. 2014). In this study, the feces of diarrheal lambs collected from sheep farms in Anhui Province were used to study the antibiotic resistance, antibiotic resistance genes, and virulence genes of isolated *E. coli*, aiming to clarify the prevalence of diarrheal *E. coli* in this area and its antibiotic resistance and pathogenicity.

MATERIALS AND METHODS

Animal Ethics. According to the requirements of the Experimental Animal Ethics Committee of Anhui Science and Technology University, mandatory restrictions on animal bodies were minimized to the greatest extent during the sampling process. In addition, this study strictly followed laboratory safety guidelines in microbiological experiments.

Sample collection and isolation and identification of *Escherichia coli*. In Anhui province, 204 fecal samples of diarrheal lambs were collected from October 2021 to October 2022 – all animals within 30 days of age. A sterile rectal swab was used to collect fecal samples, and samples were diluted in a sterilized saline solution. Samples were sterilely inoculated onto MacConkey agar and eosin methylene blue (EMB) agar and incubated for 18 hours at 37°C. The colonies with typical *E. coli* characteristics were isolated for Gram staining and microscopic examination and subcultured on nutrient agar medium for pure culture for further identification using biochemical tests, including sugar fermentation test, methyl red (MR), Voges-Proskauer (V-P) and indole test. In addition, the putative *E. coli* 16S rRNA gene was amplified and sequenced for further identification (After DNA extraction) (Table 1).

Antibiotic susceptibility testing. Antimicrobial susceptibility was determined by the standard disk diffusion method. Each isolate was tested for antibiotic susceptibility using a panel of the following antibiotics: Tetracycline (30μg/disk), cefoperazone (75μg/disk), cefoxitin (30μg/disk), ceftazidime (30μg/disk), cefotaxime (30μg/disk), netilmicin (30μg/disk), amikacin (30μg/disk), neomycin (30μg/disk), florfenicol (30μg/disk), enrofloxacin (10μg/disk), co-trimoxazole (23.75/1.25μg/disk), lincomycin (2μg/disk). The plates were incubated for 18h at 37°C, and inhibitory zone diameters were measured. Interpretation of results followed criteria recommended by the Clinical Laboratory Standard Institute (CLSI 2017).

Table 1. Details of primers used for *Escherichia coli* 16S rRNA gene and virulence genes and their PCR conditions

Primers	Sequence (5'→3')	Annealing	Extension	Product length (bp)	Reference
16S rRNA-27F	AGAGTTTGATCCTGGCTCAG	58°C for 15s (30 cycles)	72°C for 90s	1418	This study
16S rRNA-1492R	GGTTAACCTTGTTACGACTT				
mdh-F	GGTATGGATCGTTCCGACCT	58°C for 15s (30 cycles)	72°C for 30s	304	This study
mdh-R	GGCAGAATGGTAACACCAGAGT				
hlyF-F	GGCCACAGTCGTTTAGGGTGCTTACC	66°C for 15s (30 cycles)	72°C for 30s	450	This study
hlyF-R	GGCGGTTTAGGCATTCCGATACTCAG				
iss-F	CAGCAACCCGAACCACTTGATG	65°C for 15s (30 cycles)	72°C for 30s	323	This study
iss-R	AGCATTGCCAGAGCGGCAGAA				
ompA-F	AGCTATCGCGATTGCAGTG	58°C for 15s (30 cycles)	72°C for 45s	919	This study
ompA-R	GGTGTTGCCAGTTAACCGG				
fimC-F	GGGTAGAAAATGCCGATGGTG	59°C for 15s (30 cycles)	72°C for 30s	477	This study
fimC-R	CGTCATTTTGGGGGTAAGTG				
iucD-F	ACAAAAAGTTCTATCGCTTCC	55°C for 15s (30 cycles)	72°C for 30s	692	This study
iucD-R	CCTGATCCAGATGATGCTC				
st-F	ATTTTTMTTTCTGTATTRTCTT	54°C for 15s (30 cycles)	72°C for 30s	190	Ghanbarpour et al. (2017)
st-R	CACCCGGTACARGCAGGATT				
lt-F	GGCGACAGATTATACCGTGC	58°C for 15s (30 cycles)	72°C for 30s	450	Ghanbarpour et al. (2017)
lt-R	CGGTCTCTATATTCCCTGTT				
stx1-F	TGTAACTGGAAAGGTGGAGTATAC	56°C for 15s (30 cycles)	72°C for 30s	210	Ghanbarpour et al. (2017)
stx1-R	GCTATTCTGAGTCAACGAAAAATAAC				
stx2-F	GTTTTTCTTCGGTATCCTATTCCC	59°C for 15s (30 cycles)	72°C for 45s	484	Ghanbarpour et al. (2017)
stx2-R	GATGCATCTCTGGTCATTGTATTAC				

PCR = Polymerase chain reaction, M = (A/C).

DNA extraction. The purified colonies were suspended in 150μL of sterile distilled water and boiled at 100°C for 10 min and cooled, then cell debris was removed by centrifugation for 10 min at 12,000rpm, and 3μL of DNA supernatant was used for 16S rRNA gene amplification, the rest was stored at −20°C until others gene PCR amplification.

Detection of virulence genes and antibiotic resistance genes using PCR. PCR amplification was used for the detection of virulence genes in 178 isolates, including *mdh, hlyF, iss, ompA, fimC, iucD, st, lt, stx1, stx2* (Table 1), and antibiotic resistance genes, including *tetA, tetB, aac (6′)-II, blaCMY-2, qnr, aadA1, sul1 blaTEM, blaCTX-M* (Table 2). PCR reactions were performed in 25μL, including 12.5μL of 2×Taq Mix, 1μL (0.4μM) of each primer, 2μL of each DNA template, and 8.5μL of sterile ultrapure water. The PCR conditions were performed on a thermocycler with the following protocol: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 15 s, annealing for 15 s and extension at 72°C, with a final extension for 10 min at 72°C, and other conditions are shown in Table 1 and 2 (the primer sequence has been validated). After amplification, the PCR products were separated by electrophoresis on 2% agarose gel stained with nucleic acid dyes, then visualized under UV light using the gel documentation system. The data was analyzed using IBM SPSS Statistics 21 software.

RESULTS

Isolation, identification and pathogenic type detection of *Escherichia coli*

In this study, 178 isolates that met the growth characteristics of *E. coli* were isolated from 204 fecal samples, and microscopic examination after the Gram stain showed that the isolated isolates were all single rod-shaped Gram-negative bacteria. Sugar fermentation tests, methyl red (MR), Voges-Proskauer (V-P), and indole tests determined the isolates as *E. coli*. Following PCR of the *E. coli* 16S rRNA gene with specific primers, the products were sequenced and compared in the National Center for Biotechnology Information (NCBI) to determine that all isolates were *E. coli*. The age of these diarrhea lambs and the strain information of the pathogenic type determined according to our detection are shown in Table 3.

Antibiotic resistance

A CLSI classification was applied based on the zone diameters of susceptibility testing results. The antibiotic resistance results showed that the isolated isolates were at a high level of resistance to five antibiotics: 88.76% (158/178) to

Table 2. Details of primers used for *Escherichia coli* resistance genes and their PCR conditions

Primers	Sequence (5′→3′)	Annealing	Extension	Product length (bp)	Reference
tetA-F	CGAAAGGCGGGCACTCAT	60°C for 15s (30 cycles)	72°C for 30s	326	Tuo et al. (2020)
tetA-R	CGGCAGGCAGAGCAAGTAGAG				
tetB-F	ACGTGATAATACAGATACCGAA	55°C for 15s (30 cycles)	72°C for 30s	232	Tuo et al. (2020)
tetB-R	CACAAAGGCTTGGAATACTGA				
aac (6′)-II -F	CACAGTCGTACGTTGCKCTBGG	64°C for 15s (30 cycles)	72°C for 30s	235	Fouhy et al. (2014)
aac (6′)-II -R	CCTGCCTTCTCGTAGCAKCGDAT				
blaCMY-2-F	CCACTTTGCTGTCGCTGCCG	66°C for 15s (30 cycles)	72°C for 45s	662	This study
blaCMY-2-R	CGTGTTGGGCGGCGATGCTAT				
qnr-F	CACCGCTTGCACATTCATTCGC	65°C for 15s (30 cycles)	72°C for 30s	450	This study
qnr-R	ACCGTCGAGTTCGGCGTGG				
aadA1-F	GCCCATCTCGAACCGACGTT	53°C for 15s (30 cycles)	72°C for 30s	573	This study
aadA1-R	GCCACTCGGCAGCGACATC				
sul1-F	TGGCGTCGCGACTGCGAAAT	65°C for 15s (30 cycles)	72°C for 45s	813	Van et al. (2008)
sul1-R	TGGTGACGGTGTTCGGCATTCT				
blaTEM-F	TTTCGTGTCGCCCTTATTC	58°C for 15s (30 cycles)	72°C for 45s	692	This study
blaTEM-R	CCGGCTCCAGATTTATCAGC				
blaCTX-M-F	ATGTGCAGYACCAGTAA	51°C for 15s (30 cycles)	72°C for 45s	536	This study
blaCTX-M-R	ACCGCRATATCRTTGGT				

PCR = Polymerase chain reaction, K = (G/T), D = (A/G/T), Y = (C/T), R = (A/G).

Table 3. The prevalence of different pathotypes of *Escherichia coli* in diarrheic lambs according to their age

	Age (day)	Invalid sample (n)	ETEC (n)	STEC (n)	ETEC /STEC (n)	Un-classified (n)	Total (n)
Sheep	0-7	1	5	4	11	6	27
	8-14	6	4	1	35	16	62
	15-21	8	1	6	14	10	39
	21-30	3	-	1	6	1	11
Goat	0-7	-	1	5	5	3	14
	8-14	2	3	5	7	4	21
	15-21	4	-	1	14	5	24
	21-30	2	-	1	3	-	6
TOTAL	-	26	14	24	95	45	204

ETEC = Enterotoxigenic *E. coli,* STEC = Shiga toxin-producing *E. coli.*

tetracycline, 70.22% (125/178) to florfenicol and enrofloxacin, 80.34% (143/178) to co-trimoxazole, 96.63% (172/178) to lincomycin. No isolates sensitive to all studied antibiotics were observed (Table 4).

The results of the MDR spectrum analysis of 178 isolated *E. coli* are shown in Table 5. Only 11 (6.18%, 11/178) isolates were resistant to one or two antibiotics, while the remaining 167 (93.82%, 167/178) isolates were all MDR; in addition, 32.58% (58/178) were resistant to all seven antibiotics.

PCR detection of virulence genes

In this study, 109 (61.24%, 109/178) isolates possessed *st* and/or *lt* genes, which were categorized into ETEC pathotype, 119 (66.85%, 119/178) isolates were positive for *stx1* and/or *stx2* genes which categorized into STEC pathotype, 95 (53.37%, 95/178) were hybrid STEC/ETEC isolates containing *st*/*lt* and *stx1*/*stx2* genes and 45 (25.28%, 45/178) were un-classified isolates without *st*/*lt* and *stx1*/*stx2* genes (Table 3). As the housekeeping gene of *E. coli*, the detection rate of *mdh* in isolates was 100%. In addition, the prevalence rates of *ompA* and *fimC* were the highest (80.90%, 144/178 and 67.98%, 121/178, respectively), while the prevalence rate of *iucD* was the lowest (8.99%, 16/178) (Table 6). The distribution of virulence genes did not show significant differences in ETEC, STEC, and un-classified isolates (P>0.05). Some typical representative PCR results are shown in Figure 1.

PCR detection antibiotic resistance genes

The highest prevalence was observed for the *tetA*/*tetB* and *qnr* genes, 92.70% (165/178), and 75.84% (135/178), respectively. Among 165 *tetA*/*tetB* positive isolates, 155 showed tetracycline resistance (155/165, 93.94%), and 117 out of 135 *qnr* positive isolates showed enrofloxacin resistance (117/135, 86.67%). The incidence of *blaCMY-2* was the lowest, at 32.58% (58/178), similar to the proportion of resistance to cephalosporins. Additionally, the *blaTEM* and *blaCTX-M* genes were not detected. In this study, the distribution of antibiotic resistance genes did not show significant differences in ETEC, STEC, and un-classified isolates (P>0.05) (Table 7). Some typical representative PCR results are shown in Figure 2.

Table 4. The percentage of antibiotic resistance in different pathotypes of *Escherichia coli*

	FOX (n) (%)	CFP (n) (%)	TCY (n) (%)	CAZ (n) (%)	CTX (n) (%)	NET (n) (%)	AK (n) (%)	NEO (n) (%)	FLR (n) (%)	ENR (n) (%)	SXT (n) (%)	LIN (n) (%)
ETEC (n=14)	5 (35.71)	4 (28.57)	14 (100.00)	3 (21.43)	6 (42.86)	0 (0.00)	8 (57.14)	8 (57.14)	10 (71.43)	9 (64.29)	13 (92.86)	14 (100.00)
STEC (n=24)	11 (45.83)	9 (37.50)	20 (83.33)	3 (12.50)	7 (29.17)	4 (16.67)	12 (50.00)	11 (45.83)	15 (62.50)	17 (70.83)	18 (75.00)	23 (95.83)
ETEC /STEC (n=95)	38 (40.00)	36 (37.89)	85 (89.47)	27 (28.42)	46 (48.42)	19 (20.00)	50 (52.63)	56 (58.95)	69 (72.63)	70 (73.68)	80 (84.21)	93 (97.89)
Un-classified (n=45)	20 (44.44)	17 (37.78)	39 (86.67)	12 (26.67)	18 (40.00)	6 (13.33)	21 (46.67)	19 (42.22)	31 (68.89)	29 (64.44)	32 (71.11)	42 (93.33)
TOTAL (n=178)	74 (41.57)	66 (37.08)	158 (88.76)	45 (25.28)	77 (43.26)	29 (16.29)	91 (51.12)	94 (52.81)	125 (70.22)	125 (70.22)	143 (80.34)	172 (96.63)

FOX = Cefoxitin, CFP = cefoperazone, TCY = tetracycline, CAZ = ceftazidime, CTX = cefotaxime, NET = netilmicin, AK = amikacin, NEO = neomycin, FLR = florfenicol, ENR = enrofloxacin, SXT = co-trimoxazole, LIN = lincomycin, ETEC = enterotoxigenic E. coli, STEC = Shiga toxin-producing *E. coli.*

Table 5. MDR profiles of *Escherichia coli* isolate

	Patovars (n) (%)							MDR (n) (%)
	1	2	3	4	5	6	7	
ETEC (n=14)	0 (0.00)	0 (0.00)	2 (14.29)	1 (7.14)	2 (14.29)	4 (28.57)	5 (35.71)	14 (100.00)
STEC (n=24)	0 (0.00)	2 (8.33)	4 (16.67)	3 (12.50)	2 (8.33)	4 (16.67)	9 (37.50)	22 (91.67)
ETEC /STEC (n=95)	0 (0.00)	2 (2.11)	6 (6.32)	16 (16.84)	21 (22.11)	17 (17.89)	33 (34.74)	93 (97.89)
Un-classified (n=45)	4 (8.89)	3 (6.67)	0 (0.00)	8 (17.78)	9 (20.00)	10 (22.22)	11 (24.44)	38 (84.44)
TOTAL (n=178)	4 (2.25)	7 (3.93)	12 (6.74)	28 (15.73)	34 (19.10)	35 (19.66)	58 (32.58)	167 (93.82)

MDR = Multidrug resistant, ETEC = enterotoxigenic *E. coli,* STEC = Shiga toxin-producing *E. coli.*

Table 6. The distribution of virulence genes in different pathogenic types of *Escherichia coli*

	mdh (n) (%)	*hlyF* (n) (%)	*iss* (n) (%)	*ompA* (n) (%)	*fimC* (n) (%)	*iucD* (n) (%)
ETEC (n=14)	14 (100.00)	4 (28.57)	2 (14.29)	11 (78.57)	8 (57.14)	1 (7.14)
STEC (n=24)	24 (100.00)	3 (12.50)	3 (12.50)	17 (70.83)	14 (58.33)	11 (4.17)
ETEC /STEC (n=95)	95 (100.00)	25 (26.32)	12 (12.63)	78 (82.11)	68 (71.58)	12 (12.63)
Un-classified (n=45)	45 (100.00)	17 (37.78)	7 (15.56)	38 (84.44)	31 (68.89)	2 (4.44)
TOTAL (n=178)	178 (100.00)	49 (27.53)	24 (13.48)	144 (80.90)	121 (67.98)	16 (8.99)

ETEC = Enterotoxigenic *E. coli,* STEC = Shiga toxin-producing *E. coli.*

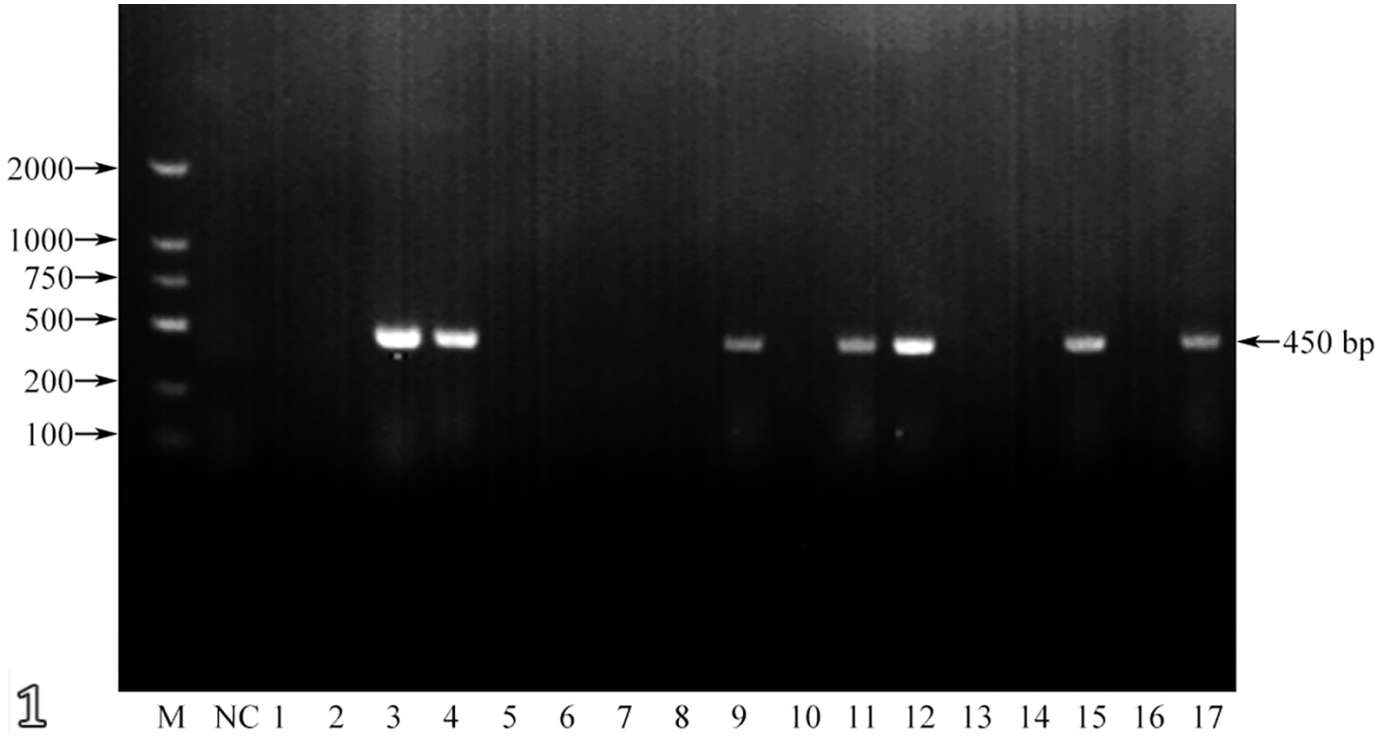

Fig.1. PCR amplification results of *Escherichia coli* virulence gene *hlyF*. NC = negative control, 1 to 17 = *E. coli*.

Table 7. The distribution of antibiotic resistance genes in different pathogenic types of *Escherichia coli*

	tetA (n) (%)	*tetB* (n) (%)	*aac (6′)-II* (n) (%)	*blaCMY-2* (n) (%)	*qnr* (n) (%)	*aadA1* (n) (%)	*sul1* (n) (%)
ETEC (n=14)	13 (92.86)	11 (78.57)	6 (42.86)	3 (21.43)	11 (78.57)	7 (50.00)	13 (92.86)
STEC (n=24)	21 (87.50)	14 (58.33)	9 (37.50)	6 (25.00)	17 (70.83)	11 (45.83)	16 (66.67)
ETEC /STEC (n=95)	73 (76.84)	64 (67.37)	51 (53.68)	32 (33.68)	75 (78.95)	46 (48.42)	60 (63.16)
Un-classified (n=45)	38 (84.44)	28 (62.22)	21 (46.67)	17 (37.78)	32 (71.11)	18 (40.00)	23 (51.11)
TOTAL (n=178)	145 (81.46)	117 (65.73)	87 (48.88)	58 (32.58)	135 (75.84)	82 (46.07)	112 (62.92)

ETEC = Enterotoxigenic *E. coli,* STEC = Shiga toxin-producing *E. coli.*

DISCUSSION

Although only some types of *Escherichia coli* are pathogenic, it is the most common and major pathogenic microorganism that causes neonatal diarrhea in animals (Muktar et al. 2015). Some studies have identified *E. coli* based on its growth characteristics and biochemical identification, and some have shown that 16S rRNA gene identification is a reliable method for accurately identifying *E. coli* (Fanjip et al. 2022). However, some closely related gut bacteria, such as *Shigella*, cannot be distinguished by the 16S rRNA gene sequence and *E. coli*. An analysis of 16S rRNA gene sequences and biochemical characteristics was used to identify *E. coli* to ensure the accuracy of strain identification.

Although ETEC and STEC strains can be isolated from healthy sheep's feces, the isolation probability is much lower (Sahoo et al. 2010). In this study, the prevalence of pathogenic *E. coli* (74.72%, 133/178) in lambs was higher than that of 71.66% (Ghanbarpour et al. 2017). We found that the frequency of ETEC isolates was 61.24% (109/178), of which 108 (60.67%, 108/178) isolates were *st* gene positive and two (1.12%, 2/178) were *lt* gene positive. *lt* gene is not generally found in isolated strains of ruminants, so its absence is not surprising (Türkyılmaz et al. 2013). 31.3%, Li et al. (2020) showed that ETEC isolates from diarrhea piglets in Shandong Province accounted for 31.3%. In addition, the detection rate of ETEC isolates in diarrhea lambs and calves in Iran was 23.72% and 28.41%, respectively (Shahrani et al. 2014, Ghanbarpour et al. 2017). Regional differences have a greater impact on the distribution of pathogenic types than species differences. Based on the importance of causing host diarrhea (Shahrani et al. 2014), this study's higher distribution of *fimC* and *ompA* is reasonable. The isolates that harbored specific genes for STEC account for 66.85 percent, of which 115 (64.61%, 115/178) isolates were *stx1* gene positive and 26 (14.61%, 26/178) were *stx2* gene positive. STEC strains are associated with many human diseases, from asymptomatic carriage to bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS) (Kumar et al. 2013, Shahrani et al. 2014), while the main source of human STEC is ruminants (Nyholm et al. 2015). According to the report by Bandyopadhyay et al. (2011), the carriers of *the stx2* gene in the STEC strain are closely related to the above human diseases. Although the distribution rate of *stx2* in our study is only 14.61%, it is enough to attract our attention. In addition, most of the collected isolates were classified as hybrid

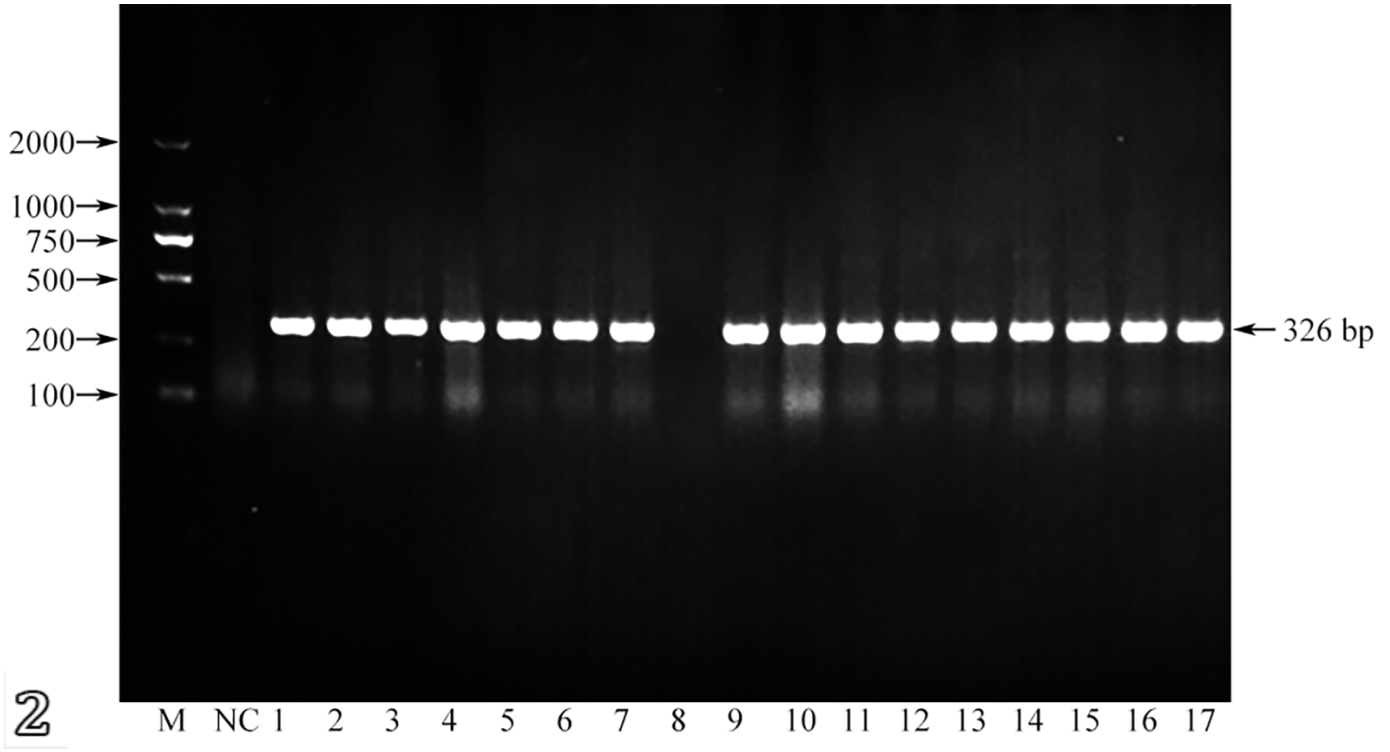

Fig.2. PCR amplification results of *Escherichia coli* of antibiotic resistance genes *tetA*. NC = negative control, 1 to 17 = *E. coli*.

STEC/ETEC isolates (53.37%, 95/178) (Brilhante et al. 2018). STEC/ETEC hybrid isolates have been isolated from human clinical samples and food and animal sources. By acquiring additional virulence genes, *E. coli* isolates already considered pathogenic may cause more severe illness (Leonard et al. 2016), representing an emerging threat.

In the study of Shahrani et al. (2014), STEC and ETEC had the highest overall resistance profiles and the highest frequency of antibiotic resistance genes. However, in our study, there was no significant difference in antibiotic resistance rate and antibiotic gene carrier rate between ETEC, STEC and un-classified isolates (P>0.05). Therefore, these un-classified isolates also have hidden dangers in storing and transmitting antibiotic resistance genes. They can secretly act as receptors and donors for antibiotic-resistance genes without symptoms and transfer their antibiotic-resistance genes to other pathogens (Poirel et al. 2018). In total, the *E. coli* isolates of our investigation had the highest level of resistance to lincomycin (96.63%), followed by tetracycline (88.76%) and co-trimoxazole (80.34%). Although China has banned the use of antibiotics as feed additives in livestock and poultry farming since 2020, the antibiotic resistance results of this study indicate that some antibiotics commonly used as feed additives still have high resistance. In addition, bacterial isolates exhibited the lowest resistance to ceftazidime (25.28%) and netilmicin (16.29%). Although the use of antimicrobial agents to treat diseases can slowly produce antibiotic-resistant isolates compared with growth promoters, the long-term use of a single antibacterial agent will undoubtedly make it ineffective.

Tetracycline is an antibiotic widely and highly tolerated by *E. coli* reported in different countries, such as the calves in Iran (98.09%) (Shahrani et al. 2014), raw meat and shellfish in Vietnam (77.8%) (Van et al. 2008), chicken in China (90%) (Wang et al. 2020). The antimicrobial pressure selected the direction of bacterial evolution to some extent and could not be changed in a short time. Therefore, we should slow this process as much as possible before the new effective antibiotic replacement method. The present study showed antibiotic resistance genes are basically consistent with the results of antibiotic resistance ratio, such as *tetA*, *tetB*, *qnr* and *sul1* were the most commonly detected. The antibiotic sensitivity test shows that treating diarrhea in sheep is more effective (data not shown). There is the possibility that antibiotic-resistance genes can be transferred from commensal and non-pathogenic bacteria to foodborne and other zoonotic pathogens (Muhie 2019). Therefore, it is necessary to guide clinical use in still correct beliefs (Aljeldah 2022) and encourage preventive strategies and targeted treatment after antibiotic sensitivity tests to avoid the further spreading of such plasmids.

CONCLUSIONS

We identified the genes encoding virulence, antibiotic resistance, and more than one antibiotic resistance from *Escherichia coli* isolates isolated from diarrheic lambs in Anhui province. As a result of our data, the incidence rate varies in different age groups, but there is no significant difference between sheep and goats.

Enterotoxigenic *E. coli* (ETEC) and/or Shiga toxin-producing *E. coli* (STEC) pathological isolates accounted for 74.72% and generally have a high carrying rate of *ompA* and *fimC* genes. ETEC and/or STEC may play a significant role in causing diarrhea in lambs in the region.

The distribution rate of *tetA*, *tetB*, *qnr,* and *sul1* is the highest, basically consistent with the antibiotic resistance rate of tetracycline, enrofloxacin, and co-trimoxazole, respectively, and the rate of multidrug-resistant (MDR) reached 93.82%. Hence, the need for clinicians to use antibiotics rationally is even more urgent.

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