**Short Report**

**Comparison of PCR and Culture Methods for Diagnosis of Enteropathogenic Campylobacter in Fowl Feces**

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**ABSTRACT**

Enteritis due to Campylobacter is the most common cause of acute bacterial diarrhea worldwide. In most cases, infection occurs as a result of consuming contaminated water or food, especially raw meat of fowls. Campylobacters are saccharolytic and fastidious bacteria. These traits limit the number of available biochemical tests by which isolates may be differentiated. These limitations might, in principle, be overcome by the use of PCR techniques, which is the aim of the present study. To compare the culture technique with PCR assay, a total of 116 fecal samples from fowls were tested using these two techniques for the presence of Campylobacters. Campylobacter strains were isolated from 11 (9.4%) out of 116 fecal cultures from fowls (8 C. jejuni and 3 C. coli). Using PCR assays, the number of positive Campylobacter increased to 27 (23%). Of these 27 positive samples, 18 were C. jejuni and 9 were C. coli. The sensitivity and specificity of PCR in comparison to the culture method were found to be 100 and 84.7%, respectively. According to the present study, it is proposed that the PCR is a reliable and sensitive method which can be used as a diagnostic technique for the detection of Campylobacter in fowls’ samples. Iran. Biomed. J. 10 (1): 47-50, 2006

**Keywords**: Campylobacter, Culture, Fowl, PCR, Diagnosis

**INTRODUCTION**

Campylobacter is a curved, rod-shaped, non-spore forming, motile, and Gram-negative organism belonging to the Campylobacteriaceae. There are several species and subspecies in this family, among them C. jejuni and C. coli are the ones identified as strains responsible for most Campylobacter infection cases in man [1]. Nowadays, Campylobacters are known to be the most common causes of bacterial diarrhea across the globe, accounting for 20 to 35% of diarrhea cases according to the world estimates [1]. This disease in humans occurs as a result of consuming contaminated milk, water, and food. Poultry meat has also been found to be the cause of up to 70% of sporadic Campylobacter infections, according to some reports [2].

Detection of the bacteria is usually carried out by culture in media containing antibiotics. There are few biochemical tests such as hippurate hydrolysis test to be used in the confirmation of Campylobacters [3]. Due to some technical difficulties such as long incubation time, uncertainly in results, and some atypical strains, which are not easy to culture, the detection of Campylobacters is more complicated. Due to these problems, genome-based detection methods like PCR have gained prominent importance in recent years. In this study, we use PCR assay to compare the results with other data, since this bacterium is important in disease transmission and the lack of well-defined method.

**MATERIALS AND METHODS**

Sampling and identification of Campylobacter. In this study, 116 fecal samples were randomly collected as rectal swap from local fowls in
industrial hatcheries in Isfahan (Iran). The samples were enriched for 1 to 2 hours on Campy-THio and then cultured linearly on a species-specific culture medium, Campylobacter selective culture medium, containing 5% defibrinated sheep blood, vancomycin, polymyxin, and trimethoprim. After incubation at 42°C for 48 hours under microaerophilic conditions, the suspected colonies were examined using Gram-staining technique, other supplementing tests like oxidase, catalase activities, hippurate hydrolysis test and also susceptibility to 30 µg discs of nalidixic acid and cephalothin were also applied. DNA extraction was performed using High Pure PCR Template Preparation Kit (Roche Diagnostic, Germany). For this purpose, 1 ml of the bacterial suspension enriched in Campy-THio medium was centrifuged at low speed (450 × g) for 10 minutes and the supernatant was removed and centrifuged at high speed (1800 × g) for 10 minutes. DNA extracted from the resulting precipitate according to the instructions recommended by the suppliers (Roche Diagnostic, Germany). The quality and quantity of the extracted DNA were determined by agarose gel electrophoresis and spectrophotometry. To run PCR, two species-specific primer sets were used: one JE1-JE2 for C. jejuni and the other COL1-COL2 for C. coli [6].

**PCR condition:**

**Sensitivity assay of PCR method.** DNA was extracted from pure culture of campylobacter and after preparation of serial dilution subjected to PCR. The sensitivity of the PCR assay was calculated based on the highest dilution of DNA in which the primer could amplify their specific target sequences.

**Specificity assay of PCR method.** Non-Campylobacter control bacteria such as E. coli spp.: Proteus mirabilis, Salmonella typhi, Streptococcus viridans, Haemophilus influenza, and H. pylori sp., Staphylococcus aureus were tested in PCR reaction with the same optimized condition.

The final modified concentrations of PCR master mix were as follow: KCl, 50 mM; Tris-HCl (pH 8.3), 10 mM; Gelatin, 0.001%; MgCl₂, 3 mM; dNTPs, 0.2 pmM; Primers, 200 Pm; Taq DNA polymerase, 0.5 U.

After the reaction mix had been prepared and distributed among the thin-walled PCR tubes, 100 nanograms of DNA extracted from samples was added to each of the tubes and 30 µl of sterile mineral oil was added. In each reaction, sets of positive controls obtained from Folkehelsa Institute (Norway) were also included. For C. jejuni, Lior 1 and 2 and for C. coli, Lior 8 was used. For negative controls, DNA extracted from non-Campylobacters (mentioned above) was used. The tubes were transferred to a HYBAID OmniGene thermal cycler unit and subjected to 30 cycles of PCR with the following thermal profile [6]: denaturation at 94°C for 30 s, primer annealing at 57°C for 30 s, and extension at 72°C for 1 min. PCR products (10 µl) were mixed with 3 µl of gel loading buffer and loaded to each well of 2% agarose gel containing 0.5 µg/ml ethidium bromide and electrophoresed for 90 minutes at 80 Volts. Tric-Boric acid-EDTA (TBE) was used as running buffer. After completion of electrophoresis, the gel transferred to a Wilber gel documentation system and the image was either printed directly or saved on a floppy disk.

**RESULTS**

**Sensitivity.** Serial dilution of genomic DNA from Campylobacter positive control tested in PCR assay. The lowest dilution compatible to PCR amplification was 20 ng of DNA equivalent to 100 organisms. The amplified fragment for C. jejuni and C. coli was 793 bp and 894 bp, respectively.

**Specificity.** For specificity evaluation, we did not get false positive with any of the other bacterial species used in this study. Therefore, the specificity for this protocol was 100 percent.

Out of 116 samples studied, 11 samples proved positive upon culture accounting for 9.4% of the total samples. The results from oxidase and catalase tests of all strains were positive. All strains were resistant to cephalotin but susceptible to nalidixic acid. The results from hippurate hydrolysis tests were positive in 8 cases but negative in the remaining 3. Thus, according to biochemical tests, 72% of the strains belonged to the C. jejuni and 28% to C. coli.

Figure 1 shows DNA extracted from all samples. Using PCR technique, 27 positive samples were detected which comprises 23.2% of all specimens. From this subset, 18 samples were amplified with the C. jejuni species-specific primer set and 9 samples with C. coli species-specific primer set (Fig. 2).

Based on PCR assay, 66.7% of the strains isolated were belonged to the C. jejuni, while the remaining 33.3% belonged to C. coli. All the culture positive samples were also PCR positive. This is why 16 cases that had been culture negative were also PCR
Fig. 1. Gel electrophoresis of purified DNA extracted from control strains and fowl feces. Lane M, Molecular weight marker; lane 1-3, DNA from control strains; lane 1, C. jejuni; lane 2, C. jejuni Lior 2; lane 3, C. coli Lior 8; lane 4-17, DNA isolated from the fowl feces.

In the present study, sensitivity and specificity of the PCR method as compared to the culture were 100% and 84.7%, respectively. Since PCR method was capable of detecting more positive cases compared to the culture method, it seems that PCR method is a good substitute for the culture method in detecting thermophilic Campylobacter in samples from chickens. The reason for culture negative cases (despite the PCR positive ones) is probably the failure of certain Campylobacter strains to grow in the species-specific medium. Some studies indicated that C. coli is more susceptible than C. jejuni to the antibiotics [14].
present in the selective agar (especially to Colistin and amphotericin). Meanwhile, enriching media sometimes promote \textit{C. coli} growth rather than \textit{C. jejuni} [15].

The long intervals between sample collection and culture may also lead to negative results in culture tests. Mahendru et al. [16] found that they were fowl culture positive samples were positive in PCR when maintained at 4°C for one week, but negative was beyond this period. For detection of this bacterium, culture with selective enrichment has been used but this method may lose sensitivity due to the non-optimal growth conditions. Based on our results, PCR found to be sensitive enough, fast and reliable that could act as appropriate substitute for culture or at least as a supplementary method, when culture yielded negative results.

REFERENCES


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