First Isolation and Molecular Characterization of bla CTX-M-121 -producing Escherichia coli O157:H7 Strain Y4-A109 from Cattle in China

Zhanqiang Su
Xinjiang Agricultural University
Panpan Tong(Former Corresponding Author)
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(New Corresponding Author) (xiejinxin198683@163.com)
Xinjiang Agricultural University

Research article

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

Posted Date: March 9th, 2020

DOI: https://doi.org/10.21203/rs.2.18636/v2

Abstract

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

Results: Of all the 27 bovine E. coli O157:H7 strains analyzed, 21 strains contained at least one virulence gene, 19 strains carried eae gene (70.4%) and 8 of them carrying stx1 + stx2 + eae + hly + tccP . Most strains were sensitive to all the antibiotics tested. However, 4 of which were antibiotic-resistant, and 2 of which possessed multi-drug resistance, including one ESBL-producing strain. This is the first report of the bla CTX-M-121 gene in bovine E. coli O157:H7. Moreover, the bla CTX-M-121 gene can be transmitted horizontally through plasmid between strains. The similarity of PFGE spectra of 27 strains was between 65.8% and 100%. Two types of PFGE were obtained through cluster analysis, including clusters and .

Conclusions: E. coli O157:H7 may have undergone clonal propagation in cattle farms as well as cross-regional transmission and horizontal transmission in different regions in Xinjiang China.

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 an investigation of an outbreak of hemorrhagic colitis associated with hamburger consumption in 1982 [2]. Since then, many outbreaks of *E. coli* O157:H7 infection have been reported in the United States, Canada, Japan, and China [3-6]. Cattle is reported to be the major reservoir and source of infection for *E. coli* O157:H7. *E. coli* O157:H7 from healthy cattle has been reported worldwide [7]. The infected cattle irregularly excreted *E. coli* O157:H7 without any pathological symptom, and transmitted the pathogen to humans through food, water, direct contact with animals or the environment [8].

The pathogenicity of *E. coli* O157:H7 is associated to genes encoding for multiple virulence factors. Shiga toxins (*stx*) is one of the major virulence factors involved in the pathogenesis of *E. coli* O157:H7 and is encoded by the *stx*1 or *stx2* genes [9]. Intimin and enterohemolysin (encoded by the *eae* gene and the *hly* gene, respectively) are two other markers that play a major role in pathogenesis [9]. The *tccP* protein encoded by the *tccP* gene is a pathogenic molecule of *E. coli* O157:H7 and is transduced into host cells through the type III secretion system to exert its pathogenic effect [10]. These genetic virulence characteristics are commonly used in epidemiological studies of strains from various origins [6,11].

Undoubtedly, antimicrobia are the main tool for the prevention and treatment of bacterial diseases in animals. However, 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 not strictly controlled [11, 12]. Diseases caused by *E.coli* usually require antimicrobial treatment, but antibiotic-resistant strains of this bacterium may cause more chronic and more severe diseases than their

antibiotic-susceptible counterparts [12]. *E. coli* O157: H7 strains isolated from humans and animals have been resistant to a variety of antibiotics [13]. The emergence of multi-drug resistant (MDR) *E. coli* O157:H7 is a public health issue.

Xinjiang has one of China's largest cattle raising industry. Effective prevention and control of bovine pathogenic microorganisms is a prerequisite to ensure the healthy and sustainable development of the cattle industry and consumer safety. To further assess the potential public health impact of *E. coli* O157:H7 isolates, we investigated the pathogenicity and antibiotic resistance of these strains originating from farms and slaughterhouses, and examined 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 cattle samples in Xinjiang, 2 of which were collected from one carcass swab sample, 4 from 3 feed samples, 8 from 8 feces samples and 13 from 5 rectal swab samples, and multiple colonies were selected from the same plate (Table 1).

Presence of virulence genes

Of the 27 *E. coli* O157:H7 isolates tested, 21 isolates carried more than one virulence genes, and 6 (22.2%) did not encode the genes evaluated in the study. Polymerase chain reaction (PCR) showed that 2 (7.4%) isolates carried *stx*1, 8 (29.6%) possessed *stx*2, and 8 (29.6%) contained both *stx1* and *stx2*. The *eae* gene and *hly* gene were detected in most (70.4%) and 17 (63.0%) *E. coli* O157:H7 strains, respectively. *TccP* in combination with *hly* and *eae* was found in 15 (55.6%) isolates, *stx*2 alone and *eae* alone were present in 2 (7.4%) isolates (Table 1).

Antibiotic resistance spectrum and distribution of antibiotic resistance genes

Twenty-three (85.2%) *E. coli* O157:H7 isolates were sensitive to all of antimicrobials investigated. Four isolates (14.8%) were resistant, 3 of which were isolated from the same cattle farm in Yili. Of the four resistant isolates, 2 were only resistant to tetracycline, and one of which carries *tetA* gene that encodes a tetracycline efflux pump. The other two were MDR strains with the resistant patterns: AMP/CHL/CIP/CTX/LEV /PIP/SXT/TET (Y4-C21-1) and AMP/CAZ/CHL/CIP/CTX/LEV/PIP/SXT/TET (Y4-A109). In particular, the ones with the Y4-A109 was an Extended Spectrum Beta-Lactamases (ESBLs)-producing strain and carrying the $bla_{\text{CTX-M-121}}$ gene.

Transferability of $bla_{\text{CTX-M}}$ genes and plasmid replicon typing

The $bla_{\text{CTX-M}}$ gene of *E. coli* O157:H7 isolate (Y4-A109) was transferred to the recipient strain (azide-resistant *E. coli* J53) by conjugation at frequencies of 10^{-6} per donor cell. Resistance to ampicillin, cefotaxime, ceftazidime, trimethoprim- sulfamethylisoxazole and tetracycline, and resistance to the

bla$_{CTX-M-121}$ gene from the *bla*$_{CTX-M}$-producing O157:H7 isolate can be transferred to the recipient. The *bla*$_{CTX-M-121}$ gene is carried by non-typeable plasmid.

Epidemiological typing

The chromosomal DNA of 27 isolates was available for PFGE typing and the isolates showed 14 different PFGE profiles (Fig. 1). The similarity among the types was higher than 65.8%, with the two dominant clusters I and II accounting for 40.7%, and 18.5%, respectively. Cluster mainly includes type p4, and cluster mainly consists of type p11 and p12. Nine strains of type p4 and 5 strains of type p12 were highly consistent in sampling time and location, which was determined as clonal propagation. Significant differences were found between p4 and p12 strains (*p* value?) with isolates were collected in different regions and years. Four drug-resistant bacterial strains belong to four different types.

Discussion

E. coli O157:H7 is an important foodborne pathogen [1]. Cattle is considered to be the major reservoir and transmitting diseases to humans primarily by eating contaminated food. In this study, a total of 2,657 cattle sourced samples were collected from Tacheng (2 farms), Bole (1 farm), Yili (4 farms and 1 slaughterhouse), Wujiaqu (1 farm), Changji (2 farms), Wulumuqi (4 farms) and Akesu (4 farms), 27 *E. coli* O157:H7 strains were isolated. *E. coli* O157:H7 was isolated from Yili, Wulumuqi and Akesu, while not from Tacheng, Bole, Wujiaqu and Changji, which indicated the presence of regional differences in bacterial distribution. We discovered that the number of *E. coli* O157:H7 isolates were low in winter and high in summer, which is consistent with the previous finding [14]. In addition, the number of *E. coli* O157:H7 isolates were lower in Xinjiang when compared to other provinces in China [6, 15]. Our previous studies have shown that the immunomagnetic separation (IMS) in practice was not statistically significant different compared to conventional method [16]. The low isolation rate of *E. coli* O157: H7 may be related to the severe dry weather conditions of Xinjiang, which needs further confirmation.

The pathogenicity of *E. coli* O157:H7 is associated with several virulence factors, including the production of Shiga toxins (*stx1* and/or *stx2*), intimin (*eae*), enterohemolysin (*hly*) and tir couple cytoskeleton protein (*tccP*). The results showed that 37.0% and 59.3% of *E. coli* O157:H7 isolates contain *stx1* and *stx2* genes, respectively. Epidemiological researches have shown that the virulence of s*tx2*-producing strains is higher than s*tx1* producers [17]. The *eae* gene which is necessary for the attaching and effacing activity encodes an intimin protein that is essential for pathogenesis [18]. In our study, this important virulence gene was detected in 70.4% of the *E. coli* O157:H7 isolates. We identified the *tccP* gene in 55.6% of the *E. coli* O157:H7 strains. Noticeably, *tccP* gene is highly correlated with both *eae* gene and *hly* gene, but not with the *stx* gene.

Although the sample size in the slaughterhouse is small, the isolation rate of carcass swab samples was higher than others samples from the cattle farms, and one of which was the MDR bacteria, which showed co-selection evidence of antibiotic resistance and virulence. In this study, two *E. coli* O157:H7 isolates were found to be resistant against new and more clinically important antimicrobial compounds such as

fluoroquinolones and cephalosporins. Beta-lactamases production is the main mechanism underlying the cephalosporin resistance in Gram-negative bacteria [19]. Broad-spectrum cephalosporins are important 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 the $bla_{CTX-M-121}$ gene in bovine *E. coli* O157:H7. The t*etA* is one of the most widespread *tet* genes found in Enterobacteria [20], and is the only tetracycline resistant gene identified in four tetracycline-resistant strains. However, to our knowledge, this is the first report about the presence of *tetA* in bovine *E. coli* O157:H7 in Xinjiang. Conjugative transfer of non-typeable plasmid was observed. Conjugation experiments successfully transduced MDR to β-lactamases, sulfonamides and tetracycline. This study highlights the importance of encouraging the appropriate use of antibiotics.

The dendrogram analysis of the PFGE results showed that the two *E. coli* O157:H7 strains isolated from the same carcass swab samples from the slaughterhouse belonged to clusters I and II, suggesting that cross-contamination may occur during the slaughter process. The Y4-A20-1, Y4-A20-3, Y4-A20-4 of cluster I, and Y4-A20-5 of cluster II from the same rectal swab, indicates that different *E. coli* O157:H7 strains have been colonized in cattle. Cluster I W1-E51-5 and cluster II W1-E51-3F were isolated from the same feed sample, suggesting that 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 the horizontal transmission was an important means of *E. coli* O157:H7 dissemination in these 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. These cattle farms were separated far away. Cross-regional transmission of bacteria may be caused by trading in live animals. Based on the analysis of virulence genes and drug resistance of *E. coli* O157:H7, we speculate that 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 slaughterhouse in Xinjiang, and most isolates carried at least one virulence gene. *E. coli* O157:H7 may have undergone clonal propagation in cattle farms and transmitted horizontally in different regions.

Methods

Sample collection

Samples (n= 2657) were collected from 18 farms and one cattle slaughterhouse in Tacheng, Bole, Yili, Wujiaqu, Changji, Wulumuqi and Akesu in Xinjiang of China between October 2012 and March 2017, including 1155 fresh feces, 1236 rectal swabs, 110 feed, 108 water and 48 carcass swabs (Table 2).

Bacterial isolate

Each 1 g or 1 ml sample (feces/feed/water) were aseptically added to 9 ml of trypticase soya broth (TSB) containing 20 mg/l novobiocin and were incubated for 6-8 h at 37 °C. A rectal swab was transferred into a separate tube containing 2 ml nutrient broth and cultured at 37 °C for 24 h [21]. One carcass swab was put into a stomacher bag and added 500 ml of modified trypticase soya broth containing 8 mg/l novobiocin. Each sponge was mixed in the stomacher bag for 2 min and then incubated for 20 h at 37 °C [22]. This was streaked out onto Sorbitol MacConkey agar supplemented with 0.01mg/l cefixime and 0.5mg/l potassium tellurite (Haibo, Qingdao,China) (CT-SMAC) and incubated for one day at 37 °C. One or more pale colonies were individually selected as presumptive *E. coli* O157 per sample. The prevalence of *E. coli* O157:H7 was assessed via polymerase chain reaction (PCR) (*rfbE* and *fliC* genes [23]) (Table 3). 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 analysis.

Virulence analysis of isolates

DNA extraction

DNA was extracted by boiling the isolates. Each colony was inoculated on CT-SMAC and incubated for 16 h at 37 °C to obtain fresh colony. Several colonies were selected and suspended separately in 200 μl of sterile distilled water in 1.5ml eppendorf tubes. The suspensions were then boiled at 95 °C for 10 min in a water bath. After centrifuging at 12000 rpm for 10 min, the supernatant containing the template DNA was transferred into 1.5ml Eppendorf tubes without nuclease and were stored at -20 °C until use.

Determination of virulence genes by PCR

To characterize the virulence genes, amplification products of *stx1*, *stx2, eae*, *hly* and *tccP* genes were used, which encode for Stx1, Stx2 toxins, intimin, enterohemolysin, and tir couple cytoskeleton protein respectively. The primers, conditions and references cited are listed in Table 3. Amplification of the targeted gene used EX Taq (TaKaRa, Dalian, China) with the following PCR program: 94 ℃ for 4 min, 30 cycles of denaturation at 94 ℃ for 30 s, annealing at 54 ℃ for 30s, and extension at 72 ℃ for 30 s, with a final extension at 72 ℃ for 10 min. Adjust annealing temperature according to primer Tm value (Table 3). The PCR amplicons (10 μl) were subjected to electrophoresis on a 1.2% agarose gel in 1× TAE buffer at 115 V for 30 min, and stained with SYBR Green (Fermentas, Germany).

Antimicrobial susceptibility tests

The susceptibility of to antibiotics was tested using the Kirby-Bauer disc diffusion technique. 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 *bla*$_{CTX-M-U}$ [27], and the entire CTX-M-type genes were amplified using the primers *bla*$_{CTX-M-1G}$ [27], *bla*$_{CTX-M-2G}$ [28] or *bla*$_{CTX-M-9G}$ [29]), *bla*$_{TEM}$ [30], and *bla*$_{SHV}$ [30] which encode β-lactamases; chloramphenicol (*cmlA1* [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 3). 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 gene.

Conjugation experiments and plasmid analysis

Sodium azide-resistant *E. coli J53* was used as a recipient and conjugated to a *bla*$_{CTX-M}$-producing isolate by filtration. Transconjugants were selected on Mac Conkey agar containing cefotaxime or ceftazidime (4 μg/ml) and sodium azide (200 μg/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 characterized by pulsed field gel electrophoresis (PFGE) using the CHEF-MAPPER System (Bio-Rad Laboratories, Hercules, CA, USA) as described by Gautom [35]. Briefly, chromosomal DNA of *E. coli* O157:H7 isolate was isolated and the inserts were digested with *Xba*I (TaKaRa Dalian, China) for 16 h at 37 ℃. The electrophoresis was performed at 6.0 V/cm for 18.5 h with an angle of 120º at 14℃. The pulse time was increased from 0.5 to 60 s. The Salmonella serotype Braenderup H9812 (ATCC BAA-664) was chosen as the molecular weight marker. Gels were then stained in ethidium bromide (1.0 mg/L). The results were interpreted according to the criteria of Tenover et al. [36].

Abbreviations

AMC: Amoxicillin-clavulanic acid

AMI: Amikacin

AMP: Ampicillin

ATM: Aztreonam,

CAZ: Ceftazidime

CHL: Chloramphenicol

CIP: Ciprofloxacin

CT-SMAC: Sorbitol MacConkey agar containing cefixime and potassium tellurite

CTX: Cefotaxime

E.coli: *Escherichia coli*

ESBLs: Extended Spectrum Beta-Lactamases

FEP: Cefepime

GEN: Gentamicin

IMS: immunomagnetic separation

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-sulfamethylisoxazole

TET: Tetracycline

TSB: Trypticase soya broth

TZP: Piperacillin-tazobactam

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.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

Funding for this study was provided by National Natural Science Foundation of China (31960695 and 31560485), 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.

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 date. P.P.T., L.N.X., and J.X.X. contributed to the writing of the manuscript. All authors read and approved the article.

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.

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 *t*ccP in clinical enterohemorrhagic and enteropathogenic *Escherichia coli* isolates. J Clin Microbiol. 2005;43:5715-20.
11. 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.
12. 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.
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. 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.
15. Ni DX, Wang H, Gu L, Guo XL, Zhuang L, Shi P, et al. Surveillance of *Escherichia coli* O157:H7 among animals in Jiangsu province in 1999. Zhonghua liuxingbingxue zazhi. 2002;23:102-4.
16. Ding H, Su ZQ, Xia LN, Wang YM, Wang D, Zhang JY, et al. Influence of immunomagnetic enrichment method for isolation and identification of *E.coli* O157:H7 from bovine. China Animal Husbandry & Veterinary Medicine. 2017;44: 1189-94. (In Chinese)
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, Geneve, 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, Herrmann 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 sulphona-mide 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. Gautom RK. Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other gram-negative organisms in 1 day. J Clin Microbiol. 1997; 35:2977-80.

36. 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

Sample place	*Farm*	*Sample source*	*Strain*	*Virulence genes*	*Collection time*
Yili	A	Feces	Y2-F25	*stx2*	2013.08
			Y2-F27	*stx2*	2013.08
	B	Feces	Y1-F166	*stx1+stx2+eae+hly+tccP*	2014.08
	C	Feces	Y3-F328	*stx2+eae+hly+tccP*	2014.08
	D	Rectalswab	Y4-A20-1	*stx1+stx2+eae+hly+tccP*	2015.09
			Y4-A20-2	*stx1+stx2+eae+hly+tccP*	2015.09
			Y4-A20-3	*eae*	2015.09
			Y4-A20-4	*stx1+stx2+eae+hly +tccp*	2015.09
			Y4-A20-5	*eae*	2015.09
			Y4-A41-2	*stx1+stx2+eae+hly+tccP*	2015.09
			Y4-A41-4	*stx1+eae+hly*	2015.09
			Y4-A103	*eae+hly*	2016.10
			Y4-A109	*stx1+stx2+eae+hly+tccP*	2016.10
	Slaughterhouse	Carcass swab	Y4-C21-1	*stx1+stx2+eae+hly+tccP*	2015.09
			Y4-C21-2	*stx1+stx2+eae+hly+tccP*	2015.09
Wulumuqi	E	Rectal swab	W2-A61-2	*stx2+eae+hly+tccP*	2016.10
			W2-A61-3	*stx2+eae+hly+tccP*	2016.10
			W2-A61-4	*stx2+eae+hly+tccP*	2016.10
			W2-A61-5	*stx2+eae+hly+tccP*	2016.10
	F	Feed	W1-E16	*stx1+eae+hly+tccP*	2016.03
			W1-E50-4	—	2017.01
			W1-E51-3	—	2017.01
			W1-E51-5	—	2017.01
Akesu	G	Feces	A2-F10	*stx2+eae+hly+tccP*	2012.10
			A2-F14	—	2012.10
	H	Feces	A1-F1	—	2015.09
			A1-F13	—	2015.09

—, No virulence genes were identified in this study.

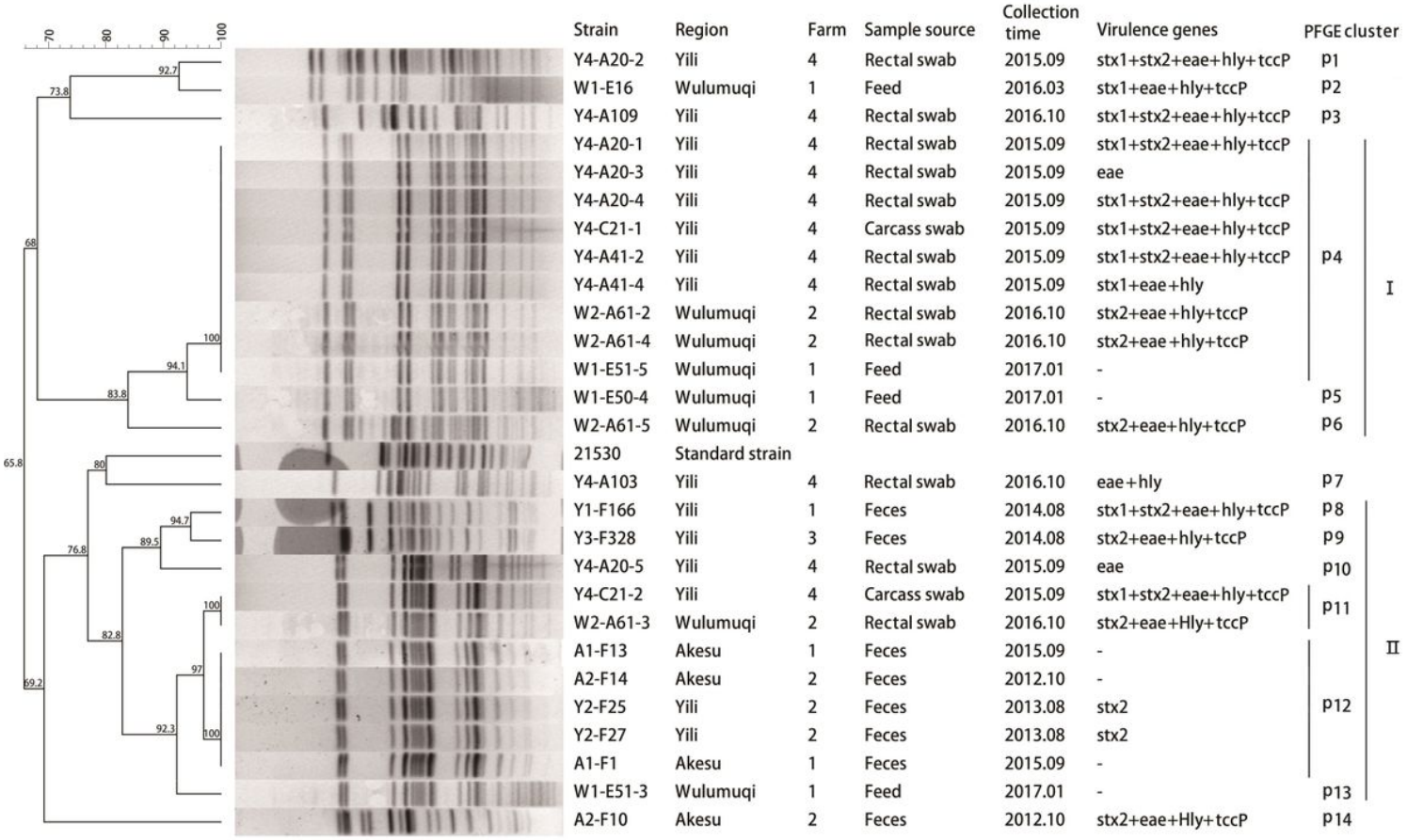
Table 2 Information on sample collection

Source of sample	*NO. of farms*	Feces	Rectal swab	Water	Feed	Carcass swab	*NO. of positive samples*	*NO. of positive farms*
Tacheng	2	134	0	0	0	0	0	0
Bole	1	43	82	10	6	0	0	0
Yili	4	480	397	42	29	0	8	4
	1 slaughterhouse	0	0	0	0	48	1	1
Wujiaqu	1	8	79	9	4	0	0	0
Changji	2	46	211	17	23	0	0	0
Wulumuqi	4	90	467	30	48	0	4	2
Akesu	4	354	0	0	0	0	4	2
Total		1155	1236	108	110	48	17	8

Table 3 Oligonucleotides of the various targeted genes

Target gene	Primer Sequence (5'-3') (Forward/reverse)	Amplicon size(bp)	Annealing Temp. (°C)	Reference
rfbE	ATTGCGCTGAAGCCTTTG/CGAGTACATTGGCATCGTG	500	54	This study
fliC	GCGCTGTCGAGTTCTATCGAGC/CAACGGTGACTTTATCGCCATTCC	625	58	[23]
*stx*1	GAAGAGTCCGTGGGATTACG/AGCGATGCAGCTATTAATAA	130	54	[24]
*stx*2	TTAACCACACCCACGGCAGT/GCTCTGGATGCATCTCTGGT	346	54	[24]
eae	CATTATGGAACGGCAGAGGT/ACGGATATCGAAGCCATTTG	375	52	This study
hly	CACACGGAGCTTATATTCTGTCA/AATGTTATCCCATTGACATCATTTGACT	319	45	[25]
tccP	CGCCATATGATTAACAATGTTTCTTCAC/CTCGAGTCACGAGCGCTTAGATGTATT	700~1000	58	This study
$bla_{\text{CTX-M-U}}$	ATGTGCAGYACCAGTAARGT/TGGGTRAARTARGTSACCAGA	593	50	[27]
$bla_{\text{CTX-M-1G}}$	GTTACAATGTGTGAGAAGCAG/CCGTTTCCGCTATTACAAAC	1018	50	[27]
$bla_{\text{CTX-M-2G}}$	ATGATGACTCAGAGCATTCG/TGGGTTACGATTTTCGCCGC	865	55	[28]
$bla_{\text{CTX-M-9G}}$	ATGGTGACAAAGAGAGTGCA/CCCTTCGGCGATGATTCTC	870	60	[29]
bla_{TEM}	ATGAGTATTCAACATTTCCGT/TTACCAATGCTTAATCAGTGA	861	48	[30]
bla_{SHV}	CCGGGTTATTCTTATTTGTCGCT/TAGCGTTGCCAGTGCTCG	1081	48	[30]
cmlA1	CCGCCACGGTGTTGTTGTTATC/CACCTTGCCTGCCCATCATTAG	698	59	[31]
sul1	CGGCGTGGGCTACCTGAACG/GCCGATCGCGTGAAGTTCCG	433	65	[32]
tetA	GCTACATCCTGCTTGCCTTC/CATAGATCGCCGTGAAGAGG	210	55	[33]
tetE	AAACCACATCCTCCATACGC/AAATAGGCCACAACCGTCAG	278	55	[33]
tetG	GCTCGGTGGTATCTCTGCTC/AGCAACAGAATCGGGAACAC	468	55	[33]

Figures



Strain	Region	Farm	Sample source	Collection time	Virulence genes	PFGE cluster	
Y4-A20-2	Yili	4	Rectal swab	2015.09	stx1+stx2+eae+hly+tccP	P1	
W1-E16	Wulumuqi	1	Feed	2016.03	stx1+eae+hly+tccP	P2	
Y4-A109	Yili	4	Rectal swab	2016.10	stx1+stx2+eae+hly+tccP	P3	
Y4-A20-1	Yili	4	Rectal swab	2015.09	stx1+stx2+eae+hly+tccP		
Y4-A20-3	Yili	4	Rectal swab	2015.09	eae		
Y4-A20-4	Yili	4	Rectal swab	2015.09	stx1+stx2+eae+hly+tccP		
Y4-C21-1	Yili	4	Carcass swab	2015.09	stx1+stx2+eae+hly+tccP		
Y4-A41-2	Yili	4	Rectal swab	2015.09	stx1+stx2+eae+hly+tccP	P4	
Y4-A41-4	Yili	4	Rectal swab	2015.09	stx1+eae+hly		I
W2-A61-2	Wulumuqi	2	Rectal swab	2016.10	stx2+eae+hly+tccP		
W2-A61-4	Wulumuqi	2	Rectal swab	2016.10	stx2+eae+hly+tccP		
W1-E51-5	Wulumuqi	1	Feed	2017.01	-		
W1-E50-4	Wulumuqi	1	Feed	2017.01	-	P5	
W2-A61-5	Wulumuqi	2	Rectal swab	2016.10	stx2+eae+hly+tccP	P6	
21530	Standard strain						
Y4-A103	Yili	4	Rectal swab	2016.10	eae+hly	P7	
Y1-F166	Yili	1	Feces	2014.08	stx1+stx2+eae+hly+tccP	P8	
Y3-F328	Yili	3	Feces	2014.08	stx2+eae+hly+tccP	P9	
Y4-A20-5	Yili	4	Rectal swab	2015.09	eae	P10	
Y4-C21-2	Yili	4	Carcass swab	2015.09	stx1+stx2+eae+hly+tccP	P11	
W2-A61-3	Wulumuqi	2	Rectal swab	2016.10	stx2+eae+Hly+tccP		
A1-F13	Akesu	1	Feces	2015.09	-		II
A2-F14	Akesu	2	Feces	2012.10	-		
Y2-F25	Yili	2	Feces	2013.08	stx2	P12	
Y2-F27	Yili	2	Feces	2013.08	stx2		
A1-F1	Akesu	1	Feces	2015.09	-		
W1-E51-3	Wulumuqi	1	Feed	2017.01	-	P13	
A2-F10	Akesu	2	Feces	2012.10	stx2+eae+Hly+tccP	P14	

Fig. 1 Dendrogram of *Xba*I pulsed-field gel electrophoresis profiles of O157:H7 isolates.

Figure 1

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