Jpn. J. Infect. Dis., 68, 216–220, 2015

Original Article

Biochemical Features and Virulence Gene Profiles of Non-O157/O26 Enterohemorrhagic *Escherichia coli* Strains from Humans in the Yamaguchi Prefecture, Japan

Mitsuhiro Kameyama, Junko Yabata, Yasuharu Nomura, and Kiyoshi Tominaga*

Department of Health Science, Yamaguchi Prefectural Institute of Public Health and Environment, Yamaguchi 753-0821, Japan

SUMMARY: The biochemical features and virulence gene profiles of 37 enterohemorrhagic *Escherichia coli* (EHEC) strains belonging to serogroups other than O157 and O26 (non-O157/O26 EHEC) were investigated. All strains were isolated from humans between 2002 and 2013 in the Yamaguchi Prefecture. Serogroup O111 strains were the most common, followed by O103, O121, and O145. Most strains (84%) were negative for sorbose fermentation, whereas only 1 and 2 were negative for sorbitol and rhamnose fermentation, respectively. Two strains lacked β-D-glucuronidase activity. *Shiga toxin* (*stx*) subtyping revealed 6 genotypes:*stx1a* (n = 20), *stx1a* + *stx2a* (n = 8), *stx2a* (n = 4), *stx2b* (n = 3), *stx2a* + *stx2c* (n = 1), and *stx2a* + *stx2d* (n = 1). Polymerase chain reaction screening of other toxin and adherence genes showed that *astA*, *subA*, and *cdtB* were present in 5, 2, and 2 strains, respectively. The intimin gene *eae* was present in 30 strains (81%). Of the 7 *eae*-negative strains, *saa* and *eibG* were found in 3 and 2 strains, respectively; no adherence factors were detected in the remaining 2 strains. The antimicrobial susceptibility profiles of the strains to 12 drugs were examined and 11 strains (30%) showed resistance to 1 or more drugs. Our results revealed that non-O157/O26 EHEC strains exhibit various biochemical phenotypes and carry several toxins and adherence factor genes.

INTRODUCTION

Enterohemorrhagic *Escherichia coli* (EHEC) is a human pathogen that causes bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS) (1). A broad range of EHEC serotypes have been associated with these illnesses, and in Japan, 2 major serogroups (O157 and O26) accounted for 57% and 23% of all human isolates reported in 2012, respectively (2). However, infections caused by EHEC strains belonging to serogroups other than O157 and O26 (non-O157/O26 EHEC) are being reported with increasing frequency (3).

EHEC strains O157, O26, and O111 generally do not ferment sorbitol, rhamnose, and sorbose, respectively (4–6). Thus, these biochemical features are often used to identify the 3 serogroups among clinical and environmental specimens. Serogroup O157 also cannot produce β-D-glucuronidase (7). However, some strains with atypical phenotypes, such as sorbitol-positive or β-D-glucuronidase-positive O157, have recently emerged (8). Although much is known about these more common serogroups, information regarding the biochemical features of other EHEC serogroups is lacking.

Shiga toxin (Stx) is a major virulence factor of EHEC and is divided into 2 major groups, Stx1 and Stx2 (9). The corresponding genes *stx1* and *stx2* are further subdivided into 3 (*stx1a*, *stx1c*, and *stx1d*) and 7 (*stx2a–g*) subtypes, respectively (10). Recent studies have revealed that in addition to Stx, a set of EHEC strains produce other toxins, such as enteroaggregative *E. coli* heat-stable toxin (EAST1) (11), subtilase cytotoxin (SubAB) (12), and cytolethal distending toxin (CDT) (13). The majority of EHEC strains belonging to serogroups O157, O26, and O111 produce intimin. Intimin is encoded by *eae*, which is located in the locus of enterocyte effacement pathogenicity island and is associated with intimate adhesion of the bacteria to cultured epithelial cells (14). However, some strains lack *eae* (15). Although almost all *eae*-negative strains have been isolated from asymptomatic carriers, some have been implicated in outbreaks of HUS (1). These *eae*-negative strains possess alternative adherence factors, such as autoagglutinating adhesin (Saa) (16) and immunoglobulin-binding protein G (EibG) (15). In addition, some *eae*-negative EHEC strains demonstrate aggregative adhesion to HEp-2 cells, in a manner similar to that of enteroaggregative *E. coli* (EAggEC) (17).

In the present study, the biochemical features and distribution patterns of virulence toxins and adhesion factor genes were investigated in non-O157/O26 EHEC strains isolated in the Yamaguchi Prefecture.

MATERIALS AND METHODS

Strains: A total of 539 EHEC isolates were sent to our laboratory from hospitals and health care centers in the Yamaguchi Prefecture, Japan. All samples were isolated between 2002 and 2013 from humans by the respective health care facilities. Cultured colonies were

Received July 9, 2014. Accepted September 9, 2014.
J-STAGE Advance Publication January 20, 2015.
DOI: 10.7883/yoken.JJID.2014.303

*Corresponding Author: Mailing address: Department of Health Science, Yamaguchi Prefectural Institute of Public Health and Environment, Yamaguchi 753-0821, Japan. Tel: 81-83-922-7630, Fax: 81-83-922-7632, E-mail address: k-tominaga@chosyudori.or.jp

inoculated into sulfide-indole motility (SIM) medium (Eiken Chemical, Co., Ltd., Tokyo, Japan) and incubated for 48 h at 25°C to confirm the motility of the isolates. The serotype was determined using commercial *E. coli* O and H antisera (Denka Seiken Co., Ltd., Tokyo, Japan). Isolates that were untypeable on the basis of O or H antigen testing were submitted to the National Institute of Infectious Diseases (Tokyo, Japan) for further identification.

Biochemical features: Carbohydrate fermentation using lactose, sorbitol, rhamnose, and sorbose was tested in semisolid media tubes containing phenol red broth base (Becton, Dickinson and Company, Sparks, MD, USA) with 0.3% (w/v) Bacto agar (Becton, Dickinson and Company) and 1.0% (w/v) of each carbohydrate. Inoculated tubes were incubated for 72 h at 37°C. Production of indole, lysine decarboxylase (LDC), and β-D-glucuronidase was confirmed in SIM medium, lysine indole motility medium (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan), and cellobiose lactose indole β-D-glucuronidase medium (Kyokuto Pharmaceutical Industrial Co., Ltd.), respectively, following incubation for 20 ± 2 h at 37°C (18).

Stx production and *stx* subtyping: Stx1 and Stx2 production was assessed using a reversed passive latex agglutination test (VTEC-RPLA; Denka Seiken Co., Ltd.), according to the manufacturer's instructions. *stx* genes were detected and subtyped by polymerase chain reaction (PCR) (10) analysis of genomic DNA prepared using a QIAamp DNA blood mini kit (Qiagen Sciences, Germantown, MD, USA), according to the manufacturer's instructions.

Detection of virulence toxins and adherence factor genes: The toxin-encoding genes *astA*, *cdtB*, and *subA* and adherence factor genes *eae, aggR, saa*, and *eibG* were detected by PCR, as described previously (11,12,15,19–22). Additional toxin genes *lt, sth*, and *stp*, coding for the heat-labile enterotoxin (LT) and heat-stable enterotoxins of the human (STh) or porcine (STp) variety, respectively, were also screened by PCR using commercial primer sets (Takara Bio, Inc., Shiga, Japan), according to the manufacturer's instructions.

Susceptibility to antimicrobial agents: The susceptibility of all strains to various antimicrobial agents was tested by employing the Kirby-Bauer disk diffusion method (23) on Mueller-Hinton agar (Oxoid, Ltd., Hampshire, UK) plates containing the following antimicrobial agents (Nippon Becton Dickinson, Fukushima, Japan): ampicillin (AMP), cephalothin (CEF), cefotaxime (CTX), streptomycin (STR), kanamycin (KAN), gentamicin (GEN), tetracycline (TET), chloramphenicol (CHL), nalidixic acid (NAL), ciprofloxacin (CIP), fosfomycin (FOM), and trimethoprim-sulfamethoxazole (SXT).

RESULTS

Non-O157/O26 EHEC strains: Of the 539 isolates collected, 423 (78.5%) were identified as serogroup O157 and 72 (13.4%) as O26. Of the remaining 44 non-O157/O26 isolates, a total of 37 epidemiologically unrelated strains, which consisted of 33 strains from sporadic cases and 1 representative strain from each of 4 each outbreaks, were subjected to further analysis (Table 1). Serogroup O111 was dominant (n = 15), followed by O103 (n = 6), O121 (n = 4), and O145 (n = 3). One strain, 09Y56, was classified as O antigen untypable (OUT) and 4 strains, 07Y16, 07Y30, 06Y27, and 06Y32, as H antigen untypable. Eighteen strains were non-motile (NM). Twenty-nine (65%) of the strains were isolated from patients, whereas the remaining isolates were from asymptomatic carriers. Strains were isolated from patients displaying the following clinical symptoms: watery diarrhea (n = 23), abdominal pain (n = 20), fever (n = 10), bloody diarrhea (n = 9), vomiting (n = 4), and intussusception (n = 1). HUS was not observed in any of the patients. Of the strains isolated from asymptomatic carriers, O91:H14 and O146:NM accounted for 2 strains each, whereas 1 strain was identified from each of the serotypes O111:NM, O112ab:H25, O169:H9, and OUT:NM.

Biochemical features: All strains were positive for lactose fermentation and indole production. Most strains (84%), including all O111 isolates, were negative for sorbose fermentation, whereas only 1 (OUT:NM) and 2 (O111:NM and O103:H11) strains were sorbitol-negative and rhamnose-negative, respectively. Seventeen strains, including all serogroup O111 isolates, were negative for LDC production, and 2 strains (O103:H2 and O103:H25) lacked β-D-glucuronidase activity (Table 1).

Characterization of virulence factors: As shown in Table 1, the all 8 O111 strains produced both Stx1 and Stx2, whereas other strains produced either Stx1 or Stx2. Six *stx* genotypes were identified: *stx1a* (n = 20) in Stx1-producing strains; *stx1a* + *stx2a* (n = 8) in Stx1 and Stx2-producing strains; and *stx2a* (n = 4), *stx2a* + *stx2c* (n = 1), *stx2a* + *stx2d* (n = 1), and *stx2b* (n = 3) in Stx2-producing strains.

Of the toxin genes, *astA* was detected in strains O121:H19 (n = 3), O146:NM (n = 1), and O169:H9 (n = 1), *subA* was detected in 2 O91:H14 strains, and *cdtB* was found in 1 strain each of O111:NM and O112ab:H25. The adherence factor gene *eae* was detected in 30 strains (81.1%). Of the 7 *eae*-negative strains, *saa* was detected in 2 O91:H14 and 1 O112ab:H25 strains, and *eibG* was found in 2 O146:NM strains. No adherence factor genes were detected in 2 strains, O169:H9 and OUT:NM.

Antimicrobial agent susceptibility: Of the 37 tested strains, 11 (29.7%) showed resistance to 1 or more antimicrobials (Table 2). The 7 O111 strains were resistant to AMP, STR, and TET, and 2 of these strains, 06Y27 and 12Y03, exhibited additional resistance to KAN and NAL and CEF and KAN, respectively. The 3 O121:H19 strains were resistant to STR and TET, and an OUT:NM strain showed resistance to TET.

DISCUSSION

We investigated various biochemical phenotypes used in identification of non-O157/O26 EHEC strains. Sorbose can be used an indicator for the isolation of EHEC O111; however, most other strains were also negative for sorbose fermentation. A few strains with sorbitol-negative or rhamnose-negative phenotypes were also found. Interestingly, strain 10Y02 (serotype O111:NM) was negative for both rhamnose and sorbose fermenta-

Table 1. Biochemical features and virulence gene profiles of non-O157/O26 EHEC strains

Strain no.	Serotype	Yr of isolation	Clinical feature	Stx		Other virulence toxin gene	Adherence factor gene	Fermentation of[1]				Lysine decar-boxylase	Indole	β-D-glucuroni-dase
				Production	Subtype			Lactose	Sorbitol	Rhamnose	Sorbose			
02Y01	O111:NM	2002	DB, F, V	1, 2	*1a*, *2a*		*eae*	+	+	+	−	−	+	+
06Y04	O111:NM	2006	AP, DW	1, 2	*1a*, *2a*		*eae*	+	+	+	−	−	+	+
07Y16	O111:HUT	2007	AP, DB, DW, F	1, 2	*1a*, *2a*		*eae*	+	+	+	−	−	+	+
07Y30	O111:HUT	2007	DB	1, 2	*1a*, *2a*		*eae*	+	+	+	−	−	+	+
09Y36	O111:NM	2009	AP, DB, DW	1, 2	*1a*, *2a*		*eae*	+	+	+	−	−	+	+
10Y02	O111:NM	2010	—	1, 2	*1a*, *2a*		*eae*	+	+	−	−	−	+	+
11Y14	O111:NM	2011	AP, DW	1, 2	*1a*, *2a*		*eae*	+	+	+	−	−	+	+
11Y18	O111:NM	2011	AP, DB	1, 2	*1a*, *2a*		*eae*	+	+	+	−	−	+	+
02Y11	O111:NM	2002	DW	1	*1a*		*eae*	+	+	+	−	−	+	+
04Y29	O111:NM	2004	AP, DB, DW	1	*1a*		*eae*	+	+	+	−	−	+	+
06Y35	O111:NM	2006	DW, F	1	*1a*	*cdtB*	*eae*	+	+	+	−	−	+	+
06Y27	O111:HUT	2006	AP, DW, F	1	*1a*		*eae*	+	+	+	−	−	+	+
09Y17	O111:NM	2009	AP, DW	1	*1a*		*eae*	+	+	(+)	−	−	+	+
12Y03	O111:NM	2012	AP, DW, F, V	1	*1a*		*eae*	+	+	+	−	−	+	+
12Y24	O111:NM	2012	DW	1	*1a*		*eae*	+	+	+	−	−	+	+
10Y24	O103:H2	2010	AP, DW, F	1	*1a*		*eae*	+	+	+	−	+	+	+
10Y42	O103:H2	2010	AP, DW, F	1	*1a*		*eae*	+	+	+	−	+	+	−
08Y07	O103:H2	2008	AP, DW	1	*1a*		*eae*	+	+	+	−	+	+	+
12Y05	O103:H2	2012	DW	1	*1a*		*eae*	+	+	+	−	+	+	+
08Y09	O103:H11	2008	AP, DB, DW, F	1	*1a*		*eae*	+	+	−	+	+	+	+
09Y07	O103:H25	2009	DW, F	1	*1a*		*eae*	+	+	(+)	−	+	+	−
07Y06	O121:H19	2007	AP, I, V	2	*2a*	*astA*	*eae*	+	+	+	+	+	+	+
07Y09	O121:H19	2007	DW	2	*2a*	*astA*	*eae*	+	+	+	+	+	+	+
07Y11	O121:H19	2007	AP, DW, V	2	*2a*	*astA*	*eae*	+	+	+	+	+	+	+
11Y11	O121:H19	2011	AP, DW	2	*2a*		*eae*	+	+	+	+	+	+	+
07Y42	O145:NM	2007	AP, DW	1	*1a*		*eae*	+	+	+	−	+	+	+
07Y46	O145:NM	2007	DW	1	*1a*		*eae*	+	+	+	−	+	+	+
11Y12	O145:NM	2011	AP, DB	1	*1a*		*eae*	+	+	(+)	−	+	+	+
06Y34	O115:H10	2006	AP, F	1	*1a*		*eae*	+	+	+	−	+	+	+
06Y32	O165:HUT	2006	AP, DB, DW	2	*2a*, *2c*		*eae*	+	+	(+)	−	−	+	+
09Y35	O91:H14	2009	—	1	*1a*	*subA*	*saa*	+	(+)	+	−	+	+	+
10Y33	O91:H14	2010	—	1	*1a*	*subA*	*saa*	+	+	+	−	+	+	+
10Y39	O112*ab*:H25	2010	—	2	*2a*, *2d*	*cdtB*	*saa*	+	+	+	−	+	+	+
11Y22	O146:NM	2011	—	2	*2b*	*astA*	*eibG*	+	(+)	+	−	+	+	+
12Y12	O146:NM	2012	—	2	*2b*		*eibG*	+	(+)	+	−	+	+	+
10Y40	O169:H9	2010	—	1	*1a*	*astA*	None	+	+	+	(+)	+	+	+
09Y56	OUT:NM	2009	—	2	*2b*		None	+	−	+	−	−	+	+

[1]: +, positive within 24 h; (+), positive after 24 to 72 h; −, negative at 72 h.
AP, abdominal pain; DB, bloody diarrhea; DW, watery diarrhea; F, fever; I, intussusception; V, vomiting; —, asymptomatic carrier.

Table 2. Antimicrobial resistance patterns of non-O157/O26 EHEC strains

Strain	Serotype	Antimicrobial resistance pattern
06Y27	O111:HUT	TET, STR, AMP, KAN, NAL
12Y03	O111:NM	TET, STR, AMP, KAN, CEF
07Y16	O111:HUT	TET, STR, AMP
11Y14	O111:NM	TET, STR, AMP
11Y18	O111:NM	TET, STR, AMP
04Y29	O111:NM	TET, STR, AMP
12Y24	O111:NM	TET, STR, AMP
07Y06	O121:H19	TET, STR
07Y09	O121:H19	TET, STR
07Y11	O121:H19	TET, STR
09Y56	OUT:NM	TET

TET, tetracyclin; STR, streptmicin; AMP, ampicillin; KAN, kanamycin; NAL, nalidicsic acid; CEF, cefalothin.

tion. Two of the 37 non-O157/O26 strains were not able to produce β-D-glucuronidase, a characteristic shared by serogroup O157 strains. We have previously encountered β-D-glucuronidase-negative EHEC O26:H11 infections (18). Therefore, these findings further confirm that it is important to consider atypical phenotypic characteristics when identifying EHEC isolates.

We identified a group of non-O157/O26 strains that produced Stx along with either EAST1, SubAB, or CDT. Although EAST1 was first discovered in an EAggEC strain, the toxin is also present in various other types of *E. coli* (11). EHEC O157 often harbors *astA*, coding for EAST1 (24), and in this study, serogroups O121, O146, and O169 also carried this toxin gene. SubAB is a newly identified toxin that was first identified in an *eae*-negative, *saa*-positive EHEC O113:H21 strain that was responsible for a small outbreak of HUS in South Australia (25). A recent study revealed that the various serogroups of *eae*-negative EHEC, including O91, produce SubAB (12). In this study, both O91:H14 strains carried the toxin gene. CDT has been detected in eae-negative EHEC strains isolated from both humans and domestic animals (13,26). In addition, 2 *cdt*-carrying strains were obtained, and of those, 1 was *eae*-negative (O112ab:H25) and the other was *eae*-positive

(O111:NM). The *cdt* gene in *E. coli* is usually located either on the chromosome or on a large plasmid (13). To elucidate the location of *cdt* in the strains used in the current study, further studies are required.

Intimin is a virulence factor of EHEC. In the present study, *eae* was detected in 30 strains (81.1%), and all strains isolated from patients (n = 29) were *eae*-positive. These observations indicate that the presence of *eae* may be strongly associated with disease in humans. However, *eae*-negative EHEC is occasionally associated with severe diseases that are indistinguishable from those caused by *eae*-positive strains (1). In this study, 7 *eae*-negative strains were found, but all were obtained from asymptomatic carriers. Meanwhile, *saa* and *eibG* are commonly found in multiple serotypes of *eae*-negative EHEC and were originally detected in serogroup O113 and O91 strains, respectively (15,16). *saa* was found in serogroup O91 and O112ab isolates, whereas *eibG* was amplified from O146:NM strains. Although no adherence factor gene was found in isolates belonging to serotypes O169:H9 or OUT:NM, an alternative adherence factor may exist in these strains.

Antimicrobial susceptibility testing showed that 29.7% of the strains showed resistance to 1 or more antibiotics. Seto et al. (27) demonstrated that 40.5% of non-O157/O26/O111 strains were resistant to at least 1 antibiotic, a resistance ratio that is much higher than that of our study. No resistance was observed towards the drugs used for treatment of EHEC infection, such as FOM and fluoroquinolones. Recently, extended-spectrum cephalosporin-resistant EHEC O26:H11 emerged in Japan (28). This finding, along with the results of the current study, indicates that continuous monitoring of antimicrobial susceptibility in EHEC strains is required.

In conclusion, non-O157/O26 strains exhibit various biochemical phenotypes and carry several toxin and adherence factor genes. The phenotypic characteristic of being negative for sorbose fermentation may be useful for the identification of non-0157/026 EHEC strains, although this requires confirmation through further screening of a large number of non-157/O26 strains. In addition, the potential role of EAST1, SubAB, and CDT in the pathogenicity of EHEC should be investigated.

Acknowledgment We thank Dr. Sunao Iyoda, Department of Bacteriology I, National Institute of Infectious Diseases, for serotyping of EHEC strains and for helpful suggestions.

Conflict of interest None to declare.

REFERENCES

1. Mellmann A, Bielaszewska M, Köck R, et al. Analysis of collection of hemolytic uremic syndrome-associated enterohemorrhagic *Escherichia coli*. Emerg Infect Dis. 2008;14:1287-90.
2. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare. Enterohemorrhagic *Escherichia coli* infections in Japan as of April 2013. Infect Agents Surveillance Rep. 2013;34: 123'-4'.
3. Akiba Y, Kimura T, Takagi M, et al. Outbreak of enterohemorrhagic *Escherichia coli* O121 among school children exposed to cattle in a ranch for public education on daily farming. Jpn J Infect Dis. 2005;58:190-2.
4. March SB, Ratnam S. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J Clin Microbiol. 1986;23:869-72.
5. Hiramatsu R, Matsumoto M, Miwa Y, et al. Characterization of shiga toxin-producing *Escherichia coli* O26 strains and establishment of selective isolation media for these strains. J Clin Microbiol. 2002;40:922-5.
6. Tanaka H, Yatsuyanagi J, Uchimura M, et al. Evaluation of sorbose MacConkey medium containing cefixime and tellurite for isolation of enterohemorrhagic *Escherichia coli* O111. J Jpn Clin Microbiol. 1999;9:48-50. Japanese.
7. Thompson JS, Hodge DS, Borczyk AA. Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* serotype O157. J Clin Microbiol. 1990;28:2165-8.
8. Gunzer F, Böhm H, Rössmann H, et al. Molecular detection of sorbitol-fermenting *Escherichia coli* O157 in patients with hemolytic-uremic syndrome. J Clin Microbiol. 1992;30:1807-10.
9. Yamasaki S, Takeda Y. Enterohemorrhagic *Escherichia coli* O157:H7 episode in Japan with a perspective on Vero toxins (Shiga-like toxins). J Toxicol Txin Rev. 1997;16:229-40.
10. Scheutz F, Teel LD, Beutin L, et al. Multicenter evaluation of a sequence-based protocol for subtyping shiga toxins and standardizing *stx* nomenclature. J Clin Microbiol. 2012;50:2951-63.
11. Veilleux S, Dubreuil D. Presence of *Escherichia coli* carrying EAST1 toxin gene in farm animals. Vet Res. 2006;37:3-13.
12. Paton AW, Paton JC. Multiplex PCR for direct detection of shiga toxigenic *Escherichia coli* strains producing the novel subtilase cytotoxin. J Clin Microbiol. 2005;43:2944-7.
13. Bielaszewska M, Fell M, Greune L, et al. Characterization of cytolethal distending toxin genes and expression in shiga toxin-producing *Escherichia coli* strains of non-O157 serogroups. Infect Immun. 2004;72:1812-6.
14. McDaiel TK, Kaper JB. A cloned pathogenicity island from enteropathogenic *Escherichia coli* confers the attaching and effacing phenotype on *E. coli* K12. Mol Microbiol. 1997;23:399-407.
15. Lu Y, Iyoda S, Satou H, et al. A new immunoglobulin-binding protein, eibG, is responsible for the chain-like adhesion phenotype of locus of enterocyte effacement-negative, shiga toxin-producing *Escherichia coli*. Infect Immun. 2006;74:5747-55.
16. Paton AW, Srimanote P, Woodrow MC, et al. Characterization of Saa, a novel autoagglutinating adhesion produced by locus of enterocyte effacement-negative shiga toxigenic *Escherichia coli* strains that are virulent for humans. Infect Immun. 2001;69: 6999-7009.
17. Morabito S, Karch H, Mariani-Kurkdjian P, et al. Enteroaggregative, shiga toxin-producing *Escherichia coli* O111:H2 associated with an outbreak of hemolytic-uremic syndrome. J Clin Microbiol. 1998;36:840-2.
18. Kameyama M, Yabata J, Nomura Y, et al. β-glucuronidase-negative enterohemorhagic *Escherichia coli* O26 infections associated with a calf. Jpn J Infect Dis. 2013;66:458-9.
19. Hinenoya A, Naigita A, Ninomiya K, et al. Prevalence and characteristics of cytolethal distending toxin-producing *Escherichia coli* from children with diarrhea in Japan. Microbiol Immunol. 2009;53:206-15.
20. Paton AW, Paton JC. Direct detection and characterization of shiga toxigenic *Escherichia coli* by multiplex PCR for stx_1, stx_2, *eae*, *ehxA*, and *saa*. J Clin Microbiol. 2002;40:271-4.
21. Narimatsu H, Ogata K, Makino Y, et al. Distribution of nonlocus of enterocyte effacement pathogenic island-related genes in *Escherichia coli* carrying eae from patients with diarrhea and healthy individuals in Japan. J Clin Microbiol. 2010;48:4107-14.
22. Cerna JF, Nataro JP, Estrada-Garcia T. Multiplex PCR for detection of three plasmid-borne genes of enteroaggregative *Escherichia coli* strains. J Clin Microbiol. 2003;41:2138-40.
23. Clinical Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; 21th informational supplement. Document M100-S21, Wayne, PA:CLSI; 2011.
24. Yamamoto T, Taneike I. The sequences of enterohemorrhagic *Escherichia coli* and *Yersinia pestis* that are homologous to the enteroaggregative *E. coli* heat-stable enterotoxin gene: cross-species transfer in evolution. FEBS Lett. 2000;472:22-6.
25. Paton AW, Woodrow MC, Doyle RM, et al. Molecular characterization of a shiga-toxigenic *Escherichia coli* O113:H21 strain lacking *eae* responsible for a cluster of cases of hemolytic-uremic syndrome. J Clin Microbiol. 1999;37:3357-61.
26. Wu Y, Hinenoya A, Taguchi T, et al. Distribution of virulence genes related to adhesins and toxins in shiga toxin-producing *Es-*

cherichia coli strains isolated from healthy cattle and diarrheal patients in Japan. J Vet Med Sci. 2010;72:589-97.
27. Seto K, Taguchi M, Kobayashi K, et al. Biochemical and molecular characterization of minor serogroups of shiga toxin-producing *Escherichia coli* isolated from humans in Osaka Prefecture. J Vet Med Sci. 2007;69:1215-22.
28. Kon M, Kurazono T, Ohshima M, et al. Cefotaxime-resistant shiga-toxin-producing *Escherichia coli* O26:H11 isolated from patient with diarrhea. J Jpn Assoc Infect Dis. 2005;79:161-8. Japanese.