

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

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## Research article

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## 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 *stx1* 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 *stx1*, 8 (29.6%) possessed *stx2*, 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, *stx2* 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*<sub>CTX-M-121</sub> gene.

### Transferability of *bla*<sub>CTX-M</sub> genes and plasmid replicon typing

The *bla*<sub>CTX-M</sub> 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-sulfamethoxazole and tetracycline, and resistance to the

*bla*<sub>CTX-M-121</sub> gene from the *bla*<sub>CTX-M</sub>-producing O157:H7 isolate can be transferred to the recipient. The *bla*<sub>CTX-M-121</sub> 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 Ⅲ mainly includes type p4, and cluster Ⅳ 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 *stx2*-producing strains is higher than *stx1* 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 ( $bla_{CTX-M}$ )  $\beta$ -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 *tetA* 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  $\beta$ -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, Wujiaku, 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) (*rfaE* 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 °C for 4 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30s, and extension at 72 °C for 30 s, with a final extension at 72 °C 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*<sub>CTX-M</sub> (the CTX-M-type genes were detected using universal primers *bla*<sub>CTX-M-U</sub> [27], and the entire CTX-M-type genes were amplified using the primers *bla*<sub>CTX-M-1G</sub> [27], *bla*<sub>CTX-M-2G</sub> [28] or *bla*<sub>CTX-M-9G</sub> [29]), *bla*<sub>TEM</sub> [30], and *bla*<sub>SHV</sub> [30] which encode β-lactamases; chloramphenicol (*cmlA1* [31]) efflux pumps; sulfonamide resistance gene (*su1* [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*<sub>CTX-M</sub>-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-MAP-PER 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 °C. The electrophoresis was performed at 6.0 V/cm for 18.5 h with an angle of 120° at 14°C. 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.

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## **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.

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## Tables

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

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

—, No virulence genes were identified in this study.

**Table 2** Information on sample collection

<i>Source of sample</i>	<i>NO. of farms</i>	Feces	Rectal swab	Water	Feed	Carcass swab	<i>NO. of positive samples</i>	<i>NO. of positive farms</i>
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	0	0	0	0	48	1	1
	slaughterhouse							
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
<i>rfbE</i>	ATTGCGCTGAAGCCTTG/CGAGTACATTGGCATCGTG	500	54	This study
<i>fliC</i>	GCGCTGTCGAGTTCTATCGAGC/CAACGGTACTTATGCCATTCC	625	58	[23]
<i>stx1</i>	GAAGAGTCCGTGGATTACG/AGCGATGCAGCTATTAATAA	130	54	[24]
<i>stx2</i>	TTAACACCACCCACGGCAGT/GCTCTGGATGCATCTCTGGT	346	54	[24]
<i>eae</i>	CATTATGGAACGGCAGAGGT/ACGGATATCGAAGCCATTG	375	52	This study
<i>hly</i>	CACACGGAGCTTATATTCTGTCA/AATGTTATCCCATTGACATCATTGACT	319	45	[25]
<i>tccP</i>	CGCCATATGATTAACAATGTTCTTCAC/CTCGAGTCACGAGCGCTTAGATGTATT	700~1000	58	This study
<i>bla</i> <sub>CTX-</sub>	ATGTGCAGYACCAGTAARGT/TGGGTRAARTARGTSACCAGA	593	50	[27]
M-U				
<i>bla</i> <sub>CTX-</sub>	GTTACAATGTGTGAGAACAG/CCGTTCCGCTATTACAAAC	1018	50	[27]
M-1G				
<i>bla</i> <sub>CTX-</sub>	ATGATGACTCAGAGCATTG/TGGGTTACGATTTGCCGC	865	55	[28]
M-2G				
<i>bla</i> <sub>CTX-</sub>	ATGGTGACAAAGAGAGTGCA/CCCTCGGCGATGATTCTC	870	60	[29]
M-9G				
<i>bla</i> <sub>TEM</sub>	ATGAGTATTCAACATTCCGT/TTACCAATGCTTAATCAGTGA	861	48	[30]
<i>bla</i> <sub>SHV</sub>	CCGGGTTATTCTTATTGTCGCT/TAGCGTTGCCAGTGCTCG	1081	48	[30]
<i>cmlA1</i>	CCGCCACGGTGTGTTATC/CACCTGCCTGCCCATCATTAG	698	59	[31]
<i>sul1</i>	CGCGTGGGCTACCTGAACG/GCCGATCGCGTGAAGTTCCG	433	65	[32]
<i>tetA</i>	GCTACATCCTGCTTGCCTTC/CATAGATGCCGTGAAGAGG	210	55	[33]
<i>tetE</i>	AAACCACATCCTCCATACGC/AAATAGGCCACAACCGTCAG	278	55	[33]
<i>tetG</i>	GCTCGGTGGTATCTCTGCTC/AGCAACAGAACATGGGAACAC	468	55	[33]

## Figures

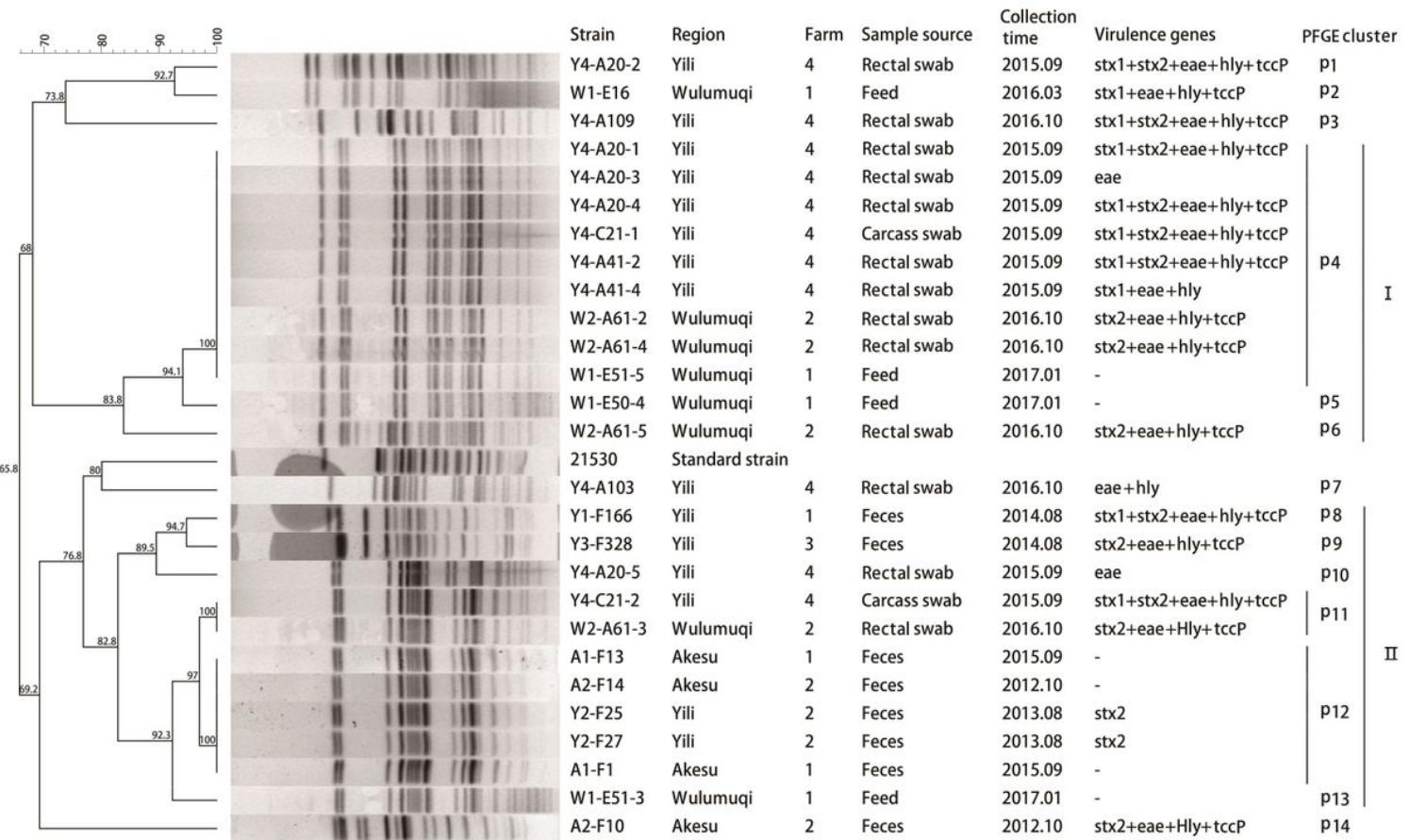


Fig.1 Dendrogram of *Xba*I pulsed-field gel electrophoresis profiles of O157:H7 isolates.

## Figure 1

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