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# Typing of *Campylobacter jejuni* isolated from poultry on the basis of *fla*A-RFLP by various restriction enzymes

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ABSTRACT

RFLP analysis of the flagellin (*fla*A) gene was compared using three different restriction endonucleases *i.e* *Dde*I, *Hinf*I and *Dpn*II to determine the genetic diversity among 43 *Campylobacter jejuni* isolates of poultry origin from the same geographical area. *fla*A gene was amplified in all the isolates and RFLP analysis showed variations. *Dde*-based RFLP was found most efficient in discriminating *C. jejuni* isolates by generating 15 different *Dde*-RFLP patterns with discriminatory index (D.I) of 0.9258 whereas *Dpn*II produced seven *Dpn*-RFLP patterns (D.I.= 0.8427). While *Hinf*I enzyme produced only six *Hinf*-RFLP patterns (D.I.= 0.6977). The discrimination of *Dpn*-RFLP was comparable to discrimination given by *Dde*-RFLP analysis, which is generally used to study *fla*A gene RFLP.

## 1. Introduction

*Campylobacter* species are the second most emerging bacterial zoonotic pathogen after *Salmonella* causing gastroenteritis (Epps et al., 2013; Silva et al., 2011). Of the many *Campylobacter* species identified, *Campylobacter jejuni* (*C. jejuni*) is the most predominant pathogen implicated in food borne infections followed by *Campylobacter coli* (*C. coli*) (Biswas, Hannon, Townsend, Potter, & Allan, 2011; Bolton, 2015; Coward et al., 2008; Wieczorek & Osek, 2013). The caeca of the chickens is frequently colonized by this organism and consumption of broiler meat and its products contaminated during production and processing is considered to be the most frequent source of human infection (Aydin et al., 2007; Pearson et al., 1993; Wirz, Overesch, Kuhnert, & Korczak, 2010).

*Campylobacter* populations that infect broiler flocks can be complex, containing multiple genotypes, as *Campylobacter* species are associated with high frequency mutation in their surface antigen *i.e.* capsule, lipooligosaccharides and flagella (Hendrixson, 2006). A wide genetic diversity of *Campylobacter* populations in poultry sources has been reported in different studies and identification of *Campylobacter* at genus or species level does not help in understanding the epidemiology of the disease (Nachamkin, Bohachick, & Patton, 1993).

Many sub typing methods have been developed to differentiate *Campylobacter* strains for epidemiological purposes. These can be grouped into phenotypic methods and genotypic methods. Various phenotypic techniques used for characterization include serotyping (based on heat stable and heat labile antigens), biotyping (based on biochemical characters), phage typing and MEE (multi locus enzyme electrophoretic typing) (Dingle et al., 2001). Genotypic methods such as restriction fragment length polymorphism of the polymerase chain reaction products (PCR-RFLP) analysis based on the flagellin genes (*fla* typing), multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), DNA microarray and amplified fragment length polymorphism (AFLP) analysis (Fitzgerald, Stanley, Andrew, & Jones, 2001; Gondo et al., 2006; Khoshbakht, Tabatabaei, Hosseinzadeh, Shekarforoush, & Aski, 2013; Vinueza-Burgos et al., 2017) are used in typing of *C. jeuni.* Although MLST and DNA microarray based techniques have been found to have high discriminatory index PCR-RFLP of flagellin gene using different restriction endonucleases is the preferred method in terms of handling, costs and time (Shi et al., 2002; El-Adawy et al., 2013).

Flagellin gene locus of *C. jejuni* contains two flagellin genes (*fla*A and *fla*B), which are highly conserved with variable regions interspersed between them (Khoshbakht, Tabatabaei, Hosseinzadeh, Aski, & Seifi, 2015; Thomrongsuwannakij, Blackall, & Chansiripornchai, 2017). This locus is suitable for restriction fragment length polymorphism (RFLP) analysis of PCR products. The conserved regions in this locus are also found in species other than *C. jejuni* making it suitable for typing other *Campylobacter* species. The level of discrimination in this technique is dependent on the restriction endonuclease chosen for the RFLP

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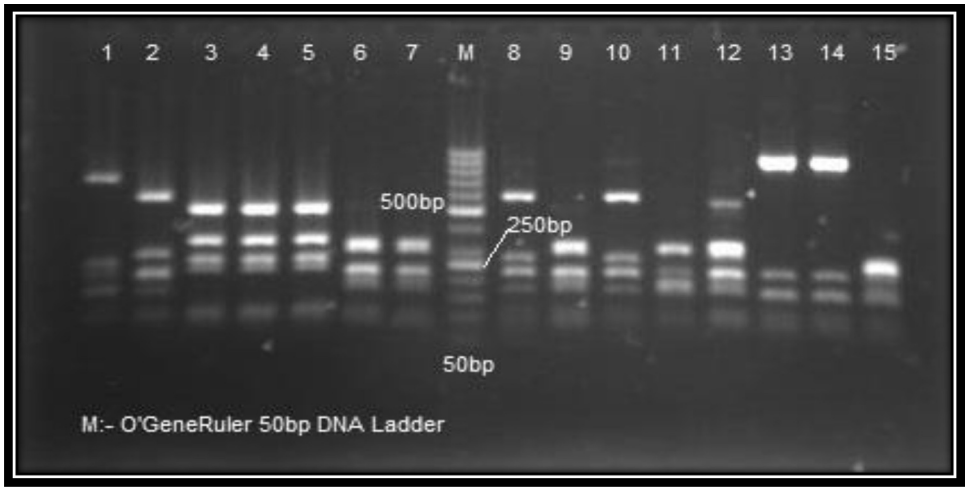


**Fig. 1.** Distribution of *Dde*-RFLP patterns of *fla*A gene amplicon (Lane M: 100 bp marker, New England Biolab; Lane 1–15 *C. jejuni* strains).

analysis (Owen & Leeton, 1999). *Dde*I restriction enzyme is the most preferred restriction enzyme for typing *C. jejuni* worldwide as it gives good discrimination for *Campylobacter* isolates within a given ecological niche (Ghorbanalizadgan, Bakhshi, & Peerayeh, 2016). The selection of a restriction enzyme for RFLP typing of any given fragment is based on the number of distinguishable fragments produced. Thus an average number of 4–8 fragments with recognizable band size are ideal for RFLP typing assay by any restriction enzyme. *In silico* analysis of the *fla*A gene led to selection of *Dpn*II restriction enzyme as one of the suitable enzyme to study. The, present study was aimed to determine the *fla*A gene RFLP patterns of *Dpn*II enzyme and compare it with *Dde*I and *Hinf*I for typing of *C. jejuni* isolates from poultry origin.

## 2. Material and methods

A total of 43 *C. jejuni* were isolated from poultry cloacal samples in and around Bikaner city (Rajasthan, India) and confirmed by amplification of genus specific 16S rRNA gene (Linton, Owen, & Stanley, 1996; Tang *et al.*, 2009) and *C. jejuni* species specific *hip*O gene (Al-Amri, Senok, Ismaeel, Al-Mahmeed, & Botta, 2007; Linton, Lawson, Owen, & Stanley, 1997) as described previously by Yadav et al. (2016).

### 2.1. Amplification of flaA gene

Amplification of *fla*A gene was carried out as per method described by Nachamkin et al. (1993) using forward primer F-GGATTTCGTATT AACACAAATGGTGC and reverse primer R-CTGTAGTAATCTTAAAAC ATTTTG. All PCR amplifications were performed in a mixture (25 µl) containing: 2.5 µl of the 10X PCR buffer, 2.5 µl of $MgCl_2$ (25 mM), 0.5 µl of dNTPs (10 mM), 1 µl of each primer (100 µM), 0.5 µl (1 U) of the *Taq* DNA polymerase (Promega), 3 µl of the bacterial template DNA and 14 µl nuclease free water. The PCR products were analyzed by electrophoresis in 1.5% agarose gel for 1 h at 100 V. The gel was then visualized under UVP gel documentation system (BioDoc-It Imaging System).

### 2.2. Restriction fragment length polymorphism (RFLP) of flaA gene

Restriction fragment length polymorphism of *fla*A PCR products was carried out with nucleotide site specific restriction endonuclease enzymes *Dde*I (3′..CˆTA..5′ and 5′ ATˆC..3′), *Hinf*I (5′..GˆATC..3′ and 3′..CTAˆG..5′) and *Dpn*II (5′.. ˆGATC..3′ and 3′..CTAGˆ..5′). Selection of the novel restriction enzyme for *fla*A gene digestion, was based on the *in silico* analysis of *fla*A gene digestion by different restrictions endonuclease enzymes (http://insilico.ehu.eus/). *Dpn*II restriction enzyme produced desired fragments (4–5) with different sizes ranging from 240 to 1500 bp with higher efficacy. Restriction enzymes were used as per the recommendation of the manufacturer (New England Biolabs). Briefly, to 10 µl of PCR product, 5 µl of nuclease free water, 2 µl of 10x buffer and 0.2 µl of restriction enzymes (*Dde*I, *Hinf*I and *Dpn*II) (0.2 µl = to 10 U/µl of Restriction enzyme) were added. The mixture was mixed gently and incubated in a water bath at 37°C for 3 h. The digest was resolved on 1.5% agarose gel and analyzed as described above. The images were analyzed with Pyelph application (Pavel & Vasile, 2012) and the resultant binary matrix was subjected to one of the agglomerative hierarchical clustering (UPGMA) method along with (Dice) similarity coefficient (Bikandi, San Millán, Rementeria, & Garaizar, 2004). Further the Discriminatory index of RFLP patterns were calculated using Discriminator power calculator tool (Bikandi et al., 2004).

## 3. Result and discussion

The flagellin gene locus of *C. jejuni* contains *fla*A gene which is arranged in tandem, is highly conserved and has short variable regions (Khoshbakht et al., 2013), therefore making it suitable for RFLP analysis. In the present study, PCR amplification of *fla*A gene sequence was performed for 43 isolates of *C. jejuni.* The amplicons were further subjected to restriction endonuclease digestion with *Dde*I, *Hinf*I and *Dpn*II.

Digestion by *Dde*I restriction enzyme produced 15 different *Dde*I-RFLP (*Dde*1 to *Dde*15) patterns with band size ranging from 200 to 1100 bp (Fig. 1) and discriminatory index of 0.9258. The discriminatory power of a tool giving discrimination above 0.5 is considered as a good method to discriminate isolates. Out of the total 15 *Dde*I-RFLP patterns, *Dde*9 was the most common pattern and found in 20.93% (9/43) isolates followed by *Dde*4 found in 9.30% (4/43) isolates while other remaining *Dde* types were found in less than four isolates (Table 1). During phylogenetic cluster analysis (on the basis of 80% genetic similarity) of *C. jejuni*, all 15 *Dde*I-RFLP patterns grouped in six clusters (Table 2). Cluster I and II had a single isolates having *Dde*1 and *Dde*15 patterns respectively. Highest numbers of 65.11% (28/43) isolates had seven *Dde* patterns (*Dde*6, *Dde*7, *Dde*8, *Dde*9, *Dde*10, *Dde*11 and *Dde*13) and were grouped in Cluster III followed by cluster

**Table 1**
RFLP analysis of *fla*A gene sequence digested by *Dde*I, *Hinf*I and *Dpn*II restriction enzymes.

| Sr. No. | Patterns | Isolate ID (total 43 isolates) | Number of isolates (%) |
|---|---|---|---|
| *Dde*I RFLP pattern | | | |
| 1 | *Dde*1 | C5 | 1 (2.32%) |
| 2 | *Dde*2 | C40,C43 | 2 (4.65%) |
| 3 | *Dde*3 | C7,C35 | 2 (4.65%) |
| 4 | *Dde*4 | C3 | 1 (2.32%) |
| 5 | *Dde*5 | C6,C8,C9 | 3 (6.97%) |
| 6 | *Dde*6 | C26,C27,C29,C31 | 4 (9.30%) |
| 7 | *Dde*7 | C33,C34 | 2 (4.65%) |
| 8 | *Dde*8 | C28,C30,C32 | 3 (6.97%) |
| 9 | *Dde*9 | C1,C4,C12,C13,C14,C17,C18,C20,C21 | 9 (20.93%) |
| 10 | *Dde*10 | C10,C11,C15,C38 | 4 (9.30%) |
| 11 | *Dde*11 | C22,C23,C24 | 3 (6.97%) |
| 12 | *Dde*12 | C16,C25,C39 | 3 (6.97%) |
| 13 | *Dde*13 | C2,C19,C36 | 3 (6.97%) |
| 14 | *Dde*14 | C41,C42 | 2 (4.65%) |
| 15 | *Dde*15 | C37 | 1 (2.32%) |
| *Hinf*I RFLP pattern | | | |
| 1 | *Hinf*1 | C5 | 1 (2.32%) |
| 2 | *Hinf*2 | C1,C4,C12,C13,C14,C17,C18,C26,C27C28,C29,C30,C31,C33,C34,C35,C36,C39,C42 | 19 (44.18%) |
| 3 | *Hinf*3 | C3,C6,C8,C9,C10,C11,C15,C16,C20, C21,C38,C43 | 12 (27.90%) |
| 4 | *Hinf*4 | C2,C7,C19,C22,C23,C24,C25,C40,C41 | 9 (20.93%) |
| 5 | *Hinf*5 | C37 | 1 (2.32%) |
| 6 | *Hinf*6 | C32 | 1 (2.32%) |
| *Dpn*II RFLP pattern | | | |
| 1 | *Dpn*1 | C4,C5,C10,C11,C15,C17,C19,C20 | 8 (18.60%) |
| 2 | *Dpn*2 | C1,C12,C13,C18,C21,C23,C26,C28,C30,C33,C34,C37 | 12 (27.90%) |
| 3 | *Dpn*3 | C2,C7,C8,C22,C24,C25,C31 | 7 (16.27%) |
| 4 | *Dpn*4 | C3,C9,C32,C40,C43 | 5 (11.62%) |
| 5 | *Dpn*5 | C6,C14,C16,C41,C42 | 5 (11.62%) |
| 6 | *Dpn*6 | C27,C29 | 2 (4.65%) |
| 7 | *Dpn*7 | C35,C36,C38,C39 | 4 (9.30%) |

**Table 2**
Phylogenetic cluster analysis of *fla*A gene RFLP patterns obtained by digestion of *Dde*I, *Hinf*I and *Dpn*II restriction enzymes.

| S. No | Clusters | *fla*A gene RFLP patterns | Number of isolates (%) |
|---|---|---|---|
| *Dde*I RFLP pattern | | | |
| 1 | Cluster I | *Dde*1 | 1 (2.32%) |
| 2 | Cluster II | *Dde*15 | 1 (2.32%) |
| 3 | Cluster III | *Dde*6,*Dde*7,*Dde*8, *Dde*9,*Dde*10,*Dde*11,*Dde*13 | 28 (65.11%) |
| 4 | Cluster IV | *Dde*3,*Dde*12,*Dde*14 | 7 (16.27%) |
| 5 | Cluster V | *Dde*2 | 2 (4.65%) |
| 6 | Cluster VI | *Dde*4,*Dde*5 | 4 (9.30%) |
| *Hinf*I RFLP pattern | | | |
| 1 | Cluster I | *Hinf*1 | 1 (2.32%) |
| 2 | Cluster II | *Hinf*3, *Hinf*4, *Hinf*6 | 22 (51.16%) |
| 3 | Cluster III | *Hinf*2, *Hinf*5 | 20 (46.51%) |
| *Dpn*II RFLP pattern | | | |
| 1 | Cluster I | *Dpn*2 | 12 (27.90%) |
| 2 | Cluster II | *Dpn*4 | 5 (11.62%) |
| 3 | Cluster III | *Dpn*3,*Dpn*5,*Dpn*6 | 14 (32.55%) |
| 4 | Cluster IV | *Dpn*1 | 8 (18.60%) |
| 5 | Cluster V | *Dpn*7 | 4 (9.30%) |

IV which consisted of 16.27% (7/43) isolates with three *Dde* patterns (*Dde*3, *Dde*12 and *Dde*14). Cluster V had 4.65% (2/43) isolates of *Dde*2 pattern and cluster VI possessed 9.30% (4/43) isolates with *Dde*4 and *Dde*5 patterns.

*Hinf*I digested *fla*A gene produced only six (*Hinf*1 to *Hinf*6) different patterns (Fig. 2) and *Dpn*II revealed seven (*Dpn*1 to *Dpn*7) different patterns (Fig. 3) with discriminatory index of 0.6977 and 0.8427 respectively. These were found less efficient than *Dde*I digested *fla*A gene RFLP patterns. Out of the total six *Hinf*-RFLP patterns obtained by digestion with *Hinf*I enzyme, *Hinf*2 pattern was the most common and observed in 44.15% (19/43) isolates. Out of seven *Dpn*-RFLP patterns observed, *Dpn*2 was the most common pattern and comprised of 27.90% (12/43) isolates followed by *Dpn*1 found in 18.60% (8/43) isolates and *Dpn*3 in 16.27% (7/43) isolates (Table 1). Remaining *Dpn* patterns had less than five isolates. The number of bands varied from 3–6 with amplicon size ranging from 75 bp to 1300 bp with *Hinf*I and *Dpn*II restriction enzymes.

*Hinf*-RFLP based phylogenetic cluster analysis of *C. jejuni* revealed three clusters (on the basis of 80% genetic similarity). Cluster I had a single isolate *i.e.* C5 having *Hinf*1 pattern while maximum number of 51.16% (22/43) isolates grouped into cluster II and had three *Hinf* patterns (*Hinf*3, *Hinf*4 and *Hinf*6). Cluster III was found in 46.51% (20/43) isolates and had two *Hinf* patterns (*Hinf*2 and *Hinf*5) (Table 2).

*Dpn*II-based RFLP patterns could be divided into five clusters on the basis of 80% genetic similarity. Cluster I had 27.90% (12/43) isolates with a single (*Dpn*2) pattern. Cluster II comprised of 11.62% (5/43) isolates and had one (*Dpn*4) pattern, cluster III had maximum 32.55% (14/43) isolates and had three *Dpn* patterns (*Dpn*3, *Dpn*5 and *Dpn*6), cluster IV possessed 18.60% (8/43) having *Dpn*1 pattern and cluster V comprised of 9.30% (4/43) isolates having *Dpn*7 pattern.

*Dde*I (D.I. − 0.9258) was found most efficient in discriminating *C. jejuni* isolates as has been reported by many workers (Hiett, Seal, & Siragusa, 2006; Khoshbakht et al., 2015; Rajagunalan et al., 2014; Vinueza-Burgos et al., 2017). The other enzymes *i.e Hinf*I (D.I. − 0.6977) and *Dpn*II (D.I. − 0.8427) were less discriminatory as compared to *Dde*I. Also, the numbers of patterns generated were highest when *Dde*I restriction enzyme was used as compared to that of *Hinf*I and *Dpn*II. *Dpn*II restriction enzyme has not been used for *fla*A gene PCR-RFLP of *C. jejuni* so far to our knowledge but the results with this enzyme were comparable to that of *Dde*I restriction enzyme in present study.


**Fig. 2.** Distribution of *Hinf*-RFLP patterns of *fla*A gene amplicon (Lane M: 100 bp marker, New England Biolab; Lane 1–15 *C. jejuni* strains).


**Fig. 3.** Distribution of *Dpn*-RFLP patterns of *fla*A gene amplicon (Lane M: 100 bp marker, New England Biolab; Lane 1–15 *C. jejuni* strains).

## 4. Conclusion

*Campylobacter jejuni* typing using RFLP was carried out in the present study using different restriction endonucleases. *Dde*-based RFLP was found most efficient in discriminating *C. jejuni* isolates compared to *Hinf*-RFLP and *Dpn*-RFLP in terms of more pattern generated, higher typeability and greater discriminatory index value, however, *Dpn*II showed comparable discrimination of isolates as with *Dde*I. Thus, the present study suggests that *Dpn*-RFLP can be used as an efficient alternative for typing of *C. jejuni* isolates.

## Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.vas.2018.06.003.

## References

Al-Amri, A., Senok, A. C., Ismaeel, A. Y., Al-Mahmeed, A. E., & Botta, G. A. (2007). Multiplex PCR for direct identification of *Campylobacter spp*. in human and chicken stools. *Journal of Medical Microbiology, 56*(10), 1350–1355.

Aydin, F., Gumussoy, K. S., Ica, T., Sumerkan, B., Esel, D., Akan, M., & Ozdemir, A. (2007). The prevalence of *Campylobacter jejuni* in various sources in Kayseri, Turkey and molecular analysis of isolated strains by PCR-RFLP. *Turkish Journal of Veterinary and Animal Sciences, 33*, 13–19.

Bikandi, J., San Millán, R., Rementeria, A., & Garaizar, J. (2004). In silico analysis of complete bacterial genomes: PCR, AFLP-PCR, and endonuclease restriction. *Bioinformatics, 20*, 798–799.

Biswas, D., Hannon, S. J., Townsend, H. G. G., Potter, A., & Allan, B. J. (2011). Genes coding for virulence determinants of *Campylobacter jejuni* in human clinical and cattle isolates from Alberta, Canada, and their potential role in colonization of poultry. *International Microbiology, 14*, 25–32.

Bolton, D. J. (2015). *Campylobacter* virulence and survival factors. *Food Microbiology, 48*, 99–108.

Coward, C., van Diemen, P. M., Conlan, A. J. K., Gog, J. R., Stevens, M. P., Jones, M. A., & Maskell, D. J. (2008). Competing isogenic *Campylobacter* strains exhibit variable population structures *in vivo*. *Applied and Environmental Microbiology, 74*, 3857–3867.

Dingle, K. E., Colles, F. M., Wareing, D. R. A., Ure, R., Fox, A. J., Bolton, F. J., Bootsma, H. J., Willems, R. J. L., Urwin, R., & Maiden, M. C. J. (2001). Multilocus sequence typing system for *Campylobacter jejuni*. *Journal of Clinical Microbiology, 39*, 14–23.

El-Adawy, H., Hotzel, H., Tomaso, H., Neubauer, H., Taboada, E. N., Ehricht, R., & Hafez,

H. M. (2013). Detection of genetic diversity in *Campylobacter jejuni* isolated from a commercial turkey flock using *fla*A typing, MLST analysis and microarray assay. *PLoS One, 8*, e51582.
Epps, S. V. R., Harvey, R. B., Hume, M. E., Phillips, T. D., Anderson, R. C., & Nisbet, D. J. (2013). Foodborne *Campylobacter*: infections, metabolism, pathogenesis and reservoirs. *International Journal of Environmental Research and Public Health, 10*, 6292–6304.
Fitzgerald, C., Stanley, K., Andrew, S., & Jones, K. (2001). Use of pulsed-field gel electrophoresis and flagellin gene typing in identifying clonal groups of *Campylobacter jejuni* and *Campylobacter coli* in farm and clinical environments. *Applied and Environmental Microbiology, 67*, 1429–1436.
Ghorbanalizadgan, M., Bakhshi, B., & Peerayeh, S. (2016). PCR-RFLP provides discrimination for total *fla*A sequence analysis in clinical *Campylobacter jejuni* Isolates. *Japanese Journal of Infectious Disease, 69*, 373–377.
Gondo, T., Sekizuka, T., Manaka, N., Murayama, O., Millar, B. C., Moore, J. E., & Matsuda, M. (2006). Demonstration of the shorter flagellin (*fla*A) gene of urease-positive thermophilic *Campylobacter* isolated from the natural environment in Northern Ireland. *Folia Microbiologica, 51*, 183–190.
Hendrixson, D. R. (2006). A phase-variable mechanism controlling the *Campylobacter jejuni Flg*R response regulator influences commensalism. *Molecular Microbiology, 61*, 1646–1659.
Hiett, K. L., Seal, B. S., & Siragusa, G. R. (2006). *Campylobacter* spp. subtype analysis using gel-based repetitive extragenic palindromic-PCR discriminates in parallel fashion to *fla*A short variable region DNA sequence analysis. *Journal of Applied Microbiology, 101*, 1249–1258.
Khoshbakht, R., Tabatabaei, M., Hosseinzadeh, S., Shekarforoush, S. S., & Aski, H. S. (2013). Distribution of nine virulence-associated genes in *Campylobacter jejuni* and *C. coli* isolated from broiler feces in Shiraz, Southern Iran. *Foodborne Pathogens and Disease, 10*, 764–770.
Khoshbakht, R., Tabatabaei, M., Hosseinzadeh, S., Aski, H. S., & Seifi, S. (2015). Genetic characterization of *Campylobacter jejuni* and *C. coli* isolated from broilers using *fla*A PCR-restriction fragment length polymorphism method in Shiraz, Southern Iran. *Jundishapur Journal of Microbiology, 8*, e18573.
Linton, D., Owen, R. J., & Stanley, J. (1996). Rapid identification by PCR of the genus *Campylobacter* and of five Campylobacter species enteropathogenic for man and animals. *Research in Microbiology, 147*, 707–718.
Linton, D., Lawson, A. J., Owen, R. J., & Stanley, J. (1997). PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. *Journal of Clinical Microbiology, 35*, 2568–2572.
Nachamkin, I., Bohachick, K., & Patton, C. M. (1993). Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. *Journal of Clinical Microbiology, 31*, 1531–1536.
Owen, R. J., & Leeton, S. (1999). Restriction fragment length polymorphism analysis of the *fla*A gene of *Campylobacter jejuni* for subtyping human, animal and poultry isolates. *FEMS Microbiology Letters, 176*, 345–350.
Pavel, A. B., & Vasile, C. I. (2012). PyElph—a software tool for gel images analysis and phylogenetics. *BMC Bioinformatics, 13*, 1–6 Article ID 9.
Pearson, A. D., Greenwood, M., Healing, T. D., Rollins, D., Shahamat, M., Donaldson, J., & Colwell, R. R. (1993). Colonization of broiler chickens by waterborne *Campylobacter jejuni*. *Applied and Environmental Microbiology, 59*, 987–996.
Rajagunalan, S., Bisht, G., Pant, S., Singh, S. P., Singh, R., & Dhama, K. (2014). Prevalence and molecular heterogeneity analysis of prevalence and molecular heterogeneity analysis of *Campylobacter jejuni* and *Campylobacter coli* isolated from human, poultry and cattle, in Pantnagar, India. *Veterinarski Arhiv, 84*, 493–504.
Shi, F., Chen, Y. Y., Wassenaar, T. M., Woods, W. H., Coloe, P. J., & Fry, B. N. (2002). Development and application of a new scheme for typing *Campylobacter jejuni* and *Campylobacter coli* by PCR-based restriction fragment length polymorphism analysis. *Journal of Clinical Microbiology, 40*, 1791–1797.
Silva, J., Leite, D., Fernandez, M., Mena, C., Gibbs, P. A., & Teixeira, P. (2011). *Campylobacter spp*. as a foodborne pathogen: a review. *Frontiers in Microbiology, 2*, 1–12 Article ID 200.
Tang, J. Y. H., Ghazali, M. F., Saleha, A. A., Nishibuchi, M., & Son, R. (2009). Comparison of thermophilic *Campylobacter spp.* occurrence in two types of retail chicken samples. *International Food Research Journal, 16*, 277–288.
Thomrongsuwannakij, T., Blackall, P. J., & Chansiripornchai, N. (2017). A Study on *Campylobacter jejuni* and *Campylobacter coli* through commercial broiler production chains in Thailand: Antimicrobial resistance, the characterization of DNA gyrase subunit A mutation, and genetic diversity by flagellin A gene restriction fragment length polymorphism. *Avian Diseases, 61*, 186–197.
Vinueza-Burgos, C., Wautier, M., Martiny, D., Cisneros, M., Damme, V. I., & De Zutter, L. (2017). Prevalence, antimicrobial resistance and genetic diversity of *Campylobacter coli* and *Campylobacter jejuni* in Ecuadorian broilers at slaughter age. *Poultry science, 96*, 2366–2374.
Wieczorek, K., & Osek, J. (2013). Antimicrobial resistance mechanisms among *Campylobacter*. *BioMed Research International, 2013*, 1–12 Article ID 340605.
Wirz, S. E., Overesch, G., Kuhnert, P., & Korczak, B. M. (2010). Genotype and antibiotic resistance analyses of *Campylobacter* isolates from ceca and carcasses of slaughtered broiler flocks. *Applied and Environmental Microbiology, 76*, 6377–6386.
Yadav, R., Gahlot, K., Yadav, J., Purva, M., Bhati, T., Deora, A., Kumar, P., Maherchandani, S., & Kashyap, S. K. (2016). Prevalence of thermophilic *Campylobacter jejuni* isolated from cloacal sample of poultry. *Haryana Veterinarian, 55*, 195–197.