Development and evaluation of a loop-mediated isothermal amplification assay for rapid and simple detection of *Campylobacter jejuni* and *Campylobacter coli*

Wataru Yamazaki,¹ Masumi Taguchi,¹ Masanori Ishibashi,¹ Miyoshi Kitazato,² Masafumi Nukina,³ Naoaki Misawa⁴ and Kiyoshi Inoue¹

Correspondence

Wataru Yamazaki
wataru@iph.pref.osaka.jp

¹Division of Bacteriology, Osaka Prefectural Institute of Public Health, Osaka, Japan

²Yodogawa Christian Hospital, Osaka, Japan

³Microbiological Department, Kobe Institute of Health, Hyogo, Japan

⁴Department of Veterinary Public Health, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan

We developed a loop-mediated isothermal amplification (LAMP) assay for the rapid and simple detection of *Campylobacter jejuni* and *Campylobacter coli*. The assay provides a specific LAMP product for each of these two species. The assay correctly identified 65 *C. jejuni* and 45 *C. coli* strains, but not 75 non-*C. jejuni/coli* strains. The sensitivity of the LAMP assay for *C. jejuni* and *C. coli* in spiked human stool specimens was 5.6×10^3 c.f.u. g⁻¹ (1.4 c.f.u. per test tube) and 4.8×10^3 c.f.u. g⁻¹ (1.2 c.f.u. per test tube), respectively. When 90 stool specimens from patients with diarrhoea were tested by LAMP and direct plating, the LAMP results showed 81.3 % sensitivity and 96.6 % specificity compared to isolation of *C. jejuni* and *C. coli* by direct plating. Further, the LAMP assay required less than 2 h for detection of *C. jejuni* and *C. coli* in stool specimens. This LAMP assay is a rapid and simple tool for the detection of *C. jejuni* and *C. coli* and will be useful in facilitating the early diagnosis of food poisoning incidents caused by these organisms.

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INTRODUCTION

Campylobacter is widely acknowledged as one of the most frequent causes of acute bacterial gastroenteritis in humans worldwide. *Campylobacter jejuni* and *Campylobacter coli* are the predominant cause of campylobacteriosis (Lawson *et al.*, 1999; Nachamkin, 2003; On, 2005). *C. jejuni* and *C. coli* diarrhoea in humans is commonly diagnosed by isolating these pathogens from a stool specimen by a bacterial culture test. The test procedure includes the plating of stool onto selective agar medium and subsequent identification of suspected colonies on the agar medium. The bacterial culture tests require at least 4 days and are time-consuming and laborious due to the fastidious and slow growth of these species. A rapid, simple and practical assay for the identification of *C. jejuni* and *C. coli* has thus been sought.

Several PCR assays offer a more sophisticated approach to the identification of *Campylobacter* species (Linton *et al.*, 1996; Yamazaki-Matsune *et al.*, 2007). Although PCR assays provide more rapid identification of *C. jejuni* and *C. coli* than conventional biochemical-based assays, they

require the use of electrophoresis to detect amplified products, which is time-consuming and tedious. Further, another real-time PCR assay recently developed for the rapid identification of *Campylobacter* species (Logan *et al.*, 2001; Sails *et al.*, 2003; Waage *et al.*, 1999) is not routinely used due to the requirement for an expensive thermal cycler with a fluorescence detector.

Among other techniques, one promising candidate is a novel nucleic acid amplification method termed loop-mediated isothermal amplification (LAMP; Notomi *et al.*, 2000). LAMP is based on the principle of autocycling strand displacement DNA synthesis performed by the *Bst* DNA polymerase large fragment for the detection of a specific DNA sequence with specific characteristics, which offers a number of advantages. First, all reactions can be carried out under isothermal conditions ranging from 60 to 65 °C. Second, its use of six primers recognizing eight distinct regions on the target nucleotides means that specificity is extremely high (Nagamine *et al.*, 2002). Third, detection is simplified by visual judgment by the unaided eye without electrophoresis (Hara-Kudo *et al.*, 2005; Song *et al.*, 2005). Thus a LAMP assay is faster and easier to perform than PCR assays, as well as being more specific

Abbreviation: LAMP, loop-mediated isothermal amplification.

(Hara-Kudo *et al.*, 2007). Furthermore, because the LAMP assay synthesizes a large amount of DNA, the products can be detected by simple turbidity. Thus, compared to PCR assays, expensive equipment is not necessary to give a high level of precision (Hara-Kudo *et al.*, 2005). These features allow simple, rapid and cost-effective detection (Iwamoto *et al.*, 2003; Hara-Kudo *et al.*, 2005). Also, the increase in the turbidity of the reaction mixture according to the production of precipitate correlates with the amount of DNA synthesized (Hara-Kudo *et al.*, 2007). A commercial LAMP assay kit for detection of *C. jejuni/coli* is, however, handicapped by the inability to discern between *C. jejuni* and *C. coli*. Although various LAMP assays for the identification of pathogenic organisms have been developed (Hara-Kudo *et al.*, 2007; Minami *et al.*, 2006; Ohtsuka *et al.*, 2005; Song *et al.*, 2005), direct detection from clinical stool specimens has not been described.

Here, we describe a rapid and simple LAMP assay for the detection of *C. jejuni* and *C. coli*. We evaluated the efficacy of this LAMP assay in the direct detection of 90 human stool specimens obtained from food poisoning incidents.

METHODS

Bacterial strains. One hundred and eighty-five bacterial strains were used: these included 65 *C. jejuni* and 45 *C. coli* strains and an additional 45 *Campylobacter* and 30 non-*Campylobacter* strains that were used as reference strains and field isolates. Details of the 155 *Campylobacter* strains are as follows (a superscript T designates a type strain). Nineteen *Campylobacter* reference strains were obtained from international culture collections: *Campylobacter coli* JCM 2529^T (Japan Collection of Microorganisms); *Campylobacter fetus* subsp. *fetus* ATCC 27374^T (American Type Culture Collection), GTC 08732 (Gifu Type Culture Collection, Gifu, Japan), GTC 08746, GTC 11236 and GTC 12267; *Campylobacter fetus* subsp. *venerealis* JCM 2528^T; *Campylobacter helveticus* ATCC 51209^T; *Campylobacter hyointestinalis* subsp. *hyointestinalis* ATCC 35217^T; *Campylobacter hyointestinalis* subsp. *lawsonii* CCIPH-1 (Culture Collection, Institute of Public Health, Osaka, Japan); *Campylobacter jejuni* subsp. *doylei* LMG 8843^T (Culture Collection of the Laboratorium voor Microbiologie, University of Ghent); *Campylobacter jejuni* subsp. *jejuni* LMG 8841^T, ATCC 33291, ATCC 33292 and JCM 2013; *Campylobacter lari* JCM 2530^T; *Campylobacter mucosalis* ATCC 43264^T; *Campylobacter sputorum* ATCC 35980^T; and *Campylobacter upsaliensis* ATCC 43954^T. One hundred and thirty-six *Campylobacter* isolates were obtained from clinical and environmental sources between 1988 and 2007 in Hyogo, Miyazaki and Osaka, Japan, as follows: 60 *C. jejuni* subsp. *jejuni* isolates from clinical sources ($n=34$), poultry ($n=18$), seagull faeces ($n=7$) and puppy faeces ($n=1$); 44 *C. coli* isolates from clinical sources ($n=36$), poultry ($n=5$) and pigs ($n=3$); 9 *C. hyointestinalis* subsp. *hyointestinalis* from simian faeces ($n=5$), cattle faeces ($n=3$) and turtle faeces ($n=1$); 9 *C. lari* isolates from seagull faeces ($n=8$) and an unknown source ($n=1$); 9 *C. upsaliensis* isolates from adult dog faeces ($n=6$) and puppy faeces ($n=3$); and 5 *C. fetus* subsp. *fetus* isolates from clinical sources ($n=4$) and cattle liver ($n=1$).

Seven non-*Campylobacter* reference strains were obtained from international culture collections (*Arcobacter butzleri* ATCC 49616^T; *Arcobacter cryaerophilus* ATCC 43158^T; *Arcobacter skirrowii* ATCC 51132^T; *Escherichia coli* ATCC 25922 and ATCC 35218; *Pseudomonas aeruginosa* ATCC 27853; and *Staphylococcus aureus* subsp. *aureus* ATCC 25923). Twenty-three non-*Campylobacter* isolates were obtained from

clinical sources (five *Helicobacter pylori* isolates; and one isolate each of *Aeromonas hydrophila*, *Aeromonas sobria*, *Citrobacter freundii*, *Enterobacter cloacae*, enterotoxigenic *Escherichia coli* O169:H41 heat-labile enterotoxin-positive, enterotoxigenic *Escherichia coli* O114:H—heat-labile enterotoxin- and heat-stable enterotoxin-positive, *Klebsiella pneumoniae*, *Morganella morganii*, *Plesiomonas shigelloides*, *Proteus mirabilis*, *Providencia alcalifaciens*, *Salmonella enterica* serovar Enteritidis, *Shigella flexneri* 1a, *Shigella sonnei*, *Vibrio cholerae* O1 Ogawa cholera toxin-positive, *Vibrio fluvialis*, *Vibrio mimicus* and *Vibrio parahaemolyticus* O3:K6 thermostable direct haemolysin-positive).

Storage and culture conditions. All *Campylobacter*, *Arcobacter* and *Helicobacter* strains were stored in brucella broth containing 10 % (v/v) horse serum and 10 % (v/v) DMSO at -80°C until use. They were grown on blood agar supplemented with 5 % (v/v) lysed horse blood and incubated for 2–3 days in a microaerobic atmosphere, except *H. pylori*, which was incubated for 7–10 days. Microaerobic conditions were generated with an AnaeroPack MicroAero (Mitsubishi Gas Chemical), which maintained an atmosphere of approximately 8 % O_2 , 7 % CO_2 and 85 % N_2 . All strains were grown at 37°C , except for *Arcobacter cryaerophilus*, which was grown at 30°C . Other bacterial strains were stored in cooked meat broth at room temperature until use, and grown on blood agar and cultured overnight at 37°C under aerobic conditions.

DNA extraction from culture. Bacterial DNA was extracted as previously described (Misawa *et al.*, 2002; Yamazaki-Matsune *et al.*, 2007). A single loopful of culture was inoculated in 50 μl NaOH (25 mmol l^{-1}) in a 1.5 ml microcentrifuge tube using a disposable loop (1 mm diameter), and the cell mixture was heated at 100°C for 10 min. After neutralization with 50 μl Tris/HCl buffer (80 mmol l^{-1} , pH 7.5), cell debris was pelleted by centrifugation at 20 000 g and 4°C for 5 min and the supernatant was used as template DNA for LAMP and multiplex PCR assays.

Identification of *Campylobacter* strains. *Campylobacter* strains were identified with biochemical-based and multiplex PCR assays according to a previously described method (Yamazaki-Matsune *et al.*, 2007).

LAMP assay. The LAMP assay was performed with a Loopamp DNA amplification kit (Eiken Chemical). The final LAMP assay comprised 5 μl template DNA, 1 μl *Bst* DNA polymerase (Eiken Chemical), 1.6 $\mu\text{mol l}^{-1}$ each of inner primers FIP and BIP, 0.2 $\mu\text{mol l}^{-1}$ each of outer primers F3 and B3, and 0.8 $\mu\text{mol l}^{-1}$ each of loop primers LoopF and LoopB, in 1 $\times$ Reaction Mix (Eiken Chemical). The final volume was adjusted to 25 μl . All primers were produced by Hokkaido System Science, and were designed from the sequence data submitted to GenBank (AL111168, *cj0414* for *C. jejuni*; and AAF101000003, *CCO0367* for *C. coli*) with Primer Explorer V4 software (Fujitsu System Solutions). The sequences and locations of each primer are shown in Table 1 and Fig. 1. Primer FIP consisted of the F1 complementary sequence and the F2 sequence. Primer BIP consisted of the B1 direct sequence and the B2 complementary sequence. Primers B3 and LF consisted of the B3 and LF complementary sequences, respectively. The mixture was incubated at 65°C for 60 min and then at 80°C for 2 min to terminate the reaction in a Loopamp real-time turbidimeter (LA-320; Teramecs). LAMP amplification was detected as the turbidity at 650 nm using a LA-320 turbidimeter in real-time. The reaction was considered to be positive when the turbidity reached 0.1 within 60 min. Turbidity visible with the unaided eye was also considered to indicate a successful LAMP procedure.

Determination of the sensitivity of the LAMP assay. To determine the sensitivity of the LAMP assay for the detection of *C. jejuni* and *C. coli* in human stool specimens, known amounts of

Table 1. LAMP primers used

Target gene	GenBank accession no.	Primer	Sequence (5'→3')	Gene location (bp)
<i>cj0414</i> *	AL111168	CJ-FIP	ACAGCACCGCCACCTATAGT-AGAAGCTTTTTTAAACTAGGGC (Flc-F2)	95–76 (Flc), 25–46 (F2)
		CJ-BIP	AGGCAGCAGAACTTACGCATT-GAGTTTGAAAAACATTCTACCTCT (B1-B2c)	101–121 (B1), 181–157 (B2c)
		CJ-F3	GCAAGACAATATTATTGATCGC (F3)	3–24
		CJ-B3	CTTTCACAGGCTGCACTT (B3c)	218–201
		CJ-LF	CTAGCTGCTACTACAGAACCAC (LFc)	74–53
		CJ-LB	CATCAAGCTTCACAAGGAAA (LB)	124–143
		CC-FIP	AAGAGATAAACACCATGATCCCAG-TCATGAATGAGCTTACTTTAGC (Flc-F2)	730–707 (Flc), 665–686 (F2)
		CC-BIP	GCGGCAAAGACTTATGATAAAGC-TACCGCCATTCCTAAAACAAG (B1-B2c)	748–770 (B1), 810–790 (B2c)
		CC-F3	TGGGAGCGTTTTTGATCT (F3)	641–658
<i>CCO0367</i> †	AAFL01000003	CC-B3	AATCAAACCTACCGCCAT (B3c)	828–811
		CC-LF	CCACTACAGCAAAGGTGATG (LFc)	706–687
		CC-LB	CCACGATAGCCTTTATGGA (LB)	771–789

*Presumed to encode an oxidoreductase from the sequence of AL111168, submitted to GenBank by Parkhill *et al.* (2000).

†Presumed to encode a *gufA* gene from the sequence of AAFL01000003, submitted to GenBank by Fouts *et al.* (2005).

C. jejuni LMG 8841^T and *C. coli* JCM 2529^T were used. Cultures were incubated at 37 °C for 48 h in a microaerobic atmosphere. Serial 10-fold dilutions of the culture were prepared in PBS and 100 µl of each was spiked into 100 mg of a *Campylobacter*-negative stool specimen, and then each of the faecal homogenates was adjusted to a 10% concentration with PBS. After mixing well, 100 µl of each was centrifuged for 5 min at 20 000 g in a 1.5 ml microcentrifuge tube.

After removal of the supernatant, the pellets were resuspended in 100 µl NaOH (25 mmol l⁻¹), and the mixture was heated at 100 °C for 10 min. After neutralization with 100 µl Tris/HCl buffer (80 mmol l⁻¹, pH 7.5), debris was pelleted by centrifugation at 20 000 g and 4 °C for 5 min. Five microlitres of each supernatant was then used as template DNA for the LAMP assay. Spiked cells in a stool specimen were diluted 1:4000 in LAMP reaction tubes. The

Fig. 1. Locations of the target sequences used as primers. The name and location of each target sequence as a primer in *C. jejuni* *cj0414* and *C. coli* *CCO0367* are shown in (a) and (b), respectively.

sensitivity tests were conducted in triplicate, and the detection limits were defined as the last positive dilutions, with the sample considered positive if all three samples tested positive. In parallel, to enumerate the bacteria, 100 µl aliquots of appropriate dilutions were spread on blood agar and incubated at 37 °C in a microaerobic atmosphere. Colonies were counted at the dilution yielding 20–200 c.f.u. after 5 days, and c.f.u. (ml suspension)⁻¹ was calculated.

Preparation of human stool specimens. A total of 90 stool specimens were obtained from 90 patients with diarrhoea derived from 22 different food poisoning incidents that occurred in Osaka Prefecture, Japan, between November 2006 and September 2007. The number of patients of individual food poisoning incidents ranged from 2 to 11. Cases were included for analysis if stool specimens were collected within 7 days from symptom onset. The specimens, all of which were diarrhoeal stool specimens, were transported to our laboratory from 13 local public health centres in Osaka Prefecture and subjected to microbiological examination for enteropathogens, including *C. jejuni* and *C. coli*, within 6 h of arrival at our laboratory. The causative agent of individual food poisoning incidents was determined according to the results of a microbiological examination. The incidents which led to the isolation of *C. jejuni/coli* were designated *C. jejuni/coli* food poisoning and the incidents which led to the isolation of non-*C. jejuni/coli* enteropathogens were designated non-*C. jejuni/coli* food poisoning. Of the 22 incidents, 13 were *C. jejuni/coli* food poisoning and 9 were non-*C. jejuni/coli* food poisoning caused by non-*C. jejuni/coli* enteropathogens. For the isolation of *C. jejuni* and *C. coli*, approximately one loopful of stool specimen was streaked onto Skirrow agar (Oxoid) and/or modified charcoal cefoperazone deoxycholate agar (mCCDA; Oxoid) using a disposable loop (10 mm diameter), and the plates were incubated at 42 °C for 48 h. Two typical campylobacter-like colonies were selected and subcultured at 42 °C for 48 h for identification. In parallel, for LAMP testing of the direct detection of *C. jejuni* and *C. coli* from faecal stool specimens, each stool specimen was adjusted to a 10% homogenate with PBS and stored at -20 °C until use, at which time 100 µl of each was used for preparation of DNA templates as described above, and the total volume was adjusted to 200 µl. Five microlitres of each was used for the LAMP assay. The LAMP assay was also carried out on the cultured *C. jejuni* and *C. coli* strains from the stool specimen to check the specificity of the LAMP reaction.

RESULTS AND DISCUSSION

Here, we report a novel and highly specific LAMP assay for the identification of *C. jejuni* and *C. coli*. This assay provides markedly more simple and rapid detection of *C. jejuni/coli* than conventional biochemical-based and PCR assays. Further, it can be applied to the direct detection of *C. jejuni/coli* in stool specimens.

A commercial LAMP assay kit for the detection of *C. jejuni/coli* is available (Furuhata *et al.*, 2006). However, this kit is unable to discern between *C. jejuni* and *C. coli*, and the sequences of its primer sets are not public. We therefore developed a new LAMP assay for *C. jejuni* and *C. coli*. A *C. jejuni* primer set based on the putative oxidoreductase gene (Wang *et al.*, 1992; Parkhill *et al.*, 2000) was designed, on the basis of the excellent specificity of this gene for *C. jejuni* in our previous study (Yamazaki-Matsune *et al.*, 2007). A *C. coli* primer set based on the putative *gufA* (heavy metal cations transporter; Asakura *et al.*, 2007; Fouts *et al.*, 2005) gene was designed since the sequence of this gene has proven to be highly conserved and specific for *C. coli* (Asakura *et al.*, 2007).

LAMP products were obtained from all 65 *C. jejuni* and 45 *C. coli* strains (Table 2). No LAMP products were obtained from any of the 75 non-*C. jejuni/coli* strains. The sensitivity of the LAMP assay for *C. jejuni* LMG 8841^T and *C. coli* JCM 2529^T in spiked human stool specimens was found to be 5.6×10^3 c.f.u. g⁻¹ (1.4 c.f.u. per test tube) and 4.8×10^3 c.f.u. g⁻¹ (1.2 c.f.u. per test tube), respectively. The LAMP product showed an increase in turbidity (Fig. 2) and was visible as white turbidity. Sensitivities determined by the two methods were constantly matched with each other.

To evaluate the efficacy of LAMP assay testing of stool specimens for the diagnosis of *C. jejuni/coli* diarrhoea, 90 stool specimens from diarrhoea patients in food poisoning

Table 2. Results of LAMP assay

Strain	No. of strains tested	Positive number by LAMP	
		<i>C. jejuni</i>	<i>C. coli</i>
<i>C. jejuni</i> subsp. <i>jejuni</i>	64	64	0
<i>C. jejuni</i> subsp. <i>doylei</i>	1	1	0
<i>C. coli</i>	45	0	45
<i>C. fetus</i> subsp. <i>fetus</i>	10	0	0
<i>C. fetus</i> subsp. <i>venerealis</i>	1	0	0
<i>C. hyointestinalis</i> subsp. <i>hyointestinalis</i>	10	0	0
<i>C. hyointestinalis</i> subsp. <i>lawsonii</i>	1	0	0
<i>C. lari</i>	10	0	0
<i>C. upsaliensis</i>	10	0	0
<i>C. helveticus</i>	1	0	0
<i>C. mucosalis</i>	1	0	0
<i>C. sputorum</i>	1	0	0
Non-Campylobacter bacteria	30	0	0

Fig. 2. Sensitivity test to detect *C. jejuni* and *C. coli* using a real-time turbidimeter. The curves from left to right indicate decreasing numbers of bacterial colonies [$14\text{--}0.14$ c.f.u. per test tube in (a) and $12\text{--}0.12$ c.f.u. per test tube in (b)]. (a) Detection of *C. jejuni*; (b) detection of *C. coli*.

incidents were tested with the LAMP assay. The LAMP results were compared with the results of a direct plating culture test for *C. jejuni/coli*. The results are summarized in Table 3. *C. jejuni* and *C. coli* were isolated from 29 and 3 of the 90 stool specimens, respectively. Of the 32 *C. jejuni/coli* culture stool specimens (29 *C. jejuni* and 3 *C. coli*) positive by direct plating, 26 were positive by LAMP assay. Of these 26 LAMP-positive specimens, 21 were *C. jejuni*-positive and 5 were both *C. jejuni*- and *C. coli*-positive. Of the remaining 58 *C. jejuni/coli* culture stool specimens negative by direct plating, 2 and 56 stool specimens yielded *C. jejuni*-positive and *C. jejuni/coli*-negative results by LAMP assay, respectively. The LAMP assay therefore showed 81.3% sensitivity (26 of 32 specimens) and 96.6% specificity (56 of 58 specimens) compared with the isolation of *C. jejuni/coli* by the conventional direct plating culture test. Further, the LAMP assay correctly identified the 29 *C. jejuni* and 3 *C. coli* strains isolated from stool specimens. Of 32 *C. jejuni/coli* culture-positive specimens,

2 and 1 were co-isolated enterohaemorrhagic *Escherichia coli* O157 verotoxin 2-positive and *Norovirus*, respectively. Among the 58 *C. jejuni/coli* culture-negative specimens, 14, 6, 4 and 2 were isolated *Salmonella* spp., *Norovirus*, *Clostridium perfringens* enterotoxin A-positive and *V. parahaemolyticus* O3:K6 thermostable direct haemolysin-positive, respectively. The bacterial culture test for the isolation and identification of *C. jejuni/coli* from the stool specimens required 4 days, with direct plating onto selective agars and sequential subculture. In contrast, the LAMP assay was markedly faster, requiring less than 2 h from the beginning of DNA extraction to final determination for the direct detection of human stool specimens.

Six culture-positive/LAMP-negative specimens were obtained as shown in Table 3. The cause of this discrepancy may have been the small number of *C. jejuni/coli* in the stool specimens, presumed lower than $4.8\text{--}5.6 \times 10^3$ c.f.u. g⁻¹, which was insufficient for LAMP detection. In a preliminary

Table 3. Comparison of results for LAMP and direct plating of *C. jejuni* and *C. coli* from 90 stool specimens

No. of specimens	Results of direct plating*		LAMP results*		Source of specimens
	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. jejuni</i>	<i>C. coli</i>	
21†	21/21	0/21	21/21	0/21	Patients with <i>C. jejuni</i> / <i>coli</i> food poisoning
3	0/3	3/3	3/3	3/3	Patients with <i>C. jejuni</i> / <i>coli</i> food poisoning
2	2/2	0/2	2/2	2/2	Patients with <i>C. jejuni</i> / <i>coli</i> food poisoning
6	6/6	0/6	0/6	0/6	Patients with <i>C. jejuni</i> / <i>coli</i> food poisoning
2	0/2	0/2	2/2	0/2	Patients with <i>C. jejuni</i> / <i>coli</i> food poisoning
24‡	0/24	0/24	0/24	0/24	Patients with <i>C. jejuni</i> / <i>coli</i> food poisoning
32§	0/32	0/32	0/32	0/32	Patients with non- <i>C. jejuni</i> / <i>coli</i> food poisoning

*Number positive/number of specimens tested.

†Of 21 specimens, enterohaemorrhagic *Escherichia coli* O157 verotoxin 2-positive and *Norovirus* were co-isolated from 2 and 1, respectively.

‡Of 24 specimens, *Norovirus* and *Salmonella* spp. were isolated from 4 and 1, respectively.

§Of 32 specimens, *Salmonella* spp., *Clostridium perfringens* enterotoxin A-positive, *Norovirus* and *Vibrio parahaemolyticus* O3:K6 thermostable direct haemolysin-positive were isolated from 13, 4, 2 and 2, respectively.

study, a LAMP assay was carried out on 10- and 100-fold diluted supernatants from the six culture-positive/LAMP-negative specimens to exclude the influence of inhibitory factors in the stool specimens (Hoshino *et al.*, 1998; Persson & Olsen, 2005; Ramamurthy *et al.*, 1993). The results were all negative, which may suggest that the number of *C. jejuni*/*coli* in the six stool specimens was small.

Of 58 specimens obtained from patients of *C. jejuni*/*coli* food poisoning incidents, 32, 2 and 24 specimens were culture-positive, culture-negative/LAMP-positive and culture-negative/LAMP-negative, respectively. All 58 specimens were potential carriers of *C. jejuni*/*coli* due to the ingestion of *C. jejuni*/*coli*-contaminated foods. However, *C. jejuni*/*coli* was not isolated from 26 of the 58 patients by direct plating culture, presumably due to any of the influence of a small ingestion amount, natural excretion, medication, rapid loss of cell viability in stools during transportation and storage, small number of cells in stool specimens and false-negative results by the overgrowth of normal flora on selective agars (Kulkarni *et al.*, 2002; Persson & Olsen, 2005; Ramamurthy *et al.*, 1993; Sails *et al.*, 2003). Of the 26 specimens, 2 were culture-negative/*C. jejuni* LAMP-positive. The remaining 24 specimens, as well as 32 control specimens obtained from patients of non-*C. jejuni*/*coli* food poisoning incidents and unrelated to *C. jejuni*/*coli*, were culture-negative/LAMP-negative. The cause of this discrepancy was thus presumed not to be false-positives but rather the detection of bacteria in stool specimens that were sufficiently stressed to resist conventional culture under standard conditions or false-negative results due to the overgrowth of normal flora contained in stool specimens (Kulkarni *et al.*, 2002; Persson & Olsen, 2005; Shirai *et al.*, 1991; Sails *et al.*, 2003) on selective agars. These results may suggest that the LAMP assay could detect the specific sequences of *C. jejuni* even when *C. jejuni* was sufficiently stressed to resist conventional culture, but with the chromosomal DNA released by autolysis or the overgrowth of normal flora inhibiting

separation of *C. jejuni* on selective agars. Further work is needed to confirm this hypothesis.

As shown in Table 3, the three *C. coli* and two *C. jejuni* culture-positive specimens were both *C. coli* and *C. jejuni* LAMP-positive. The cause of this discrepancy is probably the small number ($n=2$) of subcultures from a selective agar. As these five specimens were collected from three *C. coli* culture-positive/*C. jejuni* culture-negative and two *C. jejuni* culture-positive/*C. coli* culture-negative patients, these five patients were actually suspected of having mixed infection with *C. jejuni* and *C. coli*. Our assay may reveal mixed infection cases without the need for tedious subculture, and thereby facilitate more accurate epidemiological investigation.

In a preliminary study, 1 ml 10% *C. jejuni*/*coli* culture-positive faecal homogenate was concentrated by centrifugation to improve the yield of template DNA. The specimen was prepared as described in Methods, and finally 10% faecal homogenate was concentrated 10-fold and 5 µl was used as the template DNA. The positive rate was, however, decreased, with only 23 of 32 (71.9%) specimens being positive; 1 of 6 culture-positive/LAMP-negative specimens became LAMP-positive, while 4 of 26 culture-positive/LAMP-positive specimens became LAMP-negative. A possible explanation for this discrepancy may be the influence of inhibitory factors in the stool specimen (Hoshino *et al.*, 1998; Persson & Olsen, 2005; Ramamurthy *et al.*, 1993), concerns over which were the rationale for our use of 10% faecal homogenate without concentration.

The frequent occurrence of food poisoning incidents caused by *C. jejuni*/*coli*, and in particular the recently identified possibility of cases of Guillain-Barré or Fisher syndrome caused by certain strains of *C. jejuni* (Takahashi *et al.*, 2005), highlight the need for the rapid and accurate identification of these species. Further, the application of our LAMP assay in veterinary and environmental microbiology should

facilitate a comprehensive approach to the control of human *C. jejuni*/*coli*-associated gastroenteritis. Our LAMP assay is a powerful tool for the rapid and simple identification of *C. jejuni* and *C. coli* and will facilitate the early diagnosis of food poisoning incidents caused by these organisms.

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