International Journal of Food Microbiology 200 (2015) 31–38

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* isolated from retail raw meats in China

Xiangning Bai [a], Hong Wang [b], Youquan Xin [c], Rongjie Wei [c], Xinyuan Tang [c], Ailan Zhao [a], Hui Sun [a], Wang Zhang [a], Yan Wang [a], Yanmei Xu [a], Zhengdong Zhang [b], Qun Li [b], Jianguo Xu [a,d,*], Yanwen Xiong [a,d,*]

[a] State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping, Beijing, China
[b] Zigong Center for Disease Control and Prevention, Zigong, Sichuan Province, China
[c] Qinghai Institute for Endemic Disease Prevention and Control, Xining, Qinghai Province, China
[d] Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Hangzhou, Zhejiang Province, China

ARTICLE INFO

Article history:
Received 6 October 2014
Received in revised form 31 December 2014
Accepted 24 January 2015
Available online 30 January 2015

Keywords:
Shiga toxin-producing *Escherichia coli* (STEC)
Raw meat
Multi-locus sequence typing
Pulsed-field gel electrophoresis
Virulence gene

ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) causes diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in humans. Most human infections are attributed to consumption of STEC-contaminated foodstuffs of animal origin. In this study, we evaluated the prevalence of STEC from retail raw meats collected from two geographical regions in China. The results revealed that 166 out of 853 samples were *stx*-positive; 63 STEC isolates were recovered from 58 *stx*-positive samples including pork (4.4%, 14/318), beef (11.0%, 21/191), mutton (20.6%, 26/126), chicken (0.5%, 1/205), and duck (7.7%, 1/13). Twenty-six O serogroups and 33 O:H serotypes were identified. All three stx_1 subtypes and five stx_2 subtypes (2a to 2e) were found in the 63 STEC isolates, among which stx_{2e}-positive STEC isolates were the most predominant (39.7%), followed by stx_{1c} only (20.6%), $stx_{1c} + stx_{2b}$ (14.3%), and stx_{1a} only (9.5%). STEC isolates carried virulence genes *eae* (6.3%), *ehxA* (36.5%), *katP* (4.8%), *astA* (11.1%), and *subA* (36.5%). Of the four adherence-associated genes tested, *toxB* was absent, whereas *saa*, *paa*, and *efa1* were present in 28, three, and one STEC isolates respectively. The STEC isolates were divided into 50 PFGE patterns and 33 sequence types. STEC from different sources and geographical regions were separated by PFGE and MLST. Our results revealed that there is a high genetic diversity of STEC in retail raw meats, some of which have potential to cause human diseases.

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) represents an emerging group of food-borne zoonotic pathogens, which contribute to diarrhea, hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS) in humans (Smith et al., 2014). More than 200 virulent STEC serotypes have been isolated from human infections (Coombes et al., 2008). While STEC O157:H7 is the most common cause of STEC infections, a growing number of non-O157 STEC strains have been isolated from several clinical cases and outbreaks, including the life threatening HUS. In fact, non-O157 STECs are responsible for a larger portion of total STEC infections in the United States compared to STEC O157, especially serogroups O26, O45, O103, O111, O121 and O145 (referred to as the top six non-O157 STEC) are most frequently implicated in human infections (Brooks et al., 2005; Smith et al., 2014). Recently, methods detecting specifically STEC O157:H7 and the top six non-O157 STECs have been proposed and optimized (Conrad et al., 2014; Hegde et al., 2013; Paddock et al., 2012; Wasilenko et al., 2014). Nevertheless, the development of accurate and reliable methods for STEC detection and isolation of other serotypes has still been challenging, due to the high genetic and phenotypic variability. The public health significance of these pathogens is likely to remain under-recognized.

Among the virulence factors associated with STEC, the production of Shiga toxin (Stx) is considered to be most important (Smith et al., 2014). Human pathogenic STEC strains often possess intimin, an outer membrane protein encoded by *eae* that resides in the locus of enterocyte effacement (LEE) (Karmali et al., 2010). Enterohemolysin (*ehxA*), harbored in a 60-MDa virulent plasmid is an important virulence factor in some STEC strains (Murase et al., 2012). Besides, many other virulence and adherence factors are involved in STEC pathogenicity (Bai et al., 2013; Meng et al., 2014).

Ruminants are the most important reservoirs of STEC. The consumption of STEC-contaminated animal-derived foodstuffs especially raw meats and meat products is an important transmission route (Erickson and Doyle, 2007). STECs of various serotypes have been

* Corresponding authors at: State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping, Beijing, China. Tel.: +86 10 58900749; fax: +86 10 58900748.
E-mail addresses: xujianguo@icdc.cn (J. Xu), xiongyanwen@icdc.cn (Y. Xiong).

http://dx.doi.org/10.1016/j.ijfoodmicro.2015.01.018
0168-1605/

Table 1
Prevalence of STEC in raw pork, beef, mutton, chicken and duck samples.

Meat	No. of samples	No. of *stx* positive (%)	No. of samples with STEC isolates (%)	No. of STEC isolates (%)
Pork	318	26 (8.2)	13 (4.1)	14 (4.4)
Beef	191	63 (33.0)	19 (9.9)	21 (11.0)
Mutton	126	70 (55.6)	24 (19.0)	26 (20.6)
Chicken	205	6 (2.9)	1 (0.5)	1 (0.5)
Duck	13	1 (7.7)	1 (7.7)	1 (7.7)
Total	853	166 (19.5)	58 (6.8)	63 (7.4)

isolated from raw meat samples including beef, mutton, pork, chicken, and wild game meat (Magwedere et al., 2013). Notably, the importance of food as a potential source of human infection is emphasized by the fact that several STEC isolates from foods had the same serotypes, similar virulence patterns as STEC isolates from human patients (Werber et al., 2008). In China, STEC O157:H7 were detected in different aminals and caused a major outbreak in 1999 (Meng et al., 2013; Xiong et al., 2012). Our previous studies showed that healthy pigs and yaks are reservoirs of non-O157 STECs in China (Bai et al., 2013; Meng et al., 2014). Both O157:H7 and non-O157 STEC strains have been isolated from outpatients with acute diarrhea in southeastern China (Chen et al., 2014), and from retail meats including pork, mutton and beef in Shandong, China (Koitabashi et al., 2008). In this study, we isolated and characterized STEC from raw meat samples collected from Zigong and Beijing on a large scale and explored their pathogenic potential to humans.

2. Material and methods

2.1. Samples

Overall 853 raw meat samples were collected from supermarkets and farmer markets in Zigong and Beijing, China, from April 2013 to April 2014. The samples included pork (n = 318), chicken (n = 205), beef (n = 191), mutton (n = 126), and duck (n = 13). The samples were transported in ice as soon as possible to laboratories: Zigong Center for Disease Control and Prevention; National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention.

2.2. Detection and isolation of STEC

Twenty-five grams of each raw meat sample was enriched with 225 ml of modified Tryptone Soya Broth (mTSB) supplemented with novobiocin (10 mg/L) (Oxoid, Hampshire, UK) and incubated at 37 °C for 18–24 h on a shaking platform (220 rpm).

One microliter of each enrichment sample was centrifuged at 1500 ×*g* for 1 min. The supernatant was centrifuged at 13,000 ×*g* for 2 min and the pellet was suspended in 100 μl lysis buffer (100 mM NaCl, 10 mM Tris–HCl [pH 8.3], 1 mM EDTA [pH 9.0], and 1% Triton X-100), boiled for 10 min, and centrifuged. The resulting supernatant was used as a template to test the presence of stx_1 and stx_2 by duplex PCR (Bai et al., 2013). One loop-full of each *stx*-positive enrichment culture was directly streaked onto CHROMagar ECC and CHROMagar STEC agar (CHROMagar, Paris, France). After overnight incubation at 37 °C, approximately 10 blue or colorless, round and moist presumptive *E. coli* colonies on CHROMagar ECC and 5–10 mauve colonies with or without fluorescence on CHROMagar STEC were picked to test for the presence of stx_1 and stx_2. Finally, with the exception of different colony colors (blue or colorless), *stx* types (stx_1 or stx_2), or *stx* combinations (stx_1 only, stx_2 only, or $stx_1 + stx_2$) present in the same sample, only one STEC isolate from each *stx*-positive sample was kept for further investigation.

2.3. Biochemical tests and serotyping of STEC isolates

All *stx*-positive isolates were confirmed to be *E. coli* by biochemical tests (API 20E system, bioMérieux, Paris, France). The O:H serotype of each isolate was determined by methods as previously described (Bai et al., 2013; Meng et al., 2014).

2.4. stx subtyping

The stx_1 and/or stx_2 subtypes of all STEC isolates were determined by a PCR-based subtyping method, and then confirmed by sequencing the complete stx_1 and/or stx_2 genes of certain STEC isolates according to previously reported methods (Bai et al., 2013; Meng et al., 2014).

2.5. Detection of virulence and adherence factor genes

STEC isolates were subjected to PCR for the detection of intimin-encoding gene (*eae*), virulence-associated genes (*ehxA*, *katP*, *astA*, *subA*, *cnf1*, and *cnf2*), putative adhesin genes (*saa*, *efa1*, *paa*, and *toxB*), and HPI genes (*fyuA* and *irp*) using the primers listed in previous studies (Bai et al., 2013; Meng et al., 2014).

2.6. Pulsed-field gel electrophoresis (PFGE)

STEC isolates were digested with *Xba*I and separated by PFGE according to the protocol for non-O157 STEC from PulseNet, USA (http://www.cdc.gov/pulsenet/pathogens/index.html). The secondary enzyme *Bln*I (*Avr*II) was used in situations where the PFGE patterns obtained with the primary enzyme from two or more isolates were indistinguishable. PFGE gel images were analyzed with BioNumerics version 4.0 (Applied Maths, Sint-Martens-Latem, Belgium). An UPGMA (unweighted pair-group method with arithmetic mean) dendrogram was constructed using the BioNumerics software.

2.7. Multi-locus sequence typing (MLST)

MLST was performed according to the *E. coli* MLST website (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) using seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*). MLST data for the HUS-associated enterohemorrhagic *E. coli* (HUSEC) collection were obtained from http://campus.uni-muenster.de/hyg_klhus_husec.html?&L=1 (Mellmann et al., 2008). All human STECs sequence types (STs) of the O157, O26, O45, O103, O111, O121, and O145 serogroups were obtained from the *E. coli* MLST website. A minimum spanning tree based on these STs was generated using the BioNumerics software.

2.8. Statistical analysis

Statistical tests were performed using SAS software version 9.1 (SAS Institute Inc., Cary, NC., USA). Statistically significant differences were calculated using a $\chi 2$ test where appropriate. P values of <0.05 were considered statistically significant.

3. Results

3.1. Prevalence of STEC in raw meat samples

Out of 853 retail raw meat samples, 31 (3.6%), 58 (6.8%) and 77 (9.0%) were positive for stx_1, stx_2, both stx_1 and stx_2 respectively. Different *stx*-positive rates were observed in pork (8.2%), beef (33.0%), mutton (55.6%), chicken (2.9%), and duck (7.7%). We isolated 63 STEC isolates from 58 *stx*-positive meat samples (including 13 pork, 19 beef, 24 mutton, 1 chicken and 1 duck samples) giving a culture positive STEC rate of 34.9% (58/166) for *stx*-positive samples and 6.8% (58/853) for all samples.