Egypt. J. Vet. Sci. **Vol. 55,** No. 6, pp. 1717-1724 (2024)

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Molecular Detection of Virulence Factor of *Campylobacter Jejuni* Isolated from Organs of Chickens, Ducks, and Pigeons from Different Egyptian Provinces

Azhar G. Shalaby*, Heba F. AbdelAziz and Mai M. Morsy

Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute (AHRI), Agriculture Research Centre (ARC), Dokki, Giza 12618, Egypt

Abstract

Campylobacter Jejuni is one of the Gram-negative bacteria causing worldwide foodborne illness, especially from poultry species. Human infection may be raised as a consumption of uncooked infected poultry. This study evaluates the occurrence of *Campylobacter jejuni* in different commercial poultry species (chickens, ducks, and pigeons) and evaluates its virulence. In this study, different organs were collected and pooled together (liver intestine) from chickens, ducks, and pigeons from different Egyptian provinces. The total isolation percentage for *Campylobacter* species was 26.4% and the highest percentage was from ducks 36% followed by chickens (25%) and pigeons (22.8%), but the majority of *Campylobacter Jejuni was detected in* Pigeon samples (75%), followed by chickens (60%) and ducks (33.3%). The existence of *cdt*A, *virB*11, and *aim*A. Virulence genes were present in 81%, 33%, and 43% of the total examined *C. jejuni* strains. This study provides evidence of different virulence mechanisms that have been hidden in different poultry species.

Keywords: *Campylobacter jejuni*- isolation- PCR- Virulence genes. Egyptian provinces

Introduction

Campylobacteriosis is a severe worldwide zoonotic disease, and the primary cause is *Campylobacter jejuni*. (*C. jejuni).* Poultry plays an important role in the transmission of infection to humans [1]. Avian species, specifically chickens, establish a significant reservoir for transmitting Campylobacter spp. due to the high temperature, which is the most suitable condition for pathogen growth. [2] *Campylobacter spp.* can colonize the chicken's ceca enormously; however, the chickens rarely express these signs compared to Campylobacteriosis in humans, which fatally affects [3] It is considered a global etiological agent for gastroenteritis and diarrhea. [4] About 85% of foodborne Campylobacter enteritis in humans is caused by the consumption of contaminated food by *C. jejun.i* [5]. *C. jejuni* is found mainly in the poultry gastrointestinal system and may be found in contaminated steps of poultry [6,7]. Many pathogenic mechanisms for Campylobacter species are still incompletely understood, even with a high recovery rate. [8] found that the putative virulence factors for invasion and adhesion, toxin production, and motility are believed to be significant virulence factors [9,10]. They also added that many genes have been linked to the virulence mechanism for Campylobacter bacteria, including human infection and colonization of chickens.

The survival and different virulence mechanisms of *C. jejuni* are mediated by motility, biofilm adhesion, and quorum sensing [11]. Campylobacter Toxin consists of many cytolethal distending toxin (CDT) toxin-producing gene elements, which are three subunits (*Cdt*A, B, and C). [12]. Also, the ability of Campylobacter to adhere to the cells and colonize the intestinal wall is done through the expression of the *vir*B11, *cad*F, *pld*A, *rac*R, and *dna*J, genes. In addition, the invasion factors are mediated by *ceu*E and *cia*B genes, [13]. recent virulence gene linked to Campylobacter invasiveness is the invasion-associated marker gene (*iam*) is listed by many authors [14]. Thus, the objective of this study detection of *C. jejuni* burden in poultry as a source of human infection.

*Corresponding author: Azhar G. Shalaby, E-mail: azhargaber0@gmail.com. Tel.: +201014078741
(Received 31/12/2023, accepted 28/02/2024)
DOI: 10.21608/EJVS.2024.259612.1760

Material and Methods

Samples collection

A total of 140 poultry flocks from seven Egyptian provinces (the samples were collected in late 2021 and during 2022) were examined for carrying *Campylobacter spp*. Five individual pooled samples from each flock represented one case. Different internal organs (liver and intestine) from each case were collected on transport media (Cary Blair medium, LAB). We suspend 15 gm of the media on 1 liter of distilled water and heat it until boils. Then dispensed into a final container and sterilized by autoclaving at 121°C for 15 min according to the instructions on the label. The poultry species were chickens, ducks, and pigeons of different ages. After sample collection, it was transferred to the Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP) and examined samples under complete aseptic conditions.

Isolation and identification

Isolation and identification of *Campylobacter* species were performed according to [15]. All inoculated plates were incubated under micro-aerophilic conditions (5% O_2, 10% CO_2, and 85% N2), using an anaerobic jar with gas pack kits (Oxoid)) at 42 °C for 48 hours then were shown daily for the characteristic colonies. Direct smears from a culture that had been in existence were examined using a phase contrast microscope to show the characteristic corkscrew-like motion that is unique to Campylobacter species. Different biochemical tests were performed as catalase production, oxidase activity, and sodium hippurate hydrolysis.

Molecular detection

DNA extraction was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH Catalogue no.51304). Oligonucleotide Primers were supplied from Metabion (Germany) and PCR condition in Table 2. Conventional PCR Technique was used for the detection of the common gene for *Campylobacter jejuni* and the detection of some virulence genes (*cdtA, iamA*- and *virB*).

PCR amplification

Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Emerald, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of DNA-RNA free water, and 6 µl of template. The reaction was performed in an Applied thermal cycler.

Analysis of the PCR Products

The obtained PCR products were separated by electrophoresis [1.5% agarose gel (Applichem, Germany, GmbH) in 500ml of (1x TBE buffer] at room temperature using gradients of 5V/cm. 18 µl of PCR products were loaded in each gel slot. Gene Ruler 100 bp Ladder (Thermo Scientific) was used to determine the fragment size. The gel was photographed by a gel documentation system (applied). and the data were finally analyzed with the help of proprietary software (automatic image capture software protein simple for merly Cell Bioscience, USA).

Results

The prevalence for total isolation of Campylobacter species (spp) was 26.4% from different poultry sources, 25%, 36%, and 22.8% from chickens, ducks, and pigeons respectively. A Hippurate test was done to confirmation of the presence of *C. jejuni.* Which gave the violet color as the positive result Figure (1).

The incidence for total percentage of isolation of *C. jejuni* was 56.7% (21/37) from total *Campylobacter spp.* And it was 60%, 33.3%, and 75% chickens, ducks, and pigeons respectively as in Table 3 and Figure 2.

We noticed that some Egyptian governorates have a high percentage of *C. jejuni,* 25% of the total isolated *C. jejuni* was from Dakhlia followed by Sharkia 24%, and the absence of *C. jejuni* was observed in Menia and Kafr el Sheikh. As discussed in Table 4.

PCR results

All the positive isolates of *Campylobacter Spp* were confirmed by 23SRNA, then using mapA gene to detect the *C. jejuni* using conventional PCR. Also, PCR was used to confirm the existence of virulence genes, *cdtA, virBll,* and *aimA*. Total of 81% of poultry isolates were positive for *cdtA* gene, 43% for *virB11* while *iam* genes showed the lowest percentage of 33%. as shown in Table 5.

Discussion

Poultry meat is the main source of protein, even though it is also responsible for about 80% of human cases of foodborne and zoonotic diseases [13] Poultry is considered the chief reservoir of many bacterial infections, including Campylobacter [20]. *C. jejuni* is a Gram-negative bacterium that is considered a prominent cause of foodborne disorders worldwide [21]. Campylobacter contamination may occur during poultry plant processing as a source of contamination for humans [3]. The high occurrence of Campylobacter spp., including *C. jejuni,* which mainly invades poultry species has been conveyed as the ability to violent fowl's intestinal tracts and multiply inside them, owing to their warm-blooded body nature. In addition, *Campylobacter* contamination usually affects production [22, 23]. In this study, the prevalence of *Campylobacter* isolation was 26.4%, while C. *jejuni* was 56.7% of total isolated *Campylobacter* species, also detected

different percentages in chickens, ducks, and pigeons which were 25%, 36%, and 22.8% respectively as shown in Table 3 and Fig. 2. Many studies reported different isolation rates for Campylobacter spp. For Example, [24] mentioned that the higher C. *jejuni* percentage was 89%, while reached 94.1% [25], Other studies identified different lower percentages for Campylobacter isolation in different areas around the globe, such as in Sri Lanka [26] and in the Netherlands [27] Furthermore, many researchers as [28], noticed a lower C. *jejuni* percentage that was 11% in 2019 while, [29] the percentage was 20% by [29], but the percentage nearly reached 15% in the study of [30], our study illustrated that the most frequently *C. jejuni* was isolated from pigeons (75%), followed by chickens (60%), and lastly in ducks (33.3%), as mentioned in Tables 3 and 4, Fig. 2. Other study was agreed with the current work as [28] noted that the prevalence of *C. jejuni* in pigeons (16%) was higher than that in chickens (15.5%). Another study [29] detected the most frequently occurrence of *C. jejuni* was in ducks (26.67%), followed by chickens (20%) [30]. The current stdy was contrary to [31] that identified *the C. jejuni* in pigeon droplets with a percentage of (8.89%) and confirmed that lower than in chicken. Additionally, different studies in Canada reported lower *C. jejuni* species in pigeons (9.1%) [32]. Recent studies like [33] and [34] reported lower detection rates in pigeons than in chickens.

The genes involved in invasion and toxin production were less prevalent in *C. jejuni* strains specially from duck meat compared to chicken and the cytolethal distending toxin (CDT) is encoded by cdtA [35]. in Korea, a study has displayed that the prevalence of the virulence genes for *C. jejuni* from poultry was relatively lower than that isolated from duck meat [29]. In addition, the existence of different virulence genes. One of those genes was *cdt*A, which has been recorded in a total percentage of 81%. Our results were nearly like to [39, 40, 13 and 41] which recorded the high percentage of the *cdt*A gene (85%) in chicken *C. jejuni* isolates. but our results were in Contrary to [42] that found a low occurrence of *cdtA* gene in chickens with a percentage of 26.9%. In this study, the total detection of the *virB* gene was detected in different percentages as it appeared as 43% total percentage and its percentages were 58.3%, and 33.3% in chickens and pigeons respectively. While it was absent in ducks. many authors detected a much higher percentage (74.07%) in chickens [39]. nevertheless, the existence of the *virB* gene was detected in a lower percentage than that of the present study as in 2008 some authors as [14] confirmed the occurrence of *virB* gene with a rate of 18.5%. also, the percentage reached 5% in [43]. Our results conflicted with those of another study in Egypt conducted by [44], that recorded (77.78%) detection rate for virB11 gene in chickens, and (100%) in ducks but was absent in pigeons.

The genetic indicator *iam* has been related to the adherence and invasion of *Campylobacter* [45, 46]. there was a significant link between clinical signs, such as diarrhea, and the isolation rate of *Campylobacter* strains that invade and adhere cells. Nevertheless, the contribution of the *iam* gene to the progression of Campylobacteriosis has not been fully explained [47, 48]. in this study the total detection rate for the *iam* gene was 33%. But it was 25%, 33.3%, and 50% in chickens, ducks, and pigeons respectively. other studies revealed higher percentages of 53.8% and 66% [49, 14], Conversely, [50] mentioned the incidence of the *iam* gene was (4%) regardless of the source of isolation. Also, in Poland [51] and Canada [52] many studies were completed and clarified that the *iam* locus gene has been detected in 54.7% and 57.1% of *C. jejuni* chicken isolates, respectively, and those results appeared higher detection rate than our results (25%) for chicken. It was clear that the present data showed that pigeons have a higher percentage of some of virulence genes such as *cdtA* and *iamA* than other species like chickens and ducks. According to [41]. The occurrence of the virulence sign is not dependent only on the isolated species but also on the source of isolation.

Conclusion

The highest percentage (56.7%) of the *campylobacter* isolates were identified as *C. jejuni* among the different examined species of poultry. *C. jejuni* isolates in the current study were detected as potentially pathogenic due to the presence of several virulence genes in a high proportion. The isolates had the probability of producing a cytolethal effect from their toxin production due to the high presence of the *cdt*A gene. Correspondingly, we distinguished between different virulence factors such as adherence and invasion.

Acknowledgment

Non to declare

Conflicts of interest

The authors declared no competing interests.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

TABLE 1. The sources of examined poultry flocks for Campylobacter.

Gov./ poultry Spp	Chickens	Ducks	Pigeons	Total
Giza	16	7	9	32
Qalubia	15	2	6	23
Sharkia	17	4	8	29
Dakhlia	9	3	4	16
Benisueif	7	2	3	12
Menia	5	3	2	10
Gharbia	8	2	1	11
Kafr el sheikh	3	2	2	7
Total	80	25	35	140

TABLE 2. The primers used in this study regarding the amplicon size and references.

Gene	Primers	Oligonucleotide sequence (5'→3')	Amplicon size (bp)	Annealing temp.	References
23*SRNA*	F R	TATACCGGTAAGGAGTGCTGGAG ATCAATTAACCTTCGAGCACCG	650	59°C	**[16]**
***map*A**	MapAF MapAR	CTATTTTATTTTTGAGTGCTTGTG GCTTTATTTGCCATTTGTTTTATTA	589	55°C	**[17]**
***cdt*A**	GNW IVH	GGAAATTGGATTTGGGGCTATACT ATCACAAGGATAATGGACAAT	165	42°C	**[18]**
Iam	IAMF IAMR	GGAAATTGGATTTGGGGCTATACT ATCACAAGGATAATGGACAAT	518	52°C	
***virB*11**	VirBF VirBR	GAACAGGAAGTGGAAAAACTAGC TTCCGCATTGGGCTATATG	708	50°C	**[19]**

TABLE 3. Campylobacter species and *C. jejuni* prevalence from different poultry species

Egyptian Gov.	Chickens			Ducks			Pigeons			Total	
	No	Campylobacter species	*C. jejuni*	No	Campylobacter species	*C. jejuni*	No	Campylobacter species	*C. jejuni*	Campylobacter species	*C. jejuni*
Giza	16	4	2	7	2	1	9	2	2	8	5
Qalubia	15	2	2	2	1	0	6	1	0	4	2
Sharkia	17	4	4	4	1	1	8	3	2	8	7
Dakhlia	9	3	1	3	2	1	4	2	2	7	4
Beni Sueif	7	2	2	2	1	0	3	0	0	3	2
Menia	5	2	0	3	2	0	2	0	0	4	0
Gharbia	8	3	1	2	0	0	1	0	0	3	1
Kafr el sheikh	3	0	0	2	0	0	2	0	0	0	0
Total examined	80	20/80* 25%	12/20** 60%	25	9/25* 36%	3/9** 33.3%	35	8/35* 22.8%	6/8** 75%	37/140* 26.4%	21/37** 56.7%

. *The total percentage was calculated according to the total number of examined species from all Egyptian governorates.
** The total percentage was calculated according to the total numbers of the isolated Campylobacter spp.

TABLE 4. Percentage for *C. jejuni* in each Egyptian Governorate regardless of poultry species

Gov.	Total examined cases	*Campylobacter* species	*C. jejuni*	% of *C. jejuni* /Gov.
Giza	32	8	5	15.6
Qalubia	23	4	2	8.7
Sharkia	29	8	7	24
Dakhlia	16	7	4	25
Beni Sueif	12	3	2	16.7
Menia	10	4	0	0
Gharbia	11	3	1	9
Kafr el sheikh	7	0	0	0
Total	140	37	21	56.7

TABLE 5. Virulence genes for *C. jejuni* strains

Species/gene	Chickens (N/12)	Ducks (N/3)	Pigeons (N/6)	Total% (21)
***cdt*A**	9 (75%)	2 (66.7%)	5 (83.3%)	17(81%)
***iam*A**	3 (25%)	1 (33.3%)	3 (50%)	7(33%)
***virB*11**	7 (58.3%)	0 (0%)	2 (33.3%)	9(43%)

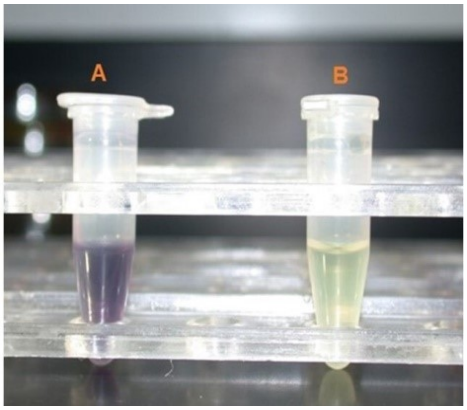

Fig. 1. Hippurate test for identification of *C. jejuni* A: the violet color indicates the presence of *C. jejuni.* B: The colorless indicates the absence of *C. jejuni*

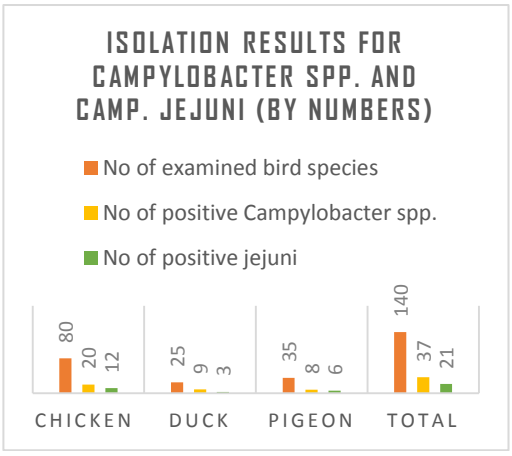

Fig. 2. Prevalence of Campylobacter species and *C. jejuni* expressed by numbers

References

1. Elmali, M. and Can, H.Y. Antimicrobial susceptibility and virulence- associated genes in *Campylobacter* isolates from milk and wastewater in Hatay, Turkey. *Ciência Rural.,* **49**(5),1-8 (2019). doi: 10.1590/0034-7167-2018-0227

2. Sibanda, N., McKenna, A., Richmond, A., Ricke, S.C., Callaway, T., Stratakos, A.C., Gundogdu, O. and Corcionivoschi, N.A. Review of the Effect of Management Practices on Campylobacter Prevalence in Poultry Farms. *Front. Microbiol.,* **9**, 1–9(2018).

3. Hermans, D., Van Deun, K., Martel, A., Van Immerseel, F., Messens, W., Heyndrickx, M., Haesebrouck, F. and Pasmans F. Colonization factors of Campylobacter jejuni in the chicken gut.*Veter. Res.,* **42**, 82(2011).

4. Kirk. M.D., Pires, S.M., Black, R.E., Caipo, M., Crump, J.A., Devlees-schauwer, B., Dopfer, D., Fazil, A., Fischer-Walker, C.L. and Hald, T. World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases. *PLoS Med.,* **2015**, 12(2010). doi: 10.1371/journal.pmed.1001921,

5. Wagenaar, J.A., French, N.P. and Havelaar, A.H. Preventing *Campylobacter* at the source: Why is it so difficult? *Clin Infect. Dis.,* **57**(11),1600–1606(2013).

6. Beiranvand, S. Piri-Gharaghie, Dehganzad, B., Khedmati, F, Jalali, F, Alizadeh, AM. and Momtaz, H. Novel NAD-independent Avibacterium paragallinarum: Isolation, characterization and molecular Identification in Iran. *Veterinary Medicine and Science*, **8**,1157–1165 (2022).

7. De Melo, F. P, da Silva, P.O., dos Santos Clemente, S.M, de Melo R P da Silva J.G, Júnior, J W, Fonseca, B.B., Mendonça, M/ and Barros, M.R. Detection of Campylobacter jejuni, Campylobacter coli, and virulence genes in poultry products marketed in Northeastern. *Brazil. Research, Society and Development.,* **10(**10),(2021). DOI: http://dx.doi.org/10.33448/rsd-v10i10.19224.,

8. Wilson, D.L., Rathinam, V.A., Qi W., Wick, L.M., Landgraf, J. and Bell, J.A. Genetic diversity in *Campylobacter jejuni* is associated with differential colonization of broiler chickens and C57BL/6J IL10-deficient mice. *Microbiology,* **156**(7), 2046–2057, (2010).

9. Younis, G., Awad, A., Khairy, M. Molecular characterization and virulence of *Campylobacter jejuni* isolated from broiler chickens. *Int. J. Poult. Sci.,* **17**(10),499–506. (2018).

10. Kalantar, M., Dallal, M., Fallah, F. and Yektaie, F. Monitoring the Virulence Genes in Campylobacter coli Strains Isolated from Chicken Meat in Tehran, *Iran. Infect Epidemiol Med.,* **3**(1), 12-15(2017). DOI: 10.18869/modares.iem.3.1.1.2,

11. Li, Y., Zhou, G., Gao, P., Gu, Y., Wang, H., Zhang, S., Zhang,Y., Wang, Y., Jing, H., He ,C.. and Zhen, G. Gastroenteritis outbreak caused by Campylobacter jejuni - Beijing, china. *China CDC Wkly*, **2**(23), 422–425 (2020).

12. Lara-Tejero, M. and Galan, J.E. A bacterial toxin that controls cell cycle progression as a deoxyribonuclease i-like protein. *Science,* **290**, 354–357(2000).

13. Gharbi, M., Béjaoui, A. and Ben Hamda, C. Distribution of virulence and antibiotic resistance genes in Campylobacter jejuni and Campylobacter coli isolated from broiler chickens in Tunisia. https://doi.org/10.1016/j.jmii.2021.07.001 2021).

14. Wieczorek, K. and Osek, J. Identifcation of virulence g,enes in Campylobacter jejuni and C. coli isolates by PCR. Bull. *Vet. Inst. Pulawy,* **52**,211–216(2008).

15. ISO 10272-1. Microbiology of food and animal feeding stuffs; Horizontal method for the detection of thermotolerant *Campylobacter*. International Standards Organization, Geneva, (2006).

16. Wang, G., Clark, C.G., Taylor, T.M., Pucknell, C., Barton, C., Price, L., Woodward, D.L.and Rodgers, F.G. Colony Multiplex PCR Assay for Identification and Differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *Fetus. J. of Clin. MicrobiI.*, **40,** 4744–4747(2002).

17. Stuckiv, U., Frey, J., Nicolet, J. and Burnens, A.P. Identification of *Campylobacter jejuni* on the basis of a species-specific gene that encodes a membrane protein. *J. Clin. Microbiol.,* **33**, 855-859(1995).

18. Rapabelli, G., Tamburro, M., Minelli, F .and Leone, A., Sammarco, M.L. Prevalence of virulence-associated genes and cytolethal distending toxin production in *Campylobacter* spp. isolated in Italy. *Comp. Immunol. Microbiol Infect. Dis.,* **33**, 355–364(2010).

19. Bacon, D.J,Alm, R.A., Burr, D.H., Kopecko, D.J., Ewing, C.P., Trust T.J. and Guerry, P.. Involvement of a Plasmid in Virulence of *Campylobacter jejuni* 81-176. *Infect. Immun*., **68** (8), 4384– 4390 (2000).

20. Mileng, K., Ramatla, T.A., Ndou, R.V., Thekisoe, O.M.M., Syakalima, M. Isolation and antibiotic sensitivity of Campylobacter species from fecal samples of broiler chickens in North West Province, South Africa," *Veterinary World*, **14**(11) 2929–2935. (2021).

21. Meurer, L., Payne, W. and Guffey, J.S. Visible light as an inhibitor of *Campylobacter jejuni*. *Int. J. Antimicrob. Agent.* **55**, 105818(2020).

22. Gianatale, E.D., Calistri, P., Donato, G.D., Dectelli, L., Goffredo, E. and Adriano, D., Thermotolerant spp. in chicken and bovine meat in Italy: prevalence, level of contamination and molecular characterization of isolates. *PLoS One* **14**, 0225957(2019). DOI: 10.1371/journal.pone.022 5957.

23. Tang. Y., Jiang. Q., Tang. H., Wang. Z., Yin, Y. and Ren, F. Characterization and prevalence of Campylobacter spp. from broiler chicken rearing period to the slaughtering process in Eastern China. *Front. Vet. Sci*., **7**,227. doi: 10.3389/fvets.2020.00227., (2020).

24. Rama, E. N., Bailey, M., Jones, D. R, Gast, R.K., Anderson, K. and Brar, J. Prevalence, persistence, and antimicrobial resistance of Campylobacter spp. from eggs and laying hens housed in five commercial housing systems. *Foodborne Pathog. Dis.,* **15**, 506–516(2018). doi: 10.1089/fpd.2017.2404.

25. Andrzejewskan, M., Grudlewska-Buda, K., S´pica1 D., SkowronK, C´ wiklin M´,skaJurkowska Szady-Grad M., Indykiewicz, P, Wiktorczyk-Kapischke, N and Klawe, J.J. Genetic relatedness, virulence, and drug susceptibility of Campylobacter isolated from water and Front. *Cell. Infect. Microb*., **12** (2022). Sec. Virus and Host https://doi.org/10.3389/fcimb.2022.1005085.,

26. Kalupahana, R.S., Kottawatta, K.S.A., Kanankege, K.S.T., Bergen, M. A. P., van Abeynayake, P., and Wagenaar, J.A. Colonization of Campylobacter spp. in broiler chickens and laying hens reared in tropical climates with low Biosecurity housing. *Appl. Environ. Microbiol.,* **79**,393–395(2013). doi: 10.1128/AEM. 02269-12.,

27. Schets, F.M., Jacobs-Reitsma,, W. F., van der Plaats R.Q. J, Heer, L. K. D., van Hoek, A.H.A.M. and Hamidjaja, R.A. Prevalence and types of Campylobacter on poultry farms and in their direct environment. *J. Water Health* **15**, 849–862(2017). doi: 10.2166/wh.2017.119.

28-. Abd El-Hamid, M.I., Abd El-Aziz, N.K, El-Sayed, M.S.Y, Etab, ME and Abo Remela, M.M Genetic Diversity of *Campylobacter jejuni* Isolated From Avian and Human Sources in Egypt Front. *Microbiol., Sec Food Microbiology,* **10** (2019). | http//doi.org/10.3389/fmicb.2019.02353.

29. Kim, J., Park, H. and Kim, J. Comparative Analysis of Aerotolerance, Antibiotic Resistance, and Virulence Gene Prevalence in Campylobacter jejuni Isolates from Retail Raw Chicken and Duck Meat in South Korea. *Microorganisms,* **7**(10), 433(2019). https://doi.org/10.3390/microorganisms7100433.,

30. Ghoneim, N., Abdel-Moein, K and Barakat, A. Isolation and molecular characterization of Campylobacter jejuni from chicken and human stool samples in Egypt. *Food Science and Technology*, **41**(1),195–202 (2020).

31. Ahmed, H, El Hofy, F. I., Ammar, A. M., Abd El Tawab, A. A. and Hefny, A.A. ERIC-PCR genotyping of some *Campylobacter jejuni* isolates of chicken and human origin in Egypt. *Vector Borne Zoonotic Dis.,* **15** 713–717(2015). https://doi.org/10.1089/vbz.2015.1836.,

32. Gabriele-Rivet, V., Fairbrother, J.H., Tremblay, D., Harel, J., Côté N .and Arsenault, J. Prevalence and risk factorsfor *Campylobacter* spp., *Salmonella* spp., *Coxiella burnetii*, and Newcastle disease virus in feral pigeons (Columba livia) in public areas of Montreal, Canada. *Can. J. Vet. Res.,* **80**, 81–85(2016).

33.Sayed, A.S.M., Ibrahim, A.I., Sobhy, M.S., Elmahallawy, E.K., Alsowayeh, N., Alarjani, K.M., El-khadragyn, M.F and Youseef, A.G. Circulation of thermophilic Campylobacter in pigeons, turkeys, and humans at live bird markets in Egypt *Front. Vet. Sci.,* **10**,1150077(2023). doi.org/10.3389/fvets.2023.1150077.

34. Yang, H., Li, Y., Zhangn, Y., Dong, B., Duan, B., Guo, L., Wang,T., Xiaoling ,L.V., Zheng, M., Cui, X. and Bai ,R. Prevalence, drug resistance spectrum and virulence gene analysis of Campylobacter jejuni in broiler farms in central Shanxi, *China Poultry Science* **102**, 102419(2023). doi.org/10.1016/j.psj.2022.102419,

35. Pickett, C. L. and Whitehouse, C. A. The cytolethal distending toxin family. *Trends Microbiol.,* **7,** 292–297(1999). doi: 10.1016/S0966-842X (99)01537-1,

36. Martinez, I., Mateo, E., Churruca, E., Girbau, C., Alonso, R. and Fernández- Astorga, A. Detection of cdtA, cdtB, and cdtC genes in Campylobacter jejuni by multiplex PCR. *Int. J. Med. Microbiol.,* **296**, 45–49, (2006).

37. Kabir, S.M.L., Kikuchi, K. and Asakura, M. Evaluation of a cytolethal distending toxin (cdt) gene-based species-specific multiplex PCR assay for the identification of Campylobacter strains isolated from diarrheal patients in Japan. *Japanese J. of Infectious Diseases,* **64**(1), 19–27 (2011).

38. Bissong, M. E.A and Ateba, C.N. Detection of virulent thermophilic Campylobacter species in communal chickens. *South African Journal of Science,* **115**(10), 1–5 (2019).

39. Krutkiewicz, A. and Klimuszko, D. Genotyping and PCR detection of potential virulence genes in Campylobacter jejuni and Campylobacter coli isolated from different sources in Poland. *Folia Microbiologica*, **55**(2), 167–175(2010).

40. Wieczorek, K, Wolkowicz, T and Osek, J. Antimicrobial Resistance and Virulence-Associated Traits of *Campylobacter jejuni* Isolated from Poultry Food Chain and Humans with Diarrhea. *Front. Microbiol., Sec. Food Microbiology*, **9**, 1508(2018). https://doi.org/10.3389/fmicb.2018.01508,

41. Sierra–Arguello, Y.M., Perdoncini, G., Rodrigues, L.B., dos Santos, L.R., Borges, K.A., Furian, T.Q., Salle, C.T.P., Moraes, H.L.S. and Nascimento, V.P. Identification of pathogenic genes in Campylobacter jejuni isolated from broiler carcasses and broiler slaughterhouses. *Sci. Rep.,* **11**, 4588 (2021).

42. Ramatla, T., Mileng, K., Ndou, R., Tawana,, M., Mofokeng, L., Syakalima, M., Lekota, E.K. and Thekisoe, O. Campylobacter jejuni from Slaughter Age Broiler Chickens: Genetic Characterization, Virulence, and Antimicrobial Resistance Genes. *Inter. J. of Article*, I, 1713213,13pages (2022). https://doi.org/10.1155/2022/1713213.

43. Mizel, S. B. and Bates, J. T. Flagellin as an adjuvant: Cellular mechanisms and potential. *J. Immunol*., **185**, 5677–5682 (2010).

44. Abdallah, M., Abaza, M.A., Fathy, R.R., Youseef, A.G., Sobhy, M., Abd Elhamid, H.S. and Ahmed W. Detection of Some Virulence and Antibiotic Resistance Genes in Campylobacter jejuni isolated from Poultry and Human the Egyptian. *Journal of Hospital Mede* **89** (2), 6373:6381, (2022).

45. Carvalho, A.C., Ruiz-Palacios, GM, Ramos-Cervantes, P., Cervantes, L.E., Jiang, X. and Pickering, L.K. Molecular characterization of invasive and non-invasive Campylobacter jejuni and Campylobacter coli isolates. *J. Clin. Microbiol.,* **39**, 1353–1359 (2001).

46. Wysok, B., Wojtacka, J. and Kivistö, R. Pathogenicity of Campylobacter strains of poultry and human origin from Poland. *Int. J. Food Microbiol.,* **334**, 108830, (2020).

47. Koga, M. Campylobacter gene polymorphism as a determinant of clinical features of Guillain-Barré syndrome. *Neurol*., **65**, 1376–1381 (2005).

48.. Rizal, A., Kumar, A. and Vidyarthi, A. S. Prevalence of pathogenic genes in Campylobacter jejuni isolated from poultry and human. *Int. J. Food Saf*., **12**,29–34 (2010).

49. Rozynek, E. Prevalence of potential virulence markers in Polish Campylobacter jejuni and Campylobacter coli isolates obtained from hospitalized children and from chicken carcasses. *J. Med. Microbiol*., **54**,615–619, (2005).

50. Lima, L.M., Perdoncini, G., Borges, K.A., Furian T.Q., Salle C.T.P., Moraes H.L.S. and do Nascimento, V.P. Prevalence and distribution of pathogenic genes in Campylobacter jejuni isolated from poultry and human sources. *J. Infect. Dev. Ctries* **16**(9),1466-1472 (2022).

51. Melo, R.T., Nalevaiko, P.C., Mendonca, E.P., Borges, L.W., Fonseca, B.B., Beletti, M.E. and Rossi, D.A. *Campylobacter* jejuni strains isolated from chicken meat harbor several virulence factors and represent a potential risk to humans. *Food Control*, **33,** 227–231 (2013).

52. Oh, E., McMullen, L.M., Chui, L. and Jeon, B. Differential survival of hyper-aerotolerant *Campylobacter jejuni* under different gas conditions. *Front. Microbiol.* **8**, 954. 36 (2017).

الكشف الجزيئي لعوامل الضراوة لبكتيريا *Campylobacter jejuni* المعزولة من أعضاء الدجاج والبط والحمام من مختلف محافظات مصر

أزهار جابر شلبى*، هبة فاروق عبد العزيز و مي محمود مرسى

المعمل المرجعى للرقابة البيطرية على الانتاج الداجنى - معهد بحوث الصحة الحيوانية - مركز البحوث الزراعية – القاهرة - مصر.

الكامبيلو باكتر جوجينای (Campylobacter jejuni) هي إحدى البكتيريا سالبة الجرام المسببة للأمراض المنقتلة عن طريق الغذاء في جميع أنحاء العالم، وخاصة في أنواع الدواجن. هدفت هذه الدراسة إلى تقييم تواجد بكتيريا Campylobacter jejuni في أنواع الدواجن التجارية المختلفة وتقييم مدى ضراوتها. في هذه الدراسة تم جمع أعضاء مختلفة (أمعاء الكبد) من الدجاج والبط والحمام من مختلف المحافظات المصرية. بلغت نسبة العزل الكلية لعزل الميكروب 26.4% وكانت أعلى نسبة في البط 36% يليها الدجاج (25%) والحمام (22.8%)، ولكن أغلبية بكتيريا Campylobacter jejuni تم اكتشافها في عينات الحمام (75%)، يليها بنسبة الدجاج (60%) والبط (33.3%). وجود cdtA، virB11، و aimAكانت جينات الضؤاوة موجودة في 81%، 33%، و43% من إجمالي سلالات *C. jejuni* التي تم فحصها. تقدم هذه الدراسة دليلاً على أليات الضراوة المختلفة التي تم اكتشافها في أنواع الدواجن المختلفة.

الكلمات الدالة : الكامبيلوباكتر جوجينای - العزل - اختبار الPCR - جينات الضراوة - المحافظات المصرية.