SHIGA-TOXIN PRODUCING *ESCHERICHIA COLI* IN VIETNAM

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Abstract: Shiga-toxin producing *Escherichia coli* isolated in Vietnam were examined. The 9 isolates included 5 from 400 diarrheal patients and 4 from 42 cows in 2003. No isolate carried *eae* gene. All human isolates except one and one of four animal isolates carried both *stx1* and *stx2*. One of the human isolates could not be definitely identified as a Shiga-toxin producing *Escherichia coli*, because it showed positive for PCR using a common primer set for *stx* gene family and also positive for *stx2d* subtype, but negative for *stx1*, *stx2* and for expression of the toxin. The serotypes of the isolates were various, but major serogroups such as O157, O26 and O111 were not found.

Enterohemorrhagic *Escherichia coli* (EHEC) has been recognized as a distinct class of diarrheagenic *E. coli* since 1982, when outbreaks of hemorrhagic colitis were observed in the United States and Canada [1, 2]. Prior to these outbreaks, in 1980, verocytotoxin-producing *E. coli* was reported in classical enteropathogenic *E. coli*, EPEC [3]. In the early 1980s, O'Brien *et al.* investigated an *E. coli* O26 strain and purified a toxin closely related to Shiga-toxin of *Shigella dysenteriae* type1 [4, 5]. EHEC was once called Verotoxin producing *E. coli* (VTEC), and recently it is commonly called Shiga-toxin-producing *E. coli* (STEC). Readers in the other research fields thus have to keep in mind that the terms EHEC, VTEC and STEC are basically the same. We use the term STEC hereafter. After the initial outbreaks of STEC infection in 1982, STEC attracted worldwide attention because the illness is clinically severe and can be followed by serious sequels such as hemolytic uremic syndrome (HUS) and central nervous disorder. Outbreaks involving hundreds of individuals cause a sensation, but sporadic infections are frequently overlooked unless the illness is serious. Nevertheless, the sporadic infection represents the major impact of this pathogen. The epidemiology of the illness and the distribution of STEC are well studied

in industrialized countries [6], but there are not many reports from the developing world. STEC strains were actively sought in a study on Bangladeshi children, but no child was infected with STEC [7]. In Lao People's Democratic Republic, 880 diarrheal patients randomly collected in 1996 and 1997 were examined, but STEC with O111 serogroup was detected in only 1 patient. Also, 1 strain without *eae* gene was detected among 278 patients examined in 2002 [8, 9]. Detection of STEC in Thailand was also reported but serotype O157:H7 strains were not found [10]. In the present study, we intensively examined human diarrheal stools and cattle stools for STEC in Vietnam.

E. coli strains were isolated from human diarrheal stools and cow's stools. The human diarrheal stools were collected from 400 patients in Nam Dinh province, and the cow stools were collected from 42 animals at a stock farm in a suburb of Hanoi in 2003. The isolated *E. coli* were first examined by multiplex PCR as described by Toma *et al.* [11]. *stx* positive strains were examined again using a single primer set (v1/v5 and v3/v4) to determine the toxin type. Primer set v1 (5'AGTTAATGTGGTGGCGAA3') and v5 (5'GACTCTTCCATCTGCCG3') was used to amplify 811 base pairs of *stx1*. Primer set v3 (5'TTCGGTATCCTATTCCCG

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3') and v4 (5'TCTCTGGTCATTGTATTA3') was used to amplify 471 base pairs of *stx*₂ and its variants [12]. Further classification of the *stx*₂ gene family was performed using primers reported by Wang *et al.* [13]. DNAs were extracted from the organisms as described by Yokoyama [14]. The PCR mixture used with a single primer set consisted of a total volume of 30 µl containing 10 mM Tris-HCl (pH8.3), 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl₂, 0.75 U of *Taq* DNA polymerase (Toyobo, Osaka, Japan), 0.2 mM deoxynucleoside triphosphate, a 0.25 µM concentration of each primer and 3 µl of the DNA template. The PCR program was 95 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min, for 25 cycles. *stx* gene positive *E. coli* were examined for production of Shiga toxin using reversed passive latex agglutination kit VTEC-RPLA (DENKA-SEIKEN Co. LTD., Tokyo). The organisms were cultured in CAYE medium as indicated in the manual attached to the kit. O:H serotyping of the strains was performed by bacterial agglutination as described previously by Orskov F., and Orskov I. [15].

STEC was detected in 5 of the 400 patients examined (1 of the 5 was questionable). Therefore, the frequency of the definite isolation of STEC was 4/400 (1.0%). STEC was also detected in 4 of the 42 cows examined (9.5%). Se-

rotype O157:H7 was not found. Three of the 9 isolates belonged to serotype O44:H16. In one isolate, O-antigen was untypable but H8; the other 5 isolates belonged to serotypes O8:H8, O100:H40, O159:H21, O55:H11 and O2:H42 (Table1). The toxin type was variable and all 9 isolates were negative for the *eae* gene. PCR for toxin typing showed that the 4 human isolates (O1-A, O3-D, O4-C, O6-B) carried both *stx*₁ and *stx*₂ and that the one questionable isolate was positive for primer set VTcom-u / VTcom-d, common to *stx*₁ and *stx*₂, but did not react with the primer set v1/v5 and v3/v4 specific to *stx*₁ and *stx*₂, respectively. Nevertheless, using the primers for detecting various genes belonging to the *stx*₂ family reported by Wang *et al.*, this isolate (O2-C) produced a relevant amplicon compatible with *stx*_{2d}. Moreover, it did not produce the toxin when examined by RPLA. Since the details are not available at present, it is questionable whether this isolate can be regarded as STEC. Among the animal isolates, one (93-6) carried both *stx*₁ and *stx*₂, and the other three carried one of them (Table1 and Fig.1). The production of toxins was compatible with the genotype, except for the one questionable isolate.

To the best our knowledge, this is the first study on STEC isolated in Vietnam and the first to suggest that STEC strains have already spread in this country. However,

Figure 1

Lanes, M:100-bp DNA ladder (upper broad band indicates 1000 bp and lower broad band indicates 500 bp), 1:O1-A, 2:O2-C, 3:O3-D, 4:O4-C, 5:O6-B, 6:5-5, 7:18a., 8:93-6, 9:2245-2 Primer sets v1/v5 and v3/v4 were used to detect 811 bp product of *stx*₁ and 471 bp product of *stx*₂, respectively. Lane2 (questionable strain O2-C as mentioned in the text)

Table 1

No.	strains	PCR			Sub.	RPLA			serotype
		<i>stx</i>	<i>stx1</i>	<i>stx2</i>		<i>eae</i>	Stx-1	Stx-2	
1	O1-A	+	+	+	2c, 2d	-	+	+	O44:H16
(2)*	(O2-C)*	(+)*	(-)*	(-)*	(2d)*	(-)*	(-)*	(-)*	(O8:H8)*
3	O3-D	+	+	+	2	-	+	+	O100:H40
4	O4-C	+	+	+	2	-	+	+	OUT:H8
5	O6-B	+	+	+	2c	-	+	+	O159:H21
6	5-5	+	-	+	2c, 2d	-	-	+	O44:H16
7	18a	+	+	-	-	-	+	-	O55:H11
8	93-6	+	+	+	2c	-	+	-	O2:H42
9	2245-2	+	-	+	2d	-	-	+	O44:H16

No. 1-5: human isolates, No. 6-9: cattle isolates, OUT: O-antigen untypable, Products from the primer set VTcom-u and VTcom-d (*stx*), v1 and v5 (*stx1*), v3 and v4 (*stx2*), subtypes of *stx2* family identified using primer sets described by Wang *et al.* (12). (*) : to be discussed

it is unknown whether these STEC originally developed in Vietnam or came from somewhere else. Serotypes of STEC in Vietnam are different from those in neighboring countries [8, 9, 10], and the organisms did not carry *eae* gene. Since the clinical data were not obtained in the present study, the pathogenicity of these isolates remains unclear. But the fact that the isolates lacked an important pathogenic gene (*eae*) indicates the necessity for further studies on the clinical findings and pathogenicity of these organisms. The adhesive factors encoded outside of the locus enterocyte effacement are now under intensive investigation in our laboratory.

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