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Research Article
Molecular Characterization and Virulence of *Campylobacter jejuni* Isolated from Broiler Chickens

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Abstract

Background and objective: *Campylobacter jejuni (C. jejuni)* is a commensal microorganism in birds that causes diarrhea due to intestinal inflammation, which leads to feet damage from standing on wet litter. This study aimed to investigate the prevalence and virulence of *C. jejuni* in broiler chickens, as well as determine histopathological changes in the chicken gut following infection. **Materials and Methods:** A total of 200 infected broiler chicken samples (100 cecal contents, 50 livers and 50 spleens) were collected from different farms at Dakahlia Governorate in Egypt. Broilers samples were subjected to *Campylobacter* isolation procedures. *Campylobacter jejuni* isolates were distinguished from other *Campylobacter* spp. using the uniplex PCR-targeting *mapA* gene. *Campylobacter jejuni* isolates were also evaluated for the presence of *cdtA* and *flaA* genes. Evaluation of gut histopathological changes after *C. jejuni* infection was carried out in twenty day-old broiler chicks. **Results:** In total, sixty-eight *C. jejuni* isolates (18 isolates from the liver samples, 12 from spleen samples and 38 from cecal contents) were recovered with an overall prevalence rate of 34%. The *flaA* gene was successfully identified in all *Campylobacter* isolates, while *cdtA*, was identified in only 62 (91.17%) isolates. Several pathological changes and inflammatory responses were found in the chicken gut as a result of *C. jejuni* infection. **Conclusion:** The prevalence of *flaA* and *cdtA* revealed a high rate of adherence and cytotoxicity-associated genes. In addition, several histopathological changes were found in the chicken gut. These findings confirmed that *C. jejuni* infection has a significant impacton poultry health and welfare and *C. jejuni* is a harmful gut commensal microorganism.

Key words: *Campylobacter jejuni*, broiler chickens, virulence genes, colonization, histomorphological changes

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Campylobacter is one of the most important bacterial sources of human enteritis worldwide and it has been isolated from a variety of animal species, including, poultry, cattle, pigs, sheep, pets, wild birds and rodents[1-3]. Chicken is considered the main source of *Campylobacter* and may responsible for approximately 70.9% of human cases, as contaminated chicken meat and meat by-products often cause human infections[4,5]. It is difficult to control the spread of *Campylobacter* in the slaughterhouse and in kitchens; however, controlling *Campylobacter* on the farm may help reduce the number of bacteria in food processing plants[6,7].

The mechanisms of disease of *C. jejuni* starts with colonization in the chicken gut and adhesion and invasion of the gut epithelial cells followed by cytotoxin production[8]. Once colonization is established, *Campylobacter* can multiply rapidly in the cecal contents[9]. While *C. jejuni* colonizes chicken ceca and small intestines, it may also be isolated from different places in the chicken gut, as well as from internal organs such as spleen and liver[10]. *Campylobacter* may present in poultry internal organs tissues via bacterial translocation by which it can cross the intestinal barrier of humans and animals and invade internal organs; the lymphatic path is thought to be the primary route of translocation[11].

Detection of virulence in *C. jejuni* can be used to evaluate the potential risk of poultry as a source of *Campylobacter* infection[12]. Motility is one of the main factors of *Campylobacter* pathogenicity and it can be identified by its spiral form and flagella bundles on cell tips. Due to these features, *Campylobacter* can move against peristaltic movements and colonize intestinal cells. The flagellum is built from a protein known as flagellum which is encoded by the *flaA* and *flaB* genes. The *flaA* gene is expressed at higher rates than *flab* and thus is essential to *Campylobacter* motility[13]. Most *C. jejuni* strains have relatively higher cytolethal distending toxin (CDT) activity comparing to *C. coli* strains; the *C. jejuni* CDT is encoded by a three-gene operon (*cdtABC*)[14-16].

The aims of this study were to characterize *C. jejuni* isolated from chicken cecal contents, determine whether *Campylobacter* can be isolated from the liver and spleen and determine the prevalence of virulence genes. In addition, we examined intestinal epithelial changes caused by *C. jejuni* infection.

MATERIALS AND METHODS

Sample collection: A total of 200 infected broiler chicken samples (100 cecal contents, 50 livers and 50 spleens) were collected from different broiler farms at Dakahlia Governorate, Egypt from October 2016 to February 2017. Cecal contents and internal organs from each chicken were individually packed into sterile plastic bags. All samples were immediately transported to the laboratory in an ice box to isolate *C. jejuni*.

Isolation and identification of *C. jejuni* isolates: Campylobacter isolation procedures were performed according to ISO 10272-1:2006[17]. Samples from cloaca swabs were dissolved into Bolton broth (CM0983, Oxoid) with *Campylobacter* growth supplement (SR0183, Oxoid) and incubated at 42°C for 24 h under microaerobic conditions followed by streaking into mCCDA agar plates (CM0739; Oxoid/UK) with a selective supplement (SR0155, Oxoid) according to the manufacturer's instructions. For internal organs (liver and spleen), 25 g of each sample were homogenized for 1min in stomacher (Stomacher 400 Lab Blender) with Bolton broth; then, the homogenate was transferred to screw-capped sterile bottle. The bottles were then incubated aerobically at 37°C for 4 h followed by 42°C for 48 h. Approximately 0.1-0.2 mL of each samples' enrichment broth was streaked into mCCDA agar plates and then incubated at 42°C for 48 h under microaerobic conditions using Oxoid Campy Gen (CN035A, Oxoid). Presumptive *Campylobacter* colonies were tested biochemically by catalase and oxidase and subjected to Gram staining[18].

PCR-based confirmation of *C. jejuni*: Genomic DNA were obtained using a conventional boiling method according to De Lamballerie *et al.*[19]. *Campylobacter jejuni* was distinguished from other *Campylobacter* spp. by the uniplex PCR-targeting *mapA* gene (Table1). PCR reactions consisted

Table 1: Oligonucleotide primers used in the study

Target gene	Primer sequence (5'-3')	Length of amplified product	Reference
mapA	F-CTA TTT TAT TTT TGA GTG CTT GTG R-GCT TTA TTT GCC ATT TGT TTT ATT A	589 bp	Shin and Lee[20]
flaA	F-AATAAAAATGCTGATAAAACAGGTG R-TACCGAACCAATGTCTGCTCTGATT	855 bp	Datta *et al.*[21]
cdtA	F-GGAAATTGGATTTGGGGCTATACT R-ATCAACAAGGATAATGGACAAT	165 bp	Wieczorek *et al.*[22]

of 12. 5 µL of 2×PCR master mix (Takara RR310A), 6 µL DNA template and 1 µL of each primer; the volume was completed to 25 µL with nuclease-free water. PCR conditions were followed according to the referenced study (Table 2).

Molecular characterization of cdt*A* and *flaA* genes: *Campylobacter jejuni* isolates were examined for the presence of *cdtA* and *fla*A genes (Table 1). The PCR reaction mixture for both genes consisted of 12.5 µL of 2×PCR master mix (Takara RR310A), 6 µL DNA template and 1 µL of 20 pmol of forward and reverse primer, the mixture was completed to 25 µL with DNA/RNA-free water (Table 2).

Poultry infections: Twenty one-day-old broiler chicks were used in this study. At one and 14 days of age, cloacal swabs were taken from all chickens and directly plated on mCCDA for *Campylobacter* determination to ensure absence of *C. jejuni*. At 14 days of age, each chicken was orally dosed with 1×10^8 *C. jejuni* in 0.5 mL of phosphate buffer saline[23]. Chicks were reared under strict biosecure conditions. At 7 days post-infection, the chicks were killed by cervical dislocation. One gram of cecal content was collected for *C. jejuni* analysis. Cecal contents were streaked on the surface of mCCDA and incubated under microaerobic condition at 42°C for 48 h[24].

Histopathology: For histomorphological analysis, tissue samples were taken from the jejunum, liver and spleen and fixed in 4% buffered formalin for 48 h. The formalin fixed tissue was processed and stained using hematoxylin and eosin according to Lynch *et al.*[25].

RESULTS

A total of 200 samples were collected from 100 chickens as follows: 50 samples from the liver, 50 samples from the spleen and 100 cecal swab samples. Each sample was tested for the presence of *C. jejuni* by isolation on mCCDA followed by the confirmatory PCR-targeting *mapA* gene (Fig. 1). Sixty eight *C. jejuni* isolates were recovered (18 from the liver samples, 12 from spleen samples and 38 from cecal swab samples), with an overall prevalence frequency of 34% (68/200).

Virulence genes were determined by uniplex PCR-targeting *flaA* and *cdtA* genes. The *fla* A gene was identified in all *Campylobacter* isolates used in this study, while *cdtA* was identified in only 62 (91.17%) *Campylobacter* isolates (Table 3, Fig. 2-3).

Campylobacter were successfully isolated from chicken ceca after infection in 2-week-old chicks. We identified several histopathological changes in the chicken gut from infection. There were alterations in the small intestinal architecture including, loss of tips, thickening and shortening of villi, inflammatory cell infiltration, sub-epithelial edema and edema and hemorrhage in serosal blood vessels (Fig. 4-6). However, no pathological changes were found in the liver and spleen.

Table 2: Cycling conditions of the primers during PCR

	Primary denaturation		Secondary denaturation		Annealing		Extension			Final extension	
Genes	°C	Min	°C	Sec	°C	Sec	°C	Sec	No. of cycles	°C	Min
mapA	94	5	94	30	55	45	72	45	35	72	10
flaA	94	5	94	30	53	45	72	50	35	72	10
cdtA	94	5	94	30	55	30	72	30	35	72	7

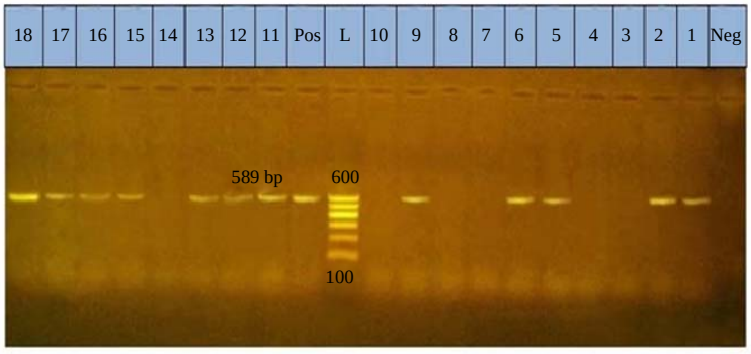

Fig. 1: Agarose gel electrophoresis showing amplification of 589 bp fragment *mapA* gene
L: Ladder, Lane: 1, 2, 5, 6.9, 11, 12, 13, 15, 16, 17 and 18 positive for *map*A gene, Neg: Negative control, Pos: Positive control

Table 3: Prevalence of *Campylobacter jejuni* in chicken samples

		Positive samples		*flaA*		*cdtA*	
Type of samples	No. of samples	No.	Percentage	No.	Percentage	No.	Percentage
Cloaca swabs	100	38	38	38	100	37	97.36
Liver	50	18	36	18	100	16	88.88
Spleen	50	12	24	12	100	9	75
Total	200	68	34	68	100	62	91.17

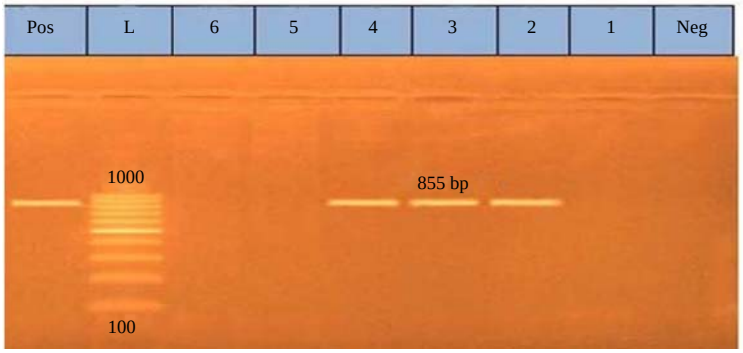

Fig. 2: Agarose gel electrophoresis showing amplification of 855 bp fragment *flaA* primer
L: Ladder, Lane: 2, 3 and 4 positive for *flaA* gene, Neg: Negative control, Pos: Positive control

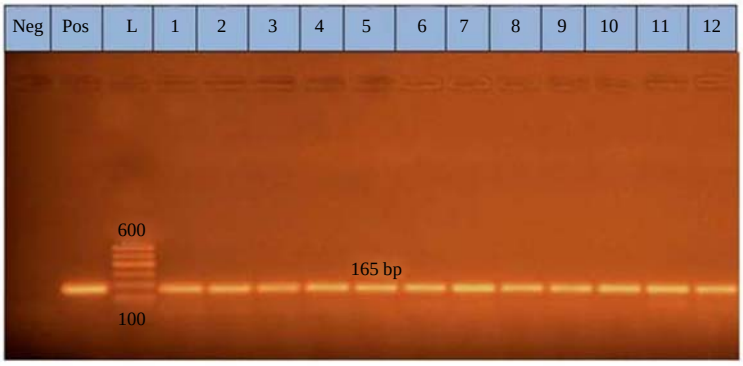

Fig. 3: Agarose gel electrophoresis showing amplification of 165 bp fragment of *cdtA* gene
L: Ladder, Lane: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 positive for cdt A gene, Neg: Negative control, Pos: Positive control

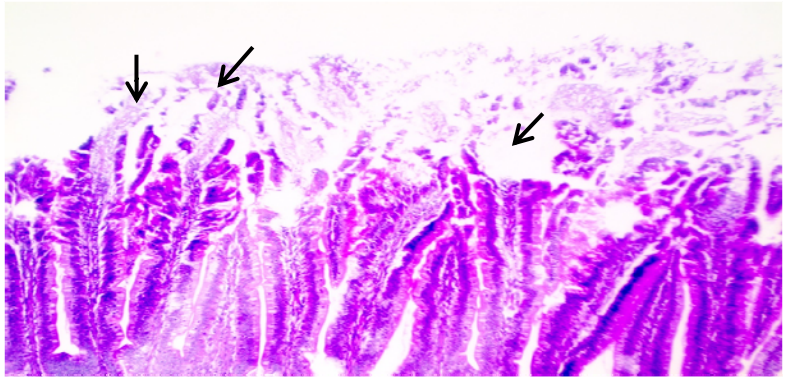

Fig. 4: Small intestine shows loss of tips of villi HE, ×: 100 arrows

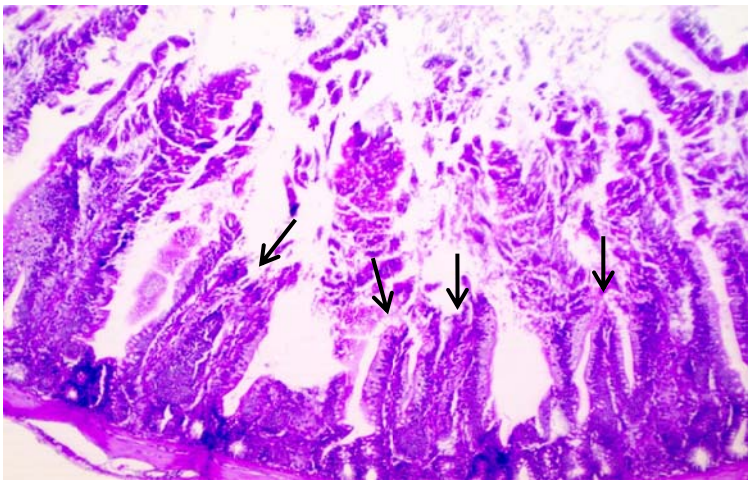

Fig. 5: Small intestine in broiler showing thickening and shortening of some villi HE, ×: 200

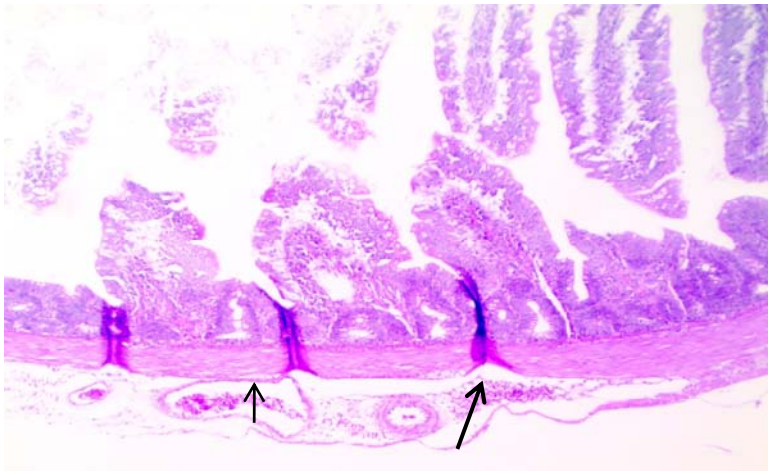

Fig. 6: Small intestine showing: Congestion and edema asterisk and hemorrhage in serosal blood vessels. HE, ×: 100

DISCUSSION

The presence of *C. jejuni* in internal organs indicates that *Campylobacter* can cross the intestinal barrier of animals and humans through bacterial translocation, which may occur via the lymphatic path or venous system[11]. In this study, the isolation rate of *C. jejuni* (34%) was relatively high, which indicates that this may be a significant avian pathogen; these results are consistent with previous studies[26-29]. *Campylobacter jejuni* was isolated from cloacal swabs, as well as the liver and spleen, which is also consistent with previous studies[30-32]. *Campylobacter* frequency may vary from one country to the other depending on many factors, such as hygienic measures and seasonal variations.

The *flaA* gene is involved in the invasion of the intestinal mucusa[33]. Therefore, its absence indicates severe reduction in motility and colonization of the human and chicken gut[34]. In this study, PCR showed that the *flaA* gene was present in all isolated *C. jejuni* strains, which is consistent with previous studies[35-37].

Cytolethal distending toxin is conserved among *Campylobacter* strains and causes direct DNA damage, which leads to induction of DNA damage checkpoint pathways[38,39]. Three genes, (*cdtA*, *cdtB* and *cdtC*), are required to produce CDT and complete cellular toxicity[40]. In this study, 62 (91.17%) *C. jejuni* isolates carried the *cdtA* gene, which is in agreement with Bang *et al.*[37], who found that the frequency of these genes exceeds 90% in *Campylobacter* isolates from

different sources[37]. In addition, Hanning *et al.*[36] reported that all *Campylobacter* isolates test were positive for the presence of *cdtA* and *flaA*[36].

The role of CDT in pathogenesis is undistinguishable; however, there are many studies on its toxic effect on cultured mammalian cells[16,41,42]. According to a study conducted in Bangladesh by Talukder *et al.*[43], CDT was identified as a virulence determinant which lead to increase fluid secretion in the intestine that causes diarrhea.

In the current study, experimental infection of commercial chickens with *C. jejuni* was associated with intestinal inflammation and pathological changes which is a harmful pathogen. Histopathological examination revealed many pathological changes including villi atrophy, which could explained by production of cytolethal distending toxin[44]. However, the role of other bacteria in the intestinal microbiota cannot be excluded[45,46].

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SIGNIFICANCE STATEMENT

This study shows that *Campylobacter* infection is associated with intestinal inflammation and other pathological changes. This study revealed significant effects of *C. jejuni* infection on commercial chickens. These findings confirm that *C. jejuni* can have a significant effecton poultry health and welfare and is a harmful gut commensal microorganism. Thus, strict control measures are required to reduce its colonization.

REFERENCES

1. Chaban, B., M. Ngeleka and J.E. Hill, 2010. Detection and quantification of 14 Campylobacter species in pet dogs reveals an increase in species richness in feces of diarrheic animals. BMC Microbiol., Vol. 10, 10.1186/1471-2180-10-73.
2. Zhao, S., S.R. Young, E. Tong, J.W. Abbott, N. Womack S.L. Friedman and P.F. McDermott, 2010. Antimicrobial resistance of *Campylobacter* isolates from retail meat in the United States between 2002 and 2007. Applied Environ. Microbiol., 76: 7949-7956.
3. Meerburg, B.G., W.F. Jacobs-Reitsma, J.A. Wagenaar and A. Kijlstra, 2006. Presence of *Salmonella* and *Campylobacter* spp. in wild small mammals on organic farms. Applied Environ. Microbiol., 72: 960-962.
4. Kittl, S., G. Heckel, B.M. Korczak and P. Kuhnert, 2013. Source attribution of human *Campylobacter* isolates by MLST and fla-typing and association of genotypes with quinolone resistance. PloS One, Vol. 8, No. 11. 10.1371/journal.pone. 0081796
5. Alterkruse, S.F., N.J. Stern, P.I. Fields and D.L. Swerdlow, 1999. *Campylobacter jejuni* an emerging food borne pathogen. Emerg. Infect. Dis., 5: 28-35.
6. Jones, F.T., R.C. Axtell, D.V. Rivers, S.E. Scheideler, F.R. Tarver, R.L. Walker and M.J. Wineland, 1991. A survey of *Campylobacter jejuni* contamination in modern broiler production and processing systems. J. Food Prot., 54: 259-262.
7. Cogan, T.A., S.F. Bloomfield and T.J. Humphrey, 1999. The effectiveness of hygiene procedures for prevention of cross contamination from chicken carcases in the domestic kitchen. Lett. Applied Microbiol., 29: 354-358.
8. Wassenaar, T.M. and M.J. Blaser, 1999. Pathophysiology of *Campylobacter jejuni* infections of humans. Microbes Infect., 1: 1023-1033.
9. Newell, D.G. and C. Fearnley, 2003. Sources of *Campylobacter* colonization in broiler chickens. Applied Environ. Microbiol., 69: 4343-4351.
10. Cox, N.A., L.J. Richardson, R.J. Buhr, P.J. Fedorka-Cray, J.S. Bailey, J.L. Wilson and K.L. Hiett, 2006. Natural presence of *Campylobacter* spp. in various internal organs of commercial broiler breeder hens. Avian Dis., 50: 450-453.
11. Balzan, S., C. de Almeida Quadros, R. de Cleva, B. Zilberstein and I. Cecconello, 2007. Bacterial translocation: Overview of mechanisms and clinical impact. J. Gastroenterol. Hepatol., 22: 467-471.
12. Melo, R.T., P.C. Nalevaiko, E.P. Mendonca, L.W. Borges, B.B. Fonseca, M.E. Beletti and D.A. Rossi, 2013. *Campylobacter jejuni* strains isolated from chicken meat harbour several virulence factors and represent a potential risk to humans. Food Control, 33: 227-231.
13. Wieczorek, K. and J. Osek, 2008. Identification of virulence genes in *Campylobacter jejuni* and *C. coli* isolates by PCR. Bull. Vet. Inst. Pulawy, 52: 211-216.
14. Pickett, C.L., E.C. Pesci, D.L. Cottle, G. Russell, A.N. Erdem and H. Zeytin 1996. Prevalence of cytolethal distending toxin production in *Campylobacter jejuni* and relatedness of *Campylobacter* sp. cdtB gene. Infect. Immun., 64: 2070-2078.
15. Whitehouse, C.A., P.B. Balo, E.C. Pesci, D.L. Cottle, P.M. Mirabito and C.L. Pickett, 1998. *Campylobacter jejuni* cytolethal distending toxin causes a G_2-phase cell cycle block. Infect. Immun., 66: 1934-1940.

16. Purdy, D., C.M. Buswell, A.E. Hodgson, K. McAlpine, I. Henderson and S.A. Leach, 2000. Characterisation of cytolethal distending toxin (CDT) mutants of *Campylobacter jejuni*. J. Med. Microbiol., 49: 473-479.
17. ISO., 2006. ISO 10272-1:2006. Microbiology of Food and Animal Feeding Stuffs-Horizontal Method for Detection and Enumeration of *Campylobacter* spp. Part 1: Detection Method. International Organization for Standardization, Geneva.
18. ISO., 2006. ISO 10272-1:2006. Microbiology of Food and Animal Feeding Stuffs- Horizontal Method for Detection and Enumeration of *Campylobacter* spp. Part 1: Detection Method. International Organization for Standardization, Geneva.
19. De Lamballerie, X., C. Zandotti, C. Vignoli, C. Bollet and P. de Micco, 1992. A one-step microbial DNA extraction method using "Chelex 100" suitable for gene amplification. Res. Microbiol., 143: 785-790.
20. Shin, E. and Y. Lee, 2009. Comparison of three different methods for *Campylobacter* isolation from porcine intestines. J. Microbiol. Biotechnol., 19: 647-650.
21. Datta, S., H. Niwa and K. Itoh, 2003. 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., 52: 345-348.
22. Wieczorek, K., R. Szewczyk and J. Osek, 2012. Prevalence, antimicrobial resistance and molecular characterization of *Campylobacter jejuni* and *C. coli* isolated from retail raw meat in Poland. Vet. Med., 57: 293-299.
23. Van Deun, K., F. Pasmans, R. Ducatelle, B. Flahou and K. Vissenberg *et al*., 2008. Colonization strategy of *Campylobacter jejuni* results in persistent infection of the chicken gut. Vet. Microbiol., 130: 285-297.
24. Firlieyanti, A.S., P.L. Connerton and I.F. Connerton, 2016. *Campylobacters* and their bacteriophages from chicken liver: The prospect for phage biocontrol. Int. J. Food Microbiol., 237: 121-127.
25. Lynch, M.J., S.S. Raphael, L.D. Mellor, P.D. Spare and M.J.H. Inwood, 1969. Medical Laboratory Technology and Clinical Pathology. 2nd Edn., WB Saunders Company, London, ISBN: 0721658261.
26. Van Asselt, E.D., W.F. Jacobs-Reitsma, R. Van Brakel, H. Van Der Voet and H.J. van der Fels-Klerx, 2008. *Campylobacter* prevalence in the broiler supply chain in the Netherlands. Poult. Sci., 87: 2166-2172.
27. Nguyen, T.N.M., H. Hotzel, H. El-Adawy, H.T. Tran and M.T. Le *et al*., 2016. Genotyping and antibiotic resistance of thermophilic *Campylobacter* isolated from chicken and pig meat in Vietnam. Gut Pathogens, Vol. 8, No. 1. 10.1186/s13099-016-0100-x.
28. Rahimi, E. and E. Tajbakhsh, 2008. Prevalence of *Campylobacter* species in poultry meat in the Esfahan city, Iran. Bulgarian J. Vet. Med., 11: 257-262.
29. Avrain, L., F. Humbert, R. L'Hospitalier, P. Sanders, C. Vernozy-Rozand and I. Kempf, 2003. Antimicrobial resistance in *Campylobacter* from broilers: Association with production type and antimicrobial use. Vet. Microbiol., 96: 267-276.
30. Awadallah, M.A.I., H.A. Ahmed, A.A. El-Gedawy and A.M. Saad, 2014. Molecular identification of *C. jejuni* and *C. coli* in chicken and humans, at Zagazig, Egypt, with reference to the survival of *C. jejuni* in chicken meat at refrigeration and freezing temperatures. Int. Food Res. J., 21: 1801-1812.
31. Ghazwan, J., 2006. Public health importance of *Campylobacter jejuni* in poultry. Ph.D. Thesis, Arabian Gulf University, Manama, Bahrain.
32. Ilida, M.N. and M.S. Faridah, 2012. Prevalence of *Campylobacter jejuni* in chicken meat and chicken-based products. J. Trop. Agric. Food Sci., 40: 63-69.
33. Wassenaar, T.M., N.M. Bleumink-Pluym and B.A. van der Zeijst, 1991. Inactivation of *Campylobacter jejuni* flagell in genes by homologous recombination demonstrates that *flaA* but not *flaB* is required for invasion. EMBO J., 10: 2055-2061.
34. Konkel, M.E., J.D. Klena, V. Rivera-Amill, M.R. Monteville, D. Biswas, B. Raphael and J. Mickelson, 2004. Secretion of virulence proteins from *Campylobacter jejuni* is dependent on a functional flagellar export apparatus. J. Bacteriol., 186: 3296-3303.
35. Rizal, A., A. Kumar and A.S. Vidyarthi, 2010. Prevalence of pathogenic genes in *Campylobacter jejuni* isolated from poultry and human. Internet J. Food Saf., 12: 29-34.
36. Hanning, I., D. Biswas, P. Herrera, M. Roesler and S.C. Ricke, 2010. Prevalence and characterization of *Campylobacter jejuni* isolated from pasture flock poultry. J. Food Sci., 75: M496-M502.
37. Bang, D.D., E.M. Nielsen, F. Scheutz, K. Pedersen, K. Handberg and M. Madsen, 2003. PCR detection of seven virulence and toxin genes of *Campylobacter jejuni* and *Campylobacter coli* isolates from Danish pigs and cattle and cytolethal distending toxin production of the isolates. J. Applied Microbiol., 94: 1003-1014.
38. Fouts, D.E., E.F. Mongodin, R.E. Mandrell, W.G. Miller and D.A. Rasko *et al*., 2005. Major structural differences and novel potential virulence mechanisms from the genomes of multiple *Campylobacter* species. PLoS Biol., Vol. 3, No. 1. 10.1371/journal.pbio.0030015
39. Lee, R.B., D.C. Hassane, D.L. Cottle and C.L. Pickett, 2003. Interactions of *Campylobacter jejuni* cytolethal distending toxin subunits *CdtA* and *CdtC* with HeLa cells. Infect. Immun., 71: 4883-4890.
40. Lara-Tejero, M. and J.E. Galan, 2001. CdtA, CdtB and CdtC form a tripartite complex that is required for cytolethal distending toxin activity. Infect. Immunity, 69: 4358-4365.

41. Okuda, J., M. Fukumoto, Y. Takeda and M. Nishibuchi, 1997. Examination of diarrheagenicity of cytolethal distending toxin: Suckling mouse response to the products of the cdtABC genes of *Shigell adysenteriae*. Infect. Immun., 65: 428-433.
42. Albert, M.J., S.M. Faruque, A.S. Faruque, K.A. Bettelheim, P.K.B. Neogi, N.A. Bhuiyan and J.B. Kaper, 1996. Controlled study of cytolethal distending toxin-producing *Escherichia coli* infections in Bangladeshi children. J. Clin. Microbiol., 34: 717-719.
43. Talukder, K.A., M. Aslam, Z. Islam, I.J. Azmi and D.K. Dutta *et al*., 2008. Prevalence of virulence genes and cytolethal distending toxin production in *Campylobacter jejuni* Isolates from diarrheal patients in Bangladesh. J. Clin. Microbiol., 46: 1485-1488.
44. Awad, W.A., A. Molnar, J.R. Aschenbach, K. Ghareeb and B. Khayal*et al*., 2015. *Campylobacter* infection in chickens modulates the intestinal epithelial barrier function. Innate Immun., 21: 151-160.
45. Humphrey, S., G. Chaloner, K. Kemmett, N. Davidson and N. Williams *et al*., 2014. *Campylobacter jejuni* is not merely a commensal in commercial broiler chickens and affects bird welfare. MBio, Vol. 5, No. 4. 10.1128/ mBio.01364-14
46. Smith, C.K., P. Kaiser, L. Rothwell, T. Humphrey, P.A. Barrow and M.A. Jones, 2005. *Campylobacter jejuni*-induced cytokine responses in avian cells. Infect. Immun., 73: 2094-2100.