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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 specifity 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 specifity 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 -86ºC were grown in Bolton broth (Oxoid CM983 with supplement SR208, Hampshire, UK) and incubated at 42ºC for 24 h under microaerophilic conditions (CampyGen, Gas Generating Kit, Oxoid). From enrichment's one ml of broth was centrifuged at 12.000 × *g* for 3 minutes and then solid phase was transferred into Chelex 100 (200 µl of 6%) before the addition of proteinase K (2 µl of 20 mg/ml). Mixture was incubated for 40 minutes at 55ºC in thermomixer (Eppendorf Thermomixer 5437). The suspensions were heated in a boiling water bath for 8 minutes and then centrifuged at 12.000 × *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 µl of master mix (Promega, Madison USA) that contains, 5 µl DNA extract, 1 × PCR Buffer, 0.2 mmol/L of each dNTP, 1.5 mmol/L $MgCl_2$, 2 U *Taq* DNA polymerase, 1 µmol/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 µl of master mix (Promega) that contains, 5 µl DNA extract, 1 × PCR Buffer, 0.2 mmol/L of each dNTP, 1.5 mmol/L $MgCl_2$, 2 U *Taq* DNA polymerase and 0.4 µmol/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 µg/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 specify and sensitivity than *ceuE* gene specific PCR assay for the detection of *C. jejuni*.

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

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

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 *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 then *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*.

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