

Development and application of a real-time polymerase chain reaction method for *Campylobacter jejuni* detection

Mao-Jun Zhang, Bo Qiao, Xue-Bin Xu, Jian-Zhong Zhang

Mao-Jun Zhang, Bo Qiao, Jian-Zhong Zhang, State Key Laboratory for Infectious Disease Prevention and Control, and National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China

Xue-Bin Xu, Shanghai Municipal Center for Disease Control and Prevention, Shanghai 200336, China

Author contributions: Zhang MJ designed the research, analyzed the data and wrote the paper; Qiao B performed the research and analyzed the data; Xu XB collected the clinical samples and analyzed the data; Zhang JZ analyzed the data.

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Correspondence to: Dr. Mao-Jun Zhang, State Key Laboratory for Infectious Disease Prevention and Control, and National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, PO Box 5, Changping District, Beijing 102206, China. zhangmaojun@icdc.cn

Telephone: +86-10-58900755 Fax: +86-10-58900700

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Abstract

AIM: To develop a real-time polymerase chain reaction (PCR) method to detect and quantify *Campylobacter jejuni* (*C. jejuni*) from stool specimens.

METHODS: Primers and a probe for real-time PCR were designed based on the specific DNA sequence of the *hipO* gene in *C. jejuni*. The specificity of the primers and probe were tested against a set of *Campylobacter spp.* and other enteric pathogens. The optimal PCR conditions were determined by testing a series of conditions with standard *C. jejuni* template. The detection limits were obtained using purified DNA from bacterial culture and extracted DNA from the stool specimen. Two hundred and forty-two specimens were analyzed for the presence of *C. jejuni* by direct bacterial culture and real-time PCR.

RESULTS: The optimal PCR system was determined using reference DNA templates, 1 × uracil-DNA glycosylase, 3.5 mmol/L MgCl₂, 1.25 U platinum *Taq* polymerase, 0.4 mmol/L PCR nucleotide mix, 0.48 μmol/L of each primer, 0.2 μmol/L of probe and 2 μL of DNA template in a final volume of 25 μL. The PCR reaction was carried as follows: 95 °C for 4 min, followed by 45 cycles of 10 s at 95 °C and 30 s at 59 °C. The detection limit was 4.3 CFU/mL using purified DNA from bacterial culture and 10³ CFU/g using DNA from stool specimens. Twenty (8.3%, 20/242) *C. jejuni* strains were isolated from bacterial culture, while 41 (16.9%, 41/242) samples were found to be positive by real-time PCR. DNA sequencing of the PCR product indicated the presence of *C. jejuni* in the specimen. One mixed infection of *C. jejuni* and *Salmonella* was detected in one specimen and the PCR test for this specimen was positive.

CONCLUSION: The sensitivity of detection of *C. jejuni* from stool specimens was much higher using this PCR assay than using the direct culture method.

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Key words: *Campylobacter jejuni*; Real time polymerase chain reaction; Application

Core tip: In the present study, we developed an effective real-time polymerase chain reaction method based on the specific DNA sequence of the *hipO* gene in *Campylobacter jejuni* (*C. jejuni*). The detection limit of this assay is 4.3 CFU/mL. A study of 242 clinical stool specimens from diarrheal patients indicated that this method is more sensitive than direct bacterial culture for the identification of *C. jejuni* from stool specimens.

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INTRODUCTION

Campylobacter spp., *Salmonella* spp., *Yersinia* spp., *Shigella* spp., and *Escherichia coli* (*E. coli*) O157 are the leading causes of human bacterial gastroenteritis worldwide^[1]. *Campylobacter jejuni* (*C. jejuni*) is the main species of *Campylobacter* that affects humans^[2,3]. *Campylobacter* infections have been investigated and followed with considerable interest since the early 1980s in China, and there have been numerous reports on *C. jejuni* infection in patients with diarrhea. However, reports and the frequency of isolation of *C. jejuni* from diarrheal patients have decreased since the late 1990s. Improvement in sanitary conditions may explain this decline in numbers; however, the difficulty in detecting this fastidious pathogen might be another contributor to the decreased number of reports and frequency of isolation. Our recent pilot study revealed that the ratio of isolation of this pathogen from stool specimens of diarrheal patients was considerably different among surveillance spots in different laboratories (2%-15%, unpublished). Sensitive and accurate detection of this pathogen is important, both for the treatment of patients and for prompt epidemiological investigation.

With the development of genomic DNA sequencing, online databases, and bioinformatic analyses, nucleic acid-based methods, particularly polymerase chain reaction (PCR) methods, have become promising tools for the rapid, reliable, and sensitive detection and diagnosis of pathogenic infection^[4-8]. In this study, we developed a real-time PCR assay to detect *C. jejuni*. We then combined this PCR assay with DNA sequence analysis and compared this method with the direct culture method to detect *C. jejuni* in stool specimens obtained from patients with diarrhea. The results obtained in this study provide a proof of concept for PCR-based detection of *C. jejuni* infection in patients with diarrhea, may lead to the development of a pre-screening approach for isolating *C. jejuni*.

MATERIALS AND METHODS

Sample collection and culture-based detection

Two hundred and forty-two stool specimens were collected from diarrheal patients (with ages ranging from 6 to 72 years) in the outpatient facilities of 11 hospitals. The specimens were transferred to a laboratory within 4 h for bacterial culture. The remainder of the samples was frozen at -80 °C for DNA extraction. A sterile cotton swab was twisted in each stool sample and streaked directly on selective Skirrow agar plates consisting of Columbia agar base (CM331, Oxoid, Basingstoke, United Kingdom), selective supplement (SR0069, Oxoid), and 5% defibrinated sheep blood. The plates were incubated in a microaerobic atmosphere (5% O₂, 10% CO₂ and 85% N₂) at 42 °C for

48 h. Suspected colonies were picked and identified by gram staining, oxidase and catalase tests, and hippurate hydrolysis analysis, according to our previous report^[9]. All specimens were also examined for the presence of *Shigella* spp., *Salmonella* spp., *Yersinia* spp. and *E. coli* O157 by culture on xylose lysine deoxycholate agar, deoxycholate hydrogen sulfide lactose agar, cefsulodin-irgasan-novobiocin agar, and sorbitol MacConkey agar, respectively. Suspected colonies were identified biochemically using API 20E strips, and the cultures were confirmed up to the species level by further serotype or PCR analysis, as previously described^[10,11].

DNA preparation from bacterial culture

Reference genomic DNA was extracted from the *Campylobacter* isolates cultured in this study by using a QIAamp DNA mini kit (Qiagen, Düsseldorf, Germany). DNA templates from other reference pathogens were gifts from Drs. Huaiqi Jing and Biao Kan. The reference DNA templates are shown in Table 1.

Primers and probe design

Specific DNA fragments of the *hipO* gene were compared using BLASTn and Vector NTI suite 6.0 software. The primers and probe sets were designed and synthesized by Shanghai Huirui Biotechnology Co., Ltd. The sequences of the primers and probe used in this study were: *hipO*-F, 5'-CGGATAGTTATAGTATTGAAGT-TATTGG-3', *hipO*-R, 5'-GAAGCAGCATAAATAG-GATCTTTT-3', and *hipO*-P, 5'-FAM-TTCTGGAG-CACTTCCATGACCACC-BHQ1-3'.

Optimization for real-time PCR

The optimal PCR conditions were determined by testing a series of conditions with standard *C. jejuni* template. The standard PCR curve was constructed using genomic DNA from *C. jejuni* strain NCTC11168. The detection limits of this assay from pure culture and stool specimens were determined using 10-fold dilutions of quantified reference *C. jejuni* genomic DNA templates (1 × 10⁰ to 1 × 10⁶ CFU/mL) and the same amount of bacteria inoculated into stool specimens that were previously confirmed to contain no *Campylobacter*. The specificity of this assay was verified using genomic DNA from other enteric pathogens. The *C. jejuni*-inoculated samples were also cultured to compare the detection limits.

Evaluation of repeatability

To evaluate the reproducibility of the assay, five serial dilutions with three replicates per concentration were performed for two separate *C. jejuni* genomic DNA templates (NCTC11168 and ATCC33560). The serial dilutions were also performed on three different *C. jejuni* strains (81116, ATCC49349 and ICDCCJ07001) simultaneously. The curves were constructed on the basis of the log pg/mL of the genomic DNA and threshold cycle (Ct). Reproducibility was assessed using the SD of the Ct value and the SD of the slope of each dilution curve, respectively.

PCR detection of *C. jejuni* from stool specimens and DNA sequencing of PCR products

After direct culture, the stool specimens were stored at -80 °C until DNA extraction using a QIAamp DNA stool mini kit (Qiagen), which was performed in accordance with the manufacturer's instructions. Conventional PCR with the universal primers targeting conserved bacterial 16s rRNA gene sequences was carried out to evaluate the validity of the template used in this study. Real-time PCR was performed in a 25- μ L volume with 2 μ L of purified DNA obtained directly from stool specimens. All the samples with Ct < 35 were considered positive. Each of the positive PCR products, except for the ones from the culture-positive specimens, was confirmed with conventional PCR using the same primers, and the PCR products were purified and sequenced. Online sequence BLAST was performed using the NCBI BLASTN suite (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome).

RESULTS

Bacterial culture

Twenty *C. jejuni* (8.3%, 20/242) isolates, 34 (14%, 34/242) *Salmonella* isolates, and one (0.4%, 1/242) *Shigella* isolate were obtained by direct plating of the 242 stool specimens. *Salmonella* was the most frequently isolated pathogenic genus from the stool specimens and *C. jejuni* was the second most frequently isolated. No *Yersinia* or *E. coli* O157 isolates were found. In another laboratory, serotyping methods were used to further identify *Salmonella* and *Shigella* colonies.

Optimized PCR assay

The optimal PCR system was determined using reference DNA templates, 1 \times uracil-DNA glycosylase, 3.5 mmol/L MgCl₂, 1.25 U platinum *Taq* polymerase, 0.4 mmol/L PCR nucleotide mix, 0.48 μ mol/L of each primer, 0.2 μ mol/L of probe, and 2 μ L of DNA template in a final volume of 25 μ L run with the following parameters: 95 °C for 4 min, followed by 45 cycles of 10 s at 95 °C and 30 s at 59 °C. Fluorescence signals were measured at the end of the annealing step of every cycle. PCR products were verified using DNA electrophoresis through 2% agarose gels. The size of the PCR product was consistent with the expected size (85 bp) in each case. The specificity of the primers and probe were tested against *Campylobacter spp.* and other enteric pathogens. Only *C. jejuni* showed positive reactions. All the other templates gave negative results, including other *Campylobacter spp.*

Detection limit and stability of the PCR assay

Serial dilutions from 10⁰ to 10⁶ CFU/ml of the target template were subjected to the real-time PCR assay (Figure 1A). The standard curve based on the dilutions of genomic DNA showed a linear relationship between Log

Table 1 Reference DNA templates used in this study

Bacteria	Strain name
<i>Campylobacter jejuni</i>	NCTC11168
<i>Campylobacter jejuni</i>	81116
<i>Campylobacter jejuni</i>	ATCC33560
<i>Campylobacter jejuni</i> sub <i>doylei</i>	ATCC49349
<i>Campylobacter jejuni</i>	ICDCCJ07001
<i>Campylobacter coli</i>	ATCC33559
<i>Campylobacter coli</i>	WHO-10.2
<i>Campylobacter fetus</i>	ATCC27374
<i>Campylobacter lari</i>	ATCC35221
<i>Escherichia coli</i> O157:H7	Isolate-1
Enterotoxigenic <i>Escherichia coli</i>	ETEC10407
Enterotoxigenic <i>Escherichia coli</i>	EAECO42
Enteroinvasive <i>Escherichia coli</i>	EIEC44825
Enteropathogenic <i>Escherichia coli</i>	Isolate-2
<i>Salmonella</i> Serovar <i>typhi</i>	CT18
Typhoid-paratyphoid A	Isolate-3
<i>Salmonella typhimurium</i>	Isolate-4
Cholera O1	Isolate-5
<i>Shigella</i>	Isolate-6
<i>Salmonella enteritidis</i>	Isolate-7
<i>Yersinia enterocolitica</i> O3	52203
<i>Vibrio parahaemolyticus</i>	Isolate-8
<i>Enterococcus</i>	Isolate-9
<i>Listeria monocytogenes</i>	Isolate-10

CFU/mL and threshold cycles (Ct; Figure 1B). Copy numbers could be obtained by the following equation using the standard curve: $Y(Ct) = -3.993X (\log \text{CFU/mL}) + 37.51$ ($R^2 = 0.998$). When Ct = 35, the detection limit of the PCR assay for pure culture was approximately 4.3 CFU/mL. However, when Ct = 35, the detection limit for *C. jejuni* in the stool specimens was 10³ CFU/g, and we could not obtain a linear relationship between log CFU/g and Ct using serial bacterial inoculation in different stool specimens. The curves constructed from the serial dilution replicates of the individual *C. jejuni* templates and those generated from the three different *C. jejuni* templates are shown in Figure 2. The variation of the Ct value and the slopes of the curves indicated that this assay could be stably reproduced.

PCR detection from stool specimens

The detection limit from both bacterial culture and real-time PCR (with Ct < 35) for the inoculated stool samples was 10³ CFU/g; therefore, a Ct value of ≤ 35 was considered positive for the real-time PCR assay in this study. Forty-one (16.9%, 41/242) samples were found to be positive by the real-time PCR method. All culture-positive specimens showed positive PCR results (Ct < 34). DNA sequencing of the positive PCR products for the culture-negative samples indicated the presence of *C. jejuni*. The samples containing *Salmonella* and *Shigella* isolates tested negative by real-time PCR (Ct > 40), except for one sample that contained both *C. jejuni* and *Salmonella* (Ct = 27).

DISCUSSION

C. jejuni is one of the major causes of food borne disease

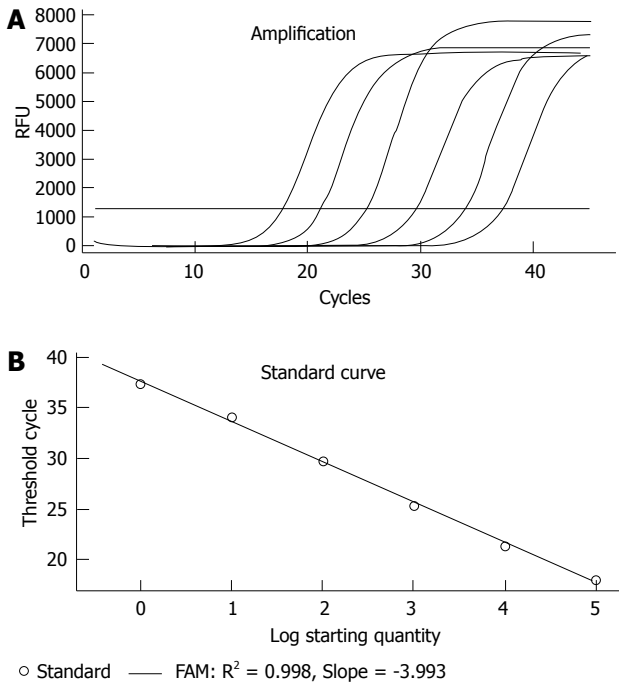


Figure 1 Real time polymerase chain reaction standard curve and the linear relationship between *Campylobacter jejuni* quantity and polymerase chain reaction cycle threshold. A: The standard curve was constructed using genomic DNA from *Campylobacter jejuni* isolates. Serial dilutions ranging from 100 to 10⁶ CFU/mL of target template were subjected to real time polymerase chain reaction assay; B: The linear relationship between Log CFU/mL and threshold cycles. RFU: Relative fluorescence units.

worldwide^[12]. It is an important pathogen of acute bacterial diarrhea in humans and has been associated with the development of Guillain-Barré syndrome, a post-infectious polyneuropathy^[13,14]. Routine detection of *Campylobacter* species in the clinical setting is currently based on culture-based detection and subsequent phenotypic identification. Its detection is difficult because of its special growth requirements, low infectious doses, and potential for entering a viable, but not cultivable, state^[15,16]. The traditional “gold standard” diagnostic methods for *Campylobacter* infection include bacterial culture and culture-based biochemical tests. These methods are both time-consuming and laborious. In addition to the fastidious culture requirements, the discrimination and differentiation of *Campylobacter* spp. are complicated and error prone. For example, distinguishing *C. jejuni* from *C. coli* is usually done on the basis of the biochemistry test for hippurate hydrolysis. Only *C. jejuni* gives a positive reaction, but previous studies have found that 10% of *C. jejuni* isolates failed to hydrolyze hippurate under laboratory conditions^[17,18]. The accurate identification of *Campylobacter* up to species level is an essential prerequisite for many epidemiological studies.

In the present study, we developed a real-time PCR assay based on the specific DNA fragment of the *hipO* gene from *C. jejuni*, which could identify *C. jejuni* directly and differentiate it from other pathogens, especially from other *Campylobacter* spp., in stool specimens from diarrheal patients. The specificity of the PCR system was

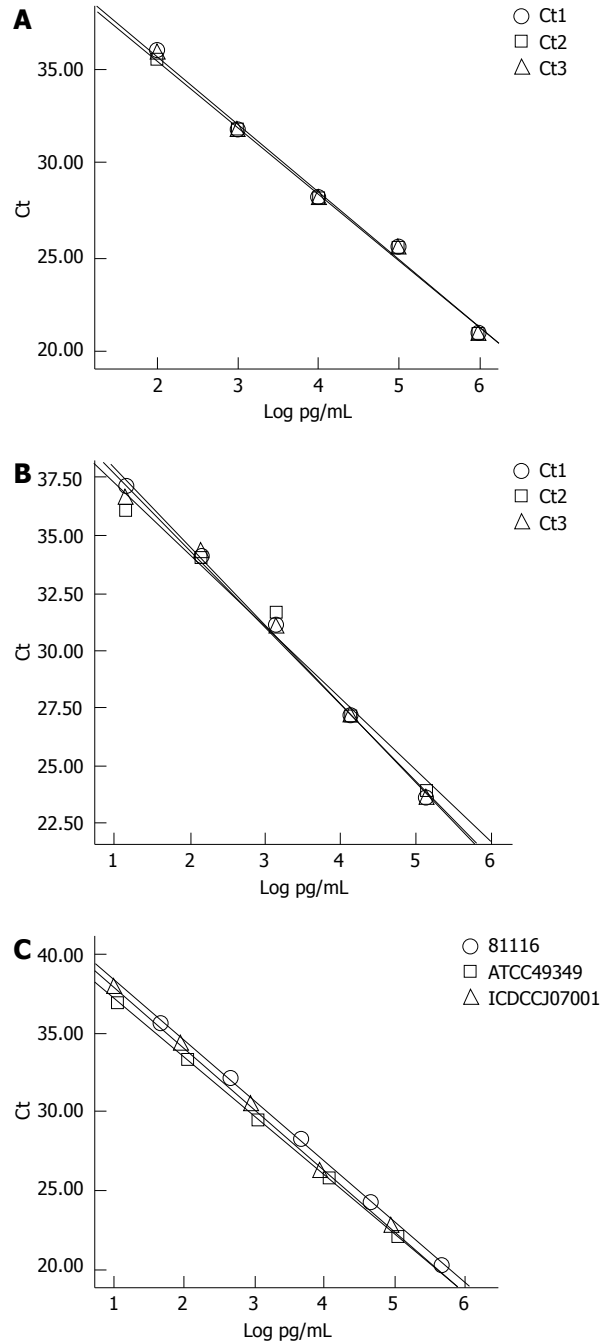


Figure 2 Stability tests. A: Curves constructed based dilutions of the genomic DNA of *Campylobacter jejuni* (C. jejuni) NCTC11168; B: Curves constructed based on the dilution of the genomic DNA of *C. jejuni* ATCC33560; C: Curves generated from the serial dilution of the genomic DNA of *C. jejuni* 81116, ATCC49349 and ICDCCJ07001. Ct: Threshold cycle.

verified with 27 reference enteric pathogens (Table 1). The detection limit of this PCR assay is less effective for stool samples than for pure culture. Loss of template during DNA extraction or inhibitors present in the stool specimens could reduce the efficiency^[19]. Two hundred and forty-two stool specimens from patients with diarrhea were tested by the direct culture and by the PCR assay. All the culture-positive samples gave positive PCR results and samples containing other pathogens tested negative by PCR (Ct > 40). The DNA sequence of the

positive PCR products indicated the presence of *C. jejuni* in the specimens. The sensitivity of this PCR assay (100%, 41/41) was much higher than that of direct bacterial culture (49%, 20/41). The result from the mixed infection sample indicated that this PCR assay is considerably accurate. The results presented in this study contrast with those reported by certain previous studies, in which PCR results were found to have the same or less sensitivity compared with the direct culture method for the detection of *C. jejuni* from stool specimens^[20,21]. However, Maher *et al*^[22] used a DNA probe-based PCR assay and were able to identify 94% (17/18) and 32% (35/109) of *Campylobacter* infections from culture-positive and culture-negative stool specimens, respectively. Furthermore, Bessède *et al*^[23] proved the weakness of the culture methods compared with PCR and immunoenzymatic methods of detection of *Campylobacter*. Notably, many aspects of the culture-based method, such as the choice of the selective media used for culture, need to be improved^[24]. Although the sensitivity of the PCR system was high in this study, false-negative PCR results may still occur because of the presence of inherent inhibitors or because of very low numbers of organisms.

Interestingly, when *C. jejuni* were inoculated into stool specimens, the detection limit by the PCR assay was 10³ CFU/g. The PCR detection limit from stool samples was similar to that reported in a previous study, but the detection limit of the direct culture methods mostly depended on the culture methods used, particularly the choice of plating medium, which may influence the efficiency of *C. jejuni* isolation during direct plating of fecal samples^[25,26]. Instant plating of the inoculated samples in this study might increase the laboratory isolation capacity. Meanwhile, the non-uniformity of the clinical stool specimens in terms of physical matter, target organisms, the associated fecal flora and transportation status would result in variability of the culture results. In this study, although we used selective medium under specific microaerobic atmosphere conditions, some samples were contaminated with *Proteus* bacilli, and the suspected colonies could not be picked because of the rapid spread of the contaminants. *Proteus* bacilli may be one of the agents that reduced culture sensitivity, and the use of cotton swabs rather than inoculating loops for streaking on plates might have increased this contamination. In this study, we used QIAamp DNA stool mini kits to extract the DNA; the QIAamp kit has the highest sensitivity and requires the least manipulation of template DNA prior to PCR. More research is required to develop an in-house procedure for DNA extraction from different specimens and for optimizing the extraction process to increase the desired yield.

In conclusion, the real-time PCR assay developed in this study is sufficiently sensitive and accurate for *C. jejuni* detection from stool specimens. Although the PCR results do not permit its use as the standard method of diagnosing *C. jejuni* infection in the clinical setting, this newly developed method will benefit large-scale epidemiology

investigations and pre-screening for bacterial isolation.

COMMENTS

Background

Campylobacter jejuni (*C. jejuni*) is a major food-borne pathogen. In addition to enteritis, *C. jejuni* infection can also cause Guillain-Barré syndrome, an autoimmune neuropathy in humans. The "gold standard" diagnostic methods for *C. jejuni* infection include bacterial culture and culture-based biochemical tests. Primary *in vitro* culture of *C. jejuni* is difficult because of its special growth requirements, low infectious doses, and potential for entering a viable, but not cultivable, state. In addition to its fastidious culture conditions, current methods for discrimination and differentiation of *Campylobacter* spp. are complicated and fallible. Specific DNA detection, particularly real-time polymerase chain reaction (PCR), is a promising tool for the rapid, reliable, and more sensitive detection and diagnosis of *C. jejuni* infection. In this study, the authors developed and validated a real-time PCR assay method for the detection of *C. jejuni* from stool specimens.

Research frontiers

C. jejuni is a fastidious organism and requires microaerophilic conditions for *in vitro* culture. Nucleic acid-based methods for the detection of *C. jejuni* infection are research hotspots, particularly PCR methods, which are promising tools for the rapid, reliable, and more sensitive detection of *C. jejuni* for laboratory-based diagnosis.

Innovations and breakthroughs

In the present study, the authors developed a sensitive and specific real-time PCR assay using a DNA fragment of the *hipO* gene in *C. jejuni*, which can directly differentiate *C. jejuni* from other pathogens and other *Campylobacter* spp. from stool specimens obtained from diarrheal patients.

Applications

The real-time PCR assay developed in this study is sensitive and accurate for the detection of *C. jejuni* from stool specimens. This method will benefit both large-scale epidemiology investigations and the clinical diagnosis of *C. jejuni* infection in humans.

Peer review

This is an excellent and well-written manuscript. The authors are to be congratulated on developing and validating their own in-house PCR-based technique to detect *C. jejuni* in stool specimens. The *hipO* gene is specific to *C. jejuni*; therefore, by definition, this is a highly specific test.

REFERENCES

- 1 Centers for Disease Control and Prevention (CDC). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--selected sites, United States, 2003. *MMWR Morb Mortal Wkly Rep* 2004; **53**: 338-343 [PMID: 15123986]
- 2 Allos BM. *Campylobacter jejuni* Infections: update on emerging issues and trends. *Clin Infect Dis* 2001; **32**: 1201-1206 [PMID: 11283810 DOI: 10.1086/319760]
- 3 Dasti JL, Tareen AM, Lugert R, Zautner AE, Gross U. *Campylobacter jejuni*: a brief overview on pathogenicity-associated factors and disease-mediating mechanisms. *Int J Med Microbiol* 2010; **300**: 205-211 [PMID: 19665925 DOI: 10.1016/j.ijmm.2009.07.002]
- 4 Fredricks DN, Relman DA. Application of polymerase chain reaction to the diagnosis of infectious diseases. *Clin Infect Dis* 1999; **29**: 475-486; quiz 487-488 [PMID: 10530433 DOI: 10.1086/598618]
- 5 Holland JL, Louie L, Simor AE, Louie M. PCR detection of *Escherichia coli* O157: H7 directly from stools: evaluation of commercial extraction methods for purifying fecal DNA. *J Clin Microbiol* 2000; **38**: 4108-4113 [PMID: 11060076]
- 6 Josefsen MH, Löfström C, Hansen TB, Christensen LS, Olsen JE, Hoorfar J. Rapid quantification of viable *Campylobacter* bacteria on chicken carcasses, using real-time PCR and propidium monoazide treatment, as a tool for quan-

- titative risk assessment. *Appl Environ Microbiol* 2010; **76**: 5097-5104 [PMID: 20562292 DOI: 10.1128/AEM.00411-10]
- 7 **Liu J**, Gratz J, Maro A, Kumburu H, Kibiki G, Taniuchi M, Howlader AM, Sobuz SU, Haque R, Talukder KA, Qureshi S, Zaidi A, Haverstick DM, Houtp ER. Simultaneous detection of six diarrhea-causing bacterial pathogens with an in-house PCR-luminex assay. *J Clin Microbiol* 2012; **50**: 98-103 [PMID: 22075596 DOI: 10.1128/JCM.05416-11]
 - 8 **Wiemer D**, Loderstaedt U, von Wulffen H, Priesnitz S, Fischer M, Tannich E, Hagen RM. Real-time multiplex PCR for simultaneous detection of *Campylobacter jejuni*, *Salmonella*, *Shigella* and *Yersinia* species in fecal samples. *Int J Med Microbiol* 2011; **301**: 577-584 [PMID: 21855409 DOI: 10.1016/j.ijmm.2011.06.001]
 - 9 **Zhang M**, Li Q, He L, Meng F, Gu Y, Zheng M, Gong Y, Wang P, Ruan F, Zhou L, Wu J, Chen L, Fitzgerald C, Zhang J. Association study between an outbreak of Guillain-Barre syndrome in Jilin, China, and preceding *Campylobacter jejuni* infection. *Foodborne Pathog Dis* 2010; **7**: 913-919 [PMID: 20455754 DOI: 10.1089/fpd.2009.0493]
 - 10 **Swanson EC**, Collins MT. Use of the API 20E system to identify veterinary Enterobacteriaceae. *J Clin Microbiol* 1980; **12**: 10-14 [PMID: 6999012]
 - 11 **Gómez-Duarte OG**, Bai J, Newell E. Detection of *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Vibrio cholerae*, and *Campylobacter* spp. enteropathogens by 3-reaction multiplex polymerase chain reaction. *Diagn Microbiol Infect Dis* 2009; **63**: 1-9 [PMID: 18990527 DOI: 10.1016/j.diagmicrobio.2008.09.006]
 - 12 **Bullman S**, O'Leary J, Corcoran D, Sleator RD, Lucey B. Molecular-based detection of non-culturable and emerging campylobacteria in patients presenting with gastroenteritis. *Epidemiol Infect* 2012; **140**: 684-688 [PMID: 21676357 DOI: 10.1017/S0950268811000859]
 - 13 **Nachamkin I**, Allos BM, Ho T. *Campylobacter* species and Guillain-Barré syndrome. *Clin Microbiol Rev* 1998; **11**: 555-567 [PMID: 9665983]
 - 14 **Yuki N**, Hartung HP. Guillain-Barré syndrome. *N Engl J Med* 2012; **366**: 2294-2304 [PMID: 22694000 DOI: 10.1056/NEJMra1114525]
 - 15 **On SL**. Identification methods for campylobacters, helicobacters, and related organisms. *Clin Microbiol Rev* 1996; **9**: 405-422 [PMID: 8809468]
 - 16 **Inglis GD**, Kalischuk LD. Direct quantification of *Campylobacter jejuni* and *Campylobacter lanienae* in feces of cattle by real-time quantitative PCR. *Appl Environ Microbiol* 2004; **70**: 2296-2306 [PMID: 15066825 DOI: 10.1128/AEM.70.4.2296-2306.2004]
 - 17 **Steinhauserova I**, Ceskova J, Fojtikova K, Obrovskaa I. Identification of thermophilic *Campylobacter* spp. by phenotypic and molecular methods. *J Appl Microbiol* 2001; **90**: 470-475 [PMID: 11298244 DOI: 10.1046/j.1365-2672.2001.01267.x]
 - 18 **Englen MD**, Ladely SR, Fedorka-Cray PJ. Isolation of *Campylobacter* and identification by PCR. *Methods Mol Biol* 2003; **216**: 109-121 [PMID: 12512359]
 - 19 **Stacy-Phipps S**, Mecca JJ, Weiss JB. Multiplex PCR assay and simple preparation method for stool specimens detect enterotoxigenic *Escherichia coli* DNA during course of infection. *J Clin Microbiol* 1995; **33**: 1054-1059 [PMID: 7615704]
 - 20 **Inglis GD**, Kalischuk LD. Use of PCR for direct detection of *Campylobacter* species in bovine feces. *Appl Environ Microbiol* 2003; **69**: 3435-3447 [PMID: 12788747 DOI: 10.1128/AEM.69.6.3435-3447.2003]
 - 21 **Lund M**, Nordentoft S, Pedersen K, Madsen M. Detection of *Campylobacter* spp. in chicken fecal samples by real-time PCR. *J Clin Microbiol* 2004; **42**: 5125-5132 [PMID: 15528705 DOI: 10.1128/JCM.42.11.5125-5132.2004]
 - 22 **Maher M**, Finnegan C, Collins E, Ward B, Carroll C, Cormican M. Evaluation of culture methods and a DNA probe-based PCR assay for detection of *Campylobacter* species in clinical specimens of feces. *J Clin Microbiol* 2003; **41**: 2980-2986 [PMID: 12843030 DOI: 10.1128/JCM.41.7.2980-2986.2003]
 - 23 **Bessède E**, Delcamp A, Sifré E, Buissonnière A, Mégraud F. New methods for detection of campylobacters in stool samples in comparison to culture. *J Clin Microbiol* 2011; **49**: 941-944 [PMID: 21209172 DOI: 10.1128/JCM.01489-10]
 - 24 **Gun-Munro J**, Rennie RP, Thornley JH, Richardson HL, Hodge D, Lynch J. Laboratory and clinical evaluation of isolation media for *Campylobacter jejuni*. *J Clin Microbiol* 1987; **25**: 2274-2277 [PMID: 3429621]
 - 25 **Potturi-Venkata LP**, Backert S, Lastovica AJ, Vieira SL, Norton RA, Miller RS, Pierce S, Oyarzabal OA. Evaluation of different plate media for direct cultivation of *Campylobacter* species from live broilers. *Poult Sci* 2007; **86**: 1304-1311 [PMID: 17575175]
 - 26 **Goossens H**, De Boeck M, Coignau H, Vlaes L, Van den Borre C, Butzler JP. Modified selective medium for isolation of *Campylobacter* spp. from feces: comparison with Preston medium, a blood-free medium, and a filtration system. *J Clin Microbiol* 1986; **24**: 840-843 [PMID: 3771769]

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