First Isolation and Molecular Characterization of blaCTX-M-121-producing Escherichia coli O157:H7 from China Cattle

Zhanqiang Su

Xinjiang Agricultural University

Panpan Tong (✉ tongpanpan123@163.com)

Xinjiang Agricultural University <https://orcid.org/0000-0002-0027-4437>

Ling Zhang

Xinjiang Agricultural University

Mengmeng Zhang

Xinjiang Agricultural University

Dong Wang

Xinjiang Agricultural University

Kaiqi Ma

Xinjiang Agricultural University

Yi Zhang

Xinjiang Agricultural University

Yingyu Liu

Xinjiang Agricultural University

Lining Xia

Xinjiang Agricultural University

Jinxin Xie

Xinjiang Agricultural University

Research article

Keywords: E. coli O157:H7, virulence genes, antibiotic resistance, PFGE, bovine

Posted Date: December 15th, 2019

DOI: <https://doi.org/10.21203/rs.2.18636/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: To study antibiotic resistance and the molecular epidemiology of bovine *Escherichia coli* (*E.coli*) O157:H7 and to explore the intrinsic relationship among different isolates, we collected 27 strains of bovine *E. coli* O157:H7 in Xinjiang from 2012 to 2017 and assessed virulence genes, antibiotic resistance and pulsed-field gel electrophoresis (PFGE) molecular typing.

Results: In this study, 21 strains carried at least one virulence gene, and 19 strains carried *eae* gene (70.4%) including 8 carrying *stx1+stx2+eae+hly+tccP*. Most strains were sensitive to all antibiotics tested, 4 strains were bacteria-resistant, and 2 strains possessed multi-drug resistance, including one ESBL-producing strain. This is the first report of a *bla*_{CTX-M-121} gene in bovine *E. coli* O157:H7. Moreover, *bla*_{CTX-M-121} gene can be transmitted horizontally by plasmid between strains. The PFGE spectral similarity of the 27 strains was between 65.8% and 100%. Two PFGE types including clusters A and B were obtained through cluster analysis.

Conclusions: *E. coli* O157:H7 may have undergone clonal propagation in cattle farms as well as cross-regional transmission. A horizontal transmission path with *E. coli* O157:H7 appeared in different areas.

Background

E.coli O157:H7 is a major foodborne pathogen that causes severe bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome in humans [1]. *E. coli* O157: H7 was first recognized as a pathogen in 1982 during an investigation into an outbreak of hemorrhagic colitis associated with hamburger consumption [2]. Since then, many outbreaks of *E. coli* O157:H7 infection have been reported in the United States, Canada, Japan and China [3–6]. As reported, cattle are a major reservoir and source of infection with *E. coli* O157:H7. *E. coli* O157:H7 from healthy cattle have been reported worldwide [7]. Cattle irregularly expel *E. coli* O157:H7 out of the body without suffering any pathological symptom and transmit strains to humans through food, water, direct contact with animals, or the environment [8]. Shiga toxins (*stx*), one of the major virulence factors involved in the pathogenesis of *E. coli* O157:H7, is encoded by the *stx1* or *stx2* genes [9]. Intimin and enterohemolysin (encoded by the *eae* gene and the *hly* gene respectively) are another two markers that play a major role in the pathogenesis [9]. The *tccP* protein encoded by *tccP* gene is a pathogenic molecule of *E. coli* O157:H7 and is transduced into host cells by the type III secretion system to exert its pathogenic effect [10]. These genetic virulence characteristics are often used in epidemiological studies on strains from various sources.

Undoubtedly, antimicrobials are a main tool for the prevention and treatment of bacterial diseases in animals, but the antibiotic resistance of bacteria has caused great concern. Antibiotic resistance has become a serious problem worldwide, especially in developing countries where the quality, distribution and use of antibiotics in human medicine and veterinary medicine are under limited control [11, 12]. The *E.coli*- caused diseases often require antimicrobial therapy, but antibiotic-resistant strains of this bacterium cause more chronic and more severe illnesses than their antibiotic-susceptible counterparts

[11]. Although antibiotics are not recommended for patients with suspected *E. coli* O157:H7 infection, *E. coli* O157:H7 isolated from infected humans or animals is resistant against multiple antibiotics [13]. The emergence of multi-drug resistant (MDR) *E. coli* O157:H7 is a public health problem.

Xinjiang is a big province of cattle raising industry in China. Effective prevention and control of bovine pathogenic microorganisms is the premise to ensure the healthy and sustainable development of cattle raising and the safety of consumers. To further assess the potential impacts of these isolates on public health, we investigated the pathogenicity and antibiotic resistance of *E. coli* O157:H7 in these farm- and abattoir- sourced isolates, studied the intrinsic relationship among different isolates and assessed the potential dissemination of MDR profiles in vitro.

Results

Isolation of *E. coli* O157:H7

A total of 27 *E. coli* O157:H7 strains were isolated from 2,657 bovine samples in Xinjiang, including 2 collected from 1 carcass swab sample, 4 from 3 feed samples, 8 from 8 feces samples and 13 from 5 rectal swab samples (Table 1).

Presence of virulence genes

Except for 6 *E. coli* O157:H7 strains that did not carry any virulence gene, most of the strains carried more than one type of virulence genes. Polymerase chain reaction (PCR) showed 10 (37%) isolates carried *stx1*, 16 (59%) possessed *stx2*, and 8 (30%) contained both. The *eae* gene and *hly* gene were detected in most (70%) and 17 (63%) *E. coli* O157:H7 strains respectively. *tccP* in combination with *hly* and *eae* was found in 15 (56%) isolates, and *stx2* alone and *eae* alone were each present in 2 (7%) isolates (Table 1).

Antimicrobial susceptibility

Of the 27 *E. coli* O157:H7 strains tested, the majority (92.6%) were sensitive to all antibiotics. Only 4 out of the 27 tested strains (14.8%) showed resistance against one, eight or ten antibiotics by three different patterns (Table 2). Two strains were multi-resistant; whereas one of them was an Extended Spectrum Beta-Lactamases (ESBLs)-producing strain. These three strains were isolated from the same cattle farm in Yili.

Prevalence of antibiotic resistance genes in *E. coli* O157:H7 isolates

The resistance genes identified in all of the 4 drug-resistant isolates were summarized in Table 2. One *bla*_{CTX-M-121} gene screened was detected. Four tetracycline-resistant isolates were detected, with only one isolate carrying the *tetA* gene encoding the tetracycline efflux pump.

Conjugal transfer of resistance

Resistance transfer by conjugation was observed in 1 *bla*_{CTX-M}-producing isolate (Y4-A109) that was subjected to conjugation assays. The resistance against ampicillin, cefotaxime, ceftazidime, trimethoprim-sulfamethyloxazole and tetracycline, and the *bla*_{CTX-M} gene from the *bla*_{CTX-M}-producing O157:H7 isolate can be transferred to the recipient.

Plasmid incompatibility

The *bla*_{CTX-M-121} genes were carried by untypeable plasmid.

Epidemiological typing

The chromosomal DNA of 27 isolates was available for PFGE typing and the isolates displayed 14 different PFGE profiles (Fig. 1). The similarity among the types was above 65.80%, with two dominant clusters I and II, each accounting for 41%, and other types accounted for 18%. Cluster I mainly included type p4, and cluster II mainly consisted of types p11 and p12. The 9 strains in type p4 and the 5 strains in type p12 were highly consistent in sampling time and location, which was identified as clonal propagation. Obvious differences were found between p4 and p12 strains, and the isolates were separated in different regions and years. Four strains of drug-resistant bacteria belonged to four different types.

Discussion

E. coli O157:H7 is an important foodborne pathogen and is mainly associated with cattle as the reservoir [1]. The presence of *E. coli* O157:H7 in cattle indirectly reflects its potential harm to humans. In this study, a total of 2,657 cattle sourced samples were collected from 18 cattle farms (8/18, 44.4%) and 1 slaughterhouse in Tacheng, Bole, Yili, Wujiaqu, Changji, Wulumuqi and Aksu, and 25 strains (25/2609, 0.1%) were isolated from 8 cattle farms. The positive rate of cattle farms was high and that of samples was low. *E. coli* O157:H7 was isolated from samples from Yili, Wulumuqi and Aksu, but not from Tacheng, Bole, Wujiaqu or Changji, which indicated the presence of regional differences in bacterial distribution. The distribution of *E. coli* O157:H7 is affected by many factors, including the influence factors on bacterial excretion and transmission [14]. Seasonal and geographical characteristics affect the excretion of *E. coli* O157:H7 in cattle, and sampling frequency also impacts the bacteria detection rate [15, 16]. Generally, the seasonal shedding of *E. coli* O157:H7 in cattle is well documented and characterized by higher prevalence in summer [15]. In this study, the seven regions differed not only in geographical distribution, but also in climate, environment, temperature and other aspects. These reasons may lead to the differences in the separation rate of *E. coli* O157:H7 in cattle.

The pathogenicity of *E. coli* O157:H7 is associated with several virulence factors, including production of at least Shiga toxins (*stx1* and/or *stx2*), intimin (*eae*), enterohemolysin (*hly*) and tir couple cytoskeleton protein (*tccP*). Results showed 37.0% and 59.3% of the *E. coli* O157:H7 isolates were positive for *stx1* and *stx2* genes respectively. Epidemiological research shows *stx2*-producing strains are more virulent than *stx1* producers [17]. The *eae* gene, which is necessary for the attaching and effacing activity, encodes the

intimin protein that is essential for pathogenesis [18]. In our study, this important virulence gene was detected in 70% of the *E. coli* O157:H7 isolates. We identified the *tccP* gene in 56% of the *E. coli* O157:H7 strains. Noticeably, *tccP* gene is highly correlated with both *eae* gene and *hly* gene, but not with *stx* gene.

Despite the small sample size in the slaughterhouse, the isolation rate of carcass swab samples was higher than others in cattle farms, and one of them was the MDR bacteria, which showed evidence of coselection for antibiotic resistance and virulence. In this study, *E. coli* O157:H7 was found to be resistant against newer and more clinically important antimicrobial compounds, such as fluoroquinolone and cephalosporins. Production of β -lactamases is the main mechanism underlying the cephalosporin resistance in Gram-negative bacteria [19]. Extended-spectrum cephalosporins are an important class of drugs in both human and veterinary medicine. We investigated various narrow-spectrum (*bla*_{TEM} and *bla*_{SHV}) and extended-spectrum (*bla*_{CTX-M}) β -lactamase-encoding genes, but only identified one – *bla*_{CTX-M}. This is the first report of a *bla*_{CTX-M-121} gene in bovine *E. coli* O157:H7. The *tetA*, which is one of the most widespread *tet* genes found in Enterobacteria [20], was the only tetracycline-resistant gene identified in four tetracycline-resistant strains. However, to the best of our knowledge, this is the first report about the presence of *tetA* in bovine *E. coli* O157:H7 in Xinjiang. Conjugative transfer of untypeable plasmid was observed. Conjugation experiments produced successful transconjugation with MDR to β -lactamases, sulfonamides and tetracycline. This study highlights the importance of encouraging the appropriate use of antibiotics.

Dendrogram analysis of PFGE results showed the two *E. coli* O157:H7 strains separated from the same carcass swab samples from the slaughterhouse belonged to clusters I and II respectively, suggesting cross-contamination may occur during slaughter. The Y4-A20-1, Y4-A20-3, Y4-A20-4 from cluster I, and Y4-A20-5 from cluster II were isolated from the same rectal swab, indicating different *E. coli* O157:H7 strains were colonized in cattle. Cluster I W1-E51-5 and cluster II W1-E51-3F were isolated from the same feed sample, suggesting the cattle farm feed was contaminated with different *E. coli* O157:H7 strains. Cluster II Y1-166 and Y3-F328 were isolated from different cattle farms in the same region at the same time, which further proved horizontal transmission was an important means of *E. coli* O157:H7 dissemination on the farms. Cluster I Y4-A20-1, W2-A61-2 and W1-E51-5, and cluster II Y2-F25, A1-F13 and A2-F14 were isolated at different time points and from different regions, and these cattle farms were far apart. The trans-regional spread of bacteria may be caused by cross-regional trading of live animals. Based on analysis of virulence genes and drug resistance of *E. coli* O157:H7, we speculated that the virulence and drug resistance may be acquired or lost during the evolution and transfer of the same cluster of strains.

Conclusions

In this study *E. coli* O157:H7 contamination was found in cattle farms and the slaughterhouse in Xinjiang, and most isolates carried at least one virulence gene. *E. coli* O157:H7 had horizontal transmission pathways in the two regions, during which the virulence and drug resistance of the bacterium were constantly evolving.

Methods

Sample collection

Samples (n=2657) including 1155 of fresh feces, 1236 from rectal swab, 110 of feed, 108 of water and 48 of carcass swab were collected between October 2012 and March 2017 from 18 cattle farms and 1 cattle slaughterhouse in China (Tacheng, Bole, Yili, Wujiaqu, Changji, Wulumuqi and Aksu in Xinjiang). Details about these samples are shown in Table 3.

Bacterial isolates

Samples (feces/feed/water) each either 1 g or 1 ml were aseptically added to 9 ml of a trypticase soya broth (TSB) containing 20 mg/l of novobiocin and were incubated for 6-8 h at 37 °C. One rectal swab was transferred into a separate tube containing 2 ml of a nutrient broth and cultured at 37 °C for 24 h [21]. One carcass swab was put into a stomacher bag and added with 500 ml of a modified trypticase soya broth containing 8 mg/l novobiocin. Each sponge was mixed in the stomacher bag for 2 min and incubated for 20 h at 37 °C [22]. This was streaked out onto sorbitol MacConkey agar supplemented with 1 mg/l potassium tellurite and incubated for one day at 37 °C. One or more pale colonies were each picked as presumptive *E. coli* O157 per sample. The prevalence of *E. coli* O157:H7 was assessed via PCR (*rfbE* and *fliC* genes [23]) (Table 4). The positive isolates were each inoculated into separate TSB and incubated for one day at 37 °C, from which glycerol stock was made and then stored at -80 °C for further analyses.

Virulence analysis of isolates

Each *E. coli* O157:H7 isolate was characterized for the presence of virulence genes *stx1* [24], *stx2* [24], *eae*, *hly* [25] and *tccP* by PCR as previously described (Table 3).

Antimicrobial susceptibility tests

The isolates were tested for the susceptibility to antibiotics using the Kirby-Bauer disc diffusion technique. The antibiotic discs obtained from OXOID, UK, including ampicillin (AMP), piperacillin (PIP), cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP), aztreonam (ATM), ampicillin-sulbactam (SAM), piperacillin- tazobactam (TZP), amoxicillin-clavulanic acid (AMC), gentamicin (GEN), amikacin (AMI), streptomycin (STR), trimethoprim-sulfamethylisoxazole (SXT), chloramphenicol (CHL), levofloxacin (LEV), ciprofloxacin (CIP), tetracycline (TET), and polymyxin B (PB) [26]. *E. coli* ATCC25922 was used as a quality control strain in the susceptibility tests. The ESBLs-producing isolates were determined by double-disk synergy tests according to CLSI [26].

Detection of antibiotic resistance genes

The following resistance determinants were investigated by PCR: *bla*_{CTX-M} (the CTX-M-type genes were detected using universal primers CTX-M-U [27], and the entire CTX-M-type genes were amplified by using

the primers CTX-M-1G [27], CTX-M-2G [28] or CTX-M-9G [29]), *bla*_{TEM} [30], and *bla*_{SHV} [30] which encode β -lactamases; chloramphenicol (*cmhA1* [31]) efflux pumps; sulfonamide resistance gene (*sul1* [32]); and the *tetA* [33], *tetE* [33], and *tetG* [33] tetracycline efflux pumps. Primer for the different genes are listed in (Table 4). Purified PCR products were sequenced. The DNA sequences and deduced amino acid sequences were compared with sequences reported in GenBank to confirm the subtypes of the β -lactamase genes.

Conjugation experiments and plasmid analysis

Conjugation was conducted with *bla*_{CTX-M}-producing isolates by the filter method, using a sodium azide-resistant *E. coli* J53 as the recipient. Transconjugants were selected on MacConkey agar containing cefotaxime or ceftazidime (4 μ g/ml) and sodium azide (200 μ g/ml). ESBLs and antibiotic susceptibility were also tested in selected transconjugants, and the presence of *bla* genes was determined using PCR as described above. The resistance plasmids carried by transconjugants were typed by using PCR-based replicon typing [34].

Epidemiological typing

All available isolates were referred for PFGE and analyzed according to some existing criteria [35]. *Xba*I-digested chromosomal DNA of isolates was characterized by PFGE using the CHEF-MAPPER System (Bio-Rad Laboratories, Hercules, CA, USA). A *Salmonella* serotype Braenderup H9812 (ATCC BAA-664) was chosen as the molecular weight marker. Electrophoresis was run at 6.0 V/cm for 18.5 h with an angle of 120° at 14°C.

Declarations

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments

The authors thank Xiaoli Du of State Key Laboratory for Infectious Disease Prevention and Control National Institute for Communicable Disease Control and Prevention, China CDC for her support.

Funding

Funding for this study was provided by National Natural Science Foundation of China (31560485 and 31960695), Natural Science Foundation of Xinjiang Uygur Autonomous Region (2019D01A51), Prior Period Project of Xinjiang Agricultural University (XJAU201703), and by Postdoctoral Science Foundation of Xinjiang Agricultural University and Xinjiang Uygur Autonomous Region High-Level Talent Introduction.

The funding source had no role in the study design, analysis, collection, data interpretation and manuscript writing.

Author information

Affiliations

Zhanqiang Su and Panpan Tong contributed equally to this work.

Corresponding author: *College of Veterinary Medicine, Xinjiang Agricultural University, Xinjiang Province, 830052 Wulumuqi, People's Republic of China*

Zhanqiang Su, Panpan Tong, Ling Zhang, Mengmeng Zhang, Dong Wang, Kaiqi Ma, Yi Zhang, Yingyu Liu, Lining Xia & Jinxin Xie

Corresponding author

Correspondence to Lining Xia and Jinxin Xie.

Authors' Contributions

Z.Q.S. and P.P.T. conceived and designed the experiments. L.Z., M.M.Z., D.W., and K.Q.M. performed the experiments. Y.Z. and Y.Y.L. analyzed the data. P.P.T., L.N.X., and J.X.X. contributed to the writing of the manuscript. All authors read and approved the article.

Ethics declarations

Ethics approval and consent to participate

The study was carried out on private land, no specific permissions were required for these locations. Sampling and publication of the data were approved by the farm owners. All procedures performed on the cattle were approved by the Animal Care and Use Committee of Xinjiang Agricultural University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

AMC: Amoxicillin-clavulanic acid

AMI: Amikacin

AMP: Ampicillin

ATM: Aztreonam,

CAZ: Ceftazidime

CHL: Chloramphenicol

CIP: Ciprofloxacin

CTX: Cefotaxime

E.coli: Escherichia coli

ESBLs: Extended Spectrum Beta-Lactamases

FEP: Cefepime

GEN: Gentamicin

LEV: Levofloxacin

MDR: Multi-drug resistant

PCR: Polymerase chain reaction

PFGE: Pulsed-field gel electrophoresis

PB: Polymyxin B

PIP: Piperacillin

SAM: Ampicillin-sulbactam

STR: Streptomycin

stx: Shiga toxins

SXT: Trimethoprim-sulfamethyloxazole

TET: Tetracycline

TSB: Trypticase soya broth

TZP: Piperacillin-tazobactam

References

1. Wang L, Qu K, Li X, Cao Z, Wang X, Li Z, et al. Use of bacteriophages to control *Escherichia coli* O157:H7 in domestic ruminants, meat products, and fruits and vegetables. [Foodborne Pathog Dis.](#) 2017;14:483-93.
2. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N Engl J Med.* 1983;308(12):681-5.
3. Ostroff SM, Kobayashi JM, Lewis JH. Infections with *Escherichia coli* O157:H7 in Washington State. The first year of statewide disease surveillance. [JAMA.](#) 1989; 262:355-9.
4. Orr P, Lorencz B, Brown R, Kielly R, Tan B, Holton D. An outbreak of diarrhea due to verotoxin-producing *Escherichia coli* in the Canadian Northwest Territories. [Scand J Infect Dis.](#) 1994;26:675-84.
5. Akashi S, Joh K, Tsuji A, Ito H, Hoshi H, Hayakawa T. A severe outbreak of haemorrhagic colitis and haemolytic uraemic syndrome associated with *Escherichia coli* O157:H7 in Japan. [Eur J Pediatr.](#) 1994;153:650-5.
6. Zhang J, Xia S, Shen G, Chen Z, Huang P, Fu B, et al. A study on acute renal failure after an outbreak of diarrhea in Suixian county, Henan province. [Zhonghua Liu Xing Bing Xue Za Zhi.](#) 2002;23:105-7.
7. Munns KD, Selinger LB, Stanford K, Guan L, Callaway TR, McAllister TA. Perspectives on super-shedding of *Escherichia coli* O157:H7 by cattle. [Foodborne Pathog Dis.](#) 2015;12:89-103.
8. Karmali MA. Emerging public health challenges of Shiga Toxin-producing *Escherichia coli* related to changes in the pathogen, the population, and the environment. *Clin Infect Dis.* 2017;64:371-6.
9. Nguyen TD, Vo TT, Vu-Khac H. Virulence factors in *Escherichia coli* isolated from calves with diarrhea in Vietnam. [J Vet Sci.](#) 2011;12:159-64.
10. Garmendia J, Ren Z, Tennant S, Midolli Viera MA, Chong Y, Whale A, et al. Distribution of *tccP* in clinical enterohemorrhagic and enteropathogenic *Escherichia coli* isolates. [J Clin Microbiol.](#) 2005;43:5715-20.
11. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. [Lancet Infect. Dis.](#) 2010;10:597-602.
12. Adamu MS, Ugochukwu ICI, Idoko SI, Kwabugge YA, Abubakar NS, Ameh JA. Virulent gene profile and antibiotic susceptibility pattern of Shiga toxin-producing *Escherichia coli* (STEC) from cattle and camels in Maiduguri, North- Eastern Nigeria. [Trop Anim Health Prod.](#) 2018;50:1327-41.
13. Mir RA, Kudva IT. Antibiotic-resistant Shiga toxin-producing *Escherichia coli*: An overview of prevalence and intervention strategies. [Zoonoses Public Health.](#) 2019;66:1-13.
14. LeJeune JT, Besser TE, Rice DH, Berg JL, Stilborn RP, Hancock, DD. Longitudinal study of fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle: predominance and persistence of specific clonal types despite massive cattle population turnover. [Appl Environ Microbiol.](#) 2004;70:377-84.
15. Ogden ID, Macrae M, Stechan, NJ. Is the prevalence and shedding concentrations of *E. coli* O157 in beef cattle in Scotland seasonal? [FEMS Microbiol Lett.](#) 2004; 233:297-300.

16. Money P, Kelly AF, Goud SW, Denholm-Price J, Threlfall EJ, Fielder MD. Cattle, weather and water: mapping *Escherichia coli* O157:H7 infections in humans in England and Scotland. [Environ Microbiol.](#) 2010;12:2633-44.
17. Proulx F, Seidman EG, Karpman D. Pathogenesis of Shiga Toxin-associated Hemolytic Uremic Syndrome. *Pediatr Res.* 2001;50:163-71.
18. Mora A, Blanco M, Blanco JE, Dahbi G, López C, Justel P, et al. Serotypes, virulence genes and intimin types of Shiga toxin (verocytotoxin)-producing *Escherichia coli* isolates from minced beef in Lugo (Spain) from 1995 through 2003. [BMC Microbiol.](#) 2007;7:13.
19. Bradford PA. Extended-spectrum β -lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. [Clin Microbiol Rev.](#) 2001;14:933-51.
20. Kennedy CA, Fanning S, Karczmarczyk M, Byrne B, Monaghan Á, Bolton D, et al. Characterizing the multidrug resistance of non-O157 Shiga toxin-producing *Escherichia coli* isolates from cattle farms and abattoirs. [Microb Drug Resist.](#) 2017;23:781-90.
21. ISO 16654., 1st ed. Microbiology—Horizontal method for the detection of *Escherichia coli* O157, International Organization for Standardization, Geneva, Switzerland. 2001.
22. ISO/TS 13136., Microbiology of food and animal feed-real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens- horizontal method for detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O11, O26, O103 and O145 serogroups. 2012.
23. Gannon VP, D'Souza S, Graham T, King RK, Rahn K, Read S. Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *Escherichia coli* strains. [J Clin Microbiol.](#) 1997;35:656-62.
24. Pollard DR, Johnson WM, Lior H, Tyler SD, Rozee KR. Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. [J. Clin. Microbiol.](#) 1990;28:540-5.
25. Bosllevac JM, Koohmaraie M. Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* isolates from commercial ground beef in the United States. [Appl Environ Microbiol.](#) 2011;77:2103-12.
26. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing, 26th Edn. CLSI supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute. 2016
27. Pagani L, Dell'Amico E, Migliavacca R, D'Andrea MM, Giacobone E, Amicosante G, et al. Multiple CTX-M-type extended-spectrum β -lactamases in nosocomial isolates of enterobacteriaceae from a hospital in northern Italy. [J. Clin. Microbiol.](#) 2003;41:4264-9.
28. Saladin M, Cao VT, Lambert T, Donay JL, Hermann JL, Ould-Hocine Z, et al. Diversity of CTX-M beta-lactamases and their promoter regions from Enterobacteriaceae isolated in three Parisian hospitals. [FEMS Microbiol Lett.](#) 2002;209:161-8.
29. Eckert C, Gautier V, Saladin-Allard M, Hidri N, Verdet C, Ould-Hocine Z, et al. Dissemination of CTX-M-type beta-lactamases among clinical isolates of Enterobacteriaceae in Paris, France. [Antimicrob](#)

Agents Chemother. 2004;48:1249 -55.

30. Lin CF, Hsu SK, Chen CH, Huang JR, Lo HH. Genotypic detection and molecular epidemiology of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a regional hospital in central Taiwan. J Med Microbiol. 2010;59:665-71.
31. Keyes K, Hudson C, Maurer JJ, Thayer S, White DG, Lee MD. Detection of florfenicol resistance genes in *Escherichia coli* isolated from sick chickens. Antimicrob Agents Chemother. 2000;44:421-4.
32. Kerrn MB, Klemmensen T, Frimodt-Møller N, Espersen F. Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacter-aemia, and distribution of sul genes conferring sulphonamide resistance. J Antimicrob Chemother. 2002;50:513-6.
33. Ng LK, Martin I, Alfa M, Mulvey M. Multiplex PCR for the detection of tetracycline resistant genes. Mol Cell Probes. 2001;15:209-15.
34. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods. 2005;63:219- 28.
35. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995;33: 2233-9.

Tables

Table 1 *E. coli* O157:H7 isolates and virulence genes

<i>Strain</i>	<i>Region</i>	<i>Farm</i>	<i>Sample source</i>	<i>Collection time</i>	<i>Virulence genes</i>
W1-E50-4	Wulumuqi	1	Feed	2017.01	-
W1-E51-3	Wulumuqi	1	Feed	2017.01	-
W1-E51-5	Wulumuqi	1	Feed	2017.01	-
W2-A61-2	Wulumuqi	2	Rectal swab	2016.10	<i>stx2+eae+hly+tccP</i>
W2-A61-3	Wulumuqi	2	Rectal swab	2016.10	<i>stx2+eae+hly+tccP</i>
W2-A61-4	Wulumuqi	2	Rectal swab	2016.10	<i>stx2+eae+hly+tccP</i>
W2-A61-5	Wulumuqi	2	Rectal swab	2016.10	<i>stx2+eae+hly+tccP</i>
W1-E16	Wulumuqi	1	Feed	2016.03	<i>stx1+eae+hly+tccP</i>
A1-F1	Akesu	1	Feces	2015.09	-
A1-F13	Akesu	1	Feces	2015.09	-
A2-F10	Akesu	2	Feces	2012.10	<i>stx2+eae+hly+tccP</i>
A2-F14	Akesu	2	Feces	2012.10	-
Y1-F166	Yili	1	Feces	2014.08	<i>stx1+stx2+eae+hly+tccP</i>
Y2-F25	Yili	2	Feces	2013.08	<i>stx2</i>
Y2-F27	Yili	2	Feces	2013.08	<i>stx2</i>
Y3-F328	Yili	3	Feces	2014.08	<i>stx2+eae+hly+tccP</i>
Y4-A20-1	Yili	4	Rectalswab	2015.09	<i>stx1+stx2+eae+hly+tccP</i>
Y4-A20-2	Yili	4	Rectal swab	2015.09	<i>stx1+stx2+eae+hly+tccP</i>
Y4-A20-3	Yili	4	Rectalswab	2015.09	<i>eae</i>
Y4-A20-4	Yili	4	Rectalswab	2015.09	<i>stx1+stx2+eae+hly +tccp</i>
Y4-A20-5	Yili	4	Rectalswab	2015.09	<i>eae</i>
Y4-C21-1	Yili	4	Carcass swab	2015.09	<i>stx1+stx2+eae+hly+tccP</i>
Y4-C21-2	Yili	4	Carcass swab	2015.09	<i>stx1+stx2+eae+hly+tccP</i>
Y4-A41-2	Yili	4	Rectal swab	2015.09	<i>stx1+stx2+eae+hly+tccP</i>
Y4-A41-4	Yili	4	Rectal swab	2015.09	<i>stx1+eae+hly</i>
Y4-A103	Yili	4	Rectal swab	2016.10	<i>eae+hly</i>
Y4-A109	Yili	4	Rectal swab	2016.10	<i>stx1+stx2+eae+hly+tccP</i>

Table 2 Patterns of antibiotic resistance of *E.coli* O157:H7 isolates

<i>Strains</i>	<i>Antibiotics</i>	<i>ESBLs-producing</i>	<i>Antibiotic resistance genes</i>
Y4-C21-1	AMP PIP CTX SXT CHL LEV CIP TET	-	-
W1-E16	TET	-	-
Y4-A103	TET	-	<i>tetA</i>
Y4-A109	AMP PIP CTX CAZ STR SXT CHL LEV CIP TET	+	<i>bla</i> CTX-M-121

Table 3 Information on sample collection

<i>Source of sample</i>	<i>Origin</i>	<i>NO. of samples</i>	<i>NO. of positive samples</i>
Two cattle farms in Tacheng	Feces	134	0
One cattle farm in Bole	Feces/Rectal swab/	43/82/10/6	0
	Water/Feed		
Five cattle farms and one	Feces/Rectal swab/	480/397/48/42/29	9
cattle slaughterhouse in Yili	Carcass		
	swab/Water/Feed		
One cattle farm in Wujiaqu	Feces/Rectal swab/	8/79/9/4	0
	Water/Feed		
Two cattle farms in Changji	Feces/Rectal swab/	46/211/17/23	0
	Water/Feed		
Four cattle farms in Wulumuqi	Feces/Rectal swab/	90/467/30/48	4
	Water/Feed		
Four cattle farms in Aksu	Feces	354	4
Total		2657	17

Table 4 Oligonucleotides of the various targeted genes

<i>Primers</i>	<i>Sequence (5'-3')</i>	<i>References/Accession No.</i>
rfbE-F	ATTGCGCTGAAGCCTTTG	CP044148.1
rfbE-R	CGAGTACATTGGCATCGTG	
fliC-F	GCGCTGTCGAGTTCTATCGAGC	[23]
fliC-R	CAACGGTGACTTTATCGCCATTCC	
stx1-F	GAAGAGTCCGTGGGATTACG	[24]
stx1-R	AGCGATGCAGCTATTAATAA	
stx2-F	TTAACCACACCCACGGCAGT	[24]
stx2-R	GCTCTGGATGCATCTCTGGT	
eae-F	CATTATGGAACGGCAGAGGT	CP044148.1
eae-R	ACGGATATCGAAGCCATTTG	
hly-F	CACACGGAGCTTATATTCTGTCA	[25]
hly-R	AATGTTATCCCATTGACATCATTTGACT	
tccP-F	CGCCATATGATTAACAATGTTTCTTCAC	CP044148.1
tccP-R	CTCGAGTCACGAGCGCTTAGATGTATT	
bla _{CTX-M-U} -F	ATGTGCAGYACCAAGTAARGT	[27]
bla _{CTX-M-U} -R	TGGGTRAARTARGTSACCAGA	
bla _{CTX-M-1G} -F	GTTACAATGTGTGAGAAGCAG	[27]
bla _{CTX-M-1G} -R	CCGTTTCCGCTATTACAAAC	
bla _{CTX-M-2G} -F	ATGATGACTCAGAGCATTTCG	[28]
bla _{CTX-M-2G} -R	TGGGTTACGATTTTCGCCGC	
bla _{CTX-M-9G} -F	ATGGTGACAAAGAGAGTGCA	[29]
bla _{CTX-M-9G} -R	CCCTTCGGCGATGATTCTC	
bla _{TEM} -F	ATGAGTATTCAACATTTCCGT	[30]
bla _{TEM} -R	TTACCAATGCTTAATCAGTGA	
bla _{SHV} -F	CCGGGTTATTCTTATTTGTCGCT	[30]
bla _{SHV} -R	TAGCGTTGCCAGTGCTCG	
cmIA1-F	CCGCCACGGTGTTGTTGTTATC	[31]
cmIA1-R	CACCTTGCCTGCCCATCATTAG	
sul1-F	CGGCGTGGGCTACCTGAACG	[32]
sul1-R	GCCGATCGCGTGAAGTTCCG	
tetA-F	GCTACATCCTGCTTGCCTTC	[33]
tetA-R	CATAGATCGCCGTGAAGAGG	
tetE-F	AAACCACATCCTCCATACGC	[33]
tetE-R	AAATAGGCCACAACCGTCAG	
tetG-F	GCTCGGTGGTATCTCTGCTC	[33]
tetG-R	AGCAACAGAATCGGGAACAC	

Figures

Figure 1

Dendrogram of XbaI pulsed-field gel electrophoresis profiles of E.coli O157:H7 isolates