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Phenotypic and molecular characterizations of multidrug-resistant diarrheagenic *E. coli* of calf origin

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Abstract

Escherichia coli has become one of the most important causes of calf diarrhea. The aim of this study is to determine the patterns of antimicrobial resistance of *E. coli* isolates from six cattle farms and to identify prominent resistance genes and virulence genes among the strains isolated from the diarrhea of calves. Antimicrobial susceptibility tests were performed using the disk diffusion method, and PCR was used to detect resistance and virulence genes. The prevalence of multidrug resistant (MDR) *E. coli* was 77.8% in dairy cattle and 63.6% in beef cattle. There were high resistance rates to penicillin (100%, 100%) and ampicillin (96.3%, 86.4%) in *E. coli* from dairy cattle and beef cattle. Interestingly, resistance rate to antimicrobials and distribution of resistance genes in *E. coli* isolated from dairy cattle were higher than those in beef cattle. Further analysis showed that the most prevalent resistance genes were bla_{TEM} and *aadA1* in dairy cattle and beef cattle, respectively. Moreover, seven diarrheagenic virulence genes (*irp2*, *fyuA*, *Stx1*, *eaeA*, *F41*, *K99* and *STa*) were present in the isolates from dairy cattle, with a prevalence ranging from 3.7% to 22.22%. Six diarrheagenic virulence genes (*irp2*, *fyuA*, *Stx1*, *eaeA*, *hylA* and *F41*) were identified in the isolates from beef cattle, with a prevalence ranging from 2.27% to 63.64%. Our results provide important evidence for better exploring their interaction mechanism. Further studies are also needed to understand the origin and transmission route of *E. coli* in cattle to reduce its prevalence.

Keywords: Dairy calves, Beef calves, *E. coli*, Multidrug resistant, Virulence gene

Introduction

Diarrheagenic *E. coli* (DEC) is a significant cause of gastroenteritis and a major health problem in animals and humans. *E. coli* infection in calves usually causes a variety of clinical signs, including diarrhea, respiratory infections, and sepsis, and then death due to dehydration and exhaustion because of the difficulties in treatment. Previous studies have shown that diarrhea is the most common problem in young calves, causing more than 52% of deaths in unweaned calves (Diarra et al. 2009). In cattle farms, antimicrobials are the most important therapy for bacterial infection. In dairy cattle farms worldwide, periodic treatment of mastitis after bacterial infection is very common, which not only easily leads to bacterial resistance but also raises concerns about the emergence of multidrug resistant (MDR) bacteria (Yang et al. 2021). The use of antimicrobials to treat infections in beef cattle can increase prevalence of antimicrobial resistance (AMR) in enteric pathogens (Cazer et al. 2017). In addition, antimicrobials are frequently used as growth promoters and preventive agents, which further increases the risk of *E. coli* resistance (Sivaraman et al.

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2020). AMR in bacteria of animal origin is considered a major challenge to veterinary medicine and public health (Anes et al. 2020), which not only seriously affects the healthy development of cattle breeding industry but also poses a serious threat to food safety. *E. coli* has also been used as a sentinel organism for monitoring AMR (de Moyaert et al. 2014). Hence, monitoring AMR in cattle is important to human and animal health.

Some pathogenic *E. coli* strains use different virulence factors to colonize the hosts' small intestine, avoiding immune response and stimulating the deleterious inflammatory response to produce diarrhea (Croxen and Brett Finlay 2010). Virulence genes that play significant roles in *E. coli* pathogenicity are associated with diarrhea in animals and humans have been described (Fröhlicher et al. 2008; Huehn et al. 2010). Among the many virulence genes identified in *E. coli* isolates from cattle, Shiga toxins (*Stx1* and *Stx2*), Yersinia high pathogenicity island (*irp2* and *fyuA*) and intimin (*eaeA*) were the most significant genes with great public health concerns (Momtaz et al. 2012; Olsson et al. 2003; Momtaz et al. 2013a, b). Cattle are a major reservoir of *E. coli*, particularly Shiga toxin-producing *E. coli* (STEC) O157:H7. In addition, *E. coli* has many serotypes, among which *E. coli* O157 can cause hemorrhagic colitis and hemolytic uremic syndrome (Iweriebor et al. 2015). Heat-labile enterotoxins (*LT*) and heat-stable enterotoxins (*STa* or *STb*) are the two most important virulence factors responsible for severe diarrhea in cattle (Nguyen et al. 2011; Kumar et al. 2013). The most important adhesins involved in *E. coli* host colonization are fimbriae. Well-characterized fimbriae of *E. coli* isolated from animals include *F4* (*K88*), *F5* (*K99*), *F6* (*987P*), *F41* and *F18*, are associated with *E. coli* pathotypes (Maciel et al. 2019). Previous studies have also shown that the ability of *E. coli* to acquire many different virulence factors may lead to the emergence of invasive strains, which pose a threat to human and animal health (Mellmann et al. 2011). Therefore, the aim of this study is to characterize AMR and identify different resistance genes and virulence genes in *E. coli* strains isolated from dairy cattle and beef cattle to provide a reference for clinical practice.

Results

Prevalence of AMR in *E. coli* isolated from dairy and beef cattle

A total of 71 *E. coli* isolates were obtained, including 27 isolates from dairy cattle and 44 isolates from beef cattle diarrheal fecal samples. Subsequently, susceptibility to 15 different antimicrobials was determined for these 71 *E. coli* isolates. All 27 *E. coli* isolates from dairy cattle were resistant to penicillin, followed by ampicillin (96.3%), amoxicillin and sulfamethoxydiazine (81.5%), tetracycline and compound sulfamethoxazole (77.8%), with the lowest resistance rate being observed for florfenicol (33.3%) (Fig. 1). Meanwhile, all isolates were sensitive to polymyxin B (100%). Consistent with the results of dairy cattle, the most sensitive antimicrobial was also polymyxin B in the 44 isolates from beef cattle (Fig. 2). The highest resistance rate was also observed for penicillin (100%), which may be related to the widespread use of penicillin for the treatment of *E. coli* disease. Further analysis showed that the resistance rate of *E. coli* to antimicrobials (except for florfenicol and polymyxin B) in dairy cattle was higher than that in beef cattle.

Prevalence of multidrug resistant (MDR) *E. coli*

Multidrug resistance was defined as resistance by an isolate to at least three antimicrobials of the panel belonging to different classes. Resistance of *E. coli* to seven different types of antimicrobials were analyzed. The results showed that multidrug resistance rates were 77.8% (21/27) in dairy cattle and 63.6% (28/44) in beef cattle. Most isolates from dairy cattle and beef cattle were resistant to five or six different types of antimicrobials. The prevalence of resistance to five different types of antimicrobials was 37% (10/27) in dairy cattle and 18.2% (8/44) in beef cattle. Compared with the isolates from dairy cattle, isolates from beef cattle had a higher prevalence of resistance to six different types of antimicrobials [dairy cattle 29.6% (8/27) *vs*. beef cattle 31.8% (14/44)] (Table 1). One isolate from beef cattle was resistant to all antimicrobials.

Prevalence of resistance genes in *E. coli*

Prevalence of 12 different resistance genes was analyzed in *E. coli* isolates from dairy cattle and beef cattle origins. The results showed that seven different resistance genes were detected in over 50% isolates from dairy cattle (Table 2). Resistance genes that had the highest positive rate were bla_{TEM} (100%), followed by *floR*, *tet* (A), , *aac* (3′)-*IIa* and *sul2*. Resistance gene with the lowest positive rate was *aadB* (0%). However, detection rate of seven drug resistance genes in 44 isolates from beef cattle was over 56%, with 100% positive rate of *aadA1*, followed by bla_{TEM}, *tet* (A), and *tet* (B) (Table 2). Overall, the positive rates for bla_{TEM}, *aadA1*, *tet* (A), *tet* (B), *floR* and *sul2* were relatively high in the *E. coli* isolates of both dairy and beef cattle. Consistent with the AMR results, detection rate of resistance genes in dairy cattle was higher than that in beef cattle.

Correlation between the resistance phenotype and resistance genes

Consistency analysis of resistance phenotypes and resistance genes to 11 antibiotics showed that β-lactam (penicillin) resistance phenotype had the highest consistency with β-lactam resistance genes (beef cattle K = 1),

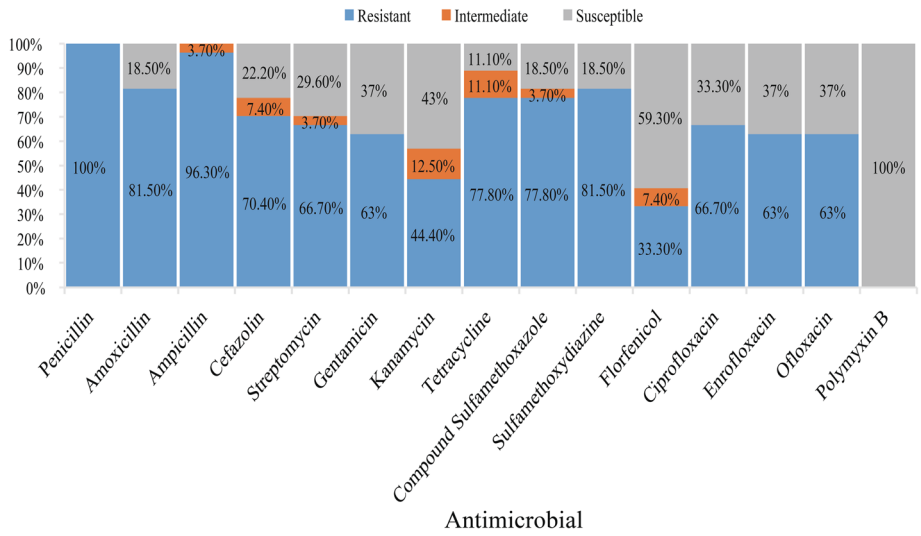

Fig. 1 Antimicrobial susceptibility profile to fifteen antimicrobials of *E. coli* isolates ($n = 27$) from dairy cattle

followed by compound sulfamethoxazole (beef cattle K = 0.59), gentamicin (beef cattle K = 0.56) and florfenico (beef cattle K = 0.41). In dairy and beef cattle, tetracycline resistance phenotype had the lowest consistency (K = – 0.55, K = – 0.77) with tetracycline resistance gene *tet* (C). Some isolates presenting drug resistance carried resistance genes, whereas some isolates carried resistance genes without manifesting a resistance phenotype (Table 3).

Prevalence of virulence genes in *E. coli*

A total of 14 virulence genes were present in *E. coli* isolates from dairy cattle and beef cattle. Seven diarrheagenesis-associated virulence genes (*irp2*, *fyuA*, *Stx1*, *eaeA*, *F41*, *K99* and *STa*) were present in isolates from dairy cattle, with a prevalence ranging from 3.7% to 22.22%. In the isolates from beef cattle, six diarrheagenesis-associated virulence genes (*irp2*, *fyuA*, *Stx1*, *eaeA*, *hylA* and *F41*) were identified, with a prevalence ranging from 2.27% to 63.64%. In addition, 5 (18.52%) isolates from dairy cattle and 19 (43.18%) isolates from beef cattle carried both *irp2* and *fyuA*. One (3.7%) isolate from dairy cattle carried *eaeA*/*Stx1*/*F41* and *F41*/*K99*/*STa* combination, but such a combination was not detected in isolates from beef cattle. In contrast, 8 (18.18%) isolates from beef cattle carried *irp2*/*fyuA*/*Stx1* combination, which were not detected in isolates from dairy cattle. *hylA*/*eaeA*/*Stx1*, *irp2*/*fyuA*/*F41* and *irp2*/*F41* combinations were detected in 1 (2.27%), 2 (4.54%) and 5 (11.36%) isolates from beef cattle, respectively. These combinations were not observed in isolates from dairy cattle (Table 4).

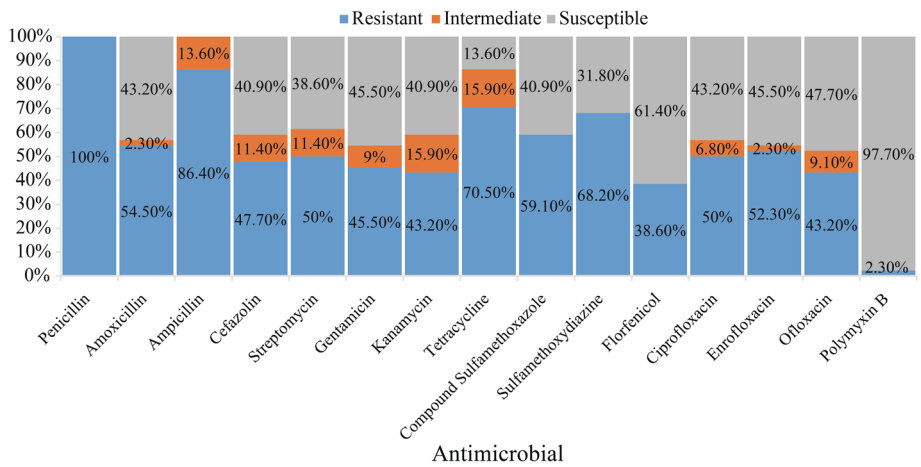

Fig. 2 Antimicrobial susceptibility profile to fifteen antimicrobials of *E. coli* isolates ($n = 44$) from beef cattle

Table 1 Various antimicrobial resistance patterns in 71 *E. coli* isolates from dairy cattle ($n = 27$) and beef cattle ($n = 44$)

Phenotypic resistance	Drug resistance spectrum	Dairy cattle (27)		Beef cattle (44)	
		Isolates	Rate	Isolates	Rate
1	PEN	0	0%	3	6.81%
	PEN-AMP	2	7.41%	7	15.91%
	PEN-AMC-AMP PEN-AMC-AMP-CFZ	1	3.7%	1	2.27%
2	PEN-SULF	1	3.7%		
	AMP-SULF			1	2.27%
	PEN-TET			2	4.55%
	PEN-AMP-COM	1	3.7%		
	PEN-AMP-TET			1	2.27%
	PEN-AMC-AMP-COM-SULF	1	3.7%		
3	PEN-AMP-TET-SULF			2	4.55%
	PEN-AMC-AMP-TET-FFC	1	3.7%		
	PEN-AMP-TET-COM-SULF			1	2.27%
	PEN-AMC-AMP-GEN-TET-COM-SULF	1	3.7%		
4	PEN-AMC-AMP-KAN-TET-COM-SULF			1	2.27%
	PEN-AMC-AMP-CFZ-STR-TET-COM-SULF PEN-AMC-AMP-CFZ-TET-COM-SULF-CIP-ENR-OFX	1	3.7%	1	2.27%
5	PEN-AMP-STR-TET-COM-SULF-FFC			1	2.27%
	PEN-AMP-KAN-GEN-TET-COM-SULF-CIP-OFZ-ENR	1	3.7%		
	PEN-AMC-AMP-CFZ-STR-TET-COM-SULF-CIP-OFX- ENR	2	7.41%		
	PEN-AMC-AMP-CFZ-STR-KAN-TET-COM-SULF-CIP-ENR-OFX	3	11.11%		
	PEN-AMC-AMP-CFZ-STR-GEN-TET-COM-SULF-CIP-ENR-OFX	2	7.41%		
	PEN-AMC-AMP-CFZ-STR-GEN-KAN-TET-COM- SULF-CIP-ENR			1	2.27%
	PEN-AMC-AMP-CFZ-KAN-GEN-TET-COM-SULF-CIP-ENR-OFX			1	2.27%
	PEN-AMC-AMP-CFZ-STR-KAN-GEN-TET-COM-SULF-CIP-ENR-OFX	2	7.41%	4	9.09%
	PEN-AMC-AMP-CFZ-STR-GEN-KAN-COM-SULF-CIP-ENR-OFX-FFC			1	2.27%
6	PEN-AMP-STR-TET--COM-SULF-ENR-FFC			1	2.27%
	PEN-AMC-AMP-CFZ-STR-TET-COM-SULF-CIP-FFC	1	3.7%		
	PEN-AMC-AMP-CFZ-STR-TET-COM-SULF-CIP- ENR-FFC			1	2.27%
	PEN-AMC-AMP-STR-GEN-TET-COM-SULF-CIP- ENR-OFX-FFC			1	2.27%
	PEN-AMC-AMP-CFZ-STR-GEN-TET-COM-SULF-CIP-ENR-OFX-FFC	1	3.7%		
	PEN-AMC-AMP-CFZ-STR-GEN-KAN-TET-COM- SULF-CIP-ENR-OFX-FFC	4	14.81%	11	25%
	PEN-AMC-AMP-CFZ-STR-TET-COM-SULF-CIP-OFZ-ENR-FFC	2	7.41%		
7	PEN-AMC-AMP-CFZ-STR-GEN-KAN-TET-COM-SULF-CIP-ENR-OFX -FFC-PB	0	0%	1	2.27%

Note: β-lactams: penicillin (PEN), amoxicillin (AMC), ampicillin (AMP), and cefazolin (CFZ); aminoglycosides: streptomycin (STR), gentamicin (GEN), and kanamycin (KAN); tetracyclines: tetracycline (TET); sulfonamides: compound sulfamethoxydiazine (SULF); fluoroquinolones: ciprofloxacin (CIP), enrofloxacin (ENR), and ofloxacin (OFX); chloramphenicol: florfenico (FFC); and polypeptides: polymyxin B (PB)

Coexistence of virulence and AMR genes in *E. coli*

Further study showed that 49 *E. coli* isolates carried at least one virulence gene, including 38 isolates from beef cattle and 11 isolates from dairy cattle. Subsequently, the coexistence of virulence genes and AMR genes in these 49 *E. coli* isolates were analyzed. The results showed that there were at least 4 AMR genes in the isolates containing virulence genes and up to 10 AMR genes (Table 5) in other isolates. Interestingly, all 49 *E. coli* isolates contained bla_{TEM} and *tet* (A) genes. In addition, most of 38 isolates from beef cattle contained bla_{TEM}, *tet* (A), *tet* (B) and *floR*

Table 2 *E. coli* resistance gene detection rate in dairy cattle and beef cattle

Classification	Gene name	Dairy cattle carry number	Positive detection rate	Beef cattle carry number	Positive detection rate
β-lactams	bla_{TEM}	27	100% (27/27)	43	97.7% (43/44)
	bla_{SHV}	5	18.5% (5/27)	4	9.1% (4/44)
	bla_{OXA}	4	14.8% (4/27)	3	6.8% (3/44)
Aminoglycosides	*aadA1*	19	70.4% (19/27)	44	100% (44/44)
	aac (3')-IIa	26	96.3% (26/27)	25	56.8% (25/44)
	aadB	0	0% (0/27)	4	9.1% (4/44)
Chloramphenicols	*floR*	26	96.3% (26/27)	26	59.1% (26/44)
Tetracyclines	*tet* (A)	26	96.3% (26/27)	43	97.7% (43/44)
	tet (B)	19	70.4% (19/27)	42	95.5% (42/44)
	tet (C)	2	7.4% (2/27)	0	0% (0/44)
Sulfonamides	*sul1*	11	40.7% (11/27)	21	47.7%(21/44)
	sul2	26	96.3% (26/27)	34	77.3%(34/44)

Table 3 Analysis of correlation between antibiotic resistance phenotype and genotype

Antibiotic (resistance gene)	Dairy cattle *E. coli* isolates ($n = 27$)				Beef cattle *E. coli* isolates ($n = 44$)			
	Genotype	Phenotype		Kappa	Genotype	Phenotype		Kappa
		S	R			S	R	
Penicillin	S	0	0	0	S	1	0	1
(bla_{TEM})	R	0	27		R	0	43	
Amoxicillin	S	0	0	0	S	1	0	0.06
(bla_{TEM})	R	5	22		R	19	24	
Ampicillin	S	1	21	0.02	S	6	34	0.03
(bla_{SHV})	R	0	5		R	0	4	
Cefazolin	S	8	15	0.13	S	23	18	0.15
(bla_{OXA})	R	0	4		R	0	3	
Streptomycin	S	4	4	0.23	S	0	0	0
(*aadA1*)	R	5	14		R	17	27	
Gentamicin	S	1	0	0.12	S	18	1	0.56
(*aac (3')-IIa*)	R	9	17		R	9	16	
Kanamycin	S	15	12	0	S	24	16	0.02
(*aadB*)	R	0	0		R	1	3	
Tetracycline	S	1	0	0.19	S	0	1	−0.04
tet (A)	R	6	20		R	13	30	
Tetracycline	S	4	4	0.29	S	2	0	0.19
tet (B)	R	4	15		R	12	30	
Tetracycline	S	5	20	−0.55	S	0	30	−0.77
tet (C)	R	1	1		R	14	0	
Compound Sulfamethoxazole	S	4	12	0.06	S	18	5	0.59
(*sul1*)	R	2	9		R	4	17	
Sulfamethoxydiazine	S	1	0	0.29	S	6	4	0.26
(*sul2*)	R	4	22		R	10	24	
Florfenico	S	1	0	0.08	S	18	0	0.41
(*floR*)	R	12	14		R	14	12	

Note: Susceptible (S and I) or Resistant (R)

Table 4 Distribution pattern of virulence genes in isolates from dairy cattle and beef cattle

Virulence Gene	Isolates from dairy cattle n (%) Total = 27	Isolates from beef cattle n (%) Total = 44	*P* value
irp2	22.22% (6/27)	63.64% (28/44)	$P < 0.01$
fyuA	22.22% (6/27)	61.36% (27/44)	$P < 0.05$
Stx1	3.70% (1/27)	22.73% (10/44)	$P < 0.05$
eaeA	3.70% (1/27)	2.27% (1/44)	$P < 0.05$
hylA	0% (0/27)	2.27% (1/44)	–
F41	14.81% (4/27)	15.91% (7/44)	$P < 0.05$
K99	3.70% (1/27)	0% (0/44)	–
STa	3.70% (1/27)	0% (0/44)	–
irp2, fyuA	18.52% (5/27)	43.18% (19/44)	$P < 0.05$
eaeA, Stx1, F41	3.70% (1/27)	0% (0/44)	–
F41, K99, STa	3.70% (1/27)	0% (0/44)	–
irp2, fyuA, Stx1	0% (0/27)	18.18% (8/44)	–
hylA, eaeA, Stx1	0% (0/27)	2.27% (1/44)	–
irp2, fyuA, F41	0% (0/27)	4.54% (2/44)	–
irp2, F41	0% (0/27)	11.36% (5/44)	–

genes, while 11 strains of isolates from dairy cattle carried *aac(3′)-IIa* and *sul2* (Table 6).

Frequency of virulence gene occurrence in isolated *E. coli* strains exhibiting antimicrobial resistance

The frequencies of virulence gene occurrence in isolated *E. coli* strains exhibiting antimicrobial resistance were detailed in Table 7. The majority of β-lactam-, aminoglycoside-, tetracycline-, sulfonamide-, fluoroquinolone- and chloramphenicol-resistant beef cattle *E. coli* isolates (more than 50%) were positive for *irp2* and *fyuA* genes with a significant association. Significant associations between the rest of virulence genes and antibiotic resistance were not observed.

Discussion

The emergence and spread of AMR bacteria have become a growing problem and a threat to global public health (WHO 2017). In veterinary practice, penicillin, ampicillin, florfenicol, sulfadiazine, streptomycin, gentamicin and tetracycline are all commonly used antimicrobials for treating *E. coli*-associated infections. Previous studies showed that all 100 *E. coli* isolates from Irish cattle farms were resistant to streptomycin, with a resistance rate of 100%, followed by resistance rates of 99% for tetracycline, 98% for sulfonamides, and 82% for ampicillin (Karczmarczyk et al. 2011). Aasmäe Birgit et al. also reported that the highest proportion of *E. coli* isolates from diseased cattle (clinical submissions) was resistant to streptomycin (Aasmäe et al. 2019). However, in this study, we showed that *E. coli* isolates from dairy cattle and beef cattle with diarrhea were highly resistant to penicillin. Similar to our results, Barigye Robert et al. reported that 23 of 23 (100%) virulent isolates from diarrheic neonatal calves were resistant to penicillin (Barigye et al. 2012). In contrast, we found that *E. coli* isolated from beef and dairy cattle were both susceptible to polymyxin B. These results indicated that *E. coli* with different origins may have undergone different evolutionary processes and thereby acquired different resistance genes. Interestingly, this research showed that the resistance rate of *E. coli* to antimicrobials (except for florfenicol and polymyxin B) from dairy cattle was higher than that of beef cattle. Multidrug resistance analysis showed that most isolates from dairy cattle and beef cattle were resistant to five or six types of antimicrobials. Similarly, multidrug resistance rate in *E. coli* isolated from dairy cattle is higher than that isolated from beef cattle. In dairy cattle, periodic treatment of mastitis after bacterial infection is very common, and antimicrobials are the most important therapies for bovine mastitis, which may be one potential reason for the high resistance rate of *E. coli* from dairy cattle (Call et al. 2008; Mazurek et al. 2013). Meanwhile, these results suggested that more rational use of antimicrobials in cattle farms was needed to prevent the development of AMR in *E. coli*.

E. coli resistance genes bla_{TEM} and bla_{SHV} were the first described extended spectrum β-lactamase (ESBL) genes in the 1980s, and they were predominant until 2000 (Poirel et al. 2018). Currently, the production of ESBL, especially bla_{TEM}, is one of the most important mechanisms of AMR from the clinical and epidemiological point of view (Poirel et al. 2018). Indeed, previous studies reported that bla_{TEM} was detected in 78.94% isolates from dairy cattle farms in the Nile Delta in Egypt, whereas bla_{SHV} and bla_{OXA} were detected only in 0.87% isolates (Braun et al. 2016). In China, previous studies

Table 5 Beef cattle *E. coli* resistance genes and virulence genes

Beef cattle strain	Virulence gene	Resistance gene
HB150601	*Stx1*	bla_{TEM}, *aadA1*, *tet* (A), *tet* (B), *floR*
HB150605	*hylA, eaeA, Stx1*	bla_{TEM}, *sul1*, *aadA1*, *tet* (A), *tet* (B), *floR*
HB150607	*irp2, fyuA*	bla_{TEM}, *aadA1*, *tet* (A), *tet* (B), *floR*
HB150608	*fyuA*	bla_{TEM}, *tet* (A), *aadA1*, *tet* (B), *floR*
HB150609	*irp2, fyuA*	bla_{TEM}, *sul1*, *aadA1*, *tet* (A), *tet* (B)
HB150610	*irp2, fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B)
HB150611	*irp2, fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet*(A), *tet* (B), *floR*
HB150614	*irp2, fyuA*	bla_{TEM}, *sul2*, *aadA1*, *tet* (A), *tet* (B)
HB150615	*fyuA*	bla_{TEM}, *sul2*, *aadA1*, *tet* (A), *tet* (B)
HB150616	*fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
ZD150501	*irp2, fyuA, Stx1*	bla_{TEM}, *sul2*, *aadA1*, *tet* (A), *tet* (B)
ZD150502	*irp2, fyuA, Stx1*	bla_{TEM}, *sul2*, *aadA1*, *tet* (A), *tet* (B)
ZD150503	*irp2, fyuA, Stx1*	bla_{TEM}, *sul2*, *aadA1*, *tet* (A), *tet* (B)
ZD150504	*irp2, fyuA, Stx1*	bla_{TEM}, *sul2*, *aadA1*, *tet* (A), *tet* (B)
ZD150505	*irp2, fyuA, Stx1*	bla_{TEM}, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
ZD150506	*irp2, fyuA, Stx1*	bla_{TEM}, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B)
ZD150507	*irp2, fyuA, Stx1*	bla_{TEM}, *sul2*, *aac(3′)-IIa*, *aadA1*, *tet* (A), *tet* (B)
ZD150508	*irp2, fyuA, Stx1*	bla_{TEM}, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
HN150801	*irp2, fyuA, F41*	bla_{TEM}, bla_{OXA}, *sul1*, *sul2*, *aac(3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
HN150802	*irp2*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *tet* (A), *tet* (B), *floR*
HN150803	*irp2, F41*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
HN150804	*irp2, F41*	bla_{TEM}, bla_{SHV}, *sul2*, *aac (3′)-IIa*, *aadA1*, *aadB*, *tet* (A), *tet* (B), *floR*
HN150805	*irp2, F41*	bla_{TEM}, bla_{SHV}, *sul2*, *aac (3′)-IIa*, *aadA1*, *aadB*, *tet* (A), *tet* (B), *floR*
HN150806	*irp2, fyuA, F41*	bla_{TEM}, bla_{OXA}, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
HN150807	*irp2, F41*	bla_{TEM}, bla_{SHV}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *aadB*, *tet* (A), *tet* (B), *floR*
HN150808	*irp2*	bla_{SHV}, *sul2*, *aac (3′)-IIa*, *aadA1*, *aadB*, *tet* (A), *tet* (B), *floR*
HN150809	*irp2*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
HN150810	*irp2*	bla_{TEM}, bla_{OXA}, *sul2*, *aac (3′)-IIa*, *tet* (A), *tet* (B), *floR*
HN150811	*irp2, F41*	bla_{TEM}, *sul2*, *tet* (A), *tet* (B), *floR*
HN150812	*irp2, fyuA*	bla_{TEM}, *sul2*, *tet* (A), *tet* (B), *floR*
DQ150401	*fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
DQ150402	*fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
DQ150403	*fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
DQ150404	*fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
DQ150505	*fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
DQ150506	*irp2, fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
DQ150507	*irp2, fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
DQ150508	*irp2, fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3′)-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*

have shown that detection rate of $bla_{TEM}$was the highest (58.7%); however, detection rate of bla_{SHV} was only 2.7% in dairy cattle farms (Yang et al. 2018). In the present study, 27 *E. coli* isolates from dairy cattle farms were tested and it was found that detection rate of bla_{TEM} was as high as 100%, and detection rates of bla_{SHV} and bla_{OXA} were also higher than previous studies. Similar to the results in dairy cattle, 44 *E. coli* isolates from beef cattle also showed the highest detection of bla_{TEM} (97.7%). In addition, a previous study reported the resistance rates of bla_{SHV} (0%) and bla_{OXA} (0%) in Japanese beef cattle (Yamamoto et al. 2014), while they were 9.1%

Table 6 Dairy cattle *E. coli* resistance genes and virulence genes

Dairy cattle strain	Virulence gene	Resistance gene
SH160413	*irp2*	bla_{TEM}, *sul1*, *sul2*, *aac (3')-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
SH160417	*irp2*, *fyuA*	bla_{TEM}, bla_{OXA}, bla_{SHV}, *sul1*, *sul2*, *aac (3')-IIa*, *aadA1*, *tet* (A), *tet* (B), *tet* (C), *floR*
SH160418	*irp2*, *fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3')-IIa*, *aadA1*, *tet* (A), *tet* (B), *tet* (C), *floR*
JS160808	*eaeA*, *Stx1*, *F41*	bla_{TEM}, bla_{OXA}, *sul2*, *aac (3')-IIa*, *tet* (A), *floR*
JS160809	*F41*, *K99*, *STa*	bla_{TEM}, *sul2*, *aac (3')-IIa*, *tet* (A), *tet* (B)
JS160810	*F41*	bla_{TEM}, *sul2*, *aac (3')-IIa*, *tet* (A)
JS160811	*F41*	bla_{TEM}, *sul2*, *aac (3')-IIa*, *tet* (A)
KD161102	*irp2*, *fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3')-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
KD161103	*irp2*, *fyuA*	bla_{TEM}, *sul2*, *aac (3')-IIa*, *aadA1*, *tet* (A)
KD161106	*irp2*, *fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3')-IIa*, *aadA1*, *tet* (A), *tet* (B), *floR*
KD161108	*fyuA*	bla_{TEM}, *sul1*, *sul2*, *aac (3')-IIa*, *aadA1*, *tet* (A), *floR*

and 6.8% in this work, respectively. These results indicated that bla_{TEM} was still the most common AMR gene in China and other countries regardless of whether the isolates were from dairy or beef cattle. Furthermore, it is worth noting that detection rates of bla_{SHV} and bla_{OXA} may have a tendency to increase. This research further showed that chloramphenicol and aminoglycoside resistance genes were present in *E. coli* isolates. Detection rates of *floR* in dairy cattle and beef cattle were 96.3% and 59.1%, respectively, which were similar to previous reports (Belaynehe et al. 2018; Wu et al. 2011). In addition, aminoglycoside (resistance) genes *aadA1* and *aadB* were detected in 70.4% and 0% of 27 *E. coli* isolates from dairy cattle and in 100% and 9.1% of 44 *E. coli* isolates from beef cattle. In Ireland, *aadA1* and *aadB* were identified in 19% and 1% of 100 (MDR) *E. coli* isolates recovered from dairy cattle (Karczmarczyk et al. 2011). In Iran, *aadA1* was detected in 26.2% of *E. coli* isolates from dairy cattle (Jamali et al. 2018). In Mexico, *aadA1* was detected in 17% of *E. coli* isolates from beef cattle (Martínez-Vázquez et al. 2018). Detection rate of *aadA1* in this study is much higher than that reported in other countries. Interestingly, the detection rate of *aac(3′)-IIa* that has not been reported in previous studies was 56.8% in beef cattle and 96.3% in dairy cattle, which is worth further investigation detection rate of tetracycline resistance gene *tet* (A) was 97.7%, followed by *tet* (B) (95.5%) and *tet* (C) (0%) in 44 *E. coli* isolates from beef cattle. In isolates from dairy cattle, detection rate of *tet* (A) was 96.33%, followed by *tet* (B) (70.4%) and *tet* (C) (7.4%). *sul1* gene was detected in 40.7% and 47.7% while *sul2* gene was detected in 96.3% and 77.3% of *E. coli* isolates from dairy cattle and beef cattle, respectively. These results are similar to those previously reported data (Karczmarczyk et al. 2011; Belaynehe et al. 2018; Shin et al. 2015; Navajas-Benito et al. 2017). Further analysis found that the overall detection rate of resistance genes in dairy cattle was higher than that of beef cattle, suggesting the widespread resistance of *E. coli* in dairy cattle.

Totally 14 different virulence genes were analyzed in *E. coli* isolates from dairy cattle and beef cattle. However, only 7 diarrheagenesis-associated virulence genes (*irp2*, *fyuA*, *stx1*, *eaeA*, *F41*, *K99* and *STa*) were detected in isolates from dairy cattle, and 6 diarrheagenesis-associated virulence genes (*irp2*, *fyuA*, *Stx1*, *eaeA*, *hylA* and *F41*) were detected in isolates from beef cattle. In beef cattle, 28 out of 44 *E. coli* isolates were positive for *irp2* (63.64%), and 27 were positive for *fyuA* (61.36%). Detection rates of *irp2* and *fyuA* in isolates from dairy cattle were also the highest, both at 22.22%. These results suggested that *irp2* and *fyuA* in *E. coli* isolates from dairy cattle and beef cattle were the main virulence genes, which was similar to the results of previous studies (Ewers et al. 2004; de Verdier et al. 2012). The results also indicated that detection rate of the main virulence genes *irp2* and *fyuA* in isolates from beef cattle was higher than that in isolates from dairy cattle. Furthermore, detection rates of *F41* and *eaeA* genes were not significantly different between beef and dairy cattle, which was consistent with the results of previous peports (Andrade et al. 2012; Hornitzky et al. 2005; Fremaux et al. 2006). However, the percentage of *stx1*-positive isolates was higher in beef cattle (22.73%) than in dairy cattle (3.7%), which was different from the results of a previous study (Bok et al. 2015). Further analysis showed that detection rate of *irp2*/*fyuA* combination in *E. coli* isolates from beef cattle was also higher than that in dairy cattle. Interestingly, *irp2*/*fyuA*/*Stx1*, *hylA*/*eaeA*/*Stx1*, *irp2*/*fyuA*/*F41* and *irp2*/*F41* combinations were not detected in dairy cattle but were detected in beef cattle. These results lay a foundation for further understanding the distribution of virulence genes in *E. coli* isolated from dairy cattle and beef cattle and provide a basis for reducing *E. coli* infections.

Table 7 Frequency of virulence genes among antibiotic-resistant *E. coli* isolates

Antibiotic resistance (beef and dairy cattle)	Beef cattle *E. coli* carry virulence genes n (%)						Dairy cattle *E. coli* carry virulence genes n (%)						
	Irp2	*fyuA*	*Stx1*	*F41*	*hylA*	*eaeA*	*Irp2*	*fyuA*	*F41*	*Stx1*	*eaeA*	*K99*	*STa*
PEN	28/43	27/43	10/43	7/43	1/43	1/43	6/27	6/27	4/27	1/27	1/27	1/27	1/27
(43) and (27)	65.1%	62.8%	23.3%	16.9%	0.02%	0.02%	22.2%	22.2%	14.8%	3.7%	3.7%	3.7%	3.7%
AMC	18/24	13/24	4/24	7/24	0/24	0/24	6/22	5/22	4/22	1/22	1/22	1/22	1/22
(24) and (22)	75%	54.2%	16.7%	25.9%	0%	0%	27.3%	22.7%	18.2%	0.05%	0.05%	0.05%	0.05%
AMP	25/38	22/38	7/38	7/38	1/38	1/38	6/26	5/26	4/26	1/22	1/22	1/22	1/22
(38) and (26)	65.8%	57.9%	18.4%	18.4%	0.03%	0.03%	23.1%	19.2%	15.4%	0.05%	0.05%	0.05%	0.05%
CFZ	17/21	11/21	3/21	7/21	0/21	0/21	4/19	4/19	4/19	1/22	1/22	1/22	1/22
(21) and (19)	80.9%	52.4%	14.3%	33.3%	0%	0%	21.1%	21.1%	21.1%	0.05%	0.05%	0.05%	0.05%
STR	16/22	12/22	4/22	7/20	0/18	0/18	4/18	4/18	4/18	1/22	1/22	1/22	1/22
(22) and (18)	72.7%	54.5%	18.2%	35%	0%	0%	22.2%	22.2%	22.2%	0.05%	0.05%	0.05%	0.05%
GEN	16/20	11/20	4/20	6/20	0/20	0/20	3/17	2/17	0/17	0/17	0/17	0/17	0/17
(20) and (17)	80%	55%	20%	30%	0%	0%	17.6%	11.8%	0%	0%	0%	0%	0%
KAN	14/19	11/19	4/19	6/19	0/19	0/19	2/12	2/12	3/12	0/12	0/12	1/22	1/22
(19) and (12)	73.7%	57.9%	21.1%	31.6%	0%	0%	16.7%	16.7%	25%	0%	0%	0.05%	0.05%
TET	22/31	17/31	7/31	7/31	1/31	1/31	6/21	5/21	4/21	1/21	1/21	1/21	1/21
(31) and (21)	70.9%	54.8%	22.6%	22.6%	0.03%	0.03%	28.6%	23.8%	19%	0.05%	0.05%	0.05%	0.05%
COM	18/26	14/26	4/26	7/26	0/26	0/26	5/21	5/21	4/21	1/21	1/21	1/21	1/21
(26) and (21)	69.2%	53.8%	15.9%	26.9%	0%	0%	23.8%	23.8%	19%	0.05%	0.05%	0.05%	0.05%
SULF	20/30	16/30	5/30	7/30	1/30	1/30	6/22	6/22	4/22	1/22	1/22	1/22	1/22
(30) and (22)	66.7%	53.3%	16.7%	23.3%	0.03%	0.03%	27.3%	27.3%	18.2%	0.05%	0.05%	0.05%	0.05%
CIP	18/22	12/22	4/22	6/22	0/22	0/22	4/18	3/18	4/18	0/18	1/18	1/18	1/18
(22) and (18)	81.8%	54.5%	18.2%	27.3%	0%	0%	22.2%	16.7%	22.2%	0%	0.06%	0.06%	0.06%
ENR	18/23	12/23	4/23	7/23	0/23	0/23	4/17	3/17	3/17	1/17	0/17	1/17	1/17
(23) and (17)	78.3%	52.1%	17.4%	30.4%	0%	0%	23.5%	17.6%	17.6%	0.06%	0%	0.06%	0.06%
OFX	17/19	13/19	4/19	6/19	0/19	0/19	3/17	3/17	3/17	0/17	0/17	0/17	0/17
(19) and (17)	89.5%	68.4%	21.1%	31.6%	0%	0%	17.6%	17.6%	17.6%	0%	0%	0%	0%
FFC	12/17	7/17	2/17	7/17	0/17	0/17	2/9	2/9	1/9	1/9	1/9	0/9	0/9
(17) and (9)	70.6%	41.2%	11.8%	41.2%	0%	0%	22.2%	22.2%	11.1%	11.1%	11.1%	0%	0%
PB	0/1	1/1	0/1	0/1	0/1	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0
(1) and (0)	0%	100%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Note: β-lactams: penicillin (PEN), amoxicillin (AMC), ampicillin (AMP), and cefazolin (CFZ); aminoglycosides: streptomycin (STR), gentamicin (GEN), and kanamycin (KAN); tetracyclines: tetracycline (TET); sulfonamides: compound sulfamethoxydiazine (SULF); fluoroquinolones: ciprofloxacin (CIP), enrofloxacin (ENR), and ofloxacin (OFX); chloramphenicol: florfenico (FFC); polypeptides: polymyxin B (PB)

Conclusions

The results of this study indicated that MDR diarrheagenic *E. coli* were more common in dairy and beef calves, with frequent MDR, ESBL and the presence of tetracycline resistance gene *tet* (A). The prevalence rate in dairy cattle is higher than that in beef cattle, which may be related to the prevalence of resistance genes and highlights the importance of the rational use of antimicrobials and strict enforcement of preventive measures in cattle farms. Furthermore, detection rate of virulence genes in the isolates from dairy cattle was lower than that in beef cattle. Although the link between resistance and virulence genes has been extensively studied and virulence genes *irp2* and *fyuA* have a high detection rate in MDR strains, it is still not conclusive. Our results provide important evidences for better exploring their interaction mechanism. Further studies are also needed to understand the origin and transmission route of *E. coli* in cattle to reduce its prevalence.

Table 8 Primers of antimicrobial resistance genes and virulence genes

Classification	Gene	Primer sequence (5′ → 3′)	Annealing temperature	Fragment length	Reference
β-lactams	bla_{OXA}	F:TTTTCTGTTGTTTGGGTTTC R:TTTCTTGGCTTTTATGCTTG	53 °C	447 bp	This work
	bla_{SHV}	F:TGTATTATCTCCCTGTTAGC R:TTAGCGTTGCCAGTGCTC	55 °C	843 bp	
	bla_{TEM}	F:CAGAAACGCTGGTGAAAG R:TTACCAATGGTTAATCAGTGAG	54 °C	788 bp	
Tetracyclines	*tet* (A)	F:GCTACATCCTGCTTGCCTTC R:CATAGATCGCCGTGAAGAGG	59.5 °C	210 bp	Ng et al. 2001
	tet (B)	F:TTGGTTAGGGGCAAGTTTTG R:GTAATGGGCCAATAACACCG	59.5 °C	659 bp	
	tet (C)	F:CTTGAGAGCCTTCAACCCAG R:ATGGTCGTCATCTACCTGCC	59.5 °C	418 bp	
Sulfonamides	*sul1*	F:TCGGACAGGGCGTCTAAG R:GGGTATCGGAGCGTTTGC	63 °C	925 bp	This work
	sul2	F:CTTGTTTCGTCCGACACAGA R:GAAGCGCAGCCGCAATTCAT	60 °C	435 bp	
Aminoglycosides	*aadA1*	F:GCAGCGCAATGACATTCTTG R:ATCCTCGGCGCGATTTTG	60 °C	282 bp	Sáenz et al. 2004
	aadB	F:GAGGAGTTGGACTATGGATT R:CTTCATCGGCATAGTAAAA	53 °C	208 bp	This work
	aac (3′)-IIa	F:GGCGACTTCACCGTTTCT R: GGACCGATCACCCTACGAG	54 °C	412 bp	
Chloramphenicols	*floR*	F: GAACACGACGCCCGCTAT R: TTCCGCTTGGCCTATGAG	54 °C	601 bp	This work
Yersinia High Pathogenicity Island	*irp2*	F:AAGGATTCGCTGTTACCGGA R:TCGGCCAGGATGATTCGTCG	60 °C	301 bp	This work
	fyuA	F:ACACGGCTTATCCTCTGGC R:GGCATCTTGACGATTAACGAA	58 °C	953 bp	This work
Intimin	*eaeA*	F:ATTACTGAGATTAAGGCTGAT R:ATTTATTTGCAGCCCCCCAT	57 °C	682 bp	This work
Fimbriae	*F41*	F:GAGGGACTTTCATCTTTTAG R:AGTCCATTCCATTTATAGGC	58 °C	431 bp	This work
	K88	F:GCTGCATCTGCTGCATCTGGTATG R:CCACTGAGTGCTGGTAGTTACAGCC	60 °C	792 bp	This work
	K99	F:TATTATCTTAGGTGGTATGG R:GGTATCCTTTAGCAGCAGTATTTC	56 °C	314 bp	This work
	987P	F:TCTGCTCTTAAAGCTACTGG R:AACTCCACCGTTTGTATCAG	55.8 °C	333 bp	This work
	F18	F:GTGAAAAGACTAGTGTTTATTTC R:CTTGTAAGTAACCGCGTAAGC	55 °C	510 bp	This work
Hemolysin	*hylA*	F:GCATCATCAAGCGTACGTTCC R:AATGAGCCAAGCTGGTTAAGCT	60 °C	534 bp	This work
Shiga toxins	*Stx1*	F:TTAGACTTCTCGACTGCAAAG R:TGTTGTACGAAATCCCCTCTG	52 °C	531 bp	This work
	Stx2	F:CCATGACAACGGACAGCAGTT R:CCTGTCAACTGAGCAGCACTTTG	58 °C	779 bp	This work
Heat-stable enterotoxins	*STa*	F:TCCCCTCTTTTAGTCAGTCAACTG R:GCACAGGCAGGATTACAACAAAGT	56 °C	163 bp	This work
	STb	F:GCAATAAGGTTGAGGTGAT R:GCCTGCAGTGAGAAATGGAC	60 °C	368 bp	This work
Heat-labile enterotoxins	*LT*	F:GGCGACAGATTATACCGTGC R:CGGTCTCTATATTCCCTGTT	54 °C	450 bp	This work

Materials and methods

Sample collection and identification of *E. coli*

From April 2016 to November 2018, we collected fecal samples from sick dairy calves with diarrhea in Suihua, Jiusan and Kedong and fecal samples from sick beef calves in Harbin, Zhaodong and Daqing in Heilongjiang Province, China. The aseptically collected intestinal and fecal samples were inoculated onto MacConkey agar and eosin methylene blue agar (Momtaz et al. 2013a, b). After overnight incubation at 37 °C, only pure pink colonies were selected and transferred to nutrient agar. The isolate was identified by 16S rDNA and stored in 50% glycerol at −80 °C.

Antimicrobial susceptibility test

The antimicrobial susceptibility of *E. coli* isolated from diarrheal dairy cattle and beef cattle was tested using the Kirby-Bauer disk diffusion method according to standards of the Clinical and Laboratory Standards Association (Clinical and Laboratory Standards Institute 2014). Nutrient agar was used to determine the susceptibility of *E. coli* to 15 different antimicrobials using commercial disks: penicillin (PEN, 10 μg), amoxicillin (AMC, 10 μg), ampicillin (AMP, 10 μg), cefazolin (CFZ, 30 μg), streptomycin (STR, 10 μg), gentamicin (GEN, 10 μg), kanamycin (KAN, 30 μg), polymyxin B (PB, 300 units), tetracycline (TET, 30 μg), compound sulfamethoxazole (COM, 23.75/1.25 μg), sulfamethoxydiazine (SULF, 5 μg), florfenico (FFC, 30 μg), ciprofloxacin (CIP, 5 μg), enrofloxacin (ENR, 5 μg), and ofloxacin (OFX, 5 μg). Laboratory-stored *E. coli* ATCC 25922 was used as a control strain.

DNA extraction and amplification of resistance genes and virulence genes

Primers used to amplify resistance genes (bla_{TEM}, bla_{SHV}, bla_{OXA}, *tet* (A), *tet* (B), *tet* (C), *sul1*, *sul2*, *aadA1*, *aadB* and *aac(3′)-IIa*, *floR*) and virulence genes (*irp2*, *fyuA*, *eaeA*, *hylA*, *K88*, *K99*, *F41*, *987P*, *F18*, *Stx1*, *Stx2*, *Sta*, *Stb* and *LT*) were shown in Table 8. Primers were synthesized by the Shanghai Bioengineering Co., Ltd. *E. coli* genomic DNA was extracted according to the manufacturer's instructions of the extraction kit (Beijing Tiangen Biotechnology Co., Ltd.). PCR was carried out in a 25 μL volume containing 12.5 μL of 2 × *Taq* MasterMix (ComWin Biotech Co., Ltd., Beijing, China), 1 μL of forward and reverse primer, 1 μL of DNA template and 9.5 μL of ddH_2O. The parameters for PCR included an initial annealing at 95 °C for 5 min and 30 cycles of 94 °C for 30 s, 53–63 °C for 45 s (the annealing temperature varied according to the primers), and 72 °C for 60 s, followed by a final extension at 72 °C for 5 min. PCR products were analyzed by electrophoresis in a 1% agarose gel.

Statistical analysis

All statistical analyses were performed using GraphPad Prism® 8.00 software (GraphPad Software, Inc., USA). For all experiments, differences were considered to be statistically significant at $P< 0.05$ values.

Abbreviations

E. coli: *Escherichia coli*; MDR: Multidrug resistant; DEC: Diarrheagenic *E. coli*; AMR: Antimicrobial resistance; STEC: Shiga-toxin producing *E. coli*; PEN: Penicillin; AMC: Amoxicillin; AMP: Ampicillin; CFZ: Cefazolin; STR: Streptomycin; GEN: Gentamicin; KAN: Kanamycin; TET: Tetracycline; COM: Compound sulfamethoxazole; SULF: Sulfamethoxydiazine; CIP: Ciprofloxacin; ENR: Enrofloxacin; OFX: Ofloxacin; FFC: Florfenico; PB: Polymyxin B; ESBL: Extended spectrum β-lactamases

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Authors' contributions

S.Y., Y.Z., and Z.Z. contributed to the conception and design of this work; S.Y., C.W., W.H., and N.C. participated in sample collection, laboratory experiments and data analysis; S.Y and Y.L. drafted the manuscript; and S.Y., Z.Z., Y.L., and Z.Z. revised the manuscript. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

All data can be shared upon reasonable request. The data can be obtained by email.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

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