Short Communication

Comparison of Enterohemorrhagic *Escherichia coli* (EHEC) O157 and EHEC Non-O157 Isolates from Patients with Diarrhea in Korea

Kyung-Hwan Oh¹, Su-Mi Jung¹, Eunkyung Shin¹, Gyung Tae Chung¹, Won-Keun Seong², and Seung-Hak Cho^{1*}

¹Division of Enteric Diseases, Center for Infectious Diseases, Korea National Institute of Health, Cheongju, and

²Center for Infectious Diseases, Korea National Institute of Health, Cheongju, Korea

SUMMARY: We compared 47 enterohemorrhagic *Escherichia coli* (EHEC) O157 isolates with 184 EHEC non-O157 isolates from Korean patients with diarrhea. In the O157 group, the strains harboring both Shiga toxin genes (*stx1* and *stx2*) were detected with highest frequency, whereas the strains harboring only *stx1* gene were most frequently detected in the non-O157 group. Eight virulence genes (*eaeA*, *hlyA*, *ehx*, *iha*, *efa1*, *tir*, *toxB*, and *espA*) were found to show a higher frequency of occurrence in the O157 group than in the non-O157 group. In addition, the symptom of bloody diarrhea was exhibited at a higher rate in the O157 group (51.1%) than in the non-O157 group (16.8%). Our findings demonstrate that EHEC O157 strains are more frequently implicated in cases of bloody diarrhea in the Korean population than EHEC non-O157 strains.

Enterohemorrhagic *Escherichia coli* (EHEC) is recognized as an important foodborne pathogen that causes several gastrointestinal illnesses, such as bloody or non-bloody diarrhea, hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS) in humans (1). Although more than 70 different pathogenic serotypes of Shiga toxin-producing *E. coli* (STEC) have been described to date, studies of EHEC prevalence have focused primarily on *E. coli* O157:H7 (2). However, recent epidemiological studies have reported that several non-O157 STEC serogroups are linked to severe disease in humans (2–5). In the present study, we compared EHEC O157 and non-O157 isolates from Korean patients with diarrhea to further characterize the EHEC isolates in these patients by serotype.

A total of 231 human clinical isolates (47 EHEC O157 strains and 184 EHEC non-O157 strains) were collected between 2003 and 2011 through a routine surveillance system, which involved laboratory testing of stool samples from patients with diarrhea, isolation of bacteria from clinical specimens, and identification of EHEC isolates. The EHEC strains were characterized for the presence of 10 virulence genes, namely the EHEC attachment-effacement gene (*eaeA*), hemolysin genes (*hlyA*, *ehx*, and *clyA*), adhesion genes (*iha*, *saa*, *efa1*, *tir*, and *toxB*), and type III secretion gene (*espA*) by PCR

(6). The EHEC strains were examined for the presence of antibiotic resistance determinants using the VITEK2 system (BioMérieux, St. Louis, MO, USA). The Clinical and Laboratory Standards Institute breakpoints were used for interpretation of susceptibility to antimicrobial agents. *E. coli* ATCC 25922 was tested as a quality control strain.

In this study, GraphPad Prism ver. 5 (La Jolla, CA, USA) was used for statistical analysis. *P*-values of ≤ 0.05 were considered to represent statistical significance.

The results of characterization of EHEC O157 and EHEC non-O157 strains are shown in Table 1. The Shiga toxin (Stx) is essential for the development of bloody diarrhea and HC; Stx2 plays a more important role than Stx1 in the development of HUS (7). The 231 EHEC strains represented 3 different enterotoxin profiles:

Table 1. Characterization of EHEC O157 and EHEC non-O157

Category		No. of isolates (%)		<i>P</i> -value
		EHEC O157	EHEC non-O157	
		(<i>n</i> = 47)	(<i>n</i> = 184)	
Toxin gene	<i>stx1</i>	4 (8.5)	85 (46.2)	< 0.0001
	<i>stx2</i>	7 (14.9)	49 (26.6)	0.0649
	<i>stx1</i> & <i>stx2</i>	36 (76.6)	50 (27.2)	< 0.0001
Age	0–9	25 (53.2)	97 (52.7)	0.5427
	10–59	10 (21.3)	70 (38.0)	0.0214
	≥ 60	12 (25.5)	17 (9.2)	0.0046
Sex	man	23 (48.9)	87 (47.3)	0.4839
	woman	24 (51.1)	97 (52.7)	0.4839
Major symptom	bloody diarrhea	24 (51.1)	31 (16.8)	< 0.0001
(excluding mere diarrhea)	fever	11 (23.4)	16 (8.7)	0.0372
	vomiting	9 (19.1)	18 (9.8)	0.1128
	abdominal pain	6 (12.8)	36 (19.6)	0.3152

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*Corresponding author: Mailing address: Division of Enteric Diseases, Center for Infectious Diseases, Korea National Institute of Health, 187 Osongsaengmyeong 2(i)-ro, Cheongju-si, Chungcheongbuk-do 363-951, Korea.

Tel: +82-43-719-8112, Fax: +82-43-719-8149,

E-mail: skcho38@korea.kr

stx1 positive EHEC (EHEC-*stx1*), *stx2* positive EHEC (EHEC-*stx2*), and *stx1* and *stx2* positive EHEC (EHEC-*stx1* & 2). The profiles of the Shiga toxin-encoding genes of the EHEC O157 strains differed from those of the EHEC non-O157 strains; in the EHEC O157 strains, *stx1* & 2 was detected most frequently, whereas the *stx1* gene was more frequently detected than *stx2* or *stx1* & 2 in the EHEC non-O157 strains. These results are in contrast with findings from Spain in 2004 (8) and Switzerland in 2011 (9). However, studies from the United States (10) and British Columbia, Canada (11) showed a similar distribution of *stx* genes in the 2 categories of EHEC.

Stratification of patients with diarrhea by age revealed similar patterns in the isolation rate of the O157 and non-O157 groups. In children < 9 years of age, the isolation rates of the 2 EHEC groups were significantly greater ($P < 0.0001$) than that of other age groups. However, in the O157 group, the isolation rates in those aged 10–59 years and ≥ 60 years were similar, while the isolation rates in those aged 10–59 year was much higher than in those aged ≥ 60 years in the non-O157 group. There was no difference between the 2 EHEC groups in terms of sex.

We identified 38 serogroups among 231 EHEC strains. Except O antigen untypeable (OUT), the most common of the 37 serogroups identified among the non-O157 EHEC strains were O103 (11.5%), O26 (10.3%), O91 (7.3%), and O8 (5.1%). These results were different from those of other studies, e.g. the major serogroups in the United States have been reported to be O26 (22%), O111 (16%), O103 (12%), O121 (8%), O45 (7%), and O145 (5%) (3). A German study reported similar results in that the most common serogroups identified were O103 (14%), O26 (14%), O91 (10%), and O145 (4%) (12).

The major symptoms of EHEC infection are diarrhea, bloody diarrhea, and fever. In the O157 group, the major symptoms reported were bloody diarrhea (51.1%), fever (23.4%), vomiting (19.1%), and abdominal pain (12.8%). However, in the non-O157 group, the major symptoms reported were abdominal pain (19.6%), bloody diarrhea (16.8%), vomiting (9.8%), and fever (8.7%). Interestingly, 93.3% of the EHEC O157 isolates causing bloody diarrhea harbored the *stx2* gene, whereas only 57.9% of the EHEC non-O157 isolates causing bloody diarrhea harbored this gene.

In addition to the Stx toxins, several other virulence factors are implicated in the pathogenesis of EHEC infections (13,14). These virulence factors have been linked to severe disease symptoms. The distribution of 10 virulence-associated genes (and 3 *int* subtypes) is shown in Fig. 1. Our findings were similar to those reported by studies in Spain (8), Switzerland (9), and Brazil (15). In the O157 group, all virulence genes, except *saa*, showed a high prevalence (85.1–100%). However, in the non-O157 group, only the *clxA* gene had a high prevalence (96.4%). The *saa* gene, whose

Fig. 1. Distribution of virulence-associated genes in EHEC O157 and EHEC non-O157 isolates. In the EHEC O157 group, 8 virulence genes, namely *hlyA*, *ehx*, *eaeA*, *iha*, *efa1*, *toxB*, *tir*, and *espA* showed a higher prevalence than those in the EHEC non-O157 group.

encoded protein is involved in adhesion, showed a much lower prevalence, in both the O157 group (2.1%) and the non-O157 group (7.6%), relative to other genes. The prevalence rate of the *eae* gene was 43 (91.5%) and 134 (72.8%) in the O157 and non-O157 groups, respectively. Interestingly, all of the EHEC O157 isolates that caused bloody diarrhea harbored hemolysin genes.

All strains were sensitive to 10 antimicrobial agents (Table 2). The most common form of antibiotic resistance in both EHEC groups was to tetracycline (19.1% and 21.7%) and ampicillin (14.9% and 13.0%). In the studies from Switzerland (9), tetracycline resistance was reported to be the most common (21.6%). Resistance to ≥ 1 of the 17 antimicrobial agents tested was detected in 14 (29.8%) O157 EHEC strains and 41 (22.3%) non-O157 EHEC strains. This result is consistent with the findings of the Swiss study, in which 25 (25.8%) of

Table 2. Antimicrobial susceptibility of EHEC O157 and EHEC non-O157 isolates

Antimicrobial agent	No. of resistant strains (%)	
	EHEC O157	EHEC non-O157
	(n = 47)	(n = 184)
ampicillin	7 (14.9)	24 (13.0)
amoxicillin-clavulanic acid	1 (2.1)	0
ampicillin-sulbactam	0	0
cefalotin	0	0
cefazolin	0	0
cefotetan	0	0
cefoxitin	0	0
cefotaxime	0	0
ceftriaxone	0	0
imipenem	0	0
amikacin	0	0
gentamicin	3 (6.4)	10 (5.4)
nalidixic acid	2 (4.3)	1 (0.5)
ciprofloxacin	0	0
tetracycline	9 (19.1)	40 (21.7)
chloramphenicol	6 (12.8)	14 (7.6)
trimethoprim-sulfamethoxazole	4 (8.5)	13 (7.1)

97 non-O157 EHEC strains were found to be resistant to ≥ 1 of the 12 antimicrobial drugs tested (9).

In conclusion, a total of 231 human clinical isolates were collected and classified as EHEC O157 and EHEC non-O157. Our results indicated numerous differences between the 2 groups. Interestingly, 93.3% of the EHEC O157 strains isolated from patients with bloody diarrhea harbored the *stx2* gene, and all EHEC O157 strains isolated from patients with bloody diarrhea carried 3 hemolysin genes tested by PCR. This finding suggests that the virulence factors encoded by *stx2*, *hlyA*, *ehx*, and *clyA* are essential for the development of bloody diarrhea in patients infected with EHEC O157. To confirm the relationship between symptoms and virulence factors, more detailed surveillance data and molecular characterization such as pulsed-field gel electrophoresis and multiple-locus variable-number tandem repeat analysis are required. This study indicates that EHEC O157 frequently links diarrhea than EHEC non-O157 in the Korean population.

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Conflict of interest None to declare.

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