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ORIGINAL ARTICLE

Epidemiological survey on *Escherichia coli* O157 in Chongqing and Three-Gorge Reservoir Areas of China

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Abstract Prevalence, presence of virulence and adherence associated genes, genetic diversity, biochemical characteristics, and antibiotic susceptibility were determined for *Escherichia coli* O157 isolated over 4 months in Chongqing city and Three-Gorge Reservoir Areas. 11 isolates of *E. coli* O157 were isolated from 1504 samples and 7 of them are O157:H7 and 4 are O157:H? All O157:H7 isolates had *eaeA*, *ehxA*, *EspA* and *Tccp* genes, but did not have *stx1* and *stx2*. All O157:H? isolates did not have *stx1*, *stx2*, *eaeA*, *ehxA*, *EspA* and *Tccp* genes except for the isolate obtained from Yunyang county which had *stx1*. When *eaeA* and *ehxA* presented in isolates were digested by restriction enzymes, the numbers and the sizes of the segments were the same as the control *E. coli* O157 strains. This suggests that *eaeA* and *ehxA* exhibit poor polymorphism. Most *E. coli* O157 isolates showed identical biochemical activities to the standard strains for sorbitol and rhamnose, and all *E. coli* O157:H7 obtained from feces at the same dairy cattle farm had similar biochemical characteristics. Antibiotic susceptibility demonstrated resistance of the isolates to penicillin, ampicillin, bacitracin, cefuroxime, erythromycin, gentamycin and tetracycline, indicating the isolates obtained in this study had a multi-drug resistance.

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Abbreviations

eaeA	attaching-and-effacing gene A subunit
EHEC	enterohaemorrhagic *Escherichia coli*
ehxA	enterohemolysin gene A subunit
EspA	*E. coli* secreted protein A subunit
HC	hemorrhagic colitis
HUS	hemolytic-uremic syndrome
mEC+n	modified *E.coli* broth supplemented containing 20 mgl^{-1} novobiocin
PCR-RFLP	polymerase chain reaction-restriction fragment length polymorphism
stx	Shiga toxin
STEC	Shiga toxin-producing *Escherichia coli*
Tccp	Tir to the actin-cytoskeleton

Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 is an important food-borne human pathogen in the world. Since it was recognized as a pathogen in 1982 in the United States (Riley et al. 1983), this serotype has been isolated in many countries (Zhao et al. 1995; Sekiya 1997; Bonardi et al. 1999; Chapman et al. 2001; Bruce et al. 2003; Rivas et al. 2006). In China, *E. coli* O157:H7 has caused two outbreaks in Jiangsu and Anhui provinces, and expanded to the Central, North and Southwest China in 1999 and 2000 (Chen et al. 2001; Ni et al. 2002; Ma et al. 2004; Zhu et al. 2004; Lu et al. 2005), respectively.

E. coli O157 might cause diarrhea, severe abdominal pain, hemorrhagic colitis (HC), and hemolytic-uremic syndrome (HUS) (Besser et al. 1993; Bruce et al. 2003; Salerno et al. 2004). The pathogenic factors of EHEC include Shiga toxins (Ludwig and Muller-Wiefel 1998), the chromosomal LEE locus that carries factors (eaeA, EspA) involved in the attaching and effacing process (Dean-Nystrom et al. 1998; Shaw et al. 2001; Neves et al. 2003), an EHEC O157:H7 type III effecter protein that couples Tir to the actin-cytoskeleton (Tccp) (Garmendia et al. 2004), and a large plasmid carrying the enterohemolysin genes (Schmidt et al. 1995).

Although some studies do not advise antibiotic treatment for infections caused by *E. coli* O157 in humans (Wong et al. 2000), others suggest that disease progression may be prevented by administrating antibiotics at an early stage (Shiomi et al. 1999; Pickering 2001). It is important to determine whether *E. coli* O157:H7 develops resistance to antibiotics in the animal reservoir and to determine if it is a possible source for the spread of resistant factors to other microorganisms in animal gastrointestinal tracts.

Chongqing is an upriver city along Yangtze River with a large population more than 30 million. If *E. coli* O157 would outbreak, not only would upriver water be polluted and the citizen's lives threatened, but also the whole Yangtze River would be polluted and the people living along Yangtze river be in danger. Therefore, it is important to carry on the epidemiological survey in Chongqing and Three-Gorge Reservoir Areas.

The purpose of this study is to collect epidemiological data of *E. coli* O157 isolated from domestic animals in Chongqing and Three-Gorge Reservoir Areas and to identify the biochemical characteristics and the antibiotic-sensitivity of the isolated bacteria. The study also aims to acquire the data of virulence and adherence associated genes in *E. coli* O157 and to investigate the level of polymorphism of the *E. coli* O157:H7.

Materials and methods

Study farms, abattoir and sampling

Fecal samples were collected directly from rectums of domestic animals and sewage samples were collected from hoggeries, according to the distributional characteristics of domestic animals in Chongqing. The places, dates, types of animals, and number of samples are listed in Table 1.

E. coli O157:H7 strains from other sources

The control *E. coli* O157 strain Sakai strain (O157-Sakai) was kindly provided by Prof. Huaiqi Jing (Chin. Center for Dis. Control and Prev.). *E. coli* O157:H7 44828 strain (O157–44828) by Dr Ming Zeng (Nat. Institute for the Cont Phar and Bio Pro). *E. coli* O157:H7 00B015 strain (O157–00B015) by Dr Hua Wang (Jiangsu CDC).

Table 1 The feces samples taken in Chongqing city and Three-gorge areas and isolates

Place	Sampling date	Farms	Types of animals	No. of samples	No. of isolates (O157:H?)	No. of isolates (O157:H7)
Hechuan	2005.3.6–2005.3.7	01–06	PN[a]	10		
			PD[b]	31		
			sow	16		
			pig	33		
Fuling	2005.3.2–2005.3.25	07–11	sow	65		
			PN	51		
			sewage	21		
			pig	12		
			PD	10		
Rongchang/Yongchuan	2005.4.1–2005.4.11	12	PD	29		
			PN	36		
			sow	110	1	
		Abattoir	pig	250	2	1
Yubei	2005.5.14–2005.5.15	13–17	DC[c]	300		6
Yunyang	2005.6.1–2005.6.14	18–24	goat	328		
		25–26	pig	202	1	
total			PN	97		
			PD	70		
			sow	191	1	
			pig	497	3	1
			MC	300		6
			goat	328		
			sewage	21		
			samples	1504	4	7

[a] PN: piglets (normal); [b] PD: piglets with diarrhea; [c] DC: dairy cattle

Primer pairs evaluating the presence of O157, H7, virulence and adherence associated genes

The primer pairs used to amplify *rfbE*, *fliC*, *stx1*, *stx2*, *eaeA*, *ehxA*, *EspA* and *Tccp* genes in this study were designed based on previous studies (Sandhu et al. 1996; Gannon et al. 1997; Boerlin et al. 1998; Desmarchelier et al. 1998; Louie et al. 1998; De Baets et al. 2004) and the sequences of the primers are shown in Table 2.

Isolation and identification of *E. coli* O157

During each farm visit, cottontipped-swabs were rubbed along the recto-anal mucosal surface. All samples were immediately transported to the laboratory in ice-cooled containers. Within 48 h of sampling, 1 g of feces from each sample was suspended in 20 ml of modified *E.coli* broth supplemented containing 20 mgl^{-1} novobiocin (mEC+n) and incubated for 6 h at 37°C (Vali et al. 2004). Following incubation, 20 μl of mEC+n was added to 980 μl of physiological saline solution (pH 7.2), and the mixture was plated on sorbitol MacConkey agar supplemented with cefixime (2.5 mgl^{-1}) and potassium tellurite (0.05 mgl^{-1}) with cottontipped-swabs and incubated for 18 h at 37°C. Five non-sorbitol-fermenting colonies were picked and plated onto triple sugar iron agar for 18 h at 37°C. Distinctive colonies were tested with anti-*E. coli* O157 and anti-*E. coli* H7 sera. Physiological saline solution was used as the negative control.

Detection of *rfbE* and *fliC* genes by PCR assay

E. coli O157 isolates were examined by polymerase chain reaction (PCR) assay to determine the presence of *rfbE* and *fliC* genes. Template DNA was prepared from pure culture of isolates, grown in LB for 18 h at 37°C. Three milliliters of culture was

Table 2 Primers used in multiplex PCR for amplification of O157, H7, virulence and adherence associated genes

Primers	Oligonucleotide sequence (5′-3′)	Amplicon size (bp)	Reference
rfbE-F	AAGATTGCGCTGAAGCCTTTG	497	Desmarchelier et al. 1998
rfbE-R	CATTGGCATCGTGTGGACAG		
fliC-F	GCAGCGAGCGAAGGTAGTGA	625	Gannon et al. 1997
fliC-R	CAGTCGCTGAAGCATACCCG		
stx1-F	ACACTGGATGATCTCAGTGG	614	Louie et al. 1998
stx1-R	CTGAATCCCCCTCCATTATC		
stx2-F	GGTCACTGGTTCGAATCCAGTAC	1400	De Baets et al. 2004
stx2-R	GGGATCCTGAATTGTGACACAGATTACACTTGTTAC		
eaeA-F	TCGTCACAGTTGCAGGCCTGGT	1110	Sandhu et al. 1996
eaeA-R	CGAAGTCTTATCCGCCGTAAAGT		
ehxA-F	CATCATCAAGCGTACGTTCC	2862	Boerlin et al. 1998
ehxA-R	ATGCTAATCGTTCATCACCT		
EspA-F	CTCGAGTTTACCAAGGGATATT	583	
EspA-R	CCATGGATACATCAAATGCAAC		
Tccp-F	CCATGGACAATGATTGTT	1014	
Tccp-R	CTCGAGGCAGCGAGATCT		

centrifuged at 8,000 rpm for 5 min, and the pellet was re-suspended in 0.2 ml ddH_2O. The suspension was heated at 100°C for 10 min and then centrifuged at 12,000 rpm for 5 min. The supernate was used for the PCR template. *Taq* polymerase (Promega, Madison, USA) was used in a total volume of 50 μl. The reaction was carried out in a Bio-Rad PCR system PTC-100 Peltier Thermal Cycler at 94°C for 5 min, with 35 cycles of 94°C for 30 s, 66°C for 30 s, and 72°C for 40 s, and final extension at 72°C for 6 min. PCR products were run on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV illumination.

Detection of virulence and adherence associated genes of *E. coli* O157

E. coli O157 isolates and three referenced strains were examined by PCR assay to determine the presence of Shiga toxin 1 and 2 genes (*stx1* and *stx2*), *E. coli* attaching-and-effacing gene A subunit *eaeA*, enterohemolysin gene A subunit *ehxA*, *E. coli* secreted protein A subunit *EspA*, and Tir-cytoskeleton coupling protein gene *Tccp*. Non-multiplex PCR was performed in a 20 μl final reaction volume containing 20 mM primers, 10 mM dNTPs, 20 mM 10×PCR buffer, 0.8 μl template DNA, 0.8 U Taq polymerase, and 15.6 μl ddH_2O. All strains were tested as described previously with primers specific to the genes encoding the *stx1* (Louie et al. 1998), *stx2* (De Baets et al. 2004), *eaeA* (Sandhu et al. 1996), and *ehxA* (Boerlin et al. 2004). *EspA* cycling condition was as follows: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 50 s; and final extension at 72°C for 6 min. *Tccp* cycling condition was as follows: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min; and final extension at 72°C for 6 min. PCR amplicons were run on a 1.5% agarose gel, strained with ethidium bromide, and visualized under UV illumination.

Analysis of EHEC O157:H7 by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

EaeA and *ehxA* of seven *E. coli* O157:H7 isolates were amplified by PCR as the above. PCR was performed in a 100 μl final reaction volume. Products of PCR were recycled by Purification Kit (Promega). 10 μl volume of the amplification products was digested with 5 to 10 U of restriction endonuclease for 6 h in a final volume of 20 μl. The enzymes *Hinc*II and *EcoR*II were used to digest the eae*A* amplification products, and the enzymes *Hae*III, *Hin*fI, *EcoR*II, and RsaI were used to digest the ehxA amplification products. The resulting DNA fragments were analyzed by electrophoresis in 2 to 4% agarose gels in TAE, and strained for 30 min in a 1 $\mu g ml^{-1}$ ethidium bromide solution. Restriction maps were constructed for each restriction pattern by using the known sequences of the eaeA and ehxA genes. The magnitude of nucleotide differences between pairs of isolates for the sequences under investigation was estimated by using the proportion of shared restriction sites.

Biochemical and motility assays

Biochemical assays of *E. coli* O157 isolates were completed with methyl red (MR), Voges-Proskauer (VP), indole, sorbitol, glucose, sulfureted hydrogen, urease, rhamnose, esculin, ONPG, DNA, dulcitol, maltose, phenylalanine deaminase, nitrate (dexidize), citrate, ornithine decarboxylase, arginine decarboxylase, and lysine decarboxylase. Motility was examined by agar stab method using motility GI medium. The results were recorded after incubating the samples for 48 h at 37°C (Tianhe, Hangzhou, China).

Antibiotic sensitivity testing

The susceptibilities of the *E. coli* O157 isolates to the different antimicrobial agents were tested using the disk-agar method standardized by the National Committee for Clinical Laboratory Standards (NCCLS 2002). The isolates were tested against 13 antibiotics (Tianhe) after incubating the samples for 24 h at 37°C. The antibiotics were penicillin, ampicillin, cefazolin sodium, cefuroxime, cefotaxime, streptomycin, gentamicin, erythromycin, bacitracin, sulfamethoxazole, tetracycline, chloramphenicol, and ofloxacin. The characterization of isolates as being susceptible, having intermediate susceptibility, or being resistant was recorded as recommended by NCCLS.

Results

Overall prevalence of *E. coli* O157-positive samples

From 1483 rectal fecal samples and 21 sewage samples, 11 isolates of *E. coli* O157 were isolated from feces and the proportion is 0.73%. The results are listed in Table 1. The number of individual positive samples from fecal samples of dairy cattle, pigs, sows, piglets with diarrhea, normal piglets, and goats were 6 (0.40%), 4 (0.27%), 1 (0.07%), 0, 0, and 0, respectively. The *E.coli* O157 isolates were isolated from four different counties in Chongqing city from April to June. In March, no *E. coli* O157 was isolated. Of the 300 fecal samples of dairy cattle, the number of positive samples of *E. coli* O157:H7 is 6 (2%); 1 *E. coli* O157:H7 isolate (0.20%) and 3 *E. coli* O157:H? isolates (0.60%) were obtained from 497 pig fecal samples; 1 *E. coli* O157:H? isolate (0.52%) was isolated from 191 sow fecal samples. No O157 isolate was detected in fecal samples of 70 piglets with diarrhea, 97 normal piglets and 328 goats.

Also, no O157 isolate was detected in the samples collected from Hechuan county and Fuling district. Six O157:H7 isolates were isolated from the same dairy cattle farm in Yubei district. One O157:H7 and three O157:H? isolates were isolated in the fecal samples of pigs from Rongchang and Yongchuan counties. In Yunyang county, one O157:H? was isolated from a hoggery, but no O157 was isolated from goats.

Genes of O157 *rfbE* and H7 *fliC*

E. coli O157 isolates were confirmed by monoclonal antibodies and PCR assay. All isolates obtained from Yubei district were positive for *rfbE* and *fliC*. Four O157 isolates from Rongchang and Yongchuan counties were serotype O157, including 1 O157:H7 isolates and 3 O157:H? isolates. The isolate obtained from Yunyang county was positive for *rfbE* but negative for *fliC* (Fig. 1).

Presence of virulence and adherence associated genes

Genetic profiling for *E. coli* O157 virulence and adherence associated genes such as *stx1*, *stx2*, *eaeA*, *ehxA*, *EspA*, and *Tccp* was performed by PCR. The results are listed in Table 3. All *E. coli* O157 isolates were negative for *stx2*. All *E. coli* O157:H7 isolates were positive for *eaeA*, *ehxA*, *EspA*, and *Tccp* except for *stx*. All *E. coli* O157:H? isolates were negative for all virulence and adherence associated genes except for one isolate obtained from Yunyang county carried *stx1*.

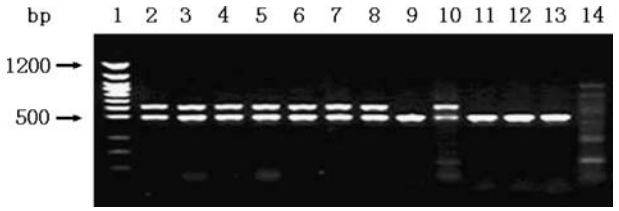

Fig. 1 Products on gel after multiplex PCR with *E. Coli* O157 strains. Lane 1: 100 bp DNA ladder marker. Lane 2: O157-Sakai. Lane 3–8: isolates obtained from Yubei. Lane 9: isolate obtained from Yunyang. Lane 10–13: isolates obtained from Rongchang and Yongchuan. Lane 14: *E. Coli* DH5α

As positive controls, all 6 genes were amplified for O157-Sakai, O157–44828 and O157–00B015 and the PCR products of the 6 genes were obtained from three strains except for the O157–00B015 that is negative for *stx1* and *stx2*. As negative control, no PCR product was amplified for *E. coli* DH5α. These results show that PCR was sensitive and specific for genic identification.

PCR-RFLP of *eaeA* and *ehxA* genes

After the PCR products of the *eaeA* genes were digested using the restriction enzymes *Hinc*IIand *EcoR*II, same restriction types were identified for 7 O157:H7 isolates and 3 control strains in this study. Three bands were obtained by electrophoresing the digested solutions because electrophoresis did not show small fragments less than 41 bp. These observations were consistent with the expected results. Similarly, we also examined the restriction types of the PCR products of the *ehxA* genes for 7 O157:H7 isolates by using the restriction enzymes *Hae*III, *Rsa* I and *EcoR*II, and our observations were in line with the results that the standard strains were digested with the same enzymes.

E. coli O157's biochemical characteristics

All isolates could ferment glucose, urease, ONPG, maltose, ornithine decarboxylase, arginine decarboxylase, lysine decarboxylase, MR, and indole, however, they could not ferment sulfureted hydrogen, esculin, phenylalanine deaminase, nitrate (dexidize), and VP

Table 3 The *stx1*, *stx2*, *eaeA, ehxA, EspA* and *Tccp* genes detected in the O157 strains isolated from Chongqing city and Three-gorges Reservoir Areas

origen of sample	Serotype	No. of isolates	PCR characterization					
			stx1	*stx2*	*eaeA*	*ehxA*	*EspA*	*Tccp*
Hechuan								
Fuling								
Rongchang\	O157:H7	1			1	1	1	1
Yongchuan	O157:H?	3						
Yubei	O157:H7	6			6	6	6	6
Yunyang	O157:H?	1	1					
total	O157:H7	7			7	7	7	7
	O157:H?	4	1					

(Table 4). Isolates obtained from Yubei district could not ferment sorbitol, rhamnose, citrate and DNA but could ferment dulcitol. Similarly, of the isolates obtained from Rongchang and Yongchuan counties, all could ferment dulcitol and citrate; one could ferment sorbitol at 37°C for 24 h, two for rhamnose and three for DNA.

Susceptibility of *E. coli* O157 to antibiotics

Eleven *E. coli* O157 isolates were tested for the susceptibility to antibiotics. All isolates were resistant to penicillin, ampicillin, and bacitracin. Most were sensitive to cefazolin (72.7%), cefotaxime (90.9%), chloramphenicol (72.7%), streptomycin (72.7%) and ofloxacin (72.7%) (Table 5). Five isolates (45.5%) were sensitive to cefuroxime, three (27.3%) were sensitive to gentamicin, six (54.5%) were sensitive to sulfamethoxazole, and only one (9.1%) was sensitive to tetracycline.

Isolates obtained from Yubei were found to be sensitive to cefotaxime, streptomycin, sulfamethoxazole, chloramphenicol and ofloxacin, and isolates obtained from Rongchang and Yongchuan counties were resistant to both sulfamethoxazole and tetracycline. One isolate obtained from Yunyang county in an enclosure with pigs on a farm was sensitive to cefazolin sodium, cefuroxime, cefotaxime and ofloxacin.

Discussion

E. coli O157 has been prevalent in the United States (Zhao et al. 1995), Japan (Sekiya 1997), Netherlands (Schouten et al. 2005), Italy (Bonardi et al. 1999), Switzerland

Table 4 Biochemical characteristics of the isolated bacteria

Biochemical reagent	Yubei		Rong/Yong		Yunyang	
	P[a]	N[b]	P	N	P	N
MR	6	0	4	0	1	0
indole	6	0	4	0	1	0
glucose	6	0	4	0	1	0
urease	6	0	4	0	1	0
ONPG	6	0	4	0	1	0
maltose	6	0	4	0	1	0
ornithine decarboxylase	6	0	4	0	1	0
arginine decarboxylase	6	0	4	0	1	0
lysine decarboxylase	6	0	4	0	1	0
VP	0	6	0	4	0	1
sulfureted hydrogen	0	6	0	4	0	1
esculin	0	6	0	4	0	1
phenylalanine deaminase	0	6	0	4	0	1
nitrate(dexidize)	0	6	0	4	0	1
motility	6	0	0	4	1	0
dulcitol	6	0	4	0	0	1
sorbitol	0	6	1	3	1	0
rhamnose	0	6	2	2	0	1
citrate	0	6	4	0	0	1
DNA	0	6	3	1	1	0

[a] :positive; [b] :negative

Table 5 Antimicrobial susceptibility to antibiotics for all *E. coli* O157 isolates

antibiotic	Yubei			Rong/Yong			Yunyang		
	S[a]	I[b]	R[c]	S	I	R	S	I	R
penicillin	0	0	6	0	0	4	0	0	1
ampicillin	0	0	6	0	0	4	0	0	1
cefazolin sodium	5	0	1	2	0	2	1	0	0
cefuroxime	3	1	2	2	2	0	1	0	0
cefotaxime	6	0	0	3	1	0	1	0	0
streptomycin	6	0	0	2	0	2	0	1	0
gentamicin	2	4	0	1	3	0	0	1	0
erythromycin	0	6	0	0	2	2	0	0	1
bacitracin	0	0	6	0	0	4	0	0	1
sulfamethoxazole	6	0	0	0	1	3	0	0	1
tetracycline	1	5	0	0	1	3	0	0	1
chloramphenicol	6	0	0	2	0	2	0	0	1
ofloxacin	6	0	0	1	0	3	1	0	0

[a]: susceptibility; [b]: intermediate susceptibility; [c]: resistance.

(Kaufmann et al. 2006), Britain (Chapman et al. 2001), Argentina (Rivas et al. 2006). Ruminants are the primary reservoir and the main sources of human infection, though pig, poultry, and wild birds have also been shown to transiently harbor O157 naturally (Zhao et al. 1995; Wallace et al. 1997; Kang et al. 2004; Schouten et al. 2005; Kaufmann et al. 2006). The results obtained from the survey on dairy cattle from Yubei district in this study showed that cattle is one of the main reservoirs of *E. coli* O157. In Jiangsu and Anhui provinces, cattle were the highest carrier of isolates (Ni et al. 2002; Lu et al. 2005). In some regions, however, other animals might be the sources of outbreaks. The survey on an outbreak in Sui county of Henan province in 2000 indicated that Boer goats introduced from foreign country had a higher level of positive samples 29.8% than the level of local goats which yielded 14.8% positive samples (Ma et al. 2004). It is obvious that goats were the reservoir of the outbreak.

Based on the survey on *E. coli* O157 in Chongqing city and Three-Gorge Reservoir Areas, we found that dairy cattle had higher positive samples (2%) than pigs which yielded 0.47% positive samples. However, *E. coli* O157 was not detected in 328 fecal samples of goats. Therefore, it is possible that dairy cattle were the main reservoir of *E. coli* O157 in Chongqing city and Three-Gorge Reservoir Areas. The majority of dairy cattle were fed in the city zone where it is highly populous. So, it is necessary to enhance the management of excrementitious disposal and the transportation and pasteurization of milk.

Hog industry is a very important part of stockbreeding in Chongqing, and pigs have also been shown to harbor O157 naturally. So, we considered pigs as the key target of this survey. One O157:H? was isolated in a fecal sample of sow but no *E. coli* O157 was isolated in fecal samples of piglets at a hoggery in Rongchang county. The possible reasons for this are the protection of the maternal antibody or the use of antibiotics that inhibit the bacteria which cause yellow scour of newborn piglets and white scour of piglets. It could be proved by the susceptibility to antibiotics of the isolates obtained in Rongchang and Yongchuan counties. In the abattoir, pigs came from diverse sources, and had a high flow rate. These are potential factors to cause the prevalence of *E. coli* O157. It is important to enhance the management of the feces in abattoirs.

The presence of *stx1*, *stx2* and *eaeA* in *E. coli* O157:H7 strains isolated from cattle has been investigated all over the world and studies performed in the USA, Japan, Taiwan of China, and the European countries have revealed that the percentage of the verotoxigenic strains varied from 0 to 100%. *Stx2* and *eaeA* genes were found to be more frequent than *stx1* in most of the studies performed in these countries (Chapman et al. 1994; Zhao et al. 1995; Sekiya 1997; Bonardi et al. 1999; Galland et al. 2001; Omisakin et al. 2003;). Some research carried out in China showed that the positive rates of *stx2* were higher in the areas where the outbreak occurred or sporadic cases existed (Guo et al. 2002; Yang et al. 2002; Zhu et al. 2004). During the outbreak in Sui county of Henan province in 2000, all *E. coli* O157:H7 isolated from patients, domestic animals, and poultry carried the gene *stx2* but not the gene *stx1* (Ma et al. 2004). In the areas where outbreak did not occur or sporadic cases did not exist, isolates did not carry *stx2*, or only a few carried the *stx2* gene (Gong et al. 2004; Zhu et al. 2005). A study carried out by Liu et al showed 5 *E. coli* O157 strains isolated from Chongqing urban district lacked the gene *stx2* (Li et al. 1999). This, together with our observation that all O157:H7 isolates had *eaeA*, *ehxA*, *EspA*, and *Tccp* genes and all O157:H? isolates did not have virulence and adherence associated genes except one isolate carried the gene *stx1*, suggested that the *E. coli* O157 isolated in Chongqing and Three-Gorge Reservoir Areas might lack the *stx2* gene.

The enzymes *Hinc*II and *EcoR*IIwere used to digest the eae*A* amplification products, and the enzymes *Hae*III, HinfI, *EcoR*II, and *Rsa*I were used to digest the ehxA amplification products. Then we could find that restriction endonuclease sites of *eaeA* and *ehxA* are conserved and that the digested fragments have the same profile as the standard isolates. In a study, Boerlin et al also did not find the polymorphism of *eaeA* and *ehxA* by PCR-RFLP (Boerlin et al. 1998). All results demonstrated that the *eaeA* and *ehxA* in Shiga toxin-producing *Escherichia coli* (STEC) were highly conserved. Seven O157:H7 isolates identified in this study showed the same genotype for the virulence factors, suggesting that *E. coli* O157:H7 has poor polymorphism.

Most of *E. coli* O157 isolates showed significantly and divergently biochemical activities from classical *E. coli* for sorbitol and rhamnose (Karch et al. 1993; Hayes et al. 1995;). *E. coli* O157:H7 obtained from feces at the same diary cattle farm had similar biochemical characteristics, and was identical to standard strains in main biochemical characteristics, especially for sorbitol and rhamnose. O157 isolates, including one O157:H7 and four O157:H?, obtained from fecal samples of pigs at different hoggeries had a diverse biochemical characteristics, especial for two isolates that could ferment sorbitol.

Some studies showed many *E. coli* O157 strains isolated from humans and cattle had high rates of resistance to ampicillin (2 to 48%), tetracycline (4 to 27%), sulfamethoxazole (10 to 26%), streptomycin (2%), and chloramphenicol (<2%) (Galland et al. 2001; Schroeder et al. 2002). In the survey on the tolerances of 63 STEC strains to 15 antimicrobial agents, Khan et al (2002) found that most strains could tolerate ampicillin (25.4%), tetracycline (23.8%), and streptomycin (14.3%) and a few could tolerate cephalothin (11.1%), co-trimoxazole (9.5%), nalidixic acid (6.4%), and neomycin (3.2%). More than one-third of the strains (35%) showed resistances to all antimicrobial agents to some extent but were not completely resistant to any of the antibiotics; 14.3% of the strains were sensitive to all the antibiotics; multi-drug resistance was found in 14 strains and there was no common resistance pattern among the strains. Our observations are consistent with these results. Furthermore, our results showed that the strains isolated in this study were resistant to ampicillin, bacitracin, sulfamethoxazole and tetracycline extensively and less resistant to cephalosporins, which is consistent with previous investigations in China (Zhu et al. 2005). This is probably due to the fact that old antibiotics were widely used in

Chinese farms while use of some new drugs is still limited because of their expensive cost and because they have not been recognized. Many strains that were sensitive to antibiotics might evolve to the resistances and the isolates obtained in this study had a wide resistance to antibiotics This may result from small diverse farms and abuse of antibiotics. In addition, the selection pressure for the use of combined antibiotics in domestic animal breeding might be responsible for the development of the resistances.

The positive rates of *E. coli* O157 isolated from domestic animals in Chongqing city and Three-Gorge Reservoir Areas is low. All the O157:H7 isolates carried the *eaeA*, *ehxA*, *EspA*, and *Tccp*, and all the O157 isolates lacked *stx* that is closely associated with pathogenesis. So, it is impossible to lead to an outbreak of *E. coli* O157 under the lack of the necessary condition. However, it is possible that same other STEC transfer *stx2* to *E. coli* O157. In addition, the positive rates of *E. coli* O157 in animals may increase under certain circumstance. Thus, *E. coli* O157 may break out. So, it is important to do epidemiological survey on *E. coli* O157 periodically.

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