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].

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.

N-benzyoylglycine 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 TGCTG TC-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°C were grown in Bolton broth (Oxoid CM983 with supplement SR208, Hampshire, UK) and incubated at 42°C 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 55°C 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 MgCl2, 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 MgCl2, 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 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 other species other than Campylobacter jejuni [18]. 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 other species other than Campylobacter jejuni [18].

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. spatorum, 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.

References