Escherichia Coli O157: H7 and Shiga-like-toxin-producing Escherichia Coli in China *

XU Jian-Guo, CHENG Bo-Kun and JING Huai-Qi

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Escherichia coli (E. coli) is one of the facultative anaerobes of the human intestinal tract, usually harmless. Infections due to pathogenic E. coli may result in urinary tract infections, sepsis, meningitis and enteric disease. Diarrheagenic E. coli has been classified into several categories, such as enterotoxigenic E. coli (ETEC), entero-invasive E. coli (EIEC), enteropathogenic E. coli (EPEC), enter-aggregative E. coli (EaggEC) and enterohemorrhagic E. coli (EHEC). A variety of virulence or potential virulence factors for diarrheagenic E. coli have been identified. The nomenclature of diarrheagenic E. coli is based on virulence factors[1,2].

In 1977 Konowalchuk et al[3] reported that some strains of pathogenic E. coli O26 : H11 produced a toxin with a profound cytopathic effect on Vero cells, and named it verotoxin (VT). O’Brien et al[4] noted[4] that the VT reported by Konowalchuk et al[3] was strikingly similar to Shiga toxin (Stx) produced by Shigella dysenteriae type 1, and it could be neutralized by anti-Stx, thus a new nomenclature, Shiga-like toxin (SLT), appeared. An alternative nomenclature is “Shiga toxin” (ST), which indicated that the specific cytotoxin described by Konowalchuk et al[3] is essentially identical at the genetic and protein levels with the Stx produced by S. dysenteriae I discovered some 100 years ago. Consequently, SLT, ST and VT have been used interchangeably, resulting in the name of verotoxin-producing E. coli (VTEC), shiga-like-toxin-producing E. coli (SLTEC) and shiga-toxin-producing E. coli (STECE) coexisted in literature[5]. However, it must be noted that E. coli O157 : H7 is the main serotype of EHEC recognized at present[1,2,6]. Hemorrhagiccolitis (HC) and hemolytic uremic syndrome (HUS) are life threatening, which are often caused by STEC or EHEC. So far as the diseases are concerned, E. coli O157 : H7 should belong to EHEC. All of the EHEC strains are believed to be pathogenic. As for its toxin, E. coli O157 : H7 should belong to VTEC, SLTEC or STEC. However, not all of the STEC strains could cause HC or HUS[7]. The confusion from the nomenclature may be clarified in future when the pathogenic mechanisms of bacteria are fully understood.

Food-borne outbreaks of SLTEC disease appear to be increasing in the world. Mass-produced and mass-distributed food can involve large numbers of people in short time. SLTEC strains belong to a very diverse range of serotypes, among which O157 : H7 is most commonly associated with large outbreaks[8]. In the summer of 1996 in Japan, a largest outbreak in the world caused by E. coli O157 : H7 was reported, in which about 10 000 cases were identified[9]. Chinese government and society became aware of the importance of E. coli O157 : H7 from the Japanese outbreak. An informal national network for detection of E. coli O157 : H7 was organized in April 1997, involving about 30 public health laboratories from different provinces and municipalities.

E. coli O157 : H7 in China

The studies of E. coli O157 : H7 in China can be divided into two phases. In phase 1, starting from 1986 up to August 1996, the bacteriologists who studied the pathogen were mainly motivated by their scientific interests, few organized projects were carried out[10]. In phase 2, starting from August 1996 up to now, the public health authorities and most of the scientists have paid more attention to E. coli O157 : H7, and a new trend of isolation of E. coli O157 : H7 has been attempted in various parts of China.

The first group of patients with HC caused by E. coli O157 : H7 were identified in Beijing in 1988,
as the etiologic agents isolated in Xuzhou city, Jiangsu Province of China\(^{[10]}\). In the three years from 1986 to 1988, 24 of 486 sporadic diarrhea patients were diagnosed as having HC, 5 strains of *E. coli* O157 : H7 were isolated, all of which were hybridized with SLT1, SLT2 and EHEC specific probe\(^{[10]}\). In 1993, two strains of *E. coli* O157 : H7 were isolated from a patient with HC and a patient with HUS, in Shandong Province. In the same period, several groups of scientists failed to isolate *E. coli* O157 : H7 in other cities of China, possibly due to lack of proper diagnostic techniques and reagents. Kain *et al.*\(^{[11]}\) reported that, in a study carried out in Beijing, about 7% of fecal samples were collected from diarrhea children hybridized with EHEC probe pCVD419 \(^{[8]}\). However, similar amount of positive samples were also observed in the control group, but without strain isolation. The 168 strains of EPEC isolated before 1982 were detected with SLT1, SLT2 and EHEC specific probes, no EHEC or STEC was found\(^{[2]}\).

In 1997, a Chinese national network for detection of *E. coli* O157 : H7 was organized, *E. coli* O157 : H7 strains were isolated from diarrhea patients in Zhejiang, Anhui Provinces and Ningxia Autonomous Region. Nine strains were isolated from pigs in Fujian Province. It was interesting to note that all of the 9 strains from pig source were negative for SLT1, SLT2, and Hly genes. In 1998, several public health laboratories in China have attempted to isolate *E. coli* O157 : H7 from various sources, such as diarrhea patients, pigs, cattle, food and cow’s milk. A total of 48 strains were isolated from cattle, pigs and milk. Some strains were found to be hybridized with Hly, SLT1 or SLT2 gene probes, most of the strains, however, were not. And, this result was demonstrated and confirmed by PCR method in the reference laboratory. The *E. coli* O157 : H7 strains data in China are summarized in Table 1.

### Table 1 *E. coli* O157 : H7 strains isolated in China

| No. Strains | Year    | Source        | Hly | SLT1 | SLT2 | Province        |
|------------|---------|---------------|-----|------|------|----------------|
| 5          | 1986-1988 | HC            | +   | +    | +    | Jiangsu        |
| 3          | 1993    | HC, HUS       | +   | +    | +    | Shandong       |
| 1          | 1997    | Diarrhea      | +   | +    | +    | Anhui          |
| 1          | 1997    | Diarrhea      | +   | -    | -    | Zhejiang       |
| 1          | 1997    | Diarrhea      | +   | ND   | ND   | Ningxia        |
| 1          | 1997    | Diarrhea      | -   | -    | -    | Passenger      |
| 9          | 1997    | Cattle        | -   | -    | -    | Fujian         |
| 2          | 1997    | Food          | +   | +    | +    | Fujian         |
| 1          | 1998    | Milk          | +   | +    | +    | Guangdong      |
| 2          | 1998    | Food          | ND  | ND   | ND   | Guangdong      |
| 4          | 1998    | Pig           | -   | -    | -    | Liaoning       |
| 1          | 1998    | Cattle        | +   | +    | +    | Liaoning       |
| 2          | 1998    | Cattle        | +   | ND   | ND   | Hebei          |
| 14         | 1998    | Cattle        | ND  | ND   | ND   | Ningxia        |

\(^{a}\)Hly: hemolysin gene.

It is shown in literature, that *E. coli* O157 : H7 strains have been isolated from samples of beef, lamb, deer, wild boar, ostrich, partridge, antelope, and reindeer\(^{[11,12]}\). Cattles have long been regarded as the principal reservoir of *E. coli* O157 : H7. STEC strains were found prevalent in the gastrointestinal tracts of other domestic animals, including sheep, pig, goat, dog, and cat\(^{[2,5]}\). Many domestic animals carrying pathogens are asymptomatic. Strains of *E. coli* O157 : H7 have also been detected in cats and dogs with diarrhea\(^{[2,5]}\). *E. coli* O157 : H7 can potentially enter the human food chain from a number of animal sources, most commonly by contamination of meat with feces or intestinal contents after slaughter. One of the most common sources of human *E. coli* O157 : H7 infections is hamburger patty, made from ground beef. Hence, most of the outbreaks of *E. coli* O157 : H7 infection all over the world have been linked to hamburgers. In the outbreak of United States in 1993, more than 700 people were infected, and over 50 cases of HUS were diagnosed. So far, this is the largest outbreak of *E. coli* O157 : H7 associated with hamburger.

In China, only few sporadic cases of *E. coli* O157 : H7 infections have been identified. No outbreak as yet has been reported. The strains of *E. coli* O157 : H7 were isolated from cattle, pigs and milk. These results suggest that risk of infection with these microbes existed in China. It occurred to us that the prevalence of *E. coli* O157 : H7 seems to be higher in pigs than one expected. It should be emphasized that the consumption of pork in China is very popular and the risk seems to be much higher than that from beef. Further investigation of *E. coli* O157 : H7 in pigs should be conducted in China. Fortunately, no known virulence gene was found in the strains isolated from pigs in China such as SLT1, SL T2 and hemolysin gene as well. However, it was reported recently that the SLT2 containing phage from sewage, as the phage containing virulence gene, could infect non-pathogenic *E. coli* rather easily. The risk of such microbe infection seems fairly high.

### SLTEC OTHER THAN O157 : H7 IN CHINA

*E. coli* O157 : H7 has not been recognized as a big public health problem in China up to now. However, STEC seems to be serious\(^{[13]}\). In clinical or public health bacteriological laboratories, only EPEC, ETEC and EIEC used to be diagnosed by serotyping techniques. Nevertheless, it has been noted not infrequently that almost pure cultures of *E. coli* were seen and new varieties of *E. coli* were isolated from certain fecal samples of diarrheal patients, which could not be serotyped with the
found that and EIEC strains[14,15]. The fragment was a diagnostic tool specific for Shigella species invasive plasmid of S.flexneri 2a, and used as probe is a 2.5-Kb fragment derived from the hybridize with SLT1 or/and SLT2 probe. INV

In general, strains of EHEC and some of EPEC were hybridized with EAggEC specific probe, which found to hybridize SLTs probes[16]. To clarify known EIEC or Shigella flexneria species was associated locus (ial). However, none of the subsequently sequenced and named invasive probes[13,14].

The DNA probes covered almost all the virulence genes reported, such as heat-stable toxin (ST), heat-labile toxin (LT), EPEC adherence factor (EAF), diffuse adherence gene (DA), EHEC specific probe pCVD419, EAggEC specific probe, 2.5 Kb specific probe for invasive plasmid (INV) of EIEC and Shigella-species, shiga-like toxin 1 or 2 (SLT1 or SLT2), EPEC attaching and effacing genes (eae). It was observed that 59.3% strains tested were hybridized with at least one of the used probes, with a higher percentage of (29.7%) E.coli strains hybridized with SLT2 and INV probes[13,14].

In general, strains of EHEC and some of EPEC hybridize with SLT1 or/and SLT2 probe. INV probe is a 2.5-Kb fragment derived from the invasive plasmid of S.flexneri 2a, and used as a diagnostic tool specific for Shigella species and EIEC strains[14,15]. The fragment was subsequently sequenced and named invasive associated locus (ial). However, none of the known EIEC or Shigella flexneria species was found to hybridize SLTs probes[16]. To clarify the relationship between EIEC and some of our strains isolated, the invasive plasmid antigen BCD (ipabCD), the key genes for invasive ability of EIEC and Shigella, were synthesized by PCR labeled by Digoxin and used as probe. The absence of DNA hybridization signals indicated a lack of ipabCD genes in E.coli F171. We also found that E.coli F171 could not provoke keratoconjunctivitis in guinea pigs. Sereny test was used as a critical marker for virulence of EIEC and Shigella species. However, with HEp-2 cell assay, the E.coli F171 is able to invade the epithelial cells. The data suggested that the genes encoding invasive ability of E.coli-F171 differed from EIEC, and E.coli F171 was therefore not a member of EIEC[13,14].

Adherence of bacteria to epithelial cells has been recognized as a virulence characteristic of enteric pathogen[11]. Three adherence patterns were defined i.e., localized adherence, diffuse adherence and aggregative adherence[17]. Many of our E.coli strains hybridized with SLT2 and INV DNA probes demonstrated HEp-2 cell aggregative adherence pattern[13,14]. However, none of them were hybridized with EAggEC specific probe, which was derived from the genes encoding EAggEC adherence factor 1 (EAF/1), and used as an identification marker for EAggEC. The aggregative adherence pattern to HEp-2 cells is the characteristic feature as EAggEC strains[18]. Under electron microscope, a unique kind of fimbria was observed on the surface of cells of E.coli F171. The subunit size of the fimbriae protein was 19KDa, and the genes encoding the fimbriae were located on a 60 MDa plasmid. E.coli HB101 cells containing the cloned genes were able to adhere onto the HEp-2 cells. The analysis of N terminal amino acid sequence indicated that E.coli F171 has its unique features[14].

The shiga-like toxins have been demonstrated as the virulence factors for E.coli strain, which could cause HC and HUS[19]. Many of EPEC and EHEC strains contain genes for SLT1 or SLT2. The toxin producing ability of E.coli F171 was studied with Vero cell assay, which was originally used for study SLTs since E.coli F171 was hybridized with SLT2 probe. Both cell culture filtrate and a crude toxin preparation of E.coli F171 were found toxic to Vero cells. The Vero cell toxicity of E.coli F171 could not be neutralized by SLT2 antibody. The fact that hybridization of E.coli F171 with SLT2 probe suggested that it has DNA fragment homologous to SLT2 gene or it has an entire SLT2 gene[14].

The invasiveness, toxin production activity and epithelial cell adherence ability have been described as key features for EIEC, ETEC and EAggEC respectively[20]. E.coli F171 could adhere onto and invade into HEp-2 cells and produce toxins. It combines many key features of EIEC, EHEC, EPEC, and EAggEC. Based on the data obtained, it seems that E.coli F171 represents a new variety of STEC. Hence the name of enteric SLTs-producing and invasive E.coli (ESIEC) was proposed. Since 31.4% of collected E.coli strains were tested in our studies shared similar features as E.coli F171, infections presumably caused by this kind of pathogenic E.coli seems to be an important public health problem in China.

In order to confirm the virulence and pathogenesis to human beings, a study in adult volunteers was carried out. By oral in take of 10^9-10^10 colony forming units (CFU) of E.coli F171, all of 8 volunteers developed diarrhea, 3 of 8 developed high fever (39.8°C). The incubation period ranged from 7 to 49 hours. Unformed stools were 3-6 times a day. The volumes of stools of 4 volunteers were above 1 000 mL a day. Antibiotic therapy was given to 5 of the 8 volunteers. No diarrhea was observed for the control group consisting of 4 volunteers, who ingested 10^9 CFU of

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non-pathogenic strain *E. coli*-HB101. Typical clinical symptoms for ESIEC in volunteers were bowel movement, diarrhea, general abdominal pain, moderate fever and unformed stool. It was revealed that ingested *E. coli* F171 could colonize and replicate for up to 7 days. By examining the stool samples of the volunteers, it was observed that the bacteria could reach an amount of \(2.74 \times 10^{12}\) CFU. The strains isolated from the patient stool samples of volunteers were confirmed as *E. coli* F171 by specific antiserum in animal against it.

Although the human pathogenic nature of *E. coli* F171 was recognized, the key virulence factors of ESIEC have not been studied in detail. The pathogenic mechanism of ESIEC, for instance, has not been understood. The “pathogenicity island”, which refers to the large chromosomal segment carrying genes involved in pathogenicity, has recently revolutionized our understanding of bacterial pathogenesis[21]. The GC content of pathogenicity islands is different from that of the other host chromosome, suggesting that they may originate from horizontal transfer between different bacterial general. The number of gram-negative bacteria species known to harbor pathogenicity islands has grown steadily, including uropathogenic *E. coli* (UPEC), EHEC, EPEC, Helicobacter pylori, salmonella typhimurium and Vibrio cholerae[21]. It is believed that there is no pathogenicity island in the non-pathogenic *E. coli*. We must investigate the pathogenicity island so as to confirm the medical significance of ESIEC. Recently, we have observed an *irp2* gene in many strains of ESIEC. The *irp2* gene is involved in iron uptake and has been considered as one of the virulence genes located on the high pathogenicity island (HPI) of Yersinia-species[22]. This gene was observed in many strains of adherent *E. coli* and in *E. coli* isolated from blood, but rarely observed in EPEC, EIEC or ETEC. No -*irp2* - was found in EHEC, Shigella and Salmonella enterica strains. It seems that pathogenicity island existed in ESIEC. The HPI of the Y. pestis is disseminated among species of the Enterobacteriaceae family which are pathogenic to humans.

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