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## Research Article

# Comparison of *hipO* and *ceuE* Gene Based PCR Assays for the Detection of *Campylobacter Jejuni*

### Abstract

The objective of this study was to find out the reproducibility and specificity of *hipO* and *ceuE* genes based PCR assays for the detection of *Campylobacter jejuni* isolated from turkey meat samples in a previous study. A total of 44 *Campylobacter* isolates including 41 *C. jejuni*, two *C. coli* and one *C. lari* were used in this study. Although all of the *C. jejuni* isolates were verified by *hipO* based PCR assay, only 18 of the 41 *C. jejuni* were detected as positive by *ceuE* based PCR assay. Both of the methods showed negative reaction with *C. coli* and *C. lari* isolates. The results showed that, *hipO* gene based PCR assay is more reproducibly and specific than *ceuE* gene specific PCR analyze for the detection and identification of *C. jejuni*.

## Introduction

*Campylobacter* infections are one of the most prevalent zoonotic bacterial foodborne diseases of humans mostly caused by *C. coli* and *C. jejuni*. In the last decade, the prevalence of gastroenteritis caused by *Campylobacter* species were in an increasing trend [1]. In addition to enteritis, extraintestinal infections and sequelae may occur, including bacteremia, urinary tract infection, reactive arthritis and “Guillain-Barre’ syndrome” affecting the peripheral nervous system [2]. As *C. jejuni* has an ability to colonize and in some cases infect poultry intestine which makes poultry meat a significant reservoir and vehicle of foodborne campylobacteriosis [3].

In order to find out the prevalence of *Campylobacter* in poultry meat, routinely, conventional culturing technique is using in many food control laboratories [4]. *Campylobacter* species are known as fastidious microorganisms, so mostly it is hard to detect with conventional method and isolate by routine media [5]. In general, detection of *Campylobacter* species especially *C. jejuni*, is difficult and time consuming using conventional techniques. Therefore specific, sensitive and rapid methods are needed for the detection of *Campylobacter* spp. from food. To overcome these concerns many detection and molecular-based typing methods including PCR have been developed and used as an important and effective tool for the detection of *Campylobacter* spp. [6-10].

In order to detect *C. jejuni* from chicken feces, hippuricase (*hipO*) [11] and the enterochelin binding lipoprotein encoded by siderophore transport (*ceuE*) genes [12] were developed for PCR. In addition, specific PCR assays based on specific primer pairs were used to differentiate and identify *C. coli* and *C. jejuni*. In a study, standard isolation procedure and PCR assay was compared for the screening of *Campylobacter* in poultry. Results of this study showed that, PCR assay was clearly more sensitive and rapid than standard isolation procedure for the detection of the pathogen [5].

N-benzoylglycine amidohydrolase (hippuricase) which is not present in *C. coli*, is an effective test to discriminate *C. jejuni* from *C. coli* phenotypically. Hippuricase activity is regulated by *hipO* gene [13] and can be detected by ninhydrin test, phenotypically [14]. Several tests which most of them are not standardized, are used in microbiology laboratories to find out the hippuricase activity [15]. After verifying that *hipO* gene is only present in *C. jejuni* among *Campylobacter* species, gene of *C. jejuni* was cloned and sequenced to develop specific primers for the identification of *C. jejuni* [15]. Also, *ceuE* gene which is an important virulence factor of *Campylobacter* spp and regulates siderophore transport system, specific primer pairs were developed for the detection both of the *C. coli* and *C. jejuni* [12,16].

Therefore, this study was aimed to compare the specificity and sensitivity of *hipO* and *ceuE* gene based primers for the detection of *C. jejuni* by PCR.

## Materials and Methods

**Campylobacter isolates:** In the present study, a total of 44 *Campylobacter* isolates including 41 *Campylobacter jejuni*, two *C. coli* and one *C. lari* were tested for the comparison of *ceuE* and *hipO* gene based PCR assays for the detection of *Campylobacter jejuni*. The isolates were recovered from turkey meat samples using conventional culture technique in a previous study [17]. *C. lari* NCTC 11352, *C. coli* ATCC 43478 and *C. jejuni* ATCC 33291 reference strains were used for the verification of the isolates tested for PCR analysis.

**PCR analysis:** In the study, *ceuE* [12] and *hipO* [11], genes based PCR assays were compared for the detection of *C. jejuni*. Primer pairs used in the *ceuE* and *hipO* genes based PCR assays were, Jej 1: 5'-CCT GCT ACG GTG AAA GTT TTG C-3', Jej 2: 5'-GAT CTT TTT GTT TTG TGC TGC-3' and Hip 400 F: 5'-GAA GAG GGT TTG GGT GGT-3', Hip 1134 R: 5'-AGC TAG CTT CGC ATA ATA ACT TG-3' (Integrated DNA Technologies, IDT, Leuven, Belgium), respectively.

## DNA extraction

Chelex-100 (Bio-Rad, Hercules, CA, USA) was used for the DNA extraction of the isolates. All isolates that stored at  $-86^{\circ}\text{C}$  were grown in Bolton broth (Oxoid CM983 with supplement SR208, Hampshire, UK) and incubated at  $42^{\circ}\text{C}$  for 24 h under microaerophilic conditions (CampyGen, Gas Generating Kit, Oxoid). From enrichment's one ml of broth was centrifuged at  $12.000 \times g$  for 3 minutes and then solid phase was transferred into Chelex 100 (200  $\mu\text{l}$  of 6%) before the addition of proteinase K (2  $\mu\text{l}$  of 20 mg/ml). Mixture was incubated for 40 minutes at  $55^{\circ}\text{C}$  in thermomixer (Eppendorf Thermomixer 5437). The suspensions were heated in a boiling water bath for 8 minutes and then centrifuged at  $12.000 \times g$  for 3 minutes. These DNA extracts were used as a template in the PCR analysis.

## DNA amplification for *ceuE* gene based PCR assay

In *ceuE* gene based PCR assay 25  $\mu\text{l}$  of master mix (Promega, Madison USA) that contains, 5  $\mu\text{l}$  DNA extract, 1  $\times$  PCR Buffer, 0.2 mmol/L of each dNTP, 1.5 mmol/L  $\text{MgCl}_2$ , 2 U *Taq* DNA polymerase, 1  $\mu\text{mol/L}$  of each primers was used. The DNA amplification was performed in a thermocycler (Biometra Personal Cycler, Goettingen, Germany) according to the protocol previously reported [12].

## DNA amplification for *hipO* gene based PCR assay

In *hipO* gene based PCR assay 25  $\mu\text{l}$  of master mix (Promega) that contains, 5  $\mu\text{l}$  DNA extract, 1  $\times$  PCR Buffer, 0.2 mmol/L of each dNTP, 1.5 mmol/L  $\text{MgCl}_2$ , 2 U *Taq* DNA polymerase and 0.4  $\mu\text{mol/L}$  of each primers was used. The DNA amplification was performed as reported previously [11].

## Electrophoresis

Resultant PCR products of each amplification process were subjected to ethidium bromide stained (0.1  $\mu\text{g/ml}$ ) 1.5% agarose gel at 100 V for 1 h. Electrophoresis gels were visualized and documented (Syngene Ingenius, Cambridge, UK). The expected PCR amplified DNA fragment sizes for *ceuE* and *hipO* genes were 793 bp and 735 bp, respectively.

## Results

In the present study, 41 *C. jejuni*, two *C. coli* and one *C. lari* isolates were analyzed for the presence of *hipO* and *ceuE* genes by PCR. By *hipO* gene based PCR analysis in all 41 (100%) *C. jejuni* isolates 735 bp DNA fragment were shown and verified as *C. jejuni*. By *ceuE* gene based PCR analysis, in only 18 (43.9%) out of 41 isolates 793 bp DNA fragment were detected and can be identified as *C. jejuni*. Both assays did not show reaction with *C. lari* and *C. coli*. The results of PCR analysis were given in Table 1.

According to the results, *hipO* gene based PCR analysis showed more specificity and sensitivity than *ceuE* gene specific PCR assay for the detection of *C. jejuni*.

## Discussion and Conclusion

It was reported that all *C. jejuni* strains harbor *hipO* gene. However thermotolerant *Campylobacter* species other than *C. jejuni* are not the carrier of this gene [13-15]. This specific character of *C. jejuni* is utilized in culture technique for discriminating *C. jejuni* from

**Table 1.** Comparison between the results with *hipO* and *ceuE* based PCR assays.

No	Bacterial isolates	Isolates code	Results of the PCR assay	
			<i>ceuE</i>	<i>hipO</i>
1.	<i>C. jejuni</i>	ATCC 33291	+	+
2.	<i>C. jejuni</i>	100-1	-	+
3.	<i>C. jejuni</i>	100-2	-	+
4.	<i>C. jejuni</i>	106-2	+	+
5.	<i>C. jejuni</i>	106-3	+	+
6.	<i>C. jejuni</i>	110-1	+	+
7.	<i>C. jejuni</i>	110-2	-	+
8.	<i>C. jejuni</i>	110-3	-	+
9.	<i>C. jejuni</i>	111-3	-	+
10.	<i>C. jejuni</i>	112-3	-	+
11.	<i>C. jejuni</i>	116-1	+	+
12.	<i>C. jejuni</i>	116-2	+	+
13.	<i>C. jejuni</i>	170-2	-	+
14.	<i>C. jejuni</i>	170-3	-	+
15.	<i>C. jejuni</i>	184-1	+	+
16.	<i>C. jejuni</i>	184-2	-	+
17.	<i>C. jejuni</i>	184-3	-	+
18.	<i>C. jejuni</i>	205-1	-	+
19.	<i>C. jejuni</i>	210-2	-	+
20.	<i>C. jejuni</i>	210-3	-	+
21.	<i>C. jejuni</i>	242-1	+	+
22.	<i>C. jejuni</i>	242-2	+	+
23.	<i>C. jejuni</i>	242-3	+	+
24.	<i>C. jejuni</i>	293-2	-	+
25.	<i>C. jejuni</i>	293-3	-	+
26.	<i>C. jejuni</i>	307-1	+	+
27.	<i>C. jejuni</i>	307-2	+	+
28.	<i>C. jejuni</i>	307-3	+	+
29.	<i>C. jejuni</i>	310-1	-	+
30.	<i>C. jejuni</i>	310-2	-	+
31.	<i>C. jejuni</i>	376-1	-	+
32.	<i>C. jejuni</i>	417-2	-	+
33.	<i>C. jejuni</i>	418-1	-	+
34.	<i>C. jejuni</i>	418-2	-	+
35.	<i>C. jejuni</i>	418-3	-	+
36.	<i>C. jejuni</i>	600-1	-	+
37.	<i>C. jejuni</i>	646-2	+	+
38.	<i>C. jejuni</i>	648-1	+	+
39.	<i>C. jejuni</i>	648-2	+	+
40.	<i>C. jejuni</i>	650-1	+	+
41.	<i>C. jejuni</i>	650-3	+	+
42.	<i>C. coli</i>	649-3	-	-
43.	<i>C. coli</i>	ATCC 43478	-	-
44.	<i>C. lari</i>	NCTC 11352	-	-

*C. coli*. Detection of the *hipO* gene which is protected in *C. jejuni* using PCR, reported as an effective tool to identify the pathogen and differentiate from the other *Campylobacter* species [15,18]. And also the strains that were analyzed as hippuricase activity negative, can differentiate from *C. coli* in order to detect the *hipO* gene in *C. jejuni* strains [18,19].

Hani and Chan [13], reported that, although 17 *C. coli* strains, *C. sputorum*, *C. upsaliensis*, *C. lari* and *Helicobacter pylori* were negative,

12 *C. jejuni* strains were found positive by hippuricase gene probe. Also they detected, hippuricase-negative *C. jejuni* strains which were verified by DNA-DNA hybridization with hippuricase probe used in the study. These findings indicated that it is possible to identify hippuricase negative *C. jejuni* strains as *C. coli* according to the phenotypic hippuricase activity test [13].

Bang et al. [20], were obtained similar results with the findings of our study. In the study, *ceuE* and *cadF* based PCR methods were used to find out the virulence factors of pig and cattle *C. jejuni* isolates and three *C. jejuni* isolates were reported as *ceuE* negative by PCR assay.

In another study, conventional cultivation method results were verified by both *ceuE* and *hipO* based PCR methods. Although three colonies were isolated as *C. coli* and three as *C. jejuni* by cultivation method, all the isolates harbored both *ceuE* and *hipO* genes. So in the study it is concluded that, *ceuE* based PCR method is able to differentiate *C. coli* and *C. jejuni* from feces of chicken [5].

In a study two different results were found with *hipO* and *ceuE* genes based PCR assay. The *C. jejuni* specific *hipO* gene was detected from 25 isolates, 10 of them were interestingly hippurate negative and 15 of them were positive. However *C. jejuni* specific *ceuE* gene was only detected from 17 isolates, five of them were hippurate negative and 12 of them were positive. In the study, 36 out of 50 hippurate negative isolates harbored *C. coli* specific *ceuE* gene. Similar to our results, in the study, three *C. jejuni* isolates were not identified correctly by *ceuE* gene based PCR assay [21].

It is concluded that, in the present study all 41 *C. jejuni* isolates harbored *hipO* but only 18 *C. jejuni* isolates showed positive reaction by *ceuE* gene based PCR assay [12]. As *C. jejuni* is one of the most important foodborne bacterial pathogen for human, laboratories have to detect this pathogen without giving false negative results. The results of this study showed that *hipO* gene based PCR assay was more reproducibly and specific than *ceuE* gene specific PCR assay for the detection and also confirmation of *C. jejuni* isolates. Although 16S rRNA method is the most commonly used for identification of the micro-organism, use of *hipO* gene-based PCR will add value to the identification of *C. jejuni*.

## References

- Anon (2010) The community summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. EFSA J 8: 1496.
- Nachamkin I, Allos BM, Ho T (1998) Campylobacter species and Guillain-Barre' syndrome. Clin Microbiol Rev 11: 555-567.
- Miller WG, Mandrell RE (2005) Prevalence of Campylobacter in the food and water supply: incidence, outbreaks, isolation and detection. 101-163. In: J Ketley, ME Konkel (Eds), Campylobacter: Molecular and Cellular Biology. Horizon Scientific Press, Norfolk, UK.
- Wedderkopp A, Rattenborg E, Madsen M (2000) National surveillance of Campylobacter in broilers at slaughter in Denmark in 1998. Avian Dis 44: 993-999.
- Bang DD, Pedersen K, Madsen M (2001) Development of a PCR assay suitable for Campylobacter spp. mass screening programs in broiler production. J Rap Met Aut Microbiol 9: 97-113.
- Debruyne L, Samyn E, De Brandt E, Vandenberg O, Heyndrickx M, et al (2008) Comparative performance of different PCR assays for the identification of Campylobacter jejuni and Campylobacter coli. Res Microbiol 159: 88-93.
- Katzav M, Isohanni P, Lund M, Hakkinen M, Lyhs U (2008) PCR assay for the detection of Campylobacter in marinated and non-marinated poultry products. Food Microbiol 25: 908-914.
- Moreno Y, Hernández M, Ferrús MA, Alonso JL, Botella S, et al. (2001) Direct detection of thermotolerant Campylobacter in chicken products by PCR and in situ hybridization. Res Microbiol 152: 577-582.
- Sallam KI (2007) Prevalence of Campylobacter in chicken and chicken by-products retailed in Sapporo area, Hokkaido, Japan. Food Cont 18: 1113-1120.
- Uyttendaele M, Schukkink R, Van Gemen B, J Devereure (1995) Detection of Campylobacter jejuni added to foods by using a combined selected enrichment and nucleic acid sequence-based amplification (NASBA). Appl Environ Microbiol 61: 1341-1347.
- Linton D, Lawson AJ, Owen RJ, Stanley J (1997) PCR detection, identification to species level, and fingerprinting of Campylobacter jejuni and Campylobacter coli direct from diarrheic samples. J Clin Microbiol 35: 2568-2572.
- Gonzalez I, Grant KA, Richardson PT, Park SF, Collins MD (1997) Specific identification of the enteropathogenic Campylobacter jejuni and Campylobacter coli by using a PCR test based on the *ceuE* gene encoding a putative virulence determinant. J Clin Microbiol 35: 759-763.
- Hani EK, Chan VL (1995) Expression and characterization of Campylobacter jejuni benzoylglycine amidohydrolase (hippuricase) gene in Escherichia coli. J Bacteriol 177: 2396-2402.
- Harvey SM (1980) Hippurate hydrolysis by Campylobacter fetus. J Clin Microbiol 11: 435-437.
- Slater ER, Owen RJ (1997) Restriction fragment length polymorphism analysis shows that the hippuricase gene of Campylobacter jejuni is highly conserved. Lett Appl Microbiol 25: 274-278.
- Park SF, Richardson PT (1995) Molecular characterization of a Campylobacter jejuni lipoprotein with homology to periplasmic siderophore-binding proteins. J Bacteriol 177: 2259-2264.
- Cakmak O, Erol I (2012) Prevalence of thermophilic Campylobacter spp. in turkey meat and antibiotic resistance of *C. jejuni* isolates. J Food Safety 32: 452-458.
- Steinhausserova I, Ceskova J, Fojtikova K, Obrovskaa I (2001) Identification of thermophilic Campylobacter spp. by phenotypic and molecular methods. J Appl Microbiol 90: 470-475.
- Casadémont I, Bizet C, Chevrier D, Guesdon JL (2000) Rapid detection of Campylobacter fetus by polymerase chain reaction combined with non-radioactive hybridization using an oligonucleotide covalently bound to microwells. Mol Cell Prob 14: 233-240.
- Bang DD, Nielsen EM, Scheutz F, Pedersen K, Handberg K, et al. (2003) PCR detection of seven virulence and toxin genes of Campylobacter jejuni and Campylobacter coli isolates from Danish pigs and cattle and cytolethal distending toxin production of the isolates. J Appl Microbiol 94: 1003-1014.
- Nakari UM, Koivumäki A, Siitonen A (2006) Species identification of human Campylobacter strains. 1681. In: Proceedings of the 16th European Congress of Clinical Microbiology and Infectious Diseases ECCMID. Nice, France.

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