Ann Microbiol
DOI 10.1007/s13213-015-1037-x

ORIGINAL ARTICLE

Prevalence and characterization of *Escherichia coli* O157 and O157:H7 in retail fresh raw meat in South China

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Received: 29 October 2014 / Accepted: 7 January 2015

Abstract *Escherichia coli* O157 is an important food-borne pathogen that can cause diarrhoea, haemorrhagic colitis, and haemolytic uraemic syndrome. The aim of this study was to investigate the prevalence, virulence genes, antibiotic resistance, and genetic diversity of *E. coli* O157 and O157:H7 in retail fresh raw meat sold in the markets of South China. Of 551 samples collected, 21 (3.81 %) were contaminated with *E. coli* O157 and seven (1.27 %) with O157:H7. The highest prevalence rate was found in beef (13.32 %), followed by pork (6.90 %), chicken (3.28 %), duck (2.54 %), and mutton (0). The virulence genes *stx1*, *stx*2, *eaeA*, and *hlyA* were detected in 10.71, 21.43, 85.71, and 25 % of 28 isolates, respectively. The isolates were highly resistant to penicillin (100 %), chloramphenicol (64.29 %), ampicillin (57.14 %), and sensitive to gentamicin (100 %), cefotazidime (96.43 %), and ciprofloxacin (96.43 %). Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) classified 28 isolates and two reference strains into 19 different profiles with a discrimination index (D) of 0.961. Four *E. coli* O157:H7 isolates from beef showed 83 % similarity with the two clinical reference strains, indicating a potential high virulence for consumers. The results of this study suggested that fresh raw meat could be potential vehicles for transmission of *E. coli* O157 to humans.

Keywords Prevalence · Virulence genes · Antimicrobial resistance · Enterobacterial repetitive intergenic consensus-PCR · *Escherichia coli* O157 · O157:H7

Introduction

Escherichia coli O157 is an important food-borne pathogen that can cause diarrhoea, haemorrhagic colitis (HC), and haemolytic uraemic syndrome (HUS) in humans (Bettelheim and Beutin 2003; Caro et al. 2006). *E. coli* O157:H7 is the predominant serotype causing severe human infections. Cattle and meat products are considered to be the main reservoir of *E. coli* O157 worldwide (Gyles 2007). However, the organism has also been isolated from other animal meat products such as chicken, pork, and lamb (Doane et al. 2007; Lenahan et al. 2007; Ateba and Mbewe 2011). Consumption of raw or undercooked contaminated meat is one of the most common means of transmitting this organism to humans, and meat and meat products have been implicated in outbreaks of *E. coli* O157:H7 in different parts of the world (Bell et al. 1994; Abong'o and Momba 2009).

The pathogenicity of *E. coli* O157 and *E. coli* O157:H7 is associated with several virulence factors. Shiga toxins 1 and 2 (encoded by *stx1* and *stx2* genes) are the most important virulence factors, and play a major role in the pathogenesis of HC and HUS (Abu-Ali et al. 2009; Seker et al. 2010). Besides shiga toxins, intimin (encoded by bacterial *eaeA* gene) and enterohaemolysin (encoded by *E-hlyA* genes) are also two important virulence factors. Intimin was found to be responsible for attachment of the bacteria to the intestinal epithelial cells, causing attaching and effacing (A/E) lesions in the intestinal mucosa (Dean-Nystrom et al. 1997). Enterohaemolysin has been demonstrated to cause enterocyte and leukocyte lysis in cattle (Bauer and Welch 1996). The

Electronic supplementary material The online version of this article (doi:10.1007/s13213-015-1037-x) contains supplementary material, which is available to authorized users.

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Published online: 23 January 2015

high virulence combined with the low infection dose make infections in humans particularly severe.

Studies revealed an increasing antibiotic resistance of *E. coli* O157 and O157:H7 in animals and meat (Magwira et al. 2005; Govaris et al. 2011). The presence of antibiotic-resistant strains in meat may represent a threat to human health because such strains can be transmitted to reach humans through the consumption of contaminated meat. Therefore, surveillance of antimicrobial resistance in *E.coli* O157 is very important for preventing the spread of antimicrobial resistance in organisms and future disease management.

Molecular typing is a useful tool for determining the genetic relationship of food-borne bacteria and identifying probable sources of infections. Among the various typing methods, pulsed field gel electrophoresis is considered to be the gold standard for fingerprinting *E. coli* O157 strains due to its high discriminatory ability (Swaminathan et al. 2001). However, this assay is labour-intensive and time-consuming. In contrast, enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) is a relatively simple and cost-effective method, which has been successfully used for genotyping of different bacterial pathogens and for tracking the bacterial source of contaminated food products (Ye et al. 2009; Chen et al. 2014).

In China, the prevalence, virulence genes, and antimicrobial resistance of *E. coli* O157 in retail fresh raw meat sold in the markets in South China has not been thoroughly investigated. Furthermore, the genetic diversity of *E. coli* O157 isolates from meat samples is unknown. The aim of this study was to investigate the presence of *E. coli* O157 and O157:H7 strains in retail fresh raw meat sold in South Chinese markets, examine the presence of *stx1*, *stx2*, *eaeA*, and *hlyA* genes, evaluate the antimicrobial resistance of the isolates, and further analyze the genetic diversity of the isolates by ERIC-PCR.

Material and methods

Sample collection

From February 2013 to January 2014, a total of 551 fresh raw meat samples, including 68 beef, 183 chicken, 118 duck, 37 mutton, and 145 pork samples, were purchased randomly from open markets and supermarkets located in four provinces (14 cities) of South China. The geographic locations of the samples sites are shown in Figure S2. Samples consisted of cut meat aseptically removed from animal carcasses (legs, neck, shoulder, breast) and minced meat. From each city, 35 to 45 samples (about 500 g per sample) were randomly collected, placed in separate sterile plastic bags to prevent spilling and cross contamination and then immediately transported to the laboratory in a cooler with ice packs and processed within 4 h.

Isolation and biochemical identification of *E. coli* O157

Escherichia coli O157 was isolated from meat samples using the USDA method with slight modifications (USDA 2002). Briefly, 25 g of each sample was placed into 225 ml of modified EC broth supplemented with 20 mg/l novobiocin (Merck, SA), homogenized for 2 min at 260 rpm using a Stomacher (Model 400 circulator, Seward Medical, Ltd., UK), and incubated for 18–24 h at 41.5 °C. The enrichment cultures were subjected to immunomagnetic separation (IMS) using beads coated with antibodies against *E. coli* O157 (Dynal, Oslo, Norway) according to the manufacturer's instructions. After IMS, a 50 μl suspension obtained from IMS was streaked onto CHROMagar O157 (CHROMagar Microbiology, France). Plates were incubated at 36 °C±1 °C for 18–24 h. Up to eight presumptively positive colonies were selected, and sub-cultured onto nutrient agar plates (Huankai, Ltd, Guangzhou, China). The isolates were identified using API 20E test strips (bioMérieux, Marcy I'Etoile, France) and by performing conventional biochemical tests, including triple sugar iron agar, sorbitol fermentation, and indole tests. All presumptive colonies were further tested using *E. coli* O157 latex agglutination (Tianrun Ltd., Ningbo, China).

Identification of *E. coli* O157 and O157:H7 by multiplex PCR

The agglutinating colonies were confirmed by multiplex PCR as described previously (Xu and others 2008). Bacterial DNA was extracted from isolates using a commercial DNA extraction kit (Dongsheng Biotech, Guangzhou, China) according to the manufacturer's instructions. The primers and PCR conditions are shown in Table 1.

PCR amplification was performed in a 25 μl reaction mixture consisting of 2.5 μl of 10× PCR buffer (500 mM KCl, 200 mM Tris–HCl), 14.9 μl of sterile distilled water, 2.5 μl of 0.2 mM dNTPs, 3.0 μl of 25 mM $MgCl_2$, 0.5 μl of 10 mM of each primer, 0.1 μl of 10 U of Taq DNA polymerase (Promega, WI, USA), and 1 μl of DNA template. The thermal cycling started with an initial incubation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 59 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. *E. coli* O157:H7 ATCC 35150 was included as positive controls, and distilled water was used as a negative control. Amplification was performed with a DNA Thermal Cycler (Applied Biosystems, CA, USA). The amplified products were then analysed by electrophoresis in a 1.2 % agarose gel containing Gold View (SBS Genetech, Beijing, China); the bands were visualized using ImageQuant 350 Capture (GE Healthcare, WI, USA).

Table 1 PCR primers for virulence gene detection

Target gene	Primer sequence (5′-3′)	Annealing temperature (°C)	Size (bp)	Reference
fliC$_{h7}$	GCGCTGTCGAGTTCTATCGAGC CAACGGTGACTTTATCGCCATTCC	59	625	Xu et al. 2008
*rfbE*O157	GGATGACAAATATCTGCGCTGC GGTGATTCCTTAATTCCTCTCTTTCC	59	291	
eaeA	GACCCGGCACAAGCATAAGC CCACCTGCAGCAACAAGAGG	60	384	Paton and Paton 1998
hlyA	GCATCATCAAGCGTACGTTCC AATGAGCCAAGCTGGTTAAGCT	60	534	
stx1	TGTAACTGGAAAGGTGGAGTATAC GCTATTCTGAGTCAACGAAAAATAA	64	210	Sarimehmetoglu et al. 2009
stx2	ATGAAGTGTATATTTAAATGG TCAGTCATTATTAAACTGCAC	49	1200	Bai et al. 2010

Determination of virulence genes by PCR assay

All the isolates were screened for the presence of *stx1*, *stx2*, *eaeA*, and *hlyA* genes using PCR methods previously described (Paton and Paton 1998; Sarimehmetoglu et al. 2009; Bai et al. 2010). The primers and PCR reaction conditions are listed in Table 1.

Bacterial DNA extraction for isolated strains was conducted as described above. *E. coli* O157:H7 ATCC 35150 (*eaeA*$^+$, *hlyA*$^+$, *stx1*$^+$, *stx2*$^+$) was included as a positive control, and distilled water was used as a negative control. The PCR products were separated by electrophoresis with 1.2 % agarose gel at 120 V for 20 min in Tris-acetate buffer. The bands were visualized using ImageQuant 350 Capture (GE Healthcare, WI, USA).

Antimicrobial resistance test

The antibiotic resistance of *E. coli* O157 and O157:H7 isolates was determined using the disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI 2006). Muller-Hinton agar and 13 antimicrobial agents were used for the assay. The 13 antibiotics (Oxoid, Hampshire, UK) tested were as follows: amikacin (30 μg), ampicillin (10 μg), cefotaxime (30 μg), ceftazidime (10 μg), cephalothin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), kanamycin (30 μg), norfloxacin (10 μg), penicillin (10 U), streptomycin (10 μg), and tetracycline (30 μg). The isolates were classified as sensitive, intermediate, and resistant using the breakpoints specified by the NCCLS, and *E. coli* ATCC 25922 was used as the reference strain.

Genetic typing of *E. coli* O157 and O157:H7 by ERIC-PCR

For ERIC-PCR, the primers ERIC-1 (5′-ATGTAAGCTCCT GGGGATTCAC-3′) and ERIC2 (5′-AAGTAAGTGACTGG GGTGAGCG-3′) were used (Versalovic et al. 1991). The PCR was performed in a 25 μl solution containing 1.0 U of Taq DNA polymerase (Dongsheng), 1.0 μM of each primer, 2.5 mM $MgCl_2$, 0.2 mM each dNTP, and 40 ng of template genomic DNA. Amplifications were performed with a DNA Thermal Cycler (Applied Biosystems, CA, USA) with the following temperature profile: an initial denaturation at 94 °C for 3 min; 35 cycles each consisting of 1 min at 94 °C, 1 min at 52 °C, 3 min at 72 °C; and a final extension at 72 °C for 10 min. The ERIC-PCR products were separated by performing electrophoresis by using a 2.0 % agarose gel with GoldView stain (0.005 %, v/v), and the gel was photographed using an UV Imaging System (GE Healthcare, WI, USA). The images were captured in TIFF file format for further analysis. Cluster analysis was performed with NTSYS-pc (Version 2.10). The index of discriminatory (DI) ability was calculated as described by Hunter and Gaston (1988).

Results and discussion

Prevalence of *E. coli* O157 and O157:H7 in meat samples

Of 551 meat samples collected, 21 (3.81 %) were contaminated with *E. coli* O157 and seven (1.27 %) with O157:H7 (Total 5.08 %, Table 2). *E. coli* O157 was isolated from four beef, six chicken, three duck, and eight pork samples; whereas, *E. coli* O157:H7 was isolated from five beef and two pork samples. The highest prevalence of *E. coli* O157 and O157:H7 was found in beef (13.32 %), followed by pork (6.90 %), chicken (3.28 %), duck (2.54 %), and mutton (0 %). Previous studies conducted in various parts of the world showed that the prevalence of *E. coli* O157 or *E. coli* O157:H7 was 2.1–28 % in beef (Jo et al. 2004; Stampi et al. 2004; Doane et al. 2007; Olatoye 2010; Rahimi et al. 2012), 1.7–4.8 % in sheep or goat meat (Hiko et al. 2008; Rahimi et al. 2012), 0–0.9 % in chicken (Jo et al. 2004; Doane et al.

Table 2 Prevalence of *E. coli* O157 and O157:H7 in beef, chicken, duck, mutton and pork samples in South China

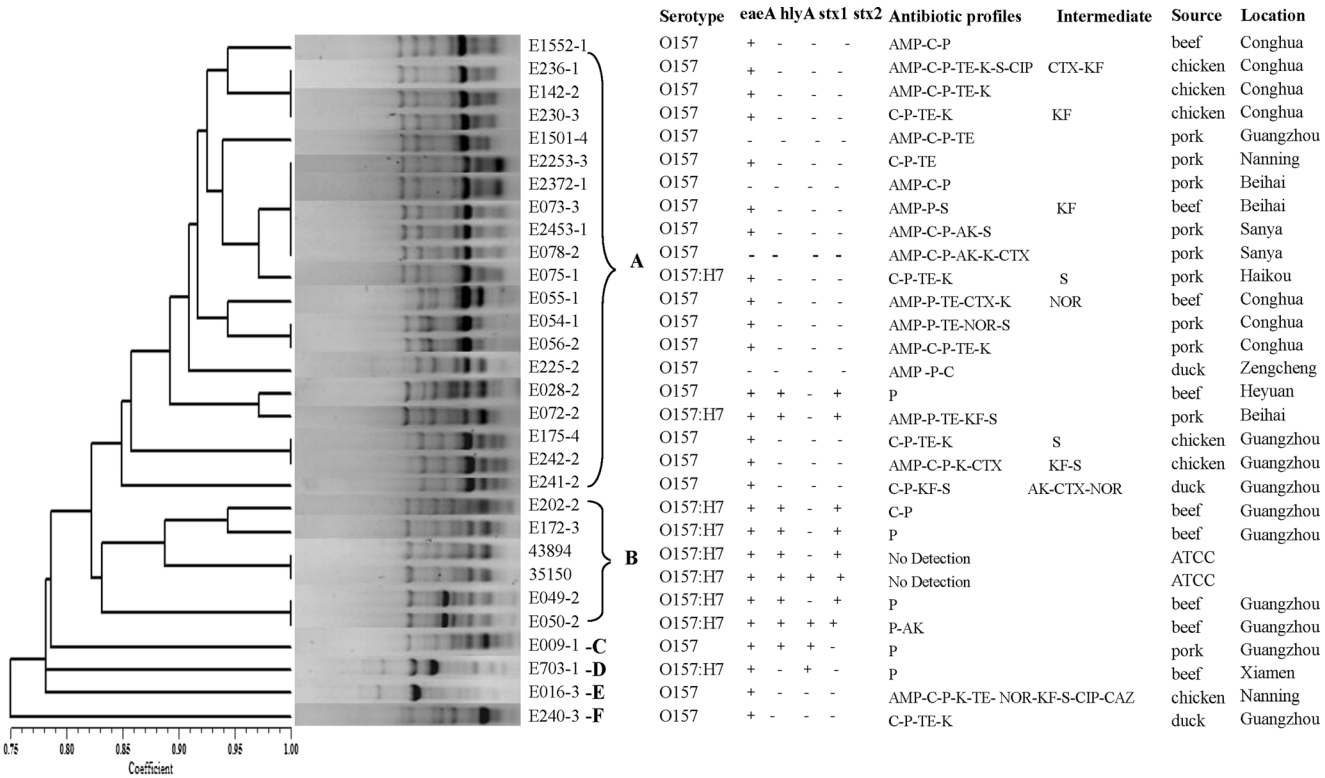
Sample	No. of samples examined	No. of *E. coli* O157 positive sample (%)	No. of *E. coli* O157:H7 positive sample (%)	Total positive sample (%)
Beef	68	4 (5.88)	5 (7.35)	9 (13.23)
Chicken	183	6 (3.28)	0 (0)	6 (3.28)
Duck	118	3 (2.54)	0 (0)	3 (2.54)
Mutton	37	0 (0)	0 (0)	0 (0)
Pork	145	8 (5.53)	2 (1.37)	10 (6.90)
Total	551	21(3.81)	7 (1.27)	28 (5.08)

2007), and 2.5–28.1 % in pork (Jo et al. 2004; Ateba and Mbewe 2011). The variation in contamination rates of meat samples from different countries could be due to the differences in sample types, study sample numbers, seasonal effects, and detection methods used.

In our investigation, *E. coli* O157 and *E. coli* O157:H7 were not detected in the mutton samples. This may be partly related to the small number of mutton samples analysed in this study. Additionally, *E. coli* O157 was isolated from 2.54 % of duck, suggesting that duck may be an important source of *E. coli* O157 strains. To our knowledge, no study on the prevalence of *E. coli* O157 in duck from China or other countries has yet been reported .

Virulence gene profiles of *E. coli* O157 and O157:H7

A total of 28 isolates (21 *E. coli* O157 and seven *E. coli* O157:H7) were identified by multiplex PCR (Figure S1). The distribution of the virulence genes in *E. coli* O157 and O157:H7 is shown in Fig. 1. The isolates had eight distinct genotype combinations and 24 (85.71 %) of the isolates carried at least one virulence gene. The *stx2* gene was detected in 21.43 % of isolates (one *E. coli* O157 and five *E. coli* O157:H7), while the *stx1* gene was detected in 10.71 % of isolates (one *E. coli* O157 and two *E. coli* O157:H7). Both *stx1* and *stx2* were detected in one *E. coli* O157:H7. The *stx2* was the predominant shiga toxin genotype. These results are in accordance with those of previous studies carried out in China and other countries (Villani et al. 2005; Bai et al. 2010). Epidemiological studies have indicated that strains carrying *stx2* are potentially more virulent than those carrying *stx1* or even strains carrying both *stx1* and *stx2* and that *stx2*-producing strains are more frequently related to HUS than those producing *stx1* or those producing both *stx1* and *stx2* (Sallam et al. 2013). Thus, it is possible to speculate that most



Serotype	eaeA	hlyA	stx1	stx2	Antibiotic profiles	Intermediate	Source	Location
O157	+	-	-	-	AMP-C-P		beef	Conghua
O157	+	-	-	-	AMP-C-P-TE-K-S-CIP	CTX-KF	chicken	Conghua
O157	+	-	-	-	AMP-C-P-TE-K		chicken	Conghua
O157	+	-	-	-	C-P-TE-K	KF	chicken	Conghua
O157	-	-	-	-	AMP-C-P-TE		pork	Guangzhou
O157	+	-	-	-	C-P-TE		pork	Nanning
O157	-	-	-	-	AMP-C-P		pork	Beihai
O157	+	-	-	-	AMP-P-S	KF	beef	Beihai
O157	+	-	-	-	AMP-C-P-AK-S		pork	Sanya
O157	-	-	-	-	AMP-C-P-AK-K-CTX		pork	Sanya
O157:H7	+	-	-	-	C-P-TE-K	S	pork	Haikou
O157	+	-	-	-	AMP-P-TE-CTX-K	NOR	beef	Conghua
O157	+	-	-	-	AMP-P-TE-NOR-S		pork	Conghua
O157	+	-	-	-	AMP-C-P-TE-K		pork	Conghua
O157	-	-	-	-	AMP -P-C		duck	Zengcheng
O157	+	+	-	+	P		beef	Heyuan
O157:H7	+	+	-	+	AMP-P-TE-KF-S		pork	Beihai
O157	+	-	-	-	C-P-TE-K	S	chicken	Guangzhou
O157	+	-	-	-	AMP-C-P-K-CTX	KF-S	chicken	Guangzhou
O157	+	-	-	-	C-P-KF-S	AK-CTX-NOR	duck	Guangzhou
O157:H7	+	+	-	+	C-P		beef	Guangzhou
O157:H7	+	+	-	+	P		beef	Guangzhou
O157:H7	+	+	-	+	No Detection		ATCC	
O157:H7	+	+	+	+	No Detection		ATCC	
O157:H7	+	+	-	+	P		beef	Guangzhou
O157:H7	+	+	+	+	P-AK		beef	Guangzhou
O157	+	+	+	-	P		pork	Guangzhou
O157:H7	+	-	+	-	P		beef	Xiamen
O157	+	-	-	-	AMP-C-P-K-TE-	NOR-KF-S-CIP-CAZ	chicken	Nanning
O157	+	-	-	-	C-P-TE-K		duck	Guangzhou

Fig. 1 ERIC-PCR DNA fingerprints analysis of *E. coli* O157 and O157:H7 isolates from fresh raw meat samples in South China. A to F refer to clusters 1 to 6 of *E .coli* O157 and O157:H7. Antibiotics: *AK* amikacin, *AMP* ampicillin, *CTX* cefotaxime, *CAZ* ceftazidime, *KF* cephalothin, *C* Chloramphenicol, *CIP* ciprofloxacin, *K* kanamycin, *NOR* norfloxacin, *P* penicillin, *S* streptomycin, *TE* tetracycline

of the *E. coli* O157:H7 isolates recovered in this study are highly virulent for humans.

The *eaeA* gene encodes a 94- to 97-kDa outer membrane protein (intimin). Researchers have emphasized the strong association between the presence of the *eaeA* gene and the pathogenesis of Shiga toxin-producing *E. coli* strains in human disease (Beutin et al. 2004). It has been reported that intimin alone can lead to diarrhea in humans by an attaching and effacing ability (Blanco et al. 2004). In our study, the *eaeA* gene was detected in 85.71 % of isolates (17 *E. coli* O157 and seven *E. coli* O157:H7). This finding is comparable to that reported in other studies. Cagney et al. (2004) reported that all the 43 (100 %) *E. coli* O57:H7 stains isolated from ground beef and beef burger carried *eae* and *hlyA* genes, whereas Cadirci et al. (2010) found that 75 % (6/ 8) of *E. coli* O57 isolates from beef and raw meatball carried *eaeA* with other virulence genes.

The *hlyA* gene encodes a plasmid-encoded enterohemolysin. The precise role of *hlyA* is unknown now, but is frequently present in LEE-negative (does not encode the locus of enterocyte effacement) Shiga toxigenic *E. coli* strains from different sources, including clinical samples from HUS patients (Pradel et al. 2008). In our study, 25 % of isolates (five *E. coli* O157:H7 and two *E. coli* O157) carried the *hlyA* gene. These results are different from previous studies (Cagney et al. 2004; Cadirci et al. 2010), in which all the *E. coli* O157:H7 isolates from food animals and beef possessed the *hlyA* gene. Our results indicated that the *hlyA* gene was present in most, but not all, of the *E. coli* O157:H7 strains isolated.

Antimicrobial resistance of *E. coli* O157 and O157:H7

Thirteen antimicrobials commonly used in human and veterinary infections were used to determine the antibiotic resistance of *E. coli* O157 and O157:H7. The results showed that all of the 28 (100 %) isolates were resistant to penicillin, 18 (64.29 %) to chloramphenicol, 16 (57.14 %) to ampicillin, 13 (46.43 %) to tetracycline, 11 (39.29 %) to kanamycin, and seven (25.00 %) to streptomycin. All the 28 (100 %) isolates were susceptible to gentamicin, 27 (96.43 %) to ceftazidime and ciprofloxacin, 24 (85.71 %) to amikacin and norfloxacin, 22 (78.57 %) to cefotaxime, and 21 (75 %) to cephalothin. Some isolates had intermediate resistance to amikacin, cefotaxime, cephalothin, and streptomycin (Fig. 1, Table 3).

Penicillin, ampicillin and tetracycline are commonly used in veterinary medicine and livestock production for disease prevention or as growth promoters in China. The high prevalence of antibiotic resistance of *E. coli* O157 isolates in this study may be related to the wide spread use of these antibiotics in animal husbandry. In previous studies, the high prevalence of tetracycline and ampicillin resistance has also been documented (Olatoye 2010; Lukásová et al. 2004). Chloramphenicol is a banned antibiotic in food animals in many countries including China. *E. coli* O157:H7 was reported to be 100 % sensitive or have intermediate resistant to this antibiotic (Hiko et al. 2008; Abong'o and Momba 2009). However, our results showed that one (14.29 %) *E. coli* O157:H7 and 17(80.95 %) *E. coli* O157 isolates were resistant to this antibiotic. The high resistance to this antibiotic indicated that irregular and unauthorized use of it might have occurred in China. Gentamicin, norfloxacin, and ciprofloxacin are commonly used as therapeutics in human medicine. Most of the isolates in this study were sensitive to these antibiotics. These results are consistent with previous studies (Hiko et al. 2008; Goncuoglu et al. 2010). However, Abong'o and Momba (2009) found 75 % (4/5) of *E. coli* O157:H7 isolates from meat were resistant to gentamicin. These findings highlight the need for continuous

Table 3 Antimicrobial resistance of *E. coli* O157 and O157:H7 isolates from retail fresh samples of beef, chicken, duck, and mutton in South China

Antibiotic	Antibiotic disc content	Susceptibility[a]		
		R No. (%)	I No. (%)	S No. (%)
Amikacin (AK)	30 μg	3 (10.7)	1 (3.57)	24 (85.72)
Ampicillin (AMP)	10 μg	16 (57.14)	0 (0)	12 (42.86)
Cefotaxime (CTX)	30 μg	3 (10.71)	3 (10.71)	22 (78.57)
Ceftazidime (CAZ)	10 μg	1 (3.57)	0 (0)	27 (96.43)
Cephalothin (KF)	30 μg	3 (10.71)	4 (14.29)	21 (75.00)
Chloramphenicol (C)	30 μg	18 (64.29)	0 (0)	10 (35.71)
Ciprofloxacin (CIP)	5 μg	1 (3.57)	0 (0)	27 (96.43)
Gentamicin (CN)	10 μg	0 (0)	0 (0)	28 (100)
Kanamycin (K)	30 μg	11 (39.29)	0 (0)	17 (60.71)
Norfloxacin (NOR)	10 μg	2 (7.14)	2 (7.14)	24 (85.72)
Penicillin (P)	10 U	28 (100)	0 (0)	0 (0)
Streptomycin (S)	10 μg	7 (25.00)	3 (10.71)	18 (64.29)
Tetracycline (TE)	30 μg	13 (46.43)	0 (0)	15 (53.57)

[a] *R* resistant; *I* intermediate; *S* susceptible

surveillance of emerging antimicrobial resistance in *E. coli* O157 and O157:H7.

Additionally, our results indicated that 22 (78.57 %) isolates were resistant to three or more antimicrobial agents. Particularly, one *E. coli* O157 isolate (016–3) was found to be resistant to ten antibiotics. The multiple antimicrobial resistance exhibited by *E. coli* O157 and O157:H7 is worrisome and indicates potential antibiotic misuse. There is an urgent need to regulate the use of antibiotics in food-producing animals and the environment in China.

Molecular typing of *E. coli* O157 and O157:H7 by ERIC-PCR

ERIC-PCR was used to study the genetic diversity of 28 isolates and two reference strains. The results are shown in Fig. 1. ERIC-PCR yielded three to nine bands ranging in size from approximately 180 to 2909 bp, which classified 30 strains into 19 different patterns with a discrimination index (DI) of 0.961. At a similarity level of 82 %, these strains were grouped into two clusters and four singletons. Cluster A included 18 *E. coli* O157 isolates and two *E. coli* O157:H7 isolates. Cluster B comprised six *E. coli* O157:H7 (four isolates and two reference strains). Singletons C, D, E, and F included only one isolate, respectively.

A correlation between the genomic profiles and the virulence genes was observed in these strains. Most of the isolates that carried *eaeA* or were without virulence genes were grouped in cluster A. In this cluster, a good correlation among ERIC patterns, virulence profiles, and the sample source was found in some isolates. Two isolates (054–1 and 056–2) obtained from pork in Conghua city showed 100 % similarity. Two isolates (175–4 and 241–2) from chicken in Guangzhou city showed 100 % similarity. Three isolates (E236-1, E142-2, E230-3) from chicken in Conghua city also showed identical ERIC patterns. These findings suggested that ERIC-PCR could be reliable in studying the relationship of isolates from different sources. Additionally, five isolates (E2253-3, E2372-1, E073-3, E2453-1, E078-2) from different sources yielded an identical pattern, suggesting that they were highly homogenous and had a close genetic relationship. In cluster B, all of the four *E. coli* O157:H7 isolates (E202-2, E172-3, E049-2, E050-2) were obtained from beef in Guangzhou city and carried *stx2*. These isolates showed 83 % similarity with the two clinical reference strains associated with HUS (ATCC 43894 and ATCC 35150), indicating that they may be highly virulent for humans. In addition, one *E. coli* O157 isolate (E009-1) and one *E. coli* O157:H7 isolate (E703-1) that carried $stx1^{+}$ showed different patterns and were grouped in singleton C and D, respectively.

No correlation was observed between the ERIC-PCR profiles and the antibiotic resistance profiles of the isolates. However, some distinguishable patterns were found in two isolates. One *E. coli* O157 (016–3) isolate (resistance to ten tested antimicrobials) from chicken was clustered as a unique singleton E. One *E. coli* O157 isolate (240–3, resistant to four antimicrobials) from duck also showed a distinctive pattern and was grouped in singleton F. These unique DNA fingerprints indicated that these isolates were genetically diverse from other isolates.

In this study, ERIC-PCR showed good discriminatory power and indicated high genetic diversity in *E. coli* O157 and O157:H7 isolates from meat samples. These results are similar to that of Ling et al. (2000). These findings suggested that ERIC-PCR is a helpful tool for characterizing the genetic relationships among isolates.

Conclusion

The results of this study revealed the presence of virulent, antibiotic resistant *E. coli* O157 and O157:H7 in raw fresh meats reaching consumers, indicating possible risks of infection to people through the consumption of raw/under-cooked meat (especially beef). ERIC-PCR revealed high genetic diversity among the *E. coli* O157 and O157:H7 isolates in fresh raw meat. Some *E. coli* O157:H7 isolates from beef showed similar patterns with the clinical reference strains, indicating a potential high virulence for consumers. This study for the first time contributed data on the prevalence and characteristics of *E. coli* O157 and O157:H7 in retail fresh raw meat in South China, providing baseline information for regulatory authorities to formulate a regulatory framework for controlling *E. coli* O157 and O157:H7 to improve the microbiological safety of meat.

Acknowledgments This work was supported by research grants from the International Science and Technology Cooperation Projects (No.2013DFH30070). The authors acknowledge colleagues in our lab for kindly technical assistance. We are also thankful to the reviewers for their valuable suggestions and comments.

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