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Morphological, Biochemical and Genotypic Analysis of Zoonotic *Campylobacter jejuni* Isolated from Chicken Meat Samples

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ABSTRACT

Background: *Campylobacter* species are a leading cause of most important food-borne diarrhoeal illness worldwide while, poultry has been identified as a significant cause of *Campylobacter* infection in humans. *C. jejuni* is highly effective in colonizing chicken intestinal mucosa without causing any clinical manifestations and the consumption of poultry meat is the major source of transmission of bacteria to humans.

Methods: The total of 19 chicken meat samples collected from retail markets in Chennai were screened by cultural examination, further subjected to phenotypic characterization using biochemical test and genotypic characterization using polymerase chain reaction assay targeting *hip O* and *map A* genes.

Result: All the isolates showed growth on modified blood free charcoal cefoperazone deoxycholate agar media (mCCDA) and 18 (94.73%) samples showed typical morphological characteristics. The 12 (63.15%) isolates showed biochemical reactions positive. The results from polymerase chain reaction showed that 10 (83.33%) isolates were positive for *C. jejuni*. This study suggested that, it is essential to investigate the incidence of *Campylobacter jejuni* infection in poultry and the risk factors at all production stages of meat production to help reducing the disease in humans in terms of food safety.

Key words: CCDA , *C. jejuni, hip O*, Hippurate, *map* A.

INTRODUCTION

Campylobacter is considered as a principal cause of most important zoonotic food-borne disease in humans for approximately 166 million diarrheal cases and 37,600 deaths per year globally (Oh *et al*., 2018). *Campylobacter* is 1 of 4 key global causes of diarrheal diseases of human gastroenteritis in the world (WHO, 2018). *C. jejuni* has been found to be the predominant species isolated from poultry samples and responsible for the majority of human Campylobacteriosis (Natsos *et al*., 2019). Poultry and poultry products are considered as the most important sources of human infections. *Campylobacter jejuni* is main foodborne pathogens and broiler meat is considered as the most important source of human Campylobacteriosis (Duque *et al*., 2021). The proliferation of *C. jejuni* in broilers suggests that the feed formulated to promote avian growth provides the nutrients required by these bacteria (Greene *et al.,* 2020).

In humans, clinical signs of Campylobacteriosis include diarrhea, abdominal pain, fever, headache, nausea and vomiting. The main recognized sequelae are Guillain-Barré Syndrome (GBS), the Reactive Arthritis (REA) and irritable bowel syndrome (IBS).Thermo tolerant *Campylobacter* which has a clinical significance are *C. jejuni* and its represents more than 90% of human infections (Mikulic *et al*., 2016).

Campylobacter jejuni showed multiple drug resistance against some antibiotics, which is of public health concern and may pose serious problems in the treatment of animals (Shekhar *et al*., 2010). The control of *Campylobacter* in poultry seems crucial for the reduction of human Campylobacteriosis cases (Natsos *et al*., 2016). Hence this study was attempted to detect the presence of *C. jejuni* using cultural, biochemical and PCR technique and compare these techniques for detection among raw chicken meat samples.

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MATERIALS AND METHODS

Collection of samples

The present study was conducted at Department of Animal Biotechnology, Madras Veterinary College, Chennai, Tamil Nadu in the year 2018-19. A total of 19 samples comprising raw chicken meat were collected from retail markets around Chennai. All the samples were collected using sterile containers and transported immediately to the laboratory under cold conditions for microbiological analysis.

Processing of samples

The isolation was performed according to Man (2011) and the isolates were identified by biochemical tests as described by (Fitzgerald and Nachamkin, 2007; Lastovica and Allos, 2008).

Phenotypic characterization

Cultural examination

Sample was enriched in modified Charcoal Cefoperazone Deoxycholate (mCCDA) broth (Hutchinson and Bolton, 1984) with CCDA supplement under microaerophillic conditions (candle jar method) by using internal gas generation system using (Microaerophilic gas pack CampyPack-BD oxoid).

Biochemical test

The isolates were identified as *C. jejuni* based on their morphological and biochemical tests. Suspected colonies were sub-cultured and confirmed by catalase, oxidase, nitrate and hippurate hydrolysis, Ninhydrin test, H_2S production.

Molecular confirmation of *Campylobacter jejuni*

The biochemically confirmed isolates employed for molecular confirmation as *C. jejuni* by polymerase chain reaction amplifying specific target gene. DNA were extracted by Phenol-Chloroform extraction method and the DNA concentration was quantified by nanodrop and stored at -20°C until further processing.

Genotypic confirmation of isolates by polymerase chain reaction for *hip O* gene and *map A* gene

The isolates were confirmed by PCR using designed primers in the study as forward primer (5-TTCCATGACCACCTC TTCC-3) and reverse primer (5-CTACTTCTTTATTGCTT GCTGC-3) for *hip O* gene. The primers used for amplification of *map A* gene were forward primer (5-CTATTTTATTTTT GAGTGCTTGTG-3) and reverse primers (5-GCTTT ATTTGCCA TTTGTTTTATTA-3) (Khoshbakht *et al*., 2015).

The PCR reactions were performed in 25 µl reaction mixture. The amplification conditions consisted of initial denaturation at 94°C for 3 min, 35 cycles with denaturation at 94°C for 1 min, annealing at 53°C for *hip O* 1 min, at 72°C for 1 min, followed by a final extension at 72°C for 5 min respectively (Al-Amri *et al*., 2007). The annealing temperature for *Map* A gene was optimized as 52°C for 1 min (Khoshbakht *et al*., 2015). The DNA from *C. jejuni* (ATCC 3329) was included as positive control for PCR identification of the isolates. The amplified products were observed and photographed using gel documentation System (Applied Biosystems).

RESULTS AND DISCUSSION

Campylobacteriosis is one of the most common bacterial infections worldwide causing economic costs (Soro *et al*., 2020). Modified blood free Charcoal cefoperazone deoxycholate agar is commonly used worldwide (Hutchinson and Bolton, 1984). In current study a total of 19 samples were processed for isolation of *Campylobacter*, all samples showed growth on mCCDA agar plates. The isolates showed typical grey/white or creamy grey in colour and moist spreading type colonies with sticky nature were confirmed phenotypically as *Campylobacter*. The suspected colonies were examined for morphological characteristics, motility, Gram's staining. Campylobacter species are Gram negative rods with characteristically curved, spiral, or S-shaped cells. The overall incidence of *Campylobacter* was found to be 18 (94.73%) by cultural examination.

Biochemical characterization

The isolates were processed for phenotypic characterization and identified by biochemical tests. The test for hippurate hydrolysis is critical for separation of *Campylobacter jejuni* and *C. coli* strains. Glycine and benzoic acid are formed when hippurate is hydrolysed by *C. jejuni* (Morris *et al*., 1985). Twelve (63.15%) isolates were positive for catalase, oxidase, nitrate and hippurate hydrolysis, Ninhydrin test (Fig 1).

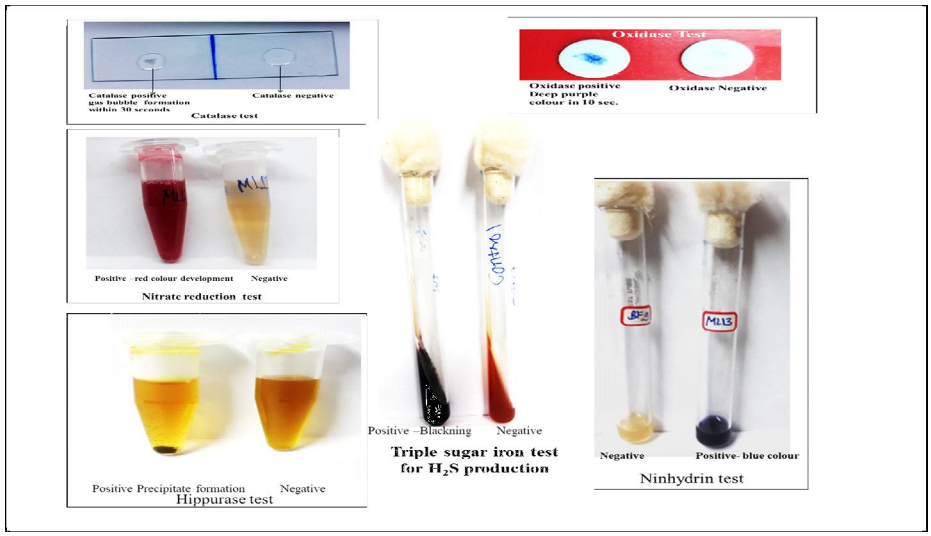

Fig 1: Characterization of *C. jejuni* isolates by biochemical tests.

The samples from raw chicken meat (9) were positive for H_2S production. *C. jejuni* biotype 2 strains are H_2S positive, whereas *C. jejuni* biotype 1 strains are H_2S negative (Penner, 1988). In this study nine isolates belong to biotyope 2 while three belong to biotype 1 of *C. jejuni*. All the samples found to be positive for ninhydrin test confirmed as *C. jejuni*. Malik *et al*., 2014 studied hundre sis test positive for two isolates, categorized as *C. jejuni* a nd negative for 30 isolates while twelve (63.15%) isolates were positive in this study.

Genotypic characterization

The size of PCR product for *hip O* gene was 270 bp and the size of the PCR product for *map A* gene was 589 bp (Fig 2). The incidence of *C. jejuni* from raw chicken meat using three screening tests was given in Table 1, Fig 3. The total of 10 (83.33%) isolates were confirmed as *C. jejuni*.

Campylobacter was detected in 76% and *Campylobacter jejuni* was the most prevalent species (64.7%) being contaminated from broiler meat products collected in retail outlets (Nicodeme *et al*., 2015). The prevalence of *Campylobacter* spp. in chicken meat was 17.33% and twenty three *Campylobacter jejuni* were identified by biochemical examination and confirmed by polymerase chain reaction collected from retail meat markets, slaughter houses (Moudgil and Kumar, 2014). In the present study higher incidence rate of 12 (63.15%) was confirmed by biochemical tests and 10 (83.33%) by polymerase chain reaction.

Sixty eight *C. jejuni* isolates were obtained with an overall prevalence rate of 34% using the uniplex PCR targeting *map A* from different farms at Dakahlia Governorate in Egypt. (Younis *et al*., 2018). The isolates were confirmed as *C. jejuni* biochemically targeting the *map A* gene of *C. jejuni*. A total of 17% and 11.87% of broiler chicken and layer chicken samples respectively, were positive for *C. jejuni* collected in Egypt (Ghoneim *et al*., 2020). While in the present study 12 (63.15%) chicken meat samples were positive on the basis of biochemical testes and 10 (83.33%) by PCR targeting *map* A gene and *hip* O genes. Chicken samples were bacteriologically positive for *Campylobacter* isolates and 285 isolates (94.37%) were identified as *C. jejuni* by polymerase chain reaction targeting the *hip* O gene specific for *C. jejuni* (Barakat *et al*., 2020).

Campylobacter jejuni (88.1%) was most prevalent species isolated from broiler meat samples by cultural method and polymerase chain reaction assay (Zendehbad *et al*., 2015). Four C. *jejuni* (3.3%) isolated from poultry meat in Northern Poland (Szczepanska *et al*., 2017), while higher incidence (63.15 %) was found in our study. *Campylobacter jejuni* (58.82%) was isolated from raw meat samples in Kolkata, India suggested that the consumption of undercooked meat possess a possible health risk for consumers (Sharma *et al*., 2016).

The most predominantly isolated species among *Campylobacter* was *C. jejuni* (69.5%) from chickens

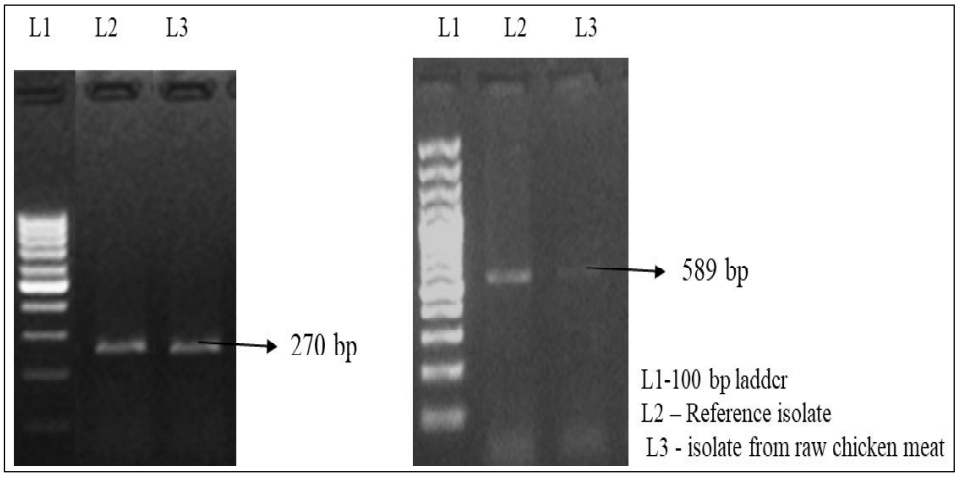

Fig 2: Agarose gel showing the amplification product of *hip O* and *map A* gene of *Campylobacter jejuni* (270 bp and 589 bp) (L=100 bp DNA ladder).

Table 1: Result of cultural, phenotypic and genotypic characterization of *C. jejuni* isolates.

Test characteristics				Results
Cultural examination				18 (94.73%)
Phenotypic characterization	Biochemical test	Catalse		12 (63.15%)
		Oxidase		12 (63.15%)
		Nitrate		12 (63.15%)
		Hippurate hydrolysis		12 (63.15%)
		Ninhydrin		12 (63.15%)
		H_2S production	Positive	9 (47.36%)
			Negative	3 (15.78%)
Genotypic characterization				10 (83.33 %)

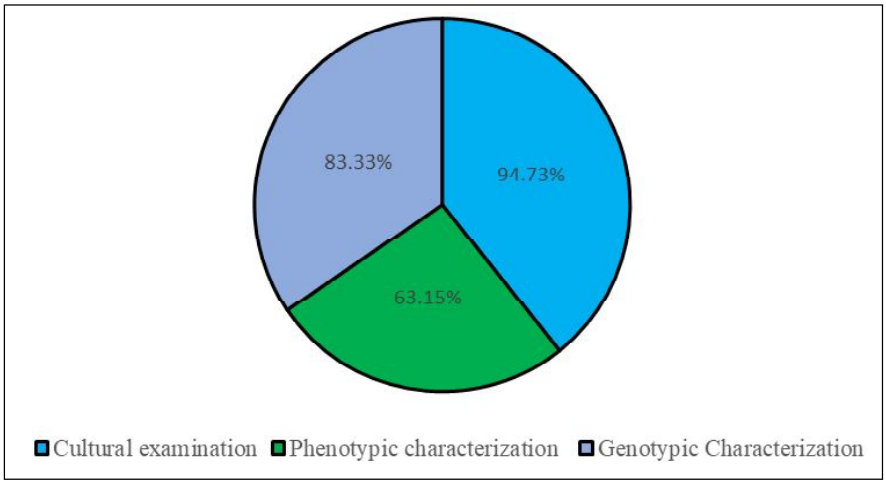

Fig 3: Comparative result of phenotypic and genotypic study of *C. jejuni* from raw chicken meat samples.

(Sinulingga *et al*., 2019). A total of 17% and 11.87% of broiler chicken and layer chicken samples respectively were positive for *C. jejuni* as the most commonly isolated species (Frosth *et al*., 2020). The risk of Campylobacteriosis in chicken meat consumers in southern Benin was studies and reported prevalence of *C. jejuni* (23.4%) from chicken meat by molecular methods (Kouglenou *et al*. 2020), while higher incidence (83.33%) was reported in our study.

Campylobacter jejuni was recognized as a main species accounting for 37 (68.5%) from chicken and turkey meat samples (Kanaan and Abdulwahid, 2019). The most frequently reported isolates were *Campylobacter jejuni* (22%) in poultry raw meat samples sold in retails. This suggested that "One Health" approach represent a strategy to control *Campylobacter* spp. and further programmes, policies, legislations and research will contribute to control contamination at primary production level and retail outlet (Gigliotti *et al*., 2019). *Campylobacter* isolates (35.4%) were recovered and analysed by multiplex PCR and four (13.8%) were identified as *C. jejuni* from broiler meat samples (Nafarrate *et al*., 2020) and their results concur with the results of our study.

C. jejuni was isolated from 17.0% of samples obtained in butcher shops and formal slaughter houses (Alaboudi *et al*., 2020). *C. jejuni* was isolated in 5 (41.7%) raw chicken samples collected from local market (Chen *et al*., 2020). Baali *et al*. (2020) reported that 65%, 55% and 70% of the cloacal swab, neck skin and caecal content were contaminated with thermotolerant *Campylobacter* strain, *Campylobacter jejuni* was the predominant species (73.5%) from broiler chickens in Batna, East Algeria and this is in accordance with the results of our study.

CONCLUSION

Poultry is an important reservoir and source of human Campylobacteriosis, although the contribution of other sources, reservoirs and transmission in reducing contamination of other meat samples. *Campylobacter* colonization in poultry and focus at the farm level has been successful in intervention strategies reducing the number of *Campylobacter* cases in several countries. Increasing farm biosecurity and education of consumers are likely to limit the risk of infection. Active on-farm biosecurity measures on chicken farms and more hygienic efforts in slaughter houses, in local chicken slaughter shops should be made for the effective control of these foodborne pathogens.

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Conflict of interest

The authors declare that they have no conflict of interest.

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: 1350-1355.

Alaboudi, A. R., Malkawia, I. M., Osailib, T. M., Abu-Bashae, E.A. and Guitiand, J. (2020). Prevalence, antibiotic resistance and genotypes of *Campylobacter jejuni* and *Campylobacter coli* isolated from chickens in Irbid governorate, Jordan. International Journal of Food Microbiology. 327: 108-656

Baali, M., Lounis, M., Al Amir, H.L., Ayachi, A. Hakem, A. and Kassah-Laouar, A. (2020). Prevalence, seasonality and antimicrobial resistance of thermo tolerant *Campylobacter* isolated from broiler farms and slaughter houses in East Algeria. Veterinary World. 13: 1221-1228.

Barakat, A.M.A., Abd El-Razik, K.A., Elfadaly, H.A., Rabie, N.S., Sadek, S.A.S. and Almuzaini, A.M. (2020). Prevalence, molecular etection and virulence gene profiles of *Campylobacter* species in human and foods of animal origin. Veterinary World.13: 1430-1438.

Chen, H., Dai, Y., Chen, J., Zhang, Y., Zhan, L., Mei, L. and Wang, H. (2020). Epidemiological and whole genomic sequencing analysis of a *Campylobacter jejuni* outbreak in Zhejiang Province, China, May 2019. Foodborne Pathogens and Disease. 17(12): 1-7.

Duque, B., Canon, J., Haddad, N., Guillou, S. and Membre, J. (2021). Quantitative approach to assess the compliance to a performance objective (PO) of *Campylobacter jejuni* in poultry meat in France. International Journal of Food Microbiology. 336: 108-916.

Fitzgerald, C. and Nachamkin, I. (2007). Campylobacter and Arcobacter. In: Manual of Clinical Microbiology, [P.R. Murray, E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (ed.)], 9th ed. ASM Press, Washington, DC. 933-946.

Frosth, S., Karlsson-Lindsjo, O., Niazi, A., Fernstrom, L.L. and Hansson, I. (2020). Identification of transmission routes of Campylobacter and on farm measures to reduce Campylobacter in chicken. Pathogens. 9: 1-17.

Ghoneim, N.H., Abdel-Moein, K.A., Barakat, A.M.A., Hegazi,A.G., El-Hamid, K.A., El-Razik, A. and Sadek, S.A. (2020). Isolation and molecular characterization of *Campylobacter jejuni* from chicken and human stool samples in Egypt. Food Science and Technology. 1-8.

Gigliotti, D., Bianchi, M.D., Galleggiante, C.A, Barbaro, A., Adriano, D., Traversa, A., Cravero, D. and Decastelli, L. (2019). Prevalence and characterization of *Campylobacter* spp. in poultry meat: Results of a year surveillance in the North-West of Italy. EC Microbiology. 15(12): 136-142.

Greene, G., Koolman, L., Whyte, P., Lynch, H., Coffey, A., Lucey, B., Egan, J., O'Connor, L. and Bolton, D. (2020). An *in vitro* investigation of the survival and/or growth of *Campylobacter jejuni* in broiler digestate from different feed types. Letters in Applied Microbiology. 72: 36-40.

Hutchinson, D.N. and Bolton, F.J. (1984). Improved blood-free selective medium for the isolation of *Campylobacter jejuni* from fecal specimens. Journal of Clinical Pathology. 37: 956-957.

Kanaan, M. and Abdulwahid, M.T. (2019). Prevalence rate, antibiotic resistance and biotyping of thermotolerant *Campylobacter* isolated from poultry products vended in wasit markets. Current Research in Nutrition and Food Science. 7(3): 905-917.

Khoshbakht, R., Tabatabaei, M., Hosseinzadeh, S., Aski, H.S. and Seifi, S. (2015). Genetic characterization of *Campylobacter jejuni* and *C. coli* isolated from broilers using flaA PCR-restriction fragment length polymorphism method in Shiraz, Southern Iran. Jundishapur Journal of Microbiology. 8: e18573.

Kouglenou, S.D., Agbankpe, A.J., Dougnon, V., Djeuda, A.D., Deguenon, E., Hidjo, M., Moussa, L.B. and Bankole, H. (2020). Prevalence and susceptibility to antibiotics from *Campylobacter jejuni* and *Campylobacter coli* isolated from chicken meat in Southern Benin, West Africa. BMC Research Notes. 13(305): 1-6.

Lastovica, A.J. and Allos, M.B. (2008). Clinical Significance of *Campylobacter* and Related Species other than *Campylobacter jejuni* and *Campylobacter coli*. In: *Campylobacter*, 3rd edn, eds. [Nachamkin, I., Szymanski, C.M., Blaser, M.J.] Washington, D.C.: American Society for Microbiology.123-50.

Malik, H., Ashok Kumar, Rajagunalan, S., Kataria, J.L., Anjay and Sachan, S. (2014). Prevalence of *jejuni* and *Campylobacter coli* among broilers in Bareilly region. Veterinary World. 7(10): 784-787.

Man, S.M. (2011). The clinical importance of emerging *Campylobacter* species. Nat. Rev. Gastroenterol. Hepato. 8: 669-685.

Mikulic, M., Humski, A., Njari, B., Ostovic, M., Duvnjak, S. and Cvetnic, Z. (2016). Prevalence of thermotolerant *Campylobacter* spp. in chicken meat in croatia and multi locus sequence typing of a small subset of *Campylobacter jejuni* and *Campylobacter coli* isolates. Food Technology and Biotechnology. 54: 475-481.

Morris, G.K., Sherbeeny, M.R., Patton, C.M., Kodaka, H., Lombard, G.L.,Edmonds,P., Hollis D.G. and Brenner, D.J. (1985). Comparison of four hippurate hydrolysis methods for identification of thermophilic *Campylobacter* spp. Journal of Clinical Microbiology. 22: 714-718.

Moudgil, P. and Kumar, A. (2014). Prevalence and antibiotic resistance pattern of *Campylobacter* species in foods of animal origin. Veterinary World. 7(9): 681-684.

Nafarrate, I., Lasagabastera, A., Sevillanob, E. and Mateob, E. (2020). Prevalence, molecular typing and antimicrobial susceptibility of *Campylobacter* spp. isolates in northern Spain. Journal of Applied Microbiology. 130(4): 1368-1379.

Natsos, G., Koutoulis, K.C., Sossidou, E., Chemaly, M. and Mouttotou, N.K. (2016). *Campylobacter* spp. infection in humans and poultry. Journal of the Hellenic Veterinary Medical Society. 67: 65-82

Natsos, G., Mouttotou, N., Ahmad, S., Kamran, Z., Ioannidis, A. and Koutoulis, K. (2019). The genus *Campylobacter*: detection and isolation methods, species identification and typing techniques. Journal of the Hellenic Veterinary Medical Society. 70: 1327-1338.

Nicodeme, M.G., Rivoal, K., Houard,E. , Rose, V., Quesne,S., Mourand, G., Rouxel, S. , Kempf, I., Guillier, L., Gauchard F. and Chemaly, M. (2015). Prevalence and characterization of *Campylobacter jejuni* from chicken meat sold in French retail outlets. International Journal of Food Microbiology. 203: 8-14.

Oh E., Katelyn andrews, J. and Jeon, B. (2018). Enhanced biofilm formation by ferrous and ferric iron through oxidative stress in *Campylobacter jejuni.* Frontiers in Microbiology. 9(1204): 1-9.

Penner, J.L. (1988). The Genus *Campylobacter*: A Decade of progress. Clinical Microbiology Reviews. 1: 157-172.

Sharma, K., Chattopadhyay, U. K. and Naskar, K. (2016). Prevalence of *Campylobacter* species in raw meat samples sold in open markets of Kolkata city. International Journal of Agriculture Environment and Biotechnology. 9(4): 5-35.

Shekhar, C., Motina, E. and Kumar, S., (2010). Microbiological quality of raw milk and its public health significance. Journal of Dairying, Foods and Home Sciences. 29 (1): 15-18.

Sinulingga, T.S., Aziz, S.A., Bitrus, A.A., Zunita, Z. and Abu, J. (2019). Occurrence of *Campylobacter* species from broiler chickens and chicken meat in Malaysia. Tropical Animal Health and Production. 52: 151-157.

Soro, A.B., Whyte, P., Bolton, D.J. and Tiwari, B.K. (2020). Strategies and novel technologies to control *Campylobacter* in the poultry chain: A review. Comprehensive Reviews in Food Science and Food Safety. 19: 1353-1377.

Szczepanska, B. andrzejewska, M., Spica D., Klawe, J.J. (2017). Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from children and environmental sources in urban and suburban areas. BMC Microbiology. 17(80): 1-9.

WHO (2018). https://www.who.int/news-room/fact-sheets/detail/*Campylobacter*, 1-5.

Younis, G., Awad, A. and Khairy, M. (2018). Molecular Characterization and virulence of *Campylobacter jejuni* isolated from broiler chickens. International Journal of Poultry Science. 17: 499-506.

Zendehbad B., Khayatzadeh, J. and Alipour, A. (2015). Prevalence, seasonality and antibiotic susceptibility of *Campylobacter* spp. isolates of retail broiler meat in Iran. Food Control. 53: 41-45.