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Research Article

Phylogenetic Grouping and Antimicrobial Resistance Profiles of *Escherichia coli* Isolated from Calves in Xinjiang, China

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Abstract

The widespread multidrug-resistant *Escherichia coli* strains have caused a severe challenge to animal health and the development of breeding industries. The purpose of this study was to investigate the phylogenetic grouping and antimicrobial resistance profiles of *E. coli* isolated from diarrheic calves in Xinjiang province, China. In this study, a total of 379 *E. coli* strains were isolated from 379 rectal swab samples of diarrheic calves. They were further analyzed their phylogenetic groupings by multiplex PCR, and were clustered into four phylogenetic groups, A (36.1%), B1 (17.4%), B2 (15.6%), and D (30.9%). All *E. coli* isolates were tested for their susceptibility to 15 antimicrobial agents by Kirby-Bauer (KB) method. The isolates showed the highest resistance rates against ampicillin (64.9%), followed by streptomycin (59.4%), tetracycline (53.8%), sulfamethoxazole/trimethoprim (50.9%), chloramphenicol (45.6%), kanamycin (44.1%) and enrofloxacin (42.0%). *E. coli* isolates exhibited lower resistance to ceftazidime (15.0%) and polymyxin (12.6%). The resistance genes *bla*$_{TEM}$, *bla*$_{OXA}$, *mcr-1*, *strA-strB*, *aadA*, *tet*(A), *tet*(B), and *tet*(C) were detected in 68.3% (168/246), 27.2% (67/246), 14.6% (7/48), 51.1% (115/225), 24.9% (56/225), 51.5% (105/204), 44.6% (91/204), and 7.8% (16/204) of *E. coli* isolates, respectively. These results demonstrate that prevalent multi-drug resistance and high level of antimicrobial resistance genes exist among *E. coli* from Xinjiang diarrheic calves and pose a potential public health concern.

Keywords: *Escherichia coli, Phylogenetic grouping, Antimicrobial resistance, Resistance genes, Calf*

Çin'in Sincan Bölgesindeki Buzağılardan İzole Edilen *Escherichia coli*'nin Filogenetik Gruplandırması ve Antimikrobiyal Direnç Profili

Öz

Yaygın çoklu ilaç dirençli *Escherichia coli* suşları, hayvan sağlığı ve üretim endüstrilerinin gelişimi için ciddi bir zorluk yaratmaktadır. Bu çalışmanın amacı, Çin'in Xinjiang eyaletindeki ishalli buzağılardan izole edilen E. coli'nin filogenetik gruplandırma ve antimikrobiyal direnç profilini araştırmaktı. Çalışmada, 379 adet ishalli buzağıdan alınan rektal sıvap örneğinden toplam 379 *E. coli* suşu izole edildi. Filogenetik gruplar ayrıca çoklu PCR ile analiz edildi ve A (%36.1), B1 (%17.4), B2 (%15.6) ve D (%30.9) olarak dört gruba kümelendi. Tüm *E. coli* izolatları, Kirby-Bauer (KB) yöntemiyle 15 antimikrobiyal maddeye karşı duyarlılıkları açısından test edildi. İzolatlar ampisiline karşı en yüksek direnç oranını gösterirken (%64.9), bunu streptomisin (%59.4), tetrasiklin (%53.8), sülfametoksazol/trimetoprim (%50.9), kloramfenikol (%45.6), kanamisin (%44.1) ve enrofloksasin (%42.0) izledi. *E. coli* izolatları seftazidime (%15.0) ve polimiksine (%12.6) daha düşük direnç gösterdi. *E. coli* izolatlarında direnç genleri *bla*$_{TEM}$, *bla*$_{OXA}$, *mcr-1*, *strA-strB*, *aadA*, *tet(A)*, *tet(B)* ve *tet(C)* sırasıyla %68.3 (168/246), %27.2 (67/246), %14.6 (7/48), %51.1 (115/225), %24.9 (56/225), %51.5 (105/204), %44.6 (91/204) ve %7.8 (16/204) olarak belirlendi. Bu sonuçlar, Xinjiang bölgesindeki ishalli buzağılardan elde edilen *E. coli* suşlarında yaygın çoklu ilaç direnci ve yüksek düzeyde antimikrobiyal direnç genlerinin bulunduğunu ve potansiyel bir halk sağlığı sorunu teşkil ettiğini gösterdi.

Anahtar sözcükler: *Escherichia coli, Filogenetik gruplama, Antimikrobiyal direnç, Direnç genleri, Buzağı*

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INTRODUCTION

Antimicrobial resistance is a serious threat to both animal breeding and human health [1]. It is estimated that more than 50% of the world's antibacterials are used in husbandry industry [2], and among them, nearly 90% of antibacterial agents have been used for prophylaxis or growth promotion [3]. Due to the long-term misuse of antibiotics in economically important animals, bacterial resistance to drugs has become an increasingly severe issue [4,5] as increased incidence of antibiotic-resistant infections coupled with a declining antibiotic pipeline is creating a global public health threat [1,6].

Escherichia coli is the most common type of abundant bacteria in human and animal intestines. Some serotypes are pathogenic and can cause diarrhea, meningitis, urinary tract infections, sepsis, or pneumonia in humans and animals [7,8]. Due to the extensive use of antibiotics in veterinary clinics, it is easy for *E. coli* to evolve resistance to drugs and become a reservoir for antibiotic resistance and resistance genes [9,10]. In recent years, numerous studies have been reported on the drug resistance of *E. coli* in cattle, including resistance phenotypes and genotypes, and also the impact of antibiotics on the selection of resistance genes [11-14]. Furthermore, drug-resistant *E. coli* isolates may not only threaten veterinary clinical treatment of infections, but also possibly spread to human via the food chain, thus posing a challenge to public health [15,16].

The Xinjiang Uygur Autonomous Region, with 1.66 million km^2, is situated in northwestern China and borders Russia, Kazakhstan, and other Central Asian countries. This region is one of the major pastoral areas in China with well-developed animal husbandry industry and an estimated cattle population of 4.2 million (Xinjiang Statistical Year-book, 2016, C832.45-54). Therefore, the aim of this study was to clarify the phylogenetic grouping, antimicrobial resistance profiles and resistance genes of *E. coli* isolates collected from calves in Xinjiang.

MATERIAL and METHODS

Ethics Statement

This study was carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Scientific Research Department of Xinjiang Academy of Agricultural and Reclamation Sciences (protocol approval number: XJNKKXY-AEP-038). This study did not involve any endangered or protected animal species. Individual oral/written informed consent for the use of samples was obtained from all the animal owners.

Sample Sources and Antimicrobial Use Histories

From May 2016 to May 2017, a total of 379 rectal swab samples were collected from 1 to 6-month-old diarrheic calves in six large-scale dairy farms and one cattle farm located in different districts (Urumqi, Wujiaqu, Changji, Shihezi, Kuitun) in Xinjiang, China, along with geographic and cattle industry representatives.The most commonly used drugs for calves in these cattle farms were: ampicillin, streptomycin, tetracycline and sulfonamides.

Isolation and Identification of E. coli Strains

After adding 2 mL of 0.85% saline to the collection tubes, the rectal swab samples were vortexed for 10 min at room temperature and allowed to stand for 5 min, according to a previously reported protocol with minor modifications [17]. Next, 10 µL supernatant was taken and inoculated on MacConkey agar (Difco Laboratories, Detroit, MI, USA) for overnight culturing at 37ºC. One colony per sample was selected for pure culturing. The suspected *E. coli* colonies were first identified by biochemical tests (Tianhe, Hangzhou, China), and they were further confirmed based on the VITEK 2 Automatic microbial analysis system (VITEK® 2 Compact 30) and 16S rRNA PCR and sequencing *(Table 1)*. The confirmed *E. coli* isolates were selected for further investigation.

Phylogenetic Grouping of E. coli Isolates

The isolated *E. coli* was identified and grouped using the triple PCR method [18]. The groups were determined based on the presence or absence of *chuA* and *yjaA* genes, as well as an unknown DNA fragment (TspE4.C2). Primers *(Table 1)* used in this assay were synthesized by Beijing Genomics Institute (BGI). PCR products were analyzed by 1% agarose gel electrophoresis and recorded by a gel imaging system and the amplicons were sequenced.

Drug Susceptibility Test

The drug susceptibility test was conducted following the Kirby-Bauer (KB) method recommended by the Clinical and Laboratory Standards Institute [19]. The bacteria were collected with a sterile loop, suspended in peptone water, and incubated at 37ºC for 2 h. The turbidity of the suspension was adjusted to 0.5 McFarland's standard (1.5×10^8 CFU/mL). The suspension was then spread onto the surface of a cation-adjusted Mueller-Hinton agar (MHA) (AOBOX, Beijing, China) plate using sterile cotton swabs. The following 15 antimicrobial agents (Oxoid, Basingstoke, England) were included in the assay: ampicillin (AMP) (10 µg), cephalexin (LEX) (30 µg), cefotaxime (CTX) (30 µg), ceftazidime (CAZ) (30 µg), streptomycin (STR) (10 µg), gentamicin (GEN) (10 µg), kanamycin (KAN) (30 µg), amikacin (AMI) (30 µg), tetracycline (TET) (30 µg), doxycycline (DOX) (30 µg), chloramphenicol (CHL) (30 µg), polymyxin B (POL) (300 IU), norfloxacin (NOR) (10 µg), enrofloxacin (EN) (10 µg), and sulfamethoxazole/trimethoprim (SXT) (23.75/1.25 µg). Test results were interpreted based on the criteria recommended by the M100, 28th edition of the CLSI (Wayne, PA, United States) (Clinical Laboratory Standards Institute) [19]. The *E. coli* strain ATCC 25922 was used for quality control.

Table 1. *The oligonucleotide sequence and predicted sizes used in the PCR*

Primer Name	Primer Sequence (5'-3')	Target Gene	Size (bp)	Reference
16S-F	GCGGACGGGTGAGTAATGT	16S rRNA	200	This study
16S-R	TCATCCTCTCAGACCAGCTA			
ChuA-F	GACGAACCAACGGTCAGGAT	chuA	279	[17]
ChuA-R	TGCCGCCAGTACCAAAGACA			
YjaA-F	TGAAGTGTCAGGAGACGCTG	yjaA	211	[17]
YjaA-R	ATGGAGAATGCGTTCCTCAAC			
TspE4C2-F	GAGTAATGTCGGGGCATTCA	TSPE4.C2	152	[17]
TspE4C2-R	CGCGCCAACAAAGTATTACG			
bla(TEM)-F	TTGGGTGCACGACTGGGT	bla_{TEM}	503	[12]
bla(TEM)-R	TAATTGTTGCCGGGAAGC			
bla(PSE)-F	CGCTTCGGGTTAACAAGTAC	bla_{PSE}	419	[12]
bla(PSE)-R	CTGGTTCATTTCAGATAGCG			
bla(OXA)-F	AGCAGCGCCAGTGCATCA	bla_{OXA}	708	[12]
bla(OXA)-R	ATTCGACCCCAAGTTTCC			
mcr-1-F	CGGTCAGTCCGTTTGTTC	mcr-1	309	[19]
mcr-1-R	CTTGGTCGGTCTGTAGGG			
tet(A)-F	GCTACATCCTGCTTGCCTTC	*tet*(A)	210	[20]
tet(A)-R	CATAGATCGCCGTGAAGAGG			
tet(B)-F	TTGGTTAGGGGCAAGTTTTG	*tet*(B)	659	[20]
tet(B)-R	GTAATGGGCCAATAACACCG			
tet(C)-F	CTTGAGAGCCTTCAACCCAG	*tet*(C)	418	[20]
tet(C)-R	ATGGTCGTCATCTACCTGCC			
strA-strB-F	TATCTGCGATTGGACCCTCTG	strA-strB	538	[21]
strA-strB-R	CATTGCTCATCATTTGATCGGCT			
AadA-F	GCAGCGCAATGACATTCTTG	*aadA1* or *aadA2*	282	[22]
AadA-R	ATCCTCGGCGCGATTTTG			

Detection of Resistance Genes by PCR Assay

Bacterial genomic DNA was extracted according to the genome DNA extraction kit manufacturer's instructions (OMEGA Bio-tek Inc., Norcross, GA, USA), and was used as template for PCR analysis. For ampicillin-resistant *E. coli*, triple PCR was used to detect three β-lactam-resistant genes: bla_{TEM}, bla_{OXA}, and bla_{PSE} [12]. For polymyxin-resistant *E. coli, mcr-1* was detected by PCR [20]. For tetracycline-resistant *E. coli*, multiplex PCR was used to detect three tetracycline-resistant genes, *tet*(A), *tet*(B), and *tet*(C) [21]. For streptomycin -resistant *E. coli*, duplex PCR was used to test two aminoglycoside-resistant genes: *strA-strB* and *aadA* [22,23]. The target gene amplified by PCR was ligated with vector pMD19-T (TaKaRa, Dalian, China) and transformed into *E. coli* DH5α competent cells, and the recombinant plasmid was sequenced (TaKaRa, Dalian, China).

Statistical Analysis

Epi Info version 7.2 (CDC) was used to perform statistical analysis. Comparison of drug resistant differences in the four phylogenetic groups (A, B1, B2, D) of *E. coli* was conducted by the $\chi 2$ –test. $P<0.05$ was considered statistically significant.

RESULTS

E. coli Isolation and Phylogenetic Characterization

A total of 379 *E. coli* strains (100% isolation rate) were isolated from calve rectal swab samples. Isolated strains were further identified and grouped by checking their PCR products with gel electrophoresis. There were three specific bands observed, 279 bp, 211 bp and (or) 152 bp, corresponding to *chuA*, *yiaA* and the DNA fragment TspE4.C2. These strains were distributed differently among the four phylogenetic groups *(Table 2)* by comparing PCR bands with the positive strains *(Fig. 1)*. group A, B1, B2 and D accounted for 36.1% (137/379), 17.4% (66/379), 15.6% (59/379) and 30.9% (117/379).

Table 2. *Phylogenetic clustering of E. coli isolated form calves*

Phylogenetic Group(s)	No. of Isolates (%) by Origin
A	137 (36.1)
B1	66 (17.4)
B2	59 (15.6)
D	117 (30.9)

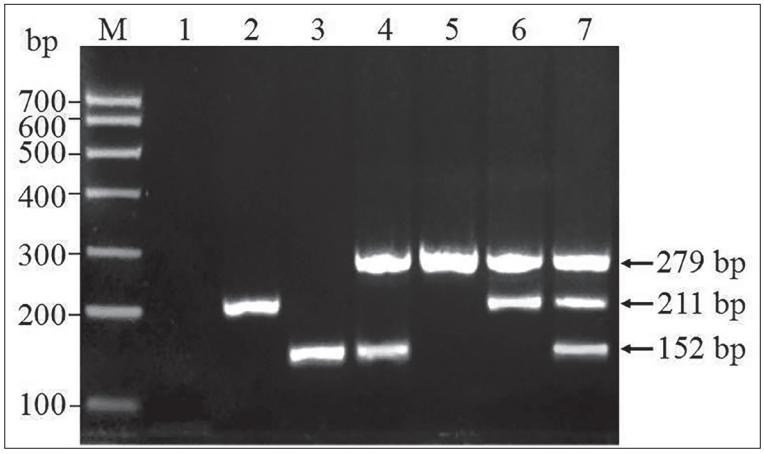
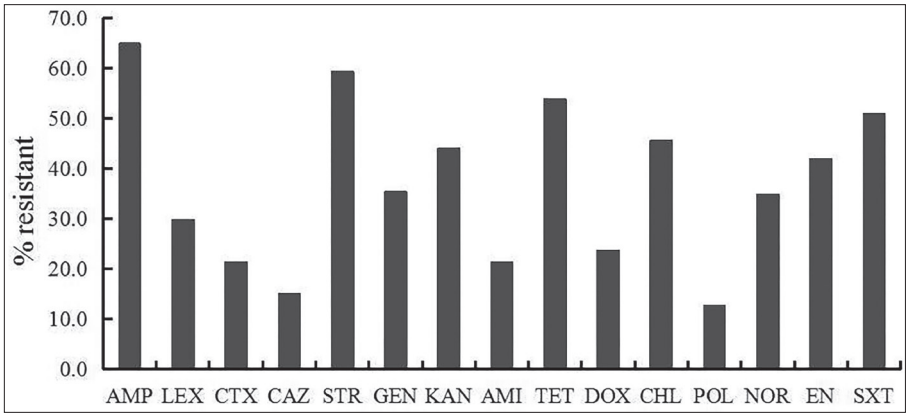


Fig 1. Phylogenetic grouping for *E. coli* isolates based on Triplex PCR method. Each combination of *chuA* and *yjaA* gene and DNA fragment TSPE4.C2 amplification allowed phylogenetic grouping of a strain. Lane M, contained markers. Lane 1 and 2, group A [*(chuA-, yjaA-,* TspE4.C2-) and *(yjaA+, chuA-,* TspE4.C2-)]; lane 3, group B1 [*chuA-, yjaA,* TspE4.C2+]; lanes 6 and 7, group B2 [*(chuA+, yjaA+,* TspE4. C2-) and (*chuA+, yjaA+,* TspE4.C2+)]; lane 4 and 5, group D [(*chuA+,yjaA-,*TspE4.C2-) and (*chuA+, yjaA-,* TspE4.C2+)]

Fig 2. The antimicrobial resistance of *E. coli* isolates. AMP: Ampicillin, LEX: Cephalexin, CTX: Cefotaxime, CAZ: Ceftazidime, STR: Streptomycin, GEN: Gentamicin, KAN: Kanamycin, AMI: Amikacin, TET: Tetracycline, DOX: Doxycycline, CHL: Chloramphenicol, POL: Polymyxin B, NOR: Norfloxacin, EN: Enrofloxacin, SXT: Sulfamethoxazole/Trimethoprim



Antimicrobial Susceptibility

Of the 379 *E. coli* strains, 64.9% (246/379) were resistant to ampicillin, which was the highest rate from the 15 antibiotics tested, followed by streptomycin (59.4%), tetracycline (53.8%), sulfamethoxazole/trimethoprim (50.9%), Chloramphenicol (45.6%), Kanamycin (44.1%), Enrofloxacin (42.0%), Gentamicin (35.4%) and Norfloxacin (34.85%). Additionally, 29.8%, 23.8%, 21.4%, 21.4%, 15.0%, and 12.6% *E. coli* isolates exhibited resistance to cephalexin, doxycycline, amikacin, cefotaxime, ceftazidime, and polymyxin B, respectively *(Fig. 2, Table 3)*.

The *E. coli* from different phylogenetic groups showed different resistance to the 15 different kinds of antibiotics. Groups A and D had relatively higher resistance rates, and group B2 showed the most susceptibility to antibiotics *(Table 3)*.

Resistance Gene Profiles from Different Resistance Phenotype of E. coli Strains

Most of the 379 *E. coli* strains had different resistance genotypes *(Fig. 3, Table 4)*. Among ampicillin-resistant strains, 91.5% (225/246) carried either bla_{TEM}, or bla_{OXA} gene, or both and. no bla_{PSE} gene was detected. Among tetracycline-resistant strains, 94.1% (192/204) had one or two of the genes *tet*(A), *tet*(B) and *tet*(C). Among streptomycin-resistant strains, 70.2% (158/225) carried the *strA-strB* or *aadA* gene, or both. Among polymyxin-resistant strains, 14.6% (7/48) had the *mcr-1* gene.

DISCUSSION

In recent years, with the development of a large-scale cattle industry in China, the incidence of cattle diseases has continued to rise, it turns to be an essential issue to understand the antibiotic resistance situation among cattles in order to provide better anti-bacterial therapy and rational use of antibiotics. Antibiotics are extensively used in animal husbandry to prevent common bacterial diseases or promote livestock growth. The antimicrobial resistance has emerged as a serious threat to both the cattle industry and public health [24]. In our study, 64.9% of *E. coli* isolates were resistant to ampicillin, and more than 50% of isolates showed resistance against streptomycin, tetracycline and sulfamethoxazole/trimethoprim, Coincidentally, these four antimicrobial agents were widely used in the local cattle farm, suggesting that antimicrobial agents used in cattle have driven the emergence and abundance of resistance.

In the United States, among *E. coli* strains taken from cattle, resistance rates have been shown to be 23.7% for tetracycline, 10.5% for sulfamethoxazole/trimethoprim, and 9.5% for streptomycin [25]. In Germany, drug resistance rates of *E. coli* from calves were 65.9% for tetracycline, 59.0% for amoxicillin, 56.5% for sulfamethoxazole/trimethoprim, and

Table 3. *Antimicrobial sensitivity of different phylogenetic groups of E. coli isolates*

Classes	Antibacterial Agents	Number of Resistant Isolates (Percentage of Resistance %)				
		A (n=137)	B1 (n=66)	B2 (n=59)	D (n=117)	Total (n=379)
Beta-lactams	*Ampicillin*	84 (61.3 %)	39 (59.1%)	32 (54.2%)	91 (77.8%)	246 (64.9)
	Cephalexin	37 (27.0%)	18 (27.7%)	16 (27.1%)	42 (35.9%)	113 (29.8)
	Cefotaxime	29 (21.2%)	13 (19.7%)	12 (20.3%)	27 (23.0%)	81 (21.4)
	Ceftazidime	20 (14.6%)	8 (12.1%)	7 (11.8%)	22 (18.8%)	57 (15.0)
Aminoglycosides	*Streptomycin*	79 (57.6%)	36 (54.5%)	29 (49.2%)	81 (69.2%)	225 (59.4)
	Gentamicin	44 (32.1%)	20 (30.3%)	18 (30.5%)	52 (44.4%)	134 (35.4)
	Kanamycin	55 (40.2%)	29 (43.9%)	18 (30.5%)	65 (55.6%)	167 (44.1)
	Amikacin	30 (21.9%)	12 (18.2%)	11 (18.6%)	28 (23.9%)	81 (21.4)
Tetracyclines	*Tetracycline*	67 (48.9%)	35 (53.0%)	32 (54.2%)	70 (59.8%)	204 (53.8)
	Doxycycline	29 (21.2%)	13 (19.7%)	12 (20.3%)	36 (30.7%)	90 (23.7)
Phenicols	*Chloramphenicol*	58 (42.3%)	25 (37.9%)	21 (35.6%)	69 (58.9%)	173 (45.6)
Polypeptides	*Polymyxin B*	15 (10.1%)	8 (12.1%)	4 (6.7%)	21 (17.9%)	48(12.6)
Quinolones	*Norfloxacin*	42 (30.7%)	22 (33.3%)	16 (27.1%)	52 (44.4%)	132 (34.8)
	Enrofloxacin	51 (37.2%)	27 (40.9%)	2 0(33.9%)	61 (52.1%)	159 (42.0)
Sulfonamides	*Sulfamethoxazole/Trimethoprim*	66 (48.1%)	32 (48.5%)	21 (35.6%)	74 (63.2%)	193 (50.9)

Table 4. *Detection of resistance genes from different resistance phenotypes of clinical isolates of Escherichia coli*

Phenotype	Resistance Gene	No. of Isolates (%)
Ampicillin (n=246)	bla_{TEM}	158 (64.2)
	bla_{OXA}	57 (23.2)
	bla_{TEM} & bla_{OXA}	10 (4.1)
	No gene detected	21 (8.5)
Tetracycline (n=204)	*tet*(A)	85(41.7)
	tet(B)	74 (36.3)
	tet(C)	13(6.4)
	tet(A)+*tet*(B)	17 (8.3)
	tet(A)+*tet*(C)	3 (1.5)
	No gene detected	12 (5.9)
Streptomycin (n=225)	*strA-strB*	102 (45.3)
	aadA	43 (19.1)
	strA-strB+aadA	13 (5.8)
	No gene detected	67 (29.8)
Polymyxin B (n=48)	*mcr-1*	7 (14.6 %)
	No gene detected	41 (85.4 %)

52.4% for streptomycin [13]. In France, the drug resistance rates of *E. coli* from calves was 79.8% for tetracycline, 68.0% for sulfa drugs, 61.0% for amoxicillin, and 60.1% for streptomycin [14]. The overall rates of drug-resistant *E. coli* in this study were higher than those in the United States but lower than them of Germany and France [13,14,25].

In this study, *E. coli* isolates were divided into four phylogenetic groups, A (36.1%), B1 (17.4%), B2 (15.6%) and D (30.9%). It has been reported that B2 and D are highly pathogenic groups [18,26], and different hosts from different regions carry distinct *E. coli* groups [27,28]. Rodriguez et al.[29] found that more group A (38%) and D (28.1%) and less group B2 (18.5%) were identified among 524 avian pathogenic *E. coli* isolates from the United States. Tetsuo Asai et al.[30] demonstrated that group B2 *E. coli* from chickens only appeared in isolates from diseased chickens. Studies in Brazil and Japan showed that healthy cattle and pigs carried more groups A and B1 *E. coli* while no group B2 [26]. Extensive antibiotic use can lead to antibiotic pressure on bacterial evolution in that niche, and selection will be directed toward to success of the most resistant pathogens [31]; Simultaneously, during colonization and infection, the most virulent pathogens will be the most successful and will therefore be the most likely to survive. Our results showed that the highly pathogenic groups D were more severely resistant than symbiotic strains and low pathogenic groups *(Table 3)* suggesting that their resistance might be related to their pathogenicity.

The resistance genes are usually located on chromosomes and mobile genetic elements [32,33], and the transference of these mobile genetic elements is an important reason for increasing numbers of multi-drug-resistant bacteria [34,35]. Among 9 genes we analyzed, our samples showed positive to bla_{TEM}, bla_{OXA}, while negative to bla_{PSE}. As comparison, bla_{TEM} and bla_{PSE} instead of bla_{OXA} were detected in *E. coli* from Canadian calves [36]. The detection rate of the *mcr-1* gene was 14.6% in this study, which is higher than that of Belgian bovine *E. coli* (11.5%) [37], but lower than that of French bovine *E. coli* (20.5%) [38]. The tetracycline resistance was mainly encoded by *tet*(A) and *tet*(B) genes, wherein *tet*(A) (51.5%) had higher prevalence than *tet*(B) (46.1%); this result is similar to the studies by Guerra et al.[12] and Van

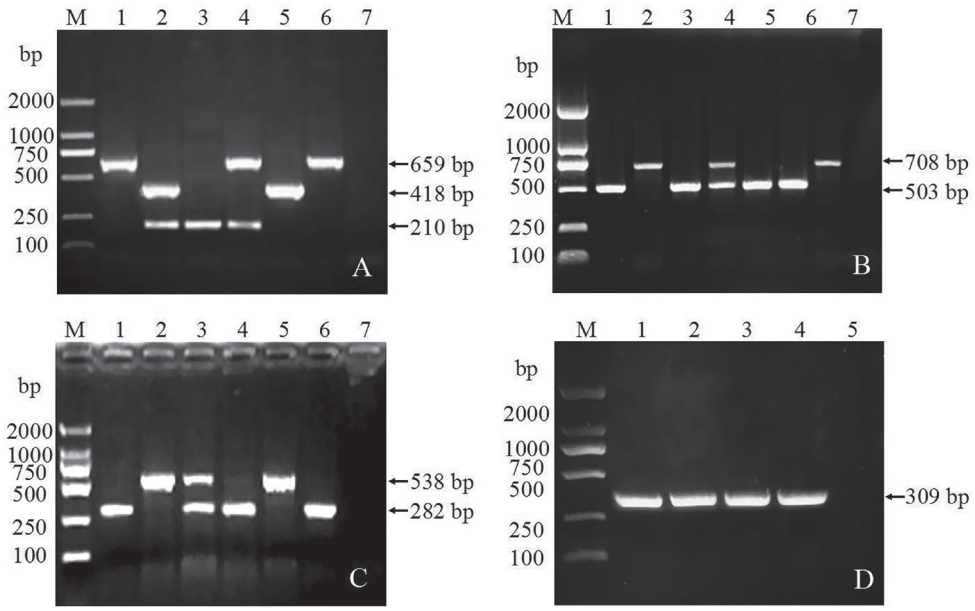


Fig 3. PCR detection of antimicrobial resistance genes in *E. coli* isolates from calves. **A:** PCR amplification of *tet*(A), *tet*(B) and *tet*(C) genes; 1-6: *tet*(A) (210 bp), *tet*(B) (659 bp) and *tet*(C) (418 bp); 7: Control negative; M: DNA Marker DL-2000; **B:** PCR amplification of bla_{TEM1}, bla_{PSE1} and bla_{OXA1} genes; 1-7: bla_{TEM1} (503 bp) and bla_{OXA1} (708 bp); 8: Control negative; M: DNA Marker DL-2000; **C:** PCR amplification of *strA-strB* and *aadA* genes; 1-6: *strA-strB* (538 bp) and *aadA* (282 bp); 7: Control negative; M: DNA Marker DL-2000; **D:** PCR amplification of *mcr-1* gene; 1-4: *mcr-1* (309 bp); 5: Control negative; M: DNA Marker DL-2000

et al.[39]. Additionally, in streptomycin-resistant *E. coli*, the *strA-strB* gene was the most common detected resistance determinant, which is consistent with previous studies [22].

In conclusion, antimicrobial resistance profiles and phylogenetic grouping of the *E. coli* clinical strains isolated from Xinjiang calves were clarified. The antibiotic resistance rates were high in diarrheal calves in Xinjiang. Therefore, the possibility of transmission of *E. coli* from calves to humans, particularly those in highly pathogenic group, can not be excluded. Also, further studies are needed to elucidate the risk of transmission to humans by analyzing the clonal relationship in *E. coli* from calves and humans.

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Conflict of Interests Statement

The authors declare that there is no conflict of interests regarding the publication of this article.

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