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Zoonotic Importance of *Campylobacter jejuni* Isolated From Chicken Farms in Egypt.

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ABSTRACT

Campylobacter spp. particularly *C. jejuni* is most often implicated as the bacterial cause of food-borne illness. Moreover, complications associated with *C. jejuni* infection—Guillian-Barré syndrome and reactive arthritis were occasionally recorded in immuno-compromised patients. For investigation the presence of *C. jejuni*, a total of 200 cloacal swabs and 5 water samples were collected from poultry farms in El-Giza Governorate, Egypt. Additionally 20 human stool specimens were gathered from persons work at the same farms. Bacteriological examination of the collected samples using mCCDA agar media, evidenced only 6 campylobacter isolates, further confirmation of these isolates was done by Polymerase Chain Reaction targeting *23S rRNA*. Only, five isolates were identified as *C. jejuni* using PCR targeting *mapA* gene with isolation rates 1.5%, 20.0% and 5.0% in cloacal swabs, water samples and human stool specimens, respectively. Furthermore, the amplified PCR product of *mapA* gene sequenced and determined by BLAST analysis at GenBank. Phylogenetic tree analysis of *mapA* sequences recovered from gene in selected *C.jejuni* isolates emphasized genetic relatedness among isolates from humans (KY473955), and chickens (KY435368). This finding implies probable sharing of this pathogen between humans and chickens, also confirming chicken as a potential source for transmitting this pathogen to closely contact humans.

Keywords: *Campylobacter jejuni, mapA,* chickens, human, Egypt.

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INTRODUCTION

Awareness of the public health implications of *Campylobacter* infections has evolved for over a century [1]. Campylobacter species are considered normal intestinal biota of animals used for food production, particularly poultry [2], whereas, commercial poultry such as broilers, layers, turkeys, and ducks as well as free living birds are considered to be natural reservoirs of thermophilic *Campylobacter* [3]. Meanwhile, this pathogen can colonize the digestive tract of humans and most warm-blooded animals [4].

Among the 17 validly named species in the genus *Campylobacter*, *C. jejuni* ssp. *jejuni*, *C. coli*, *C. fetus* ssp. *fetus*, *C. upsaliensis*, *C. lari*, and *C. hyointestinalis* ssp. *hyointestinalis* are the recognized cause of intestinal infections in humans [5]. *C. jejuni* is the most frequently reported *Campylobacter* species (80-90%) followed by *C. coli* (5-10%) [6].

Veterinarians, farmers, abattoir workers, and those engaged in meat processing are likely to be at an increased risk of exposure to *Campylobacter* species [7]. Most of the campylobacteriosis infections are acquired by ingestion of contaminated food or water, or through direct contact with animals, particularly poultry [8].

Generally, clinical symptoms of *C.jejuni* infection including diarrhea, abdominal pain, high fever, and malaise commonly occur within 2 – 5 days after ingestion of contaminated foods or water [7]. In addition to an important cause of bacterial gastroenteritis in humans, *C. jejuni* has also been associated with Guillane-Barré Syndrome (GBS) , an acute immune-mediated demyelinating disorder of the peripheral nervous system, occasionally, in immuno-compromised patients [9].

Recently, polymerase chain reaction (PCR)-based methods have been developed for rapid detection, identification, and confirmation of *Campylobacter* species as well as for typing of *Campylobacter* strains **[10].** Despite the wide-spread occurrence of Campylobacter spp. their epidemiological cycle is not well understood. Moreover, the possibility of cross-contamination as well as tracing the genuine source of Campylobacter infections is not straightforward. Therefore, the current study was undertaken to explore the occurrence of *C. jejuni* in chickens, drinking water and human in close contact with such chicken in different farms in Egypt. In addition, contribute in bridging this gap of knowledge by exploring genetic relatedness among thermophilic *Campylobacter* isolates from human and chicken populations from the same farm using gene sequencing.

MATERIALS AND METHODS

Samples collection; a total of 200 cloacal samples were collected using sterile swabs from diarrheic and apparently healthy chicken in farms in El-Giza Governorate. Additionally, human stool specimens (2-10g) were gathered from 20 persons work at poultry farms. Moreover 5 water samples were obtained from water tanks in poultry farms at the same localities of cloacal swabs and stool specimens collection. Sterile swabs directly immersed into tubes containing Cary Blair transport medium (Oxoid CM0519) then directly inoculated into tubes containing 9 ml of sterile selective enrichment thioglycollate broth [11].

All samples were collected in sterile containers, labeled and placed in an ice box then immediately transferred into laboratory of Zoonoses Department, Faculty of Veterinary Medicine, Cairo University, for bacteriological examination.

Water samples were filtered using membrane filtration method (MF) in accordance with standard procedure of American Public Health Association [12]. One litre of water was allowed to drawn through vacuum pump filter apparatus containing filter membrane with pore size 0.45 µm (Gelman Sciences, Inc., Ann Arbor, Mich.) for trapping bacteria.

Bacteriological examination; each sample was pre-enriched in *Campylobacter* enrichment broth base (thioglycollate enrichment broth Oxoid) supplemented with Campylobacter selective supplement (CCDA – Oxoid SR0155). Then, incubated at 37°C for 4 h, followed by incubation for a further 24 - 48 hrs at 42°C in a microaerophilic condition using anaerobic jars and CampyGen generating kits (Oxoid CN0025,CN0035). A loopful from sample was streaked onto mCCDA agar (Oxoid CM0739) supplemented with CCDA supplement

[11]. Afterward, the plates were incubated at 42ºC for 48 h under the same condition and the suspected *Campylobacter* colony from each selective agar plate was subcultured and tentatively identified according to morphological features and Gram's stain [13].

Biochemical identification: on mCCDA agar (Oxoid) supplemented with CCDA supplement**,** the suspected colonies were streaked and subjected to biochemical identification using catalase test, oxidase test, urea hydrolysis test, hydrogen sulphide (H2S) production, and citrate utilization test [14]. Afterward, these colonies were subjected to molecular identification.

Molecular identification: *Campylobacter* isolates (5) obtained from the examined samples were subjected for genotyping using conventional PCR (cPCR).

DNA extraction from pure suspected colonies of *Campylobacter species* was performed according to Sheedy *et al.* [15] and the extracted DNA was stored at -20°C until use. Polymerase chain reaction was performed on the extracted DNAs, whereas 2 genes were targeted as in table (1).

The amplification reaction was performed according to Wang *et al*. [16], using 2 X master mix HotStarTaq Master Mix kit, (Thermo Scientific) Cat No. K1081. The thermal profile of the reaction was initial denaturation at 94 ºC for 5 min, 35 cycles each consisting of denaturation at 94 ºC for 30 s, annealing at 55 ºC for 45 s, and extension at 72 ºC for 45s, were performed; followed by a final extension step at 72 ºC for 10 min. as *C. jejuni* ATCC 33560 obtained from Naval Medical Research Unit No.3 was used as positive control. Afterward, gel electrophoresis was done on the amplified PCR product in 1.5% agarose gel and visualized under ultraviolet light.

Gene sequencing: PCR products generated from *mapA* gene in selected isolates of C*. jejuni* were purified using GeneJET_ PCR purification kit (Thermo) and DNA sequencer was used to conduct sequencing. The sequencing step was conducted with Big Dye Terminator V3.1 Cycle sequencing Kit (Applied Biosystems). The obtained sequences were determined by BLAST analysis with sequences available at Genbank (NCBI).

Phylogenetic tree analysis was used to assess the relatedness of our gene sequences recovered from different sources (human contact and chicken). BioEdit, software was used for Clustral WMultiple alignment and sequences identity matrix as well as MEGA6 software version (6.06) was used for construction of phylogenetic by neighbor-joining method (Fig. 3).

Nucleotide sequence accession numbers; the nucleotide sequences of (*mapA*) gene recovered from *C.jejuni* isolates determined in this study have been deposited in GenBank under the following accession numbers: (cloacal swab, KY435368 *C .jejuni*) and (human stool, KY473955 *C.jejuni*).

RESULTS AND DISCUSSION

Campylobacter jejuni is one of the most zoonotic pathogens transmitted between animal and humans. It is commensal in the gastrointestinal tract of many domestic and wild animals; especially birds [18]. As well as, several studies also showed that *C. jejuni* is more commonly found in chickens [19] compared to other Campylobacter spp.

In the present study, molecular identification of thermophilic *campylobacter* spp using Campylobacter *23S rRNA* gene evidenced only 6 *campylobacter* spp. isolates in all the examined samples, Fig. (1). Moreover, using of *mapA* as a specific target gene for *C. jejuni* for further molecular identification of *campylobacter* spp, revealed that 5/6 (83.3%) was positive for *C. jejuni*. All the isolates successfully generated bands whose patterns were analyzed Fig. (2), whereas, the prevalence of *C. jejuni* in chicken was 1.5% as shown in Table (2), this finding was similar to Marinuo *et al*. [20] who recorded 1.48% as the isolation rate of *Campylobacter* spp. in the poultry farms. On the other side, our findings were lower than those isolated from 7(30%) of 23 farm chicken cloacal samples Bosnia and Herzegovina [21], as well as *C. jejuni* that isolated from 36% in chicken with diarrhea [22].

The low prevalence rate recorded in chicken might be linked to the free range system which is common in the study area as coprophagy which enhances bird to bird spread is limited. This can be supported by findings of Robino *et al.* [23] with a *Campylobacter* spp. prevalence rate of 78.4% in intensively reared poultry and 18.3% in small scale rural poultry farming in Italy.

Variations in isolation rates may be explained by the actual differences in local prevalence of *Campylobacter* in a specific region, seasonality, chicken management system, sampling techniques and laboratory methodologies employed. For example, sampling of chicken faeces by using cloacal swabs was found to be less sensitive as compared to the intestinal contents [24]. In addition, it was established that at a certain stage of colonization process *Campylobacter* species can only be found in the caecum and cannot be shed in faeces [25]. At such a stage, caecum is the best colonization site and not elsewhere.

Campylobacter species are ubiquitous in the environment and around broiler houses and may be easily transported into the human workers either in utilities, such as feed, litter and water. In an outbreak of waterborne *campylobacter*, water was a common vehicle and affected thousands of individuals [26]; sewage was also reported as the most likely contamination source [27]. Our study revealed that *C. jejuni* was 20% in water samples collected from poultry farms. Nearly similar result (18.7%) was recorded by Pagaya *et al*. [28] from groundwater in India were found positive for *C. jejuni*. lower results reported by Shimaa *et al.* [29] in Chicken farms water was 12%. Higher result (57%) was reported by Tambalo *et al.* [30] in water samples collected in 2009 in southern Saskatchewan. Also Savill *et al.* [31] found high levels of thermophilic Campylobacter in New Zealand, with detection rates of 60% in river water and 75% in shallow ground water.

The variation in results may contribute to detection methods that are still times consuming and pathogens are likely to be present only at low numbers in large volumes of drinking water. The non-detection might be related to the long lag between exposure and initiation of microbiological studies.

Although Campylobacter is insignificant for poultry health, it is a leading cause of food-borne gastroenteritis in humans worldwide, and contaminated poultry meat is recognized as the main source for human exposure. The prevalence of *Campylobacter jejuni* in human stool specimens observed in this study (5%) is comparable to 6 and 6.4% reported in Nigeria and Alexandria, Egypt, respectively, [32, 33], Table (1). Moreover, in Cairo, Egypt, Zaghloul *et al*. [34] reported that *Campylobacter* spp. was identified in 6.6% of human stool samples. Meanwhile, lower prevalence rates were obtained by Varoli *et al*. [35] and Kang *et al.* [36], were 2.3% and 2.9% respectively. Concerning the low prevalence rate of *Campylobacter* species detected in human samples during the current study could be attributed to the low number of samples collected from diarrheic patients. On the other hand, a higher isolation rate of 16.7% was reported in Giza, Egypt, this higher percentage could be attributed to the sampling of stool samples from human in contact with food animals [37]. This variation may be attributed to the different in method of sampling, procedure and locality.

Several studies recorded that keeping animals in close proximity with humans was a risk factor for human *Campylobacter* infection. Kusiluka *et al.* [38] reported that children from families that kept poultry and cattle had significantly higher prevalence of *Campylobacter* infections (27.2%) than their counterparts without animals (3.4%). Previous studies also demonstrated that toddlers in families keeping chickens had an average of 4 faeces-to-mouth episodes in 12-h [39]. The fact that poultry faeces can yield viable *C. jejuni* for at least 48 h after deposition suggests existence of a high risk of *Campylobacter* transmission in environments where there may be frequent human-animal contacts [40].

From the obtained results it is worthwhile to note that *C. jejuni* is the most predominant species detected among all the examined samples, this species is most often implicated as the causative agent of human Campylobacteriosis [6].

In order to explore the identity between the human and chickens *C. jejuni* strains isolated from the same farm, phylogenetic tree was constructed using *mapA* gene sequences (Fig.3). Analysis of the sequences demonstrated that our study sequences of chicken (KY435368) and human (KY473955) show close relation to each other with identity 100%. On the other hand, human stool sequence (CP012690.) located in South Africa and chicken sequence (CP017033) in USA show also similarity. This finding implies probable sharing of these pathogens between humans and chickens, as well as confirming chicken as a potential source for transmitting this pathogen to closely contact humans.

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Table (1): Oligonucleotide primers used for molecular identification of Campylobacter species:

Primers	Target agent & genes	Primer sequence (5'-3')	Amplified Length (bp)	Reference
23S	Campylobacter *23S rRNA*	TATACCGGTAAGGAGTGCTGGAG (F)	650	[16]
23S		ATCAATTAACCTTCGAGCACCG ®		
MDMapA1	*C. jejuni mapA*	CTATTTTATTTTTGAGTGCTTGTG (F)	589	[17]
MDMapA2		GCTTTATTTGCCATTTGTTTTATTA®		

Table (2): Occurrence of *C. jejuni* among the examined farms identified by cPCR technique.

Type of samples	Number of Examined samples	Number of Positive samples	Percentage %
Cloacal swabs	**200**	**3**	**1.5**
Water	**5**	**1**	**20.0**
Human stool specimens	**20**	**1**	**5.0**

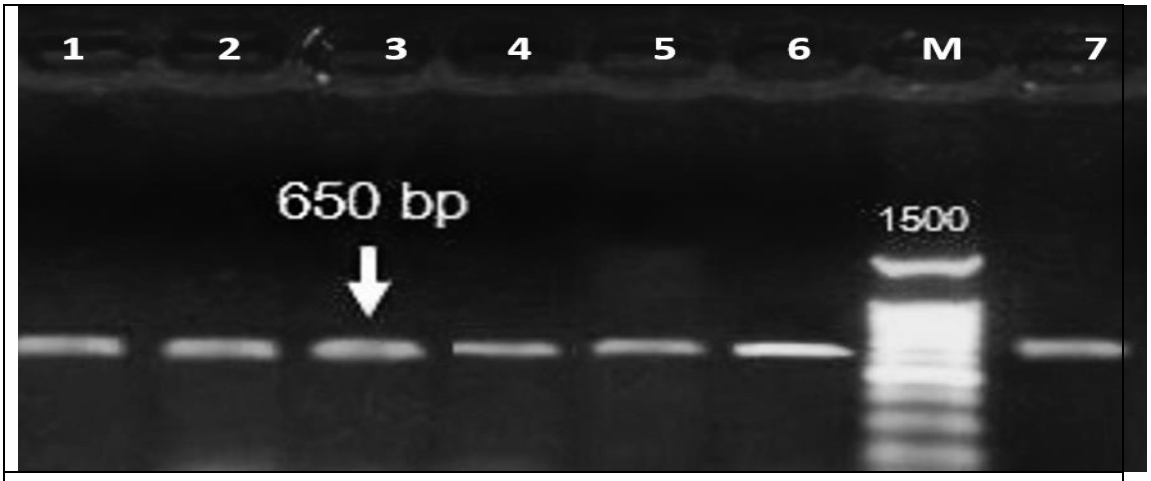

Figure (1). Agarose gel electrophoresis of conventional PCR for detection of 23S gene for *Campylobacter spp*. (650-bp fragment) in examined samples.
Lanes 1-6: positive results for *Campylobacter spp*.
Lane 7: positive control.
M: Marker (Gel Pilot 100 bp. ladder)

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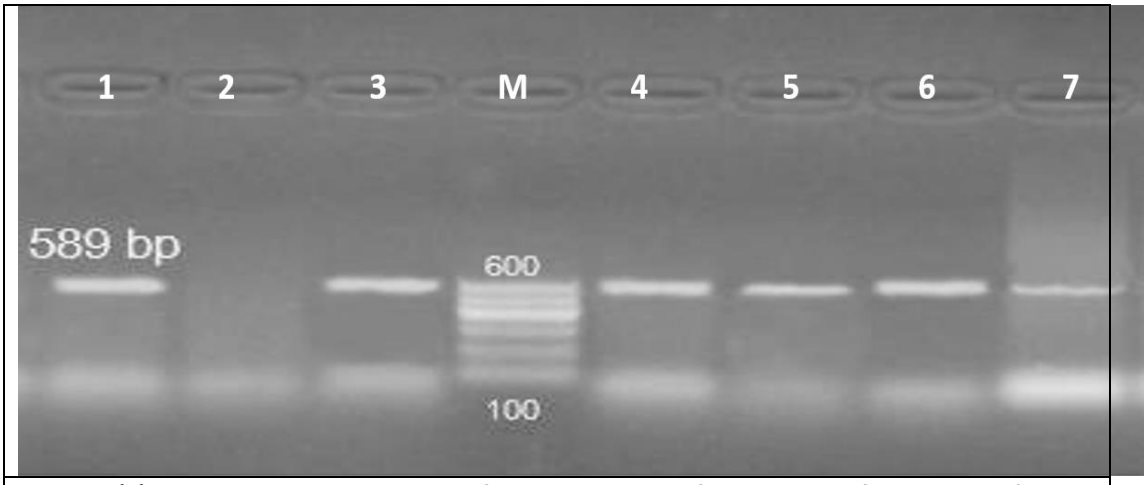

Figure (2).Agarose gel electrophoresis of conventional PCR for detection of *map A* gene for *C. jejuni.* (589-bp fragment) in examined samples.
Lane 1: positive control (*C. jejuni* (ATCC 33560).
Lane 2: negative control for *C.jejuni.* (Master Mix without any DNA).
Lanes 3-7: positive results for *C. jejuni*.
M: Marker (Gel Pilot 100 bp. ladder).

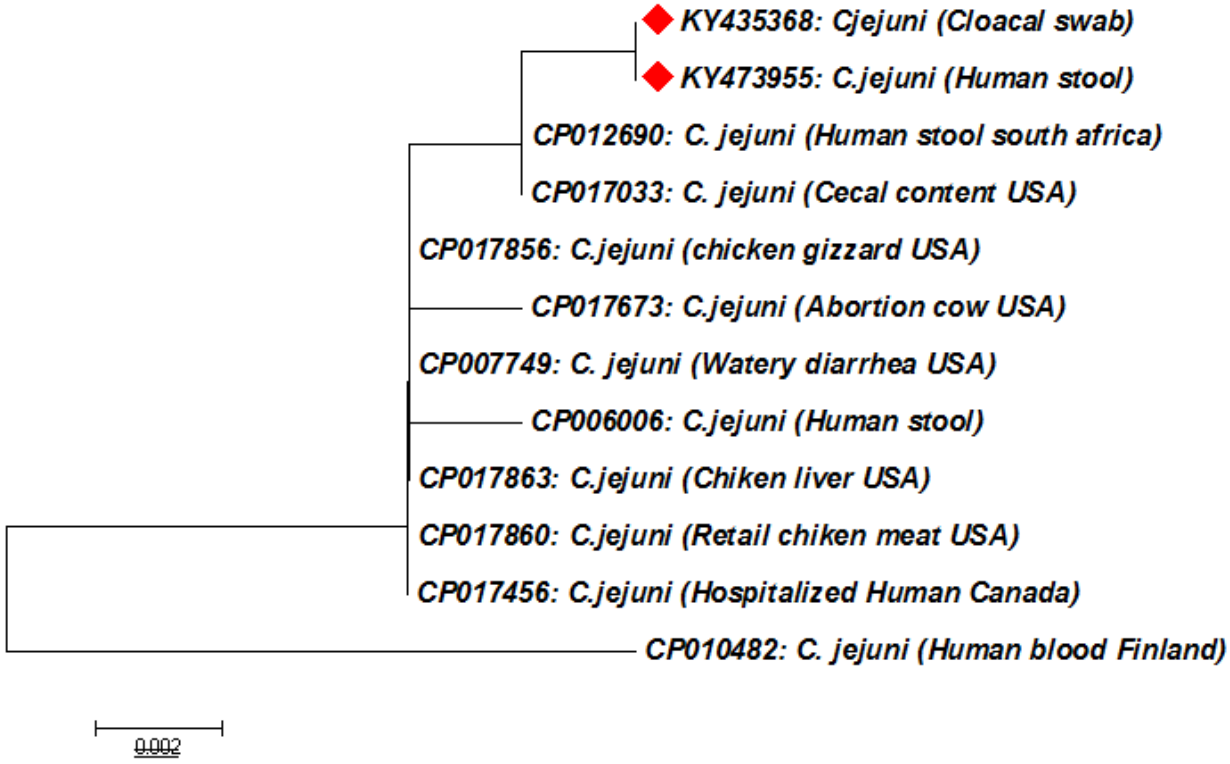

Figure (3): Phylogenetc tree analysis of *mapA* gene sequences of campylobacter isolates recovered from different sources (chicken and human). The accession numbers of our study sequences and related sequences retrieved from GenBank were shown. The tree was generated based on the neighbor-joining method.

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CONCLUSION

Our findings support the suggestion that the presence of the animals and the poor hygienic conditions, that place the human at an increased risk of *Campylobacter* disease. Additionally, the observed genetic similarity among *C. jejuni* isolates from human and chicken suggests existence of cross transmission of these pathogens between them. More genetic and epidemiological studies are recommended to further explore genetic relatedness among thermophilic *Campylobacter* isolates from humans and chickens especially those at a close proximity to humans.

REFERENCES

[1] Kist, M. (1985). The historical background of *Campylobacter* infection: new aspects. In: Pearson AD, editor. Proceedings of the 3rd International Workshop on *Campylobacter* Infections; Ottawa; 1985 Jul 7-10. London: Public Health Laboratory Service, p.23-7.

[2] Ketley, J. (1995). Virulence of Campylobacter spp.: a molecular genetic approach. Journal of Medical Microbiology, 42: 312–27.

[3] Shane, S.M. (1992). The significance of *Campylobacter jejuni* infection in poultry. a review. Avian Pathol., 21:189-213.

[4] Humphrey, T., O, Brien, S. and Madsen, M. (2007). *Campylobacter* as zoonotic pathogens: A food production perspective. Int. J.Food Microbiol.,117:237-257.

[5] Lastovica, A. J. and Allos, B. M. (2008). Clinical significance of *Campylobacter* and related species other than *Campylobacter jejuni* and *Campylobacter coli.* In Nachamkin, I., Szymanski, C., Blaser, M. (Eds.). Campylobacter, p. 123-149. Washington, DC: ASM Press.

[6] Fitzgerald, C., Whichard, J. and Nachamkin, I. (2008). Campylobacter. In Nachamkin, I., Szymanski, C., Blaser, M. (Eds.). Diagnosis and antimicrobial susceptibility of Campylobacter species, p. 227-243. Washington, DC: ASM Press.

[7] Butzler, J.P. (2004). *Campylobacter*, from obscurity to celebrity. Clin. Microbiol. Infect., 10: 868-876.

[8] Whiley, H., van den Akker. B., Giglio, S. and Bentham , R.(2013) .The role of environmental reservoirs in human campylobacteriosis. *Int J Environ Res Public Health,*10: 5886–907.

[9] Allos, B. M., (2001). *Campylobacter jejuni* infections: update on emerging issues and trends. Clin. Infect. Dis., 32:1201-1206.

[10] Englen, M. D., Ladely, S. R. and Fedorka-Cray, P. J. (2003). Isolation of Campylobacter and identification by PCR. Methods Mol. Biol., 216:109-121.

[11] ISO 10272-1:2006. AND ISO/TS 10272-2:2006. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Campylobacter* spp. Part 1: Detection method; Part 2: Colony count technique. International Organisation for Standardisation (ISO), ISO Central Secretariat.

[12] American Public Health Association (APHA), (1981). Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, Washington, D.C.

[13] Koneman, E.W., Allen S.D., Janda, W.M., Schreckenberger, P.C. and Winn, W.C. (1995).Color Atlas and Textbook of Diagnostic Microbiology (1395pp., 5th edn.) Philadelphia, New York, Lippincott.

[14] Nachamkin, I., (1999). Campylobacter and arcobacter, p. 716-726.*In* P.R. Murray, E.J. Barron, M.A. Pfaller, F.C. Tenover, and R.H .Yolken(ed) , Manual of clinical microbiology,7th ed. ASM Press, Washington, DC.

[15] Sheedy, S.A., Ingham, A., Rood, J.I. and Moore, R.J. (2004). Highly conserved alpha toxin sequences of avianisolates of C.*perfringens*. J. Clinical Microbiol., 42(3):1345-134.

[16] Wang, G., Clark, C.G., Taylor, T.M., Pucknell, C., Barton, C., Price, L., Woodward D.L. and Rodgers, F.G. (2002). Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C.coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. *J Clin Microbiol.,* 40:4744-7.

[17] Shin, E. and Lee, Y. (2009). Comparison of three different methods for campylobacter isolation from porcine intestines. J Microbiol Biotechnol.,19(7):647-50.

[18] Takamiya, M. , Ozen, A. , Rasmussen, M. , Alter, T. , Gilbert, T., Ussery, D. W. and Knøchel, S. (2011) . Genome Sequence of *Campylobacter jejuni* strain 327, a strain isolated from a turkey slaughterhouse. Standards in Genomic Sciences ,4:113-122.

[19] Humphrey,T. J., Henley, A. and Lanning, D.G. (1993). The colonization of broiler chickens with *Campylobacter jejuni*: some epidemiological investigations. Epidemiol. Infect., 110: 601-607.

[20] Marinou, I., Bersimis, S., Ioannidis, A., Nicolaou, C., Mitroussia-Ziouva, A., Legakis N. J. and Chatzipanagiotou, S. (2012). Identification and antimicrobial resistance of *Campylobacter* species isolated from animal sources. Frontiers in Microbiology, 3 (58): 1-6.

[21] Uzunovic-Kamberovic.S., Zorman, T. and Mozina, S. (2007) Role of poultry meat in sporadic *Campylobacter* infection in Bosnia and Herzegovina: Laboratory-based study. Croat. Med.J., 48 (8):842-851.

[22] Khalifa, N. O., Jehan, S.A. and Nagwa, S. R. (2013) Zoonotic and Molecular Characterizations of *Campylobacter jejuni* and *Campylobacter coli* Isolated from Beef Cattle and Children. Global Veterinaria 11 (5): 585 591.

[23] Robino, P., Tomassone, L., Tramuta, C., Rodo, M., Giammarino, M., Vaschetti, G. and Nebbia, P. (2010). Prevalence of *Campylobacter jejuni, Campylobacter coli* and enteric *Helicobacter* in domestic and free living birds in North-Western Italy. *Schweiz Arch Tierheilkd.* 152(9):425-431.

[24] Miwa, N., Takegahara, Y., Terai K, Kato H, Takeuchi T. (2003). *Campylobacter jejuni* contamination on broiler carcasses of *C. jejuni*-negative flocks during processing in a Japanese slaughterhouse. Int Food Microbiol., 84: 105–9.

[25] Kazwala, R.R., Collins, J.D., Hanna, J., Crinion, R.A.P and O'Mahony, H. (1990) Factors responsible for introduction and spread of *Campylobacter jejuni* in commercial poultry production. Vet Rec., 126:305–306.

[26] Miller, W.G. and Mandrell , R.E. (2005) Prevalence of Campylobacter in the food and water supply: incidence, outbreaks, isolation and detection. Campylobacter Mol Cell Biol., 101–63. 12.

[27] Maurer, A.M. and Sturchler, D. A. (2000).waterborne outbreak of small round structured virus, campylobacter and shigella co-infections in La Neuveville, Switzerland, 1998. Epidemiol Infect., 125(2):325– 32.

[28] Pagaya J, Massi, M. N., Limmon, G. V. and Natsir, R. (2015). Detection of flaA Virulence Genes Campylobacter jejuni, Isolated from Human Faeces and Groundwater Using PCR Method. Int.J.Curr.Microbiol.App.Sci., 4(6): 379-387.

[29] Shimaa, T. O., El Fadaly, H.A. and Barakat, A.M. (2015). Public Health Hazard of Zoonotic *Campylobacter jejuni* Reference to Egyptian Regional and Seasonal Variations. Research Journal of Microbiology, 10 (8): 343-354.

[30] Tambalo D. D., Tyler Boa, Aryal, B. and Yost C. K. (2016). Temporal variation in the prevalence and species richness of *Campylobacter* spp. in a 3 prairie watershed impacted by urban and agricultural mixed inputs Canadian Journal of Microbiology, Vol. 62, No. 5: pp. 402-410.

[31] Savill, M.G., Hudson, J,A., Ball, A, Klena, J.D., Scholes, P., Whyte, R.J. and Mccormick, R.E. (2001). Elucidation of *Campylobacter* in New Zealand recreational and drinking waters. J. Appl. Microbiol., 91:38-46.

[32] Aboderin, A. O., Smith, S. I., Oyelese, A. O., Onipede, A. O., Zailani, S. B. and Coker, A. O. (2002). Role of *Campylobacter jejuni/coli* in diarrhoea in Ile-Ife, Nigeria. East African Medical Journal, 79 (8): 423-426.

[33] Pazzaglia, G., Bourgeois, A. L., Mourad, A. S., Gaafar, T., Diab, A. S., Hebert, A., Churilla, A. and Murphy, J. R. (1995). Campylobacter diarrhea in Alexandria, Egypt. Journal of Egyptian Public Health Association, 70 (3-4): 229-241.

[34] Zaghloul, M. Z., Farouk, N. and Galal, Z. A. (2012). Detection of *Cambylobacter* spp. in stool samples by new methods in comparison to culture. Life Science Journal, 9 (4): 2566-2571.

[35] Varoli, O., Gatti, M. and Piscolla, F. (1989). A one-year study on thermophilic *Campylobacter* spp. isolated from fecal specimens. Microbiologica, 12 (3): 263-5.

[36] Kang, Y. S., Cho, Y. S., Yoon, S. K., Yu, M. A., Kim, C. M., Lee, J. O. and Pyun, Y. R. (2006). Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from raw chicken meat and human stools in Korea. Journal of Food Protection, 69 (12): 2915-2923.

[37] Hassanain, N. A., (2011). Antimicrobial resistant *Campylobacter jejuni* isolated from humans and animals in Egypt. Global Veterinaria, 6 (2): 195-200.

[38] Kusiluka, L.J.M., Karimuribo, E.D., Mdegela, R.H., Luoga, E.J., Munishi, P.K.T., Mlozi, M.R.S., Kambarage, D.M. (2005).Prevalence and impact of water-borne zoonotic pathogens in water, cattle and humans in selected villages in Dodoma Rural and Bagamoyo districts, Tanzania. Phys Chem Earth., 30:818–825.

[39] Lindblom, G.B., Ahren, C., Changalucha, J., Gabone, R., Kaijser, B., Nilsson, L.A., Sjogren, E., Svennerholm, A.M. and Temu, M. (1995). *Campylobacter jejuni/coli* and enterotoxigenic *Escherichia coli* (ETEC) in faeces from children and adults in Tanzania. Scand J Infect Dis., 27:589–593.

[40] Marquis, G.S., Ventua, G., Gilamn, R.H., Porras, E., Miranda, E., Carbajal, L. and Pentafiel, M. (1990). Faecal contamination of shanty town toddlers in households non-corralled poultry, Lima, Peru. Am J Public Health., 80:146–149.