Jpn. J. Infect. Dis., 64, 2011

Laboratory and Epidemiology Communications

Serotyping and Molecular Characterization of *eae*-Positive *Escherichia coli* Isolated in a Gastroenteritis Outbreak

Yoko Miyajima*, Yoko Matui, Reiko Adachi, Kazunari Yamamoto, Tetuya Saito, Gen Kobayashi, Mari Saito, and Jyunichi Tanabe

Niigata City Institute of Public Health and Environment, Niigata 950–2023, Japan

Communicated by Makoto Ohnishi

(Accepted May 29, 2011)

Enteropathogenic *Escherichia coli* (EPEC) causes diarrhea and represents a major health problem among infants, particularly in developing countries (1). EPEC produces characteristic attaching-and-effacing (A/E) lesions on intestinal epithelial cells. The A/E phenotype is encoded by a cluster of genes including the *eae* gene located on the locus of enterocyte effacement (2). The attachment to epithelial cells is caused primarily by type IV fimbriae known as a bundle-forming pilus (BFP), which is encoded by a cluster of *bfp* genes located on a large virulence plasmid (3). However, some EPEC strains do not have the virulence plasmid and are referred to as ''atypical EPEC'' (4,5).

We provide an overview of a gastroenteritis outbreak and discuss the results of the consequent assays in which various types of EPEC were detected.

On June 16, 2007, elementary school A in Niigata City reported that a large number of the 6th grade students were absent from school because of gastroenteritis, which seemed to be spreading among the schoolchildren. Consequently, a health center started investigating the issue. Elementary schools B and C reported similar cases: some 6th grade students had developed gastroenteritis symptoms after a 1-night school trip. All three schools had used the same hotel accommodations. Elementary school A had used the hotel on June 13 and 14, and elementary schools B and C had used it on June 14 and 15. Incidence of gastroenteritis among the students who participated in the school trips was high: school A, 71.4%; school B, 77.8%; and school C, 57.1%. However, none of the teachers who had accompanied the children developed any symptoms. Further, students in other grades did not show symptoms. The major symptoms included diarrhea and abdominal pain. Some of the students developed headache and fever, and a small number developed nausea or vomiting. Fecal samples were submitted by 59, 19, and 7 children of elementary schools A, B, and C, respectively.

Bacterial and viral assays were performed in 85 and 69 fecal samples, respectively, and both assays were performed in a vomit sample. The bacterial assays were carried out by conventional methods employing SS agar, DHL agar, MacConkey agar, CT-SMC agar, TCBS agar, CCDA agar, NGKG agar, mannitol salt agar with egg yolk, and CW agar with egg yolk. Norovirus, sapovirus, astrovirus, adenovirus, and rotavirus were examined in the viral assay. No known viruses or bacteria associated with food poisoning were detected. Although no accumulation of specific O-serogroup *E. coli* isolated from the specimens was observed, the possibility of diarrheagenic *E. coli* as a causative agent was examined by sweep PCR for the virulence factors *elt*, *estA1/2*, *invE*, *stx1/2*, *eae*, *aggR*, and *astA* using an unpurified bacterial lawn on DHL agar plates. A pilot investigation indicated that 4 of 10 samples tested were *eae*-positive, suggesting that the outbreak was caused by EPEC. Thus, we further tried to detect *eae* and *bfpA* genes from 2 or 3 *E. coli* isolates from each specimen. *E. coli* with the *eae* gene was detected in 43 of 85 fecal specimens analyzed (50.6%). In 21 specimens, multiple (2 or 3) *E. coli* isolates were *eae*-positive but of different O-serogroups. In total, 69 *E. coli* isolates were *eae*-positive, including 10 that were positive for *bfpA*. Serotyping showed that among 69 *E. coli* isolates, 63 *eae*-positive isolates belonged to 8 different serotypes; the serotypes of 6 isolates could not be determined (Table 1). As mentioned above, diversity was observed among strains derived from single specimens, indicating the possibility of mixed infection by different types of *eae*-positive *E. coli*. The predominant serotype (42 of 69 isolates, 60.9%) was OUT:H21 (Table 1), and the second most

Table 1. Number of serotypes among *eae*-positive strains

Serotype	School			Total
	A	B	C	
OUT:H21	30	11	1	42
O115:HUT[1)]	4	4	2	10
O8:HUT	2		2	4
O128:H2	1		1	2
O153:H7			2	2
O29:HUT	1			1
OUT:H2	1			1
OUT:H6	1			1
OUT:HUT	2	2	2	6
Total	42	17	10	69

1): *bfpA* gene-positive.
UT, untypable.

*Corresponding author: Mailing address: Niigata City Institute of Public Health, Koshin 2151-1, Niigata 950-2023, Japan. Tel: +81-25-231-1231, Fax: +81-25-130-5818, E-mail: eisei.rc@city.niigata.lg.jp

Table 2. Molecular and biological characterization of OUT:H21 and O115:HUT EPEC isolates

Serotype	Allele of virulence genes[1]			Biological characterization[2]			
	eae	*bfpA*	*perA*	Autoaggregation	Contact hemolysis	Cell adhesion[3]	FAS[3]
OUT:H21	θ	(−)	(−)	−	−	−	−
O115:HUT	ε	α	(−)	−	+	−	−

[1]: (−) indicates absence of the gene.
[2]: − indicates negative result.
[3]: positive after 6 h.

common serotype was O115:HUT (10 of 69 isolates, 14.5%), which carried both the *eae* and *bfpA* genes. Nucleotide sequencing was carried out using PCR products amplified from internal regions of *eae* from 2 representative strains of each serotype and *bfpA* from O115:HUT strains (6). Alleles of OUT:H21 and O115:HUT were θ and ε, respectively, and the *bfpA* of O115:HUT was typed as α (Table 2).

To reveal the genetic relatedness of the OUT:H21 and O115:HUT isolates, we performed pulsed-field gel electrophoresis (PFGE) analysis using *Xba*I digestion products. All OUT:H21 isolates showed an identical PFGE pattern. The PFGE pattern from all O115:HUT isolates was also identical but distinct from the pattern of OUT:H21 (data not shown). These patterns were observed from isolates regardless of the elementary schools from which the specimens were collected.

Although the pathogenicity of *eae*-positive *E. coli* has been controversial (4,5), some unique phenotypes are induced by typical EPEC strains. Thus, we tested the isolates of OUT:H21 (n = 42) and O115:HUT (n = 10) for 4 different phenotypes: (i) localized adherence in the HEp-2 cell assay, (ii) autoaggregation, (iii) contact hemolysis, and (iv) actin accumulation beneath cell-attached bacteria using F-actin fluorescence staining (FAS), as previously described (6–8). Isolations of the most prevalent serotype in this study OUT:H21, which were *bfp*-defective atypical EPEC, did not show any phenotype usually induced by typical EPEC. While O115:HUT isolates (*eae*- and *bfpA*-positive) could induce contact hemolysis, the isolates did not show local adherence or autoaggregation (Table 2). PCR analysis for *perA* genes using the specific primer set described previously (9) showed that O115:HUT isolates were *perA*-negative. Since *perA* is important for the expression of BFP, the absence of *perA* in the isolates might explain the negative results in cell attachment and autoaggregation assays.

This gastroenteritis outbreak was an unusual case in which different serotypes of *eae*-positive *E. coli* were isolated. The putative typical EPEC isolated in this case possessed both *eae* and *bfpA*, but *perA* was deleted or mutated to an extent to which it could not be detected using PCR. As such, these strains did not show the typical characteristics of EPEC. Furthermore, OUT:H21 isolates showed no unique phenotype of EPEC. Although the role of OUT:H21 in the pathogenesis of gastroenteritis was not proven, epidemiological data suggested that OUT:H21 could be at least one cause of this outbreak.

Detection of adhesive *E. coli* by assaying for virulence factor-related genes was effective because it provided quick results. However, the analysis is not sufficient, and additional investigation is required to phenotypically characterize the isolated strains, particularly for pathogenic traits. Further investigation is especially required to determine the phenotypes of the atypical EPEC isolated in this study. As noted previously by Nataro, the pathogenesis of atypical EPEC infection has not yet been clarified (5). Therefore, it is important to carry out epidemiological studies in conjunction with detailed analysis of the strains isolated in the event of a gastroenteritis outbreak. Further study to reveal the mechanism of pathogenesis will be needed in order to develop improved identification methods for atypical EPEC.

This article appeared in the Infectious Agents Surveillance Report (IASR), vol. 32, p. 143–144, 2011 in Japanese.

Acknowledgments We are grateful to Kenitiro Ito (Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan) for critical review of this manuscript.

Conflict of interest None to declare.

REFERENCES

1. Nataro J.P. and Kaper J.B. (1998): Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev., 11, 142–201.
2. Cravioto, A., Gross, R.J., Scotland, S.M., et al. (1979): An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Curr. Microbiol., 3, 95–99.
3. Donnenberg, M.S., Giron, J.A., Nataro, J.P., et al. (1992): A plasmid-encoded type IV fimbrial gene of enteropathogenic *Escherichia coli* associated with localized adherence. Mol. Microbiol., 6, 3427–3437.
4. Trabulsi, L.R., Keller, R., Tania, A., et al. (2002): Typical and atypical enteropathogenic *Escherichia coli*. Emerg. Infect. Dis., 8, 508–513.
5. Nataro, J.P. (2006): Atypical enteropathogenic *Escherichia coli*: typical pathogens? Emerg. Infect. Dis., 12, 696.
6. Blank, T.E., Zhong, H., Bell, A.L., et al. (2000): Molecular variation among type IV pilin (*bfpA*) genes from diverse enteropathogenic *Escherichia coli* strains. Infect. Immun., 68, 7028–7038.
7. Warawa, J., Finlay, B.B. and Kenny, B. (1999): Type III secretion-dependent hemolytic activity of enteropathogenic *Escherichia coli*. Infect. Immun., 67, 5538–5540.
8. Knutton, S., Phillips, A.D., Smith, H.R., et al. (1991): Screening for enteropathogenic *Escherichia coli* in infants with diarrhea by the fluorescent-actin staining test. Infect. Immun., 59, 365–371.
9. Iida, M., Okamura, N., Yamazaki, M., et al. (2010): Classification of *perA* sequences and their correlation with autoaggregation in typical enteropathogenic *Escherichia coli* isolates collected in Japan and Thailand. Microbiol. Immunol., 54, 184–195.