Iranian Biomedical Journal 10 (1): 47-50 (January 2006)

Short Report

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

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Received 26 December 2004; revised 14 June 2005; accepted 4 July 2005

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*s 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 Campylobacteriacae. There are several species and subspecies in this family, among them *C. jujuni* 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

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[DOR: 20.1001.1.1028852.2006.10.1.2.1]

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 agar, 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 G-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 then 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 spectro-photometry. To run PCR test, two species-specific primer sets were used: one JEJ1-JEJ2 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 mirablis*, *Salmonella typhi*, *Streptococcus viridance*, *Heamophilus influenza*, and *H. pylori* sp., *S. 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%; $MgCl_2$, 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

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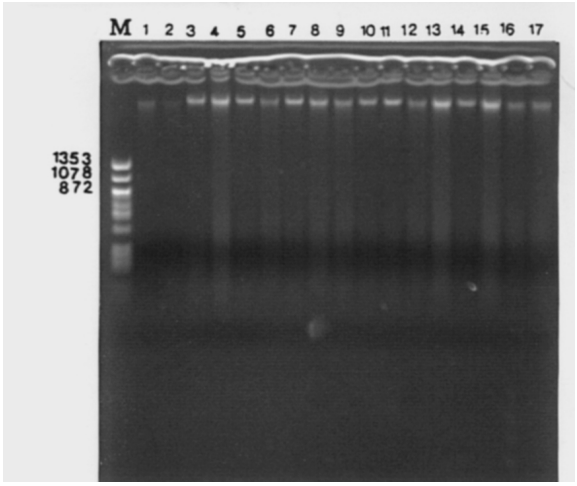


Fig. 1. Gel electrophoresis of purified DNA extracted from control strains and fowl feces Lior 1. Lane M, Molecular weight marker; lane 1-3, DNA from control strains; lane 1, *C. jejuni*; lane 2, *C. jejuni* Lior 2; lane 3, *C. coli* Lior 8; lane 4-17, DNA isolated from the fowl feces.

positive. In the remaining cases (89 samples), the results from both culture and PCR were simultaneously negative.

DISCUSSION

Based on our results, 9.4% of the samples collected from poultry farms of Isfahan (Iran) were infected with *Campylobacter* when cultured in selective medium. There are vast differences in *Campylobacter* infection reported in literature. For instance, in some studies between 30% to as high as 100% of fowls have been shown to be carriers of the *Campylobacter* as a normal flora of their digestive system [5]. On the other hand, Magistrado *et al.* [4] reported 5.9% out of 135 fowls' samples were positive in culture tests. According to our biochemical tests used for strain differentiation, 72% of positive samples were belonged to *C. jejuni* and the remaining 28% categorized as *C. coli* and there were no uniform distribution patterns of these two strains. In some studies, up to 99 percent of all *Campylobacter* infections in fowls were caused by *C. jejuni* [7] while Van Looveren *et al.* [8] reported the prevalence of *C. jejuni* and *C. coli* isolates to be 79% and 21%, respectively. Similarly, Eyigor *et al.* [9] determined the frequency of these two strains about 67% and 33%, respectively. According to Magistrado *et al.* [4], out of 8 fowls fecal isolates, 3 isolates were detected to be *C. jejuni* and 5 other isolates as *C. coli.* When PCR was used as a detection strategy, out of 27 cases, 32.2% was positive, of which 18 cases were *C. jejuni* and the other 9 cases were belonged to *C. coli* strain.

Different results have also been reported in the literature on the detection of *Campylobacter* in fowls' samples using the PCR technique. Eyigor *et al.* [9] and Grennan *et al.* [10] reported that 100% of their samples being infected when PCR was used as a detection method, while Winters *et al.* [11] and Denis *et al.* [12] reported 80% and 66.3% of their PCR samples were positive, respectively. Similarly, *Studer et al.* [13] as well reported 68% samples to be PCR positive. On the other hand, Magistrado *et al.* [4] reported only 5.9% of the samples to be PCR positive.

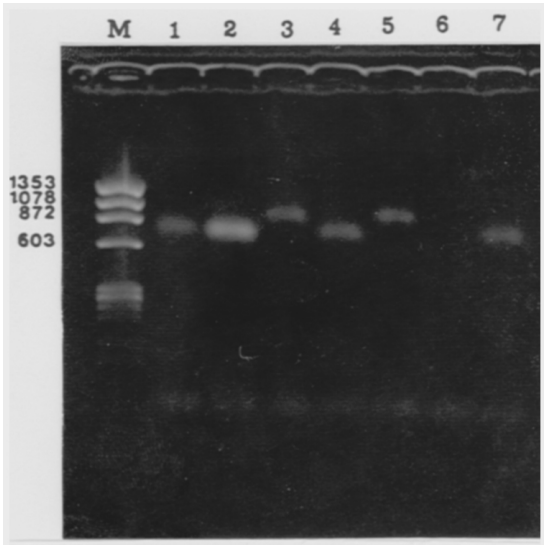


Fig. 2. Gel electrophoresis of PCR product isolated from control strains and fowl feces. Lane M, Molecular weight marker; lane 1-3: Control strains; lane 1, *C. jejuni* Lior 1; lane 2, *C. jejuni* Lior 2; lane 3, *C. coli* Liro 8; lane 4 and 7, *C. jejuni* isolated from fowl feces; lane 5, *C. coli* isolated from fowl feces; lane 6, Non-*Campylobacter jejuni* and *coli* specimen.

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*s 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]

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present in the selective agar (especially to Colistin and amphotericin). Meanwhile, enriching media sometimes promote *C. coli* growth rather than *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

1. Guerrant, R.L. (2000) Campylobacter enteritis. In: *Textbook of Medicine*. (Cecil, R.L. and Goldman, L. eds.), W.B. Saunders company, Philadelphia, USA. pp. 1687-1690.
2. Blaser, M. (2000) Campylobacter jejuni and related species. In: *Principles and Practices of Infectious Diseases*. (Mandell, G.L., Douglas, R.G., Bennett, J.E. and Dolin, R. eds.), Churchill Livingstone, Philadelphia. USA. pp. 2276-2285.
3. Moore, J.E. and Murphy, P.G. (2000) Hippurate hydrolysis and speciation of thermophilic *Campylobacter spp. Br. J. Biomed. Sci. 57 (2): 180-182*.
4. Magistrado, P.A., Garcia M.M. and Raymundo, A.K. (2001) Isolation and polymerase chain reaction-based detection of *Campylobacter jejuni* and *Campylobacter coli* from poultry in the Philippines. *Int. J. Food Microbiol. 70 (1-2): 197-206*.
5. O'sullivan, N.A., Fallon, R. and Carrol, C. (2000) Detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli* in broiler chicken samples using a PCR/DNA probe membrane based colorimetric detection assay. *Mol. Cell probes 14 (1): 7-16*.
6. Gonzalez, I., Grant, K.A. and Richardsons, P.T. (1997) Specific identification of the enteropathogens *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*.
7. Ng, L.K., Kingombe, C.I. and Yan, W. (1997) Specific detection and confirmation of *Campylobacter jejuni* by DNA hybridization and PCR. *Appl. Environ. Microbiol. 63 (11): 4558-4563*.
8. Van Looveren, M., Daube, G. and De Zutter, L. (2001) Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. *J. Antimicrob chemother. 48 (2): 235-240*.
9. Ejigor, A., Dawson, K.A., Langlois, B.E. and Pickett, C.L. (1999) Detection of cytolethal distending toxin activity and *cdt* genes in *Campylobacter spp* isolated from chicken carcasses. *Appl. Environ. Microbiol. 65 (4): 1501-1505*.
10. Grennan, B., O'sullivan, N.A. and Fallon, R. (2001) PCR-ELISAS for the detection of *Campylobacter jejuni* and *Campylobacter coli* in poultry samples. *Biotechniques 30 (3): 602-606*.
11. Winters, D., O'Leary, A.E. and Slavik, M.F. (1997) Rapid PCR with nested primers for direct detection of *Campylobacter jejuni* in chicken washes. Mol. Cell Probes. *11 (4): 267-271*.
12. Denis, M., Refregier-petton, J. and Laisney, M.J. (2001) Campylobacter contamination in french chicken production from farm to consumers. Use of a PCR assay for detection and identification of *Campylobacter jejuni* and *Campylobacter coli*. *J. Appl. Microbiol. 91 (2): 255-267*.
13. Studer, E., Luthy, J. and Hubner, P. (1999) Study of the presence of *Campylobacter jejuni* and *C. coli* in sand samples from four Swiss chicken farms. *Res. Microbiol. 150 (3): 213-219*.
14. Goossens, H., Boeck, M. and Coignau, H. (1984) 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. 24 (5): 840-843*.
15. Denis, M., Soumet, C. and Rivoal, K. (1999) Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. *Lett. Appl. Miciobiol. 29 (6): 406-410*.
16. Mahendru, M., Prasad, K.N., Dhole, T.N. and Ayyagari, A. (1997) Rapid identification of *Campylobacter jejuni* strains by polymerase chain reaction and their restriction fragment length polymorphism analysis. *Indian J. Med. Res. 105: 9-14*.

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