Int J Infect. 2016 October; 3(4):e37727. doi: 10.17795/iji-37727.

Published online 2016 August 3. **Research Article**

Prevalence of Seven Virulence Genes of *Campylobacter jejuni* Isolated from Patients with Diarrhea in Rosario, Argentina

Cecilia Casabonne,[1,*] Agustina Gonzalez,[1] Virginia Aquili,[1] Tomas Subils,[1] and Claudia Balague[1]

[1]Microbiology Department, College of Biochemical and Pharmaceutical Sciences, National University of Rosario, Rosario, Argentina

[*]*Corresponding author*: Cecilia Casabonne, Microbiology Department, College of Biochemical and Pharmaceutical Sciences, National University of Rosario, Rosario, Argentina. Tel: +54-3414804562, E-mail: ccasabonne@fbioyf.unr.edu.ar

Received 2016 March 22; **Revised** 2016 May 31; **Accepted** 2016 June 01.

Abstract

Background: *Campylobacter jejuni* (*C. jejuni*) is a major cause of human diarrheal disease.
Objectives: This study was conducted to determine the prevalence of different pathogenic genes in isolates recovered from human stool samples in Rosario, Argentina.
Methods: A total of 30 isolates were identified as *C. jejuni* on the basis of morphological and biochemical-based detection. The isolates were screened for the presence of seven pathogenic genes namely *fla*A, *cad*F, *cia*B, *cdt*B, *cgt*B, *doc*C and *wla*N, which are responsible for expression of adherence, invasion, colonization, chemotaxis and cytotoxin production in *C. jejuni*.
Results: The isolates showed a wide variation in the presence of these genes. All the isolates were positive for *fla*A, *cad*F and *cdt*B genes. Of the *C. jejuni* studied, 40.0%, 23.3%, 20.0% and 6.7% were positive for *cia*B, *doc*C, *wla*N and *cgt*C, respectively.
Conclusions: This study provides initial data on the prevalence and distribution of the *fla*A, *cad*F, *cia*B, *cdt*B, *cgt*B, *doc*C and *wla*N genes in *C. jejuni*.

Keywords: Virulence Genes, Diarrhea, *Campylobacter jejuni*

1. Background

Campylobacter is a zoonotic bacterium frequently associated with acute gastroenteritis in both developing and industrialized countries. Human campylobacteriosis is predominantly caused by *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*), although *C. jejuni* is responsible for the majority of these infections (1). The disease presentation can vary from a mild watery diarrhea to bloody dysentery as the organism colonizes the small intestine of the human host early in infection and later moves to the colon, which is the target organ (2). *Campylobacter jejuni* is also frequently associated with the development of immunoreactive complications such as polyarthralgia, Guillain- Barre and Miller Fisher syndromes and eventually death (3).

The cause of these discrepant outcomes of infection in humans is not clear although several studies have suggested that various virulence factors present in campylobacters contribute to their survival and the establishment of disease in the host. Motility, bacterial adhesion, invasion of the intestinal epithelium and production of toxin and hemolysin appear to be the main virulence factors. Bacterial adhesion and invasion are well-established early events before the initiation of the inflammatory processes and diarrheal development (4).

The invasiveness of *C. jejuni* strains plays a vital role in the pathogenesis of this organism and is often used as a measure of bacterial virulence, reflecting the involvement of multiple bacterial structures and mechanisms in this process. Although these pathogens are generally considered invasive, the level of invasion of intestinal epithelial cells in vitro varies among strains (5).

During the initial stage, *Campylobacter* adheres to the human intestinal cell lining and then is internalized within the cells causing tissue damage, inflammation and thereby gastroenteritis. The bacterial factors implicated in host cell invasion are capsular polysaccharide (CPS), flagella, sialylation of the lipooligosaccharides (LOS) outer core or *Campylobacter* invasive antigens (Cia) (6-8). It is well known that the polar flagellum present in *C. jejuni* is crucial for the initial interactions of this organism with its host and facilitates the colonization of the intestinal epithelial cells (9, 10). However, the precise invasive manner of *C. jejuni* in the disease pathogenesis in humans still needs further clarification.

Moreover, bacterial toxins play a role in the development of the disease. The most important virulence factors include cytolethal distending toxin (CDT) and hemolysin,

and the major cell defense mechanisms include superoxide dismutase and membrane factors (11).

Some genes have recently been recognized as responsible for the expression of pathogenicity. In this study, *fla*A (12), *cad*F (13) and *doc*C (14) were selected as pathogenic genes responsible for the expression of adherence and colonization, *cia*B (15) as a pathogenic gene responsible for the expression of invasion, *cdt*B as a pathogenic gene responsible for the expression of cytolethal distending toxin production (16), while *wla*N (14) and *cgt*B (17) were selected as pathogenic genes responsible for the expression of Guillain- Barre syndrome (GBS).

2. Objectives

The objective of this study was to analyze the virulence genes of *C. jejuni* in order to understand the pathogenesis of this microorganism.

3. Methods

3.1. Bacterial Isolates and Growth Conditions

Twenty-five *C. jejuni* isolates from Rosario, Argentina were tested in this study. These strains were isolated from human feces and were grown on *Campylobacter* selective agar plates at 42°C for 48 hours under microaerobic conditions.

3.2. Microbiological Tests

The isolates were identified as *C. jejuni* based on their morphological and biochemical tests, including catalase, oxidase, H_2S (TSI), hippurate hydrolysis, resistance to nalidixic acid and sensitivity towards cephalothin.

3.3. Virulence Genes Characterization

A suspension of 200 μL of 24-hour bacterial culture was treated at 100°C for 10 minutes in a hot water bath and cooled in an ice water bath. The suspension resulting from the thermal shock was centrifuged at 10000 g for five minutes. An extract of 5 μL of the supernatant was used as a DNA matrix for the detection of virulence genes. A polymerase chain reaction (PCR) technique was also used to detect the *cad*F, *fla*A, *cdt*B, *doc*C, *cia*B, *wla*N and *cgt*B genes. The primers used are recorded in Table 1. The amplification reactions of the genes encoding virulence factors were carried out in a reaction mixture of 25 μL constituting 1× buffer (Fermentans), 3 mM of Cl_2Mg, 250 μM of each deoxynucleotide triphosphate (dNTP), 20 pmoles of each primer and 1,25 U of Taq DNA polymerase (Fermentans). polymerase chain reactions were performed in a DNA thermal cycler (IVEMA, Argentina). The amplification program for these virulence markers involved an initial denaturation at 95°C for four minutes and the cyclic phase was repeated 35 times. Each cycle involved a denaturation at 94°C for one minute, a hybridization of the primers at annealing temperatures (Ta) for one minute and an elongation phase at 72°C for one minute; with a final extension at 72°C for five minutes. Details of the Ta are given in Table 1.

Proper negative controls with all components of the PCR mix except for DNA template were included with each PCR run. The revelation of the amplification products was carried out on agarose gel at 1.5% with 0.5μg/ml of ethidium bromide. The estimation of the size of the amplicon was carried out by comparing them with the bands of a molecular weight marker (Thermo Scientific).

4. Results

A total of 30 *C. jejuni* isolates were screened for the presence of seven virulence-associated genes (data not shown).

Among these *C. jejuni* strains, prevalence of *cdt*B, *cad*F and *fla*A virulence genes was 100% (30/30). The frequency of the *cia*B gene among *C. jejuni* strains was 50% (15/30). Furthermore, the presence of *doc*C, *wla*N, and *cgt*B genes was 23.3%, 20% and 6.7%, respectively. Detailed prevalence of the seven virulence genes in *C. jejuni* recovered from clinical isolates are shown in Figure 1.

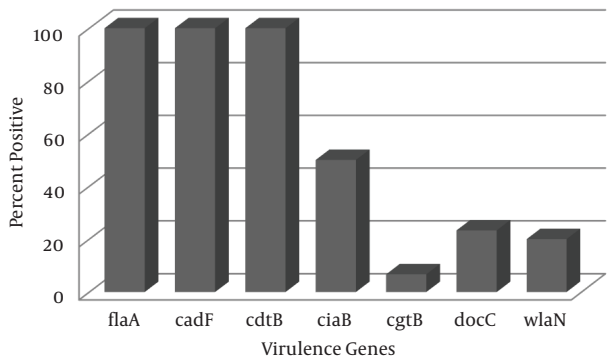

Figure 1. Prevalence of Virulence Genes in *Campylobacter jejuni* Recovered From Clinical Isolates

Compared with the gene occurrence pattern among the 30 *C. jejuni* isolates from human feces, nine gene occurrence patterns were detected. It was also shown that all the isolates tested possessed three or more of the seven virulence-associated genes (Table 2).

5. Discussion

When colonizing the intestines, enteric campylobacters are predicted to express several putative virulence fac-

Table 1. Nucleotidic Sequences of the Primers Used and Size of the Amplicons

VirulenceFactor[a]	Primers	Oligonucleotide Sequence	Ta, °C	Amplicon Size, bp	References
***cad*F**			48	400	(13)
	cadF-F	TTGAAGGTAATTTAGATATG			
	cadF-R	CTAATCCTAAAGTTGAAAC			
***fla*A**			52	1713	(12)
	flaA-F	ATGGGATTTCGTATTAACAC			
	flaA-R	CTGTAGTAATCTTAAAACATTTTG			
***cdt*B**			55	495	(16)
	cdtB-F	GTTGGCACTTGGAATTTGCAAGGC			
	cdtB-R	GTTAAAATCCCCTGCTATCAACCA			
***cia*B**			50	1165	(13)
	ciaB-F	TTTCCAAATTTAGATGATGC			
	ciaB-R	GTTCTTTAAATTTTTCATAATGC			
***doc*C**			50	1835	(14)
	docC-F	TGAGCTACGCTATCATTG			
	docC-R	GCTTACGCTATGGGTTGG			
***wla*N**			55	330	(14)
	wlaN-F	TGCTGGGTATACAAAGGTTGTG			
	wlaN-R	ATTTTGGATATGGGTGGGG			
***cgt*B**			52	561	(17)
	cgtB-F	TAAGAGCAAGATATGAAGGTG			
	cgtB-R	GCACATAGAGAACGCTACAA			

Abbreviations: bp, bases pair; Ta, annealing temperature.
[a]Gene encoding virulence factor.

Table 2. Virulence-Associated Gene Patterns of *Campylobacter jejuni* Isolated from Human Feces

Number	Patterns	No. of Isolates
1	*fla*A, *cad*F, *cdt*B, *cia*B, *doc*C, *wla*N	1
2	*fla*A, *cad*F, *cdt*B, *cia*B, *doc*C, *cgt*B	1
3	*fla*A, *cad*F, *cdt*B, *cia*B, *wla*N	3
4	*fla*A, *cad*F, *cdt*B, *cia*B, *doc*C	4
5	*fla*A, *cad*F, *cdt*B, *cia*B	6
6	*fla*A, *cad*F, *cdt*B, *wla*N	2
7	*fla*A, *cad*F, *cdt*B, *cgt*B	1
8	*fla*A, *cad*F, *cdt*B, *doc*C	1
9	*fla*A, *cad*F, *cdt*B	11
Total		30

tors. Some genes determine the expression of these virulence factors, which are generally implicated in the processes of adhesion and invasiveness of this pathogen.

As a first step, colonization of the intestine requires the ability to move into the mucus layer covering the intestinal cells. *Campylobacter* motility is conferred by the polar

flagella, which together with their 'cork-screw' shape allow them to efficiently penetrate this mucus barrier (18-20). The most important virulence factor that has been studied and well characterized in Campylobacter was the flagellin, which is encoded by the *fla*A gene (21). The *fla*A and *fla*B genes constitute the locus of flagellin; however, molecular genetics research has revealed that *fla*A is essential for colonization, whereas the *fla*B gene is not (22).

As mentioned before, it is known that *fla*A, *cad*F and *cia*B genes, studied in this work are involved in adhesion and invasiveness of *Campylobacter* spp. (23, 24). Moreover, it has been seen that *cia*B gene has been associated with cell invasion, as it encodes for the secretion of a protein necessary for the invasion of epithelial cells (8). The *cad*F gene, in turn, encodes a protein that interacts with the host's fibronectin matrix, which is necessary for colonization of the cell surface (25).

In this study, a number of putative virulence and toxin genes were studied, including *fla*A, *cad*F and *cia*B genes, that are involved in adhesion and colonization of the host's gut (13, 14). All tested *C. jejuni* isolates were positive for the *fla*A gene. Previous studies showed that the detection rate of *fla*A gene was 95% (26) and 100% (27, 28).

A study conducted by Rozynek et al. (29) on *C. jejuni* coli isolates showed that all analyzed strains possessed the *cad*F gene. In a similar study, all isolates with human origin had the *cad*F gene (30). Our results detected the *cad*F gene in 100% of the tested strains, as was observed by Biswas et al., Datta et al. and Gripp et al. (27, 31, 32). The prevalence of *fla*A and *cad*F gene in all the isolates indicates a pathogenic potential since both genes play an important role in Campylobacter pathogenesis.

On the other hand, the detection rate of *cia*B was 50%. In studies conducted by Biswas et al., Datta et al. and Hyun-Ho Cho et al., the *cia*B gene was detected in a 92.31%, 98.2% and 87.5%, respectively (27, 28, 31).

This study was performed to investigate the CDT-encoding gene (*cdt*B) of C. jejuni from human clinical samples. The *cdt* gene cluster consists of three adjacent genes (*cdt*A, *cdt*B, and *cdt*C). The CDT toxin is composed of CdtB protein, as the enzymatically-active subunit and two hetero-dimeric subunits (*Cdt*A and *Cdt*C), which are responsible for the holotoxin binding to the cell membrane (33).

The *cdt*B was detected in 100% of the isolates tested in this study, which is consistent with previous reports by Asakura et al., Thakur et al. and Gripp et al. (26, 32, 33).

Two Guillain- Barre syndromes-associated genes (*cgt*B and *wla*N) were detected by the PCR method, showing that *cgt*B gene was detected in 6.7% and *wla*N gene was detected in 20.0% of the isolates. The *cgt*B and *wla*N gene product as β-1, 3-galactotransferase is responsible for the specific LOS structure. LOS, similar to gangliosides in neurons, is thought to be a critical factor in the triggering of GBS and Miller-Fisher syndrome neuropathies after *C. jejuni* infection (3, 17). The higher prevalence of these genes might be associated with GBS in humans.

The rate of the presence of *wla*N gene is similar to that obtained in other studies (27). However, Hyun-Ho Cho et al. showed 71.7% prevalence for the *cgt*B gene among isolates from human sources (28).

Finally, in some enteric pathogens, such as *C. jejuni*, chemotaxis is important for pathogenesis and colonization of the host. Chemotaxis allows motile bacteria to navigate depending on the extracellular chemical composition. Bacteria are either attracted or repelled by chemicals sensed by trans-membrane Methyl-Accepting Chemotaxis proteins (MCP). Among the identified determinants, there was a gene encoding a MCP (docC), presumably required for proper chemotaxis to a specific environmental component (34). In our work, docC gene was present in 23.3% of the strains. Little is known about the prevalence rate of this gene. Hyun-Ho Cho et al. reported the prevalence rate of this gene in *C. jejuni* isolates from humans to be 19.6% (28).

In conclusion, several virulence factors have been documented for *Campylobacter* spp., which could contribute to its motility, intestinal colonization and invasion.

The present study showed the high prevalence of the *cad*F, *fla*A and *cgt*B genes among *C. jejuni* isolated from human feces. Furthermore, the isolates showed a pattern, which is different from other studied pathogenic genes. Moreover, this work enhances knowledge on the prevalence of virulence factors in *C. jejuni* isolated from human feces, and the contribution of these characteristics to clinico-epidemiological monitoring.

Acknowledgments

We gratefully acknowledge Victor J. Vilela hospital, Zona Norte hospital and Provincial hospital, for providing the isolates. We would like to thank Maria Robson, Mariana de Sanctis, Geraldine Raimundo, Carolina Perret and Romina Ricardo for their assistance in the language correction.

Footnotes

Authors' Contribution: Claudia Balague and Cecilia Casabonne developed the original idea and the protocol, abstracted and analyzed the data, and wrote the manuscript. Virginia Aquili, Agustina Gonzalez and Tomas Subils contributed to the development of the protocol, abstracted data, and prepared the manuscript.

Financial Disclosure: The authors declare that they have no financial interests related to the material in the manuscript.

Funding/Support: No funding was provided for this work.

References

1. Adedayo O, Kirkpatrick BD. Campylobacter jejuni infections: update on presentation, diagnosis, and management. *Hosp Physician.* 2008;**44**(7):9–15.
2. Allos BM. Campylobacter jejuni Infections: update on emerging issues and trends. *Clin Infect Dis.* 2001;**32**(8):1201–6. doi: 10.1086/319760. [PubMed: 11283810].
3. Dingle KE, Van Den Braak N, Colles FM, Price LJ, Woodward DL, Rodgers FG, et al. Sequence typing confirms that Campylobacter jejuni strains associated with Guillain-Barre and Miller-Fisher syndromes are of diverse genetic lineage, serotype, and flagella type. *J Clin Microbiol.* 2001;**39**(9):3346–9. [PubMed: 11526174].
4. Bhavsar S, Kapadnis B. Virulence factors of Campylobacter. *The Internet J Microbiol.* 2006;**3**(2).
5. van Vliet AH, Ketley JM. Pathogenesis of enteric Campylobacter infection. *Symp Ser Soc Appl Microbiol.* 2001(30):45–56. [PubMed: 11422560].
6. Watson RO, Galan JE. Campylobacter jejuni survives within epithelial cells by avoiding delivery to lysosomes. *PLoS Pathog.* 2008;**4**(1):14. doi: 10.1371/journal.ppat.0040014. [PubMed: 18225954].
7. Louwen R, Heikema A, van Belkum A, Ott A, Gilbert M, Ang W, et al. The sialylated lipooligosaccharide outer core in Campylobacter jejuni is an important determinant for epithelial cell invasion. *Infect Immun.* 2008;**76**(10):4431–8. doi: 10.1128/IAI.00321-08. [PubMed: 18644887].
8. Rivera-Amill V, Kim BJ, Seshu J, Konkel ME. Secretion of the virulence-associated Campylobacter invasion antigens from Campylobacter jejuni requires a stimulatory signal. *J Infect Dis.* 2001;**183**(11):1607–16. doi: 10.1086/320704. [PubMed: 11343209].
9. Konkel ME, Monteville MR, Rivera-Amill V, Joens LA. The pathogenesis of Campylobacter jejuni-mediated enteritis. *Curr Issues Intest Microbiol.* 2001;**2**(2):55–71. [PubMed: 11721281].
10. Hendrixson DR, DiRita VJ. Identification of Campylobacter jejuni genes involved in commensal colonization of the chick gastrointestinal tract. *Mol Microbiol.* 2004;**52**(2):471–84. doi: 10.1111/j.1365-2958.2004.03988.x. [PubMed: 15066034].
11. Fry BN, Feng S, Chen YY, Newell DG, Coloe PJ, Korolik V. The galE gene of Campylobacter jejuni is involved in lipopolysaccharide synthesis and virulence. *Infect Immun.* 2000;**68**(5):2594–601. [PubMed: 10768949].
12. Hanel I, Muller J, Muller W, Schulze F. Correlation between invasion of Caco-2 eukaryotic cells and colonization ability in the chick gut in Campylobacter jejuni. *Vet Microbiol.* 2004;**101**(2):75–82. doi: 10.1016/j.vetmic.2004.04.004. [PubMed: 15172689].
13. Konkel ME, Gray SA, Kim BJ, Garvis SG, Yoon J. Identification of the enteropathogens Campylobacter jejuni and Campylobacter coli based on the cadF virulence gene and its product. *J Clin Microbiol.* 1999;**37**(3):510–7. [PubMed: 9986804].
14. Muller J, Schulze F, Muller W, Hanel I. PCR detection of virulence-associated genes in Campylobacter jejuni strains with differential ability to invade Caco-2 cells and to colonize the chick gut. *Vet Microbiol.* 2006;**113**(1-2):123–9. doi: 10.1016/j.vetmic.2005.10.029. [PubMed: 16300911].
15. Konkel ME, Kim BJ, Rivera-Amill V, Garvis SG. Bacterial secreted proteins are required for the internaliztion of Campylobacter jejuni into cultured mammalian cells. *Mol Microbiol.* 1999;**32**(4):691–701. [PubMed: 10361274].
16. Pickett CL, Pesci EC, Cottle DL, Russell G, Erdem AN, Zeytin H. Prevalence of cytolethal distending toxin production in Campylobacter jejuni and relatedness of Campylobacter sp. cdtB gene. *Infect Immun.* 1996;**64**(6):2070–8. [PubMed: 8675309].
17. Linton D, Gilbert M, Hitchen PG, Dell A, Morris HR, Wakarchuk WW, et al. Phase variation of a beta-1,3 galactosyltransferase involved in generation of the ganglioside GM1-like lipo-oligosaccharide of Campylobacter jejuni. *Mol Microbiol.* 2000;**37**(3):501–14. [PubMed: 10931344].
18. Haag LM, Fischer A, Otto B, Grundmann U, Kuhl AA, Gobel UB, et al. Campylobacter jejuni infection of infant mice: acute enterocolitis is followed by asymptomatic intestinal and extra-intestinal immune responses. *Eur J Microbiol Immunol (Bp).* 2012;**2**(1):2–11. doi: 10.1556/EuJMI.2.2012.1.2. [PubMed: 24611115].
19. Snelling WJ, Matsuda M, Moore JE, Dooley JS. Campylobacter jejuni. *Lett Appl Microbiol.* 2005;**41**(4):297–302. doi: 10.1111/j.1472-765X.2005.01788.x. [PubMed: 16162134].
20. Szymanski CM, King M, Haardt M, Armstrong GD. Campylobacter jejuni motility and invasion of Caco-2 cells. *Infect Immun.* 1995;**63**(11):4295–300. [PubMed: 7591061].
21. Hermans D, Van Deun K, Martel A, Van Immerseel F, Messens W, Heyndrickx M, et al. Colonization factors of Campylobacter jejuni in the chicken gut. *Vet Res.* 2011;**42**:82. doi: 10.1186/1297-9716-42-82. [PubMed: 21714866].
22. Konkel ME, Klena JD, Rivera-Amill V, Monteville MR, Biswas D, Raphael B, et al. Secretion of virulence proteins from Campylobacter jejuni is dependent on a functional flagellar export apparatus. *J Bacteriol.* 2004;**186**(11):3296–303. doi: 10.1128/JB.186.11.3296-3303.2004. [PubMed: 15150214].
23. Nuijten PJ, van den Berg AJ, Formentini I, van der Zeijst BA, Jacobs AA. DNA rearrangements in the flagellin locus of an flaA mutant of Campylobacter jejuni during colonization of chicken ceca. *Infect Immun.* 2000;**68**(12):7137–40. [PubMed: 11083841].
24. Ziprin RL, Young CR, Byrd JA, Stanker LH, Hume ME, Gray SA, et al. Role of Campylobacter jejuni potential virulence genes in cecal colonization. *Avian Dis.* 2001;**45**(3):549–57. [PubMed: 11569726].
25. Monteville MR, Yoon JE, Konkel ME. Maximal adherence and invasion of INT 407 cells by Campylobacter jejuni requires the CadF outer-membrane protein and microfilament reorganization. *Microbiology.* 2003;**149**(Pt 1):153–65. doi: 10.1099/mic.0.25820-0. [PubMed: 12576589].
26. Thakur S, Zhao S, McDermott PF, Harbottle H, Abbott J, English L, et al. Antimicrobial resistance, virulence, and genotypic profile comparison of Campylobacter jejuni and Campylobacter coli isolated from humans and retail meats. *Foodborne Pathog Dis.* 2010;**7**(7):835–44. doi: 10.1089/fpd.2009.0487. [PubMed: 20367499].
27. Datta S, Niwa H, Itoh K. Prevalence of 11 pathogenic genes of Campylobacter jejuni by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. *J Med Microbiol.* 2003;**52**(Pt 4):345–8. doi: 10.1099/jmm.0.05056-0. [PubMed: 12676874].
28. Cho HH, Kim SH, Min W, Ku BK, Kim YH. Prevalence of virulence and cytolethal distending toxin (CDT) genes in thermophilic Campylobacter spp. from dogs and humans in Gyeongnam and Busan, Korea. *Korean J Vet Res.* 2014;**54**(1):39–48. doi: 10.14405/kjvr.2014.54.1.39.
29. Rozynek E, Dzierzanowska-Fangrat K, Jozwiak P, Popowski J, Korsak D, Dzierzanowska D. Prevalence of potential virulence markers in Polish Campylobacter jejuni and Campylobacter coli isolates obtained from hospitalized children and from chicken carcasses. *J Med Microbiol.* 2005;**54**(7):615–9. doi: 10.1099/jmm.0.45988-0. [PubMed: 15947425].
30. Rizal A, Kumar A, Vidyarthi AS. Prevalence of pathogenic genes in Campylobacter jejuni isolated from poultry and human. *Internet J Food Saf.* 2010;**12**:29–34.
31. Biswas D, Hannon SJ, Townsend HG, Potter A, Allan BJ. 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. *Int Microbiol.* 2011;**14**(1):25–32. doi: 10.2436/20.1501.01.132. [PubMed: 22015699].
32. Gripp E, Hlahla D, Didelot X, Kops F, Maurischat S, Tedin K, et al. Closely related Campylobacter jejuni strains from different sources reveal a

generalist rather than a specialist lifestyle. *BMC Genomics.* 2011;**12**:584. doi: 10.1186/1471-2164-12-584. [PubMed: 22122991].

33. Asakura M, Samosornsuk W, Taguchi M, Kobayashi K, Misawa N, Kusumoto M, et al. Comparative analysis of cytolethal distending toxin (cdt) genes among Campylobacter jejuni, C. coli and C. fetus strains. *Microb Pathog.* 2007;**42**(5-6):174–83. doi: 10.1016/j.micpath.2007.01.005. [PubMed: 17353111].
34. Vegge CS, Brondsted L, Li YP, Bang DD, Ingmer H. Energy taxis drives Campylobacter jejuni toward the most favorable conditions for growth. *Appl Environ Microbiol.* 2009;**75**(16):5308–14. doi: 10.1128/AEM.00287-09. [PubMed: 19542337].