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Isolation and molecular characterization of *Campylobacter jejuni* from chicken and human stool samples in Egypt

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Abstract

Two hundred broiler chicken samples, 160 laying chicken samples and 75 human stool samples were collected in Egypt. The samples were microbiologically examined, and the *C. jejuni* isolates were confirmed biochemically and by PCR targeting the *mapA* gene of *C. jejuni*. The prevalence of the *cadF* virulence gene was then determined using PCR. A total of 17.33%, 17% and 11.87% of human stool, broiler chicken and laying chicken samples, respectively, were positive for *C. jejuni*, with a total of 66 *Campylobacter jejuni* isolates (15.17%). Ten *C. jejuni* isolates (15.15%) carried the *cadF* virulence gene (7.69%, 20.58% and 10.52% of human stool samples, broiler and laying chicken samples respectively). Phylogenetic investigation demonstrated that two of the isolates from chicken had high homology with other *C. jejuni* isolates from human stool samples. Moreover, amino acid sequence alignment revealed a mutation in these isolates of zoonotic significance. The present results support the possible risk of transmitting highly virulent *C. jejuni* as a foodborne pathogen from both broiler and layer chickens to human in Egypt. 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 this foodborne disease.

Keywords: *C. jejuni*; chicken; human; PCR; *cadF* gene; Egypt.

Practical Application: This study support the high risk of transmitting *C. jejuni* as a foodborne pathogen from both broiler and layer chickens due to the high rates of virulent *C. jejuni* isolation from different chicken samples, as expressed by the percentage of virulent *C. jejuni* isolates isolated from human stool specimens. Our study showed the key of control of this pathogen outbreaks in Egypt through an effective biosecurity measures on chicken farms and more hygiene in slaughter houses and local chicken slaughter shops.

1 Introduction

Campylobacteriosis is one of the most well-characterized bacterial foodborne infections worldwide that arises chiefly due to the consumption of poultry and poultry products (Wieczorek et al., 2012). The disease is caused by numerous species within the genus *Campylobacter*, but *Campylobacter jejuni* is the most commonly isolated species from established cases of human campylobacteriosis (Eurosurveillance Editorial Team, 2012).

Campylobacteriosis causes countless outbreaks and hence a high frequency of hospitalization, occasionally causing death (Taylor et al., 2013).

Campylobacter species are the fundamental cause of the bacterial gastrointestinal malady campylobacteriosis, which causes diarrhoea, dysentery patterns, cramps, pain and fever in developing countries (European Food Safety Authority, 2010).

Foodborne illnesses are largely caused by bacteria, most notably *Campylobacter jejuni*, which accounts for 77.3% of foodborne illnesses (Doyle & Erickson, 2006).

Foodborne *Campylobacter* infections occur as a result of the consumption of undercooked or raw poultry, liver or grilled chicken meat (Edwards et al., 2014). This may be due to the thermophilic properties of *Campylobacter* species, especially *C. jejuni*, which favours poultry hosts due to their high body temperatures (Verwoerd, 2000).

The intestinal tract of chickens, particularly the caecum and colon, is considered a region of tropism for a vast number of *Campylobacter* species during processing, especially if the intestinal tract is ruptured and the contents are moved to the skin, prompting further pollution to the carcass (Vinueza-Burgos et al., 2017). Likewise, contact with faecal material on eggs results in egg contamination and the transmission of the bacteria to the inside of the egg, thus initiating the disease after consumption of eggs (Adesiyun et al., 2005).

Campylobacter species are considered the second driving aetiology of paediatric diarrhoea (Rao et al., 2001). In Egypt, it is an endemic disease, and the evaluated percentage of infections

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in children who are in direct contact with diseased poultry is 12.3% for *C. jejuni* (El-Tras et al., 2015).

The bacterial isolation and identification of *Campylobacter* is considered the gold standard for disease identification; however, it is tedious and laborious due to the complex nature of *Campylobacter* (Li et al., 2014). Thus, molecular techniques, such as polymerase chain reaction (PCR) and sequencing, can permit the simple, fast and exact identification of *C. jejuni* and reveals its epidemiological characteristics (Miller et al., 2010).

The disease seriousness relies upon the virulence of the strain and on the host's immune state (Younis et al., 2018). *CadF* is one of the reference virulence genes that encodes proteins involved in the attack and attachment of *C. jejuni* (Elmali & Can, 2019), and this gene is present at a high prevalence in *C. jejuni* isolates (Andrzejewska et al., 2015).

Thus, this study evaluates the prevalence, virulence gene profile, and molecular and phylogenetic characterization of *Campylobacter jejuni* isolated from chickens and humans from different governorates in Egypt.

2 Materials and methods

2.1 Sampling

A total of 435 samples (Table 1) were used in the present investigation and were gathered between June 2015 and December 2016 from governorates in Egypt (Cairo, Giza, Fayoum, Minya and Qalubia). Additionally, human stool specimens (n = 75) were collected at random from people with diarrhoea who were admitted to different laboratories, people in contact with backyard chickens and slaughterhouses and from diarrheic children admitted to Abul-Riesh hospital for kids in Egypt. Ten grams of each sample (chicken intestine, liver, meat, egg shell swab, cloacal swab, and human stool) was collected in a sterile sample collection vial and transported to the laboratory. All samples were quickly placed at 4 °C and handled to isolate *Campylobacter* species.

All parts of this study were approved by the Medical Research Ethics Committee, National Research Centre, Giza, Egypt, under registration number 16220.

2.2 Isolation and identification of Campylobacter jejuni

C. jejuni was isolated from the inspected samples as recently described (Penner, 1988). Briefly, 10 grams of each sample (meat, liver, intestinal content, inner egg content and stool) were homogenized in sterile thioglycollate broth. Cloacal swabs and swabs from the external egg shell were incubated in tubes containing sterile thioglycollate broth. Broth samples were incubated at 42 °C for 48 hours in a microaerophilic atmosphere (10% CO_2, 5% O_2 and 85% N_2). A loopful of enrichment broth was streaked onto mCCDA plates (Oxoid) and incubated under microaerophilic conditions at 42 °C for 48 hours (Persson & Olsen, 2005). The colonies were then subjected to microscopic examination for the identification of *C. jejuni* utilizing phase contrast microscopy after a seagull appearance was observed with Gram staining (Vandamme et al., 2008).

Refined colonies were used in the biochemical identification of *C. jejuni* as previously described (Frost et al., 1998). The recognized colonies were stored at -70 °C in thioglycolate broth containing 15% glycerol for further validation using molecular methods (Sheppard et al., 2009).

2.3 Molecular identification

The extraction of DNA from *C. jejuni* isolates was completed utilizing DNA extraction kits (GF-1, Vivantis, Selangor, Malaysia) as indicated by the producer's guidelines.

Polymerase chain reaction (mapA gene)

The amplification of the *mapA* gene for *C. jejuni* was carried out on 10 representative isolates that were biochemically confirmed utilizing the primers listed in Table 2. Amplification conditions were as follows: 6 minutes at 94 °C; 35 cycles of 50 seconds at 94 °C, 40 seconds at 57 °C, and 50 seconds at 72 °C; and a final extension of 3 minutes at 72 °C. The PCR products were analysed using 1.5% agarose gel electrophoresis and inspected with a UV transilluminator (Figure 1).

Virulence gene characterization of C. jejuni isolates

The biochemically confirmed *C. jejuni* isolates were characterized for in vitro recognition of the *cadF* virulence gene by PCR (Konkel et al., 1999) utilizing the primers listed in Table 2.

Table 1. Numbers and types of collected samples.

Sample type	Broiler (n = 200)			Layer (n = 160)				Human stool	Total
	Intestine	Liver	Meat	Intestine	Cloacal swabs	Egg			
						Inner content	Outer swabs		
Number	90	60	50	40	40	40	40	75	435

Table 2. Primer sets for PCR amplification of the *C. jejuni* genes.

Primer sequences (5′ to 3′)	Annealing temperature (°C)	PCR product (bp)	Targeted gene	Reference
F (5`- CTA TTT TAT TTT TGA GTG CTT GTG) R (5 `-GCT TTA TTT GCC ATT TGT TTT ATTA)	57	589	*mapA*	Shin & Lee (2009)
F (5`- TTG AAG GTA ATT TAG ATA TG) R (5 `-CTA ATA CCT AAA GTT GAA AC)	45	400	*cadF*	Konkel et al. (1999)

Phylogenetic tree construction

The positive PCR products were then sequenced by MACROGEN Company (Korea) on 3730XL sequencer (Applied Biosystems, USA). The precision of the data was confirmed by bidirectional sequencing with the forward and reverse primers utilized in PCR.

The nucleotide sequences acquired in this examination were analysed using the BioEdit 7.0.4.1 and Muscle (EMBL's European Bioinformatics Institute, 2020) programs. The subsequent sequences were aligned with the *cadF* virulence gene of reference sequences of *Campylobacter* spp. (Table 3) utilizing a neighbour-joining analysis of the aligned sequences executed in the program CLC Genomics Workbench 3.

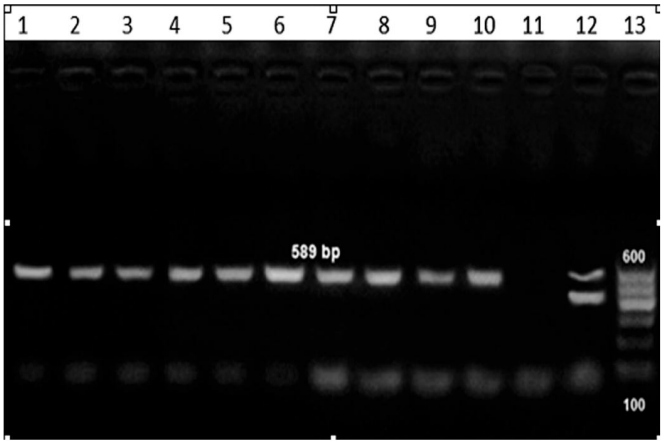

Figure 1. Amplification of the *mapA* gene of *C. jejuni* isolates. Lane 13: DNA ladder (100 bp.); lane 12: positive control; lane 11: negative control; lanes 1-10: positive *C. jejuni* isolates showing specific bands at 589 bp.

3 Results

In this investigation, 435 samples were gathered from Cairo, Giza, Fayoum, Minya and Qalubia in Egypt for the isolation and biochemical identification of *C. jejuni* from chicken and human stool samples (Table 4). Eighteen (20%), 10 (16.66%) and 6 (12%) *C. jejuni* isolates were detected in the intestinal content, liver and meat samples of broiler chickens, respectively. Additionally, 7 (17.75%), 6 (15%) and 6 (7.5%) *C. jejuni* isolates were detected in the intestinal content, cloacal swabs and egg samples of laying chickens, respectively. In the human stool samples, thirteen C. *jejuni* isolates (17.33%) were distinguished from 75 diarrheic persons, with a total of 66 (15.17%) *C. jejuni* isolates from the chicken and human stool samples. The prevalence of infection was almost the same in broiler chickens and human stool samples, as presented in Table 4.

In this investigation, ten representative *C. jejuni* isolates that were biochemically validated were further molecularly characterized through the amplification of the *mapA* gene specific to *C. jejuni*. All the isolates demonstrated the specific product (589 bp) for *C. jejuni*, as shown in Figure 1.

With respect to the virulence properties of the *C. jejuni* isolates, interestingly, ten isolates (15.15%) carried the virulence-associated *CadF* gene among the sixty-six *C. jejuni* isolates (Table 5) and generated the expected product (400 bp), as shown in Figure 2.

From our outcomes, 20.58%, 10.52% and 7.69% of isolates from broiler chickens, layer chickens and human stool samples carried the *cadF* virulence gene, respectively, with a total percentage of 15.15%. The highest prevalence of the *cadF* virulence gene was detected in the broiler intestine samples (27.77%), while the lowest was detected in eggs (0%).

Table 3. Details of the *C. jejuni* isolates, including source and country, used in the present study available in GenBank.

Ser. no	Organism	Strain	Host	Isolation Source	Country	Acccess. No
1	*Campylobacter jejuni*	CJ3	Broiler chicken	Meat	Egypt	**MN103378**
2	*Campylobacter jejuni*	CJ4	Laying chicken	Intestine	Egypt	**MN103379**
3	*Campylobacter jejuni*	CJ5	Human	Stool	Egypt	**MN103380**
4	*Campylobacter jejuni* subsp. *jejuni*	D42a	Chicken	Caecum	USA	CP007751
5	*Campylobacter jejuni*	RM1285	Chicken	Breast exudate	USA	CP012696
6	*Campylobacter jejuni*	YQ2210	Turkey	----------	USA	CP017859
7	*Campylobacter jejuni*	104	Chicken	---------	Brazil	CP023343
8	*Campylobacter jejuni*	CFSAN032806	Chicken	Breast	USA	CP023543
9	*Campylobacter jejuni*	FDAARGOS_421	Chicken	Carcass	USA	CP023866
10	*Campylobacter jejuni*	NCTC 12664	Chicken	---------	United Kingdom	CP028912
11	*Campylobacter jejuni*	FORC_083	Chicken	Meat	South Korea	CP028933
12	*Campylobacter jejuni* subsp. *jejuni*	CLB104	Chicken	Liver	United Kingdom	CP034393
13	*Campylobacter jejuni* subsp. *jejuni*	00-2425	Human	Stool	Canada	CP006729
14	*Campylobacter jejuni*	CJ074CC443	Human	----------	Finland	CP012216
15	*Campylobacter jejuni* subsp. *jejuni*	RM3196	Human	----------	South Africa	CP012690
16	*Campylobacter jejuni*	FDAARGOS_263	Human	Stool	USA	CP022077
17	*Campylobacter jejuni*	FDAARGOS_422	Human	----------	USA	CP023867
18	*Campylobacter jejuni* subsp. *jejuni*	huA17	Human	Stool	Germany	CP028372
19	*Campylobacter jejuni* subsp. *jejuni*	NCTC10983	Human	Blood	United Kingdom	LR134511

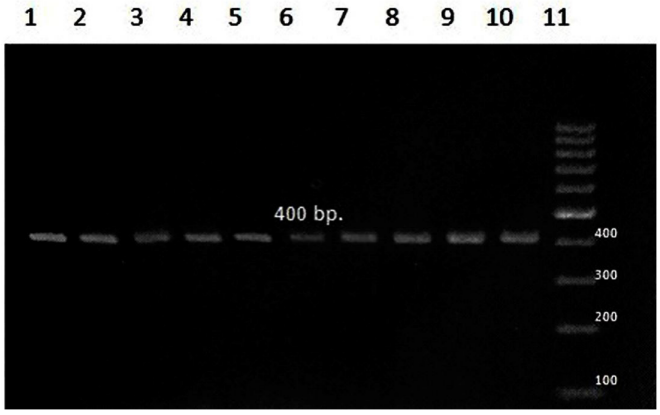

Figure 2. Agarose gel electrophoresis of *CadF* gene PCR products in *C. jejuni* isolates: Lane 11: DNA ladder (100 bp); lanes 1-10: positive *C. jejuni* isolates showing specific bands at 400 bp.

3.1 Nucleotide sequence accession numbers

Three sequences (*Campylobacter jejuni*) utilized in this investigation have been deposited in the GenBank database under accession no: MN103378-MN103380. Phylogenetic analysis affirmed that all three isolates were *Campylobacter jejuni* with homology results of 99-100%. In the phylogenetic tree, all Egyptian isolates formed two separate clusters, as shown in Figure 3. Phylogenetic investigation demonstrated that CJ3 (MN103378) and CJ4 (MN103379), which were isolated from chicken meat and intestine samples, respectively (Table 3), had high homology with the *C. jejuni* isolates (CP006729) and (CP012216) isolated from human stool samples (Figure 3).

The Egyptian isolate CJ5 (MN103380) isolated from human stool had high homology with the *C. jejuni* isolate (CP023867), which was also isolated from human stool (Figure 3).

The amino acid sequence alignment of the three Egyptian *C. jejuni* isolates revealed a mutation in the sequence of two

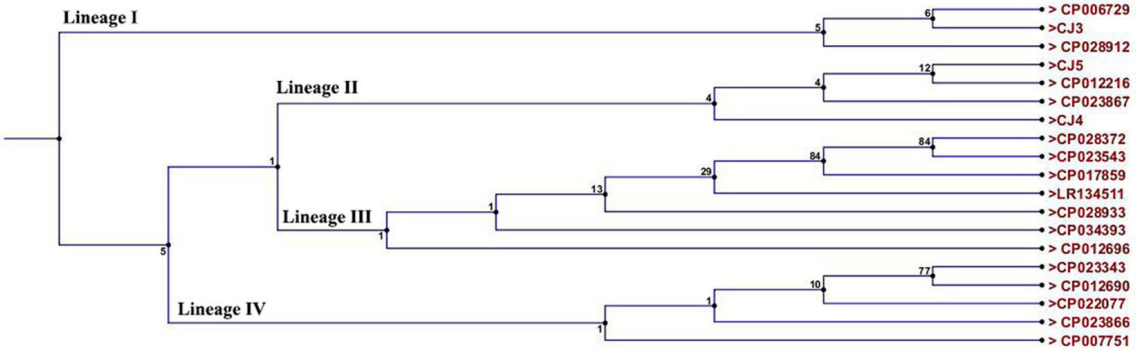

Figure 3. Phylogenetic relationship of selected strains of *Campylobacter jejuni* from human and poultry representing the four distinct lineages, based on the *cadF* virulence gene. The GenBank accession numbers of the isolates are provided.

Table 4. Incidence of *C. jejuni* in the samples examined by conventional method.

Samples		Positive *C. jejuni* Isolates	
Type	NO.	NO.	(%)
Broiler Chicken	**200**	**34**	**17**
Intestine	90	18	20
Meat	50	6	12
Liver	60	10	16.66
Laying Chicken	**160**	**19**	**11.87**
Intestine	40	7	17.75
Cloacal swabs	40	6	15
Eggs	80	6	7.5
Human Stool	**75**	**13**	**17.33**
Total	435	66	15.17

Table 5. *cadF* virulence gene profile of *C. jejuni* isolates from different sources.

Type of sample	No. of positive *C. jejuni* isolates	*CadF gene*	
		No. of *CadF*-positive *C. jejuni* isolates	(%)
1) Broiler Chicken Samples	**34**	**7**	**20.58%**
Broiler intestine	18	5	27.77%
Broiler meat	6	1	16.66%
Broiler liver	10	1	10%
2) Laying Chicken Samples	**19**	**2**	**10.52%**
Layer intestine	7	1	14.28%
Cloacal swabs	6	1	16.66%
Eggs	6	0	0%
3) Human Stool Samples	**13**	**1**	**7.69%**
Total	66	10	15.15%

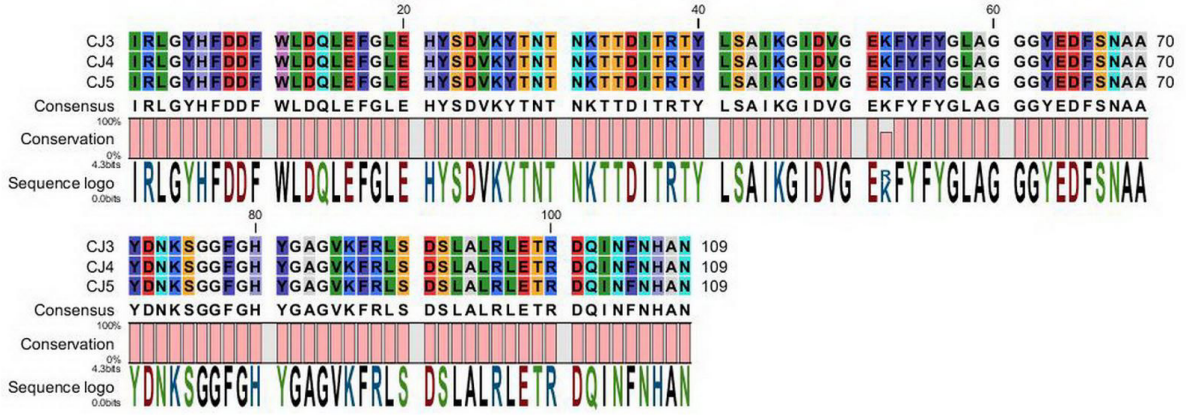

Figure 4. Amino acid sequence alignment of the three Egyptian *C. jejuni* isolates from humans and chickens.

isolates of zoonotic importance (CJ3 and CJ4) compared to the sequence of the CJ5 isolate (human isolate), where the amino acid arginine (R) number 52 was replaced with lysine (K), as shown in Figure 4.

4 Discussion

C. jejuni is considered one of the most notable and virulent foodborne pathogens, even in developed countries, and it causes severe bacterial gastroenteritis in humans who have consumed contaminated food, particularly poultry and poultry products (Eurosurveillance Editorial Team, 2012). The outcomes of the present investigation revealed that the intestinal contents of broiler and laying chickens were contaminated with *C. jejuni* at rates of 20% and 17.75%, respectively. This prevalence could be due to the intestinal tract of chickens, especially the caecum and colon, which is considered an area of tropism for a large number of *Campylobacter* species (Jokinen et al., 2011). Lower rates of contamination were detected in Egypt in previous studies Omara et al. (2015), Youseef et al. (2017), Elsayed et al. (2019), with *C. jejuni* isolation rates of 12.8%, 4% and 14.7%, respectively. However, higher rates of isolation (33.3%, 72.1%) were revealed by Abd El-Tawab et al. (2018) and El Fadaly et al. (2016) respectively.

In our study, *C. jejuni* isolates (16.66%) were derived from liver samples because the liver *is considered an organ of tropism for C. jejuni* (Boukraa et al., 1991). A lower prevalence (4% & 6.6%) was reported by Youseef et al. (2017) and Hafez et al. (2018) respectively, while higher rates (37.5% and 52.8%) were found by Barakat et al. (2015) and El Fadaly et al. (2016) in Egypt respectively.

Six meat samples (12%) also harboured *C. jejuni*. Meat contamination during the slaughtering and processing of chickens carriers a higher risk of contamination when the intestinal tract is ruptured and the contents are transferred to the carcass (Berrang et al., 2001). This incidence was in close agreement with that (12.82%) detected by Abd El-Tawab et al. (2015) from chicken breast meat. Lower rates (10%) were revealed by Chatur (2014) and in Egypt (9.6% &10%) by Omara et al. (2015) and Hafez et al. (2018) respectively. However, higher prevalence (46.9%, 16.6% &18.33%) were found by Wieczorek et al. (2012), Elgabry et al. (2016) and Modirrousta et al. (2016) respectively.

In our study, the cloacal swabs from six laying hens yielded *C. jejuni* at a rate of 17.75%. This finding was in close agreement with the finding (17.8%) of Fonseca et al. (2006). Lower isolation levels of *C. jejuni* (8%&7%) have been recorded by Chatur (2014) and Akosua et al. (2017) respectively. Higher rates were reported in Italy by Parisi et al. (2007) and others in Egypt (Stojanov et al., 2007; Hedawey &Youssef, 2014) with isolation rates of 21.1%, 26% and 25%, respectively.

Additionally, the egg samples contained internal contents that did not reveal *C. jejuni* isolates and swabs from eggshells carried *C. jejuni* at a rate of 15%. This could be due to the poor ability of *C. jejuni* to penetrate the egg albumin or yolk, while it was confined to the inner egg membranes (Neill et al., 1985). The result obtained from egg content samples was comparable with that of Fonseca et al. (2006), Jones et al. (2012) and Ge et al. (2016), whereas, one isolate (4.28%) of *C. jejuni* was retrieved from egg samples as reported by Shane et al. (1986). The eggshell sample results were consistent with those (16.66%) reported by Modirrousta et al. (2016). However, Hedawey & Youssef (2014) found a lower isolation rate (1%).

Generally, *Campylobacter* is the most common bacterium that causes gastroenteritis globally in humans and can be lethal to young children, geriatric patients and immunocompromised patients (Sainato et al., 2018).

In the current study, the total isolation rate of *C. jejuni* in human stool samples was 17.33% (16.66% in adults and 17.77% in children), which could result from the ingestion of undercooked or raw *C. jejuni*-contaminated chicken meat, liver or eggs (Edwards et al., 2014). This finding was comparable to that (16.66%) of Hassanain (2011) in Egypt. Lower *C. jejuni* isolation levels (14%, 5.19% & 12.3%) were reported by Khalifa et al. (2013), Girgis et al. (2014) and El-Tras et al.(2015) respectively in Egypt and 4.39% as reported by Di Giannatale et al. (2014) in Italy. However, higher isolation rates (51.5%, 27.5%, and 33.33%) were detected by El Fadaly et al. (2016), Abushahba et al. (2018) and Rouby et al. (2019) correspondingly.

Several genes are related to *Campylobacter* virulence, which could lead to human infection and chicken colonization

(Kalantar et al., 2017). The most virulent is the gene *Campylobacter* adhesin to fibronectin F (*cadF*) (Elsayed et al., 2019).

From our results, 20.58%, 10.52% and 7.69% of isolates from broiler chickens, layer chickens and human stool samples carried the *cadF* virulence gene, respectively, with a total of 15.15%. The highest prevalence of the *cadF* virulence gene was isolated from the broiler intestine samples (27.77%), while the lowest prevalence was from eggs (0%).Regarding the prevalence of *CadF* gene, our results were lower than that of Elmali & Can (2019) that reported a prevalence of 41.6% and so lower than that of Al Amri et al.(2007), Abu-Madi et al.(2016) and Samad et al.(2019) who described nearly 100% of *CadF* gene prevalence.

In the present study, the relatively high prevalence rate of the *cadF* gene from broiler chicken isolates suggests that many poultry-derived strains have possible pathogenic properties for humans (Frasao et al., 2017; Kalantar et al., 2017).

Our results confirm the extensive prevalence of virulence genes, especially in broiler chickens and human stool samples, which indicates that strict control, public health, and food safety policies are required to prevent consumers from contracting this zoonotic pathogen. The accessibility of pathogen virulence data should increase the awareness of these clinically and economically important pathogen isolates in Egypt.

The detection of Campylobacter species was verified by DNA sequencing of representative samples. The collected sequences were BLAST searched with those in the database, and a phylogenetic analysis was performed (Table 1). Clear sequences of the *cadF* virulence gene were obtained from three isolates. Homology findings (99-100%) showed that the three isolates were *C. jejuni*. The neighbour-joining (NJ) phylogenetic analysis based on the *cadF* gene (Figure 3) showed that all three Egyptian isolates clustered with specific sequences of human origin and not poultry origin (other clusters), while two of them (CJ3 and CJ4) were isolated from chickens. Human stool was a primary source of infection. This shows the zoonotic importance of these two *C. jejuni* isolates and the continuous pathogen loop from chicken to human and vice versa. This result is important in understanding the epidemiology of *C. jejuni* in Egypt.

In addition, the amino acid sequence alignment of the three Egyptian *C. jejuni* isolates from humans and chickens (Figure 4) revealed a mutation in the sequence of two isolates of zoonotic significance (CJ3 and CJ4) relative to the sequence of the CJ5 isolate (human isolate), where the amino acid arginine (R) number 52 was substituted with lysine (K). Therefore, when we speak of this virulence gene (*Campylobacter* adhesin to fibronectin F), the shift in the amino acid sequence of the two *C. jejuni* isolates (CJ3 and CJ4) compared to the human isolate (CJ5) may be explained by increased pathogen virulence and relatively easier transmission in Egypt between humans and chickens. In the future, this concept will be further researched.

5 Conclusions

The present results support the possible risk of transmitting *C. jejuni* as a foodborne pathogen from both broiler and layer chickens due to the high rates of *C. jejuni* isolation from different chicken samples, as expressed by the percentage of *C. jejuni* isolated from human stool specimens. The use of PCR and next-generation sequencing is important to ensure that this pathogen is quickly identified, characterized and examined epidemiologically. Active on-farm biosecurity measures in chicken farms and more hygienic efforts in slaughter houses, in local chicken slaughter shops and by those who rear backyard chickens should be made for the effective control of this foodborne disease.

References

Abd El-Tawab, A., Ammar, A., Ahmed, H., El Hofy, F. I., & Hefny, A. (2018). Bacteriological and molecular identification of some *Campylobacter* species in broilers and their macrolide resistance profile. *Benha Veterinary Medical Journal*, 34(1), 374-391. http://dx.doi.org/10.21608/bvmj.2018.54483.

Abd El-Tawab, A. A., El Hofy, F. I., Ammar, A. M., Ahmed, H. A., & Hefny, A. A. (2015). Bacteriological and molecular identification of Campylobacter species in chickens and humans, at Zagazig city, Egypt. *Benha Veterinary Medical Journal*, 28(1), 17-26. http://dx.doi.org/10.21608/bvmj.2015.32523.

Abu-Madi, M., Behnke, J. M., Sharma, A., Bearden, R., & Al-Banna, N. (2016). Prevalence of virulence/stress genes in *Campylobacter jejuni* from chicken meat sold in Qatari retail outlets. *PLoS One*, 11(6), e0156938. http://dx.doi.org/10.1371/journal.pone.0156938. PMid:27258021.

Abushahba, M., Ahmed, S., Ibrahim, A., & Mosa, H. (2018). Prevalence of zoonotic species of *Campylobacter* in broiler chicken and humans in Assiut Governorate, Egypt. *Approaches in Poultry, Dair y& Veterinary Sciences*, 3(4), 1-9.

Adesiyun, A., Offiah, N., Seepersadsingh, N., Rodrigo, S., Lashley, V., Musai, L., & Georges, K. (2005). Microbial health risk posed by table eggs in Trinidad. *Epidemiology and Infection*, 133(6), 1049-1056. http://dx.doi.org/10.1017/S0950268805004565. PMid:16274501.

Akosua, B. K., Kwasi, O. D., Enoch, H. F., & Karen, A. K. (2017). Multidrug resistant *Campylobacter* in faecal and carcasses of commercially produced poultry. *African Journal of Microbiological Research*, 11(7), 271-277. http://dx.doi.org/10.5897/AJMR2015.8329.

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(10), 1350-1355. http://dx.doi.org/10.1099/jmm.0.47220-0. PMid:17893173.

Andrzejewska, M., Szczepańska, B., Śpica, D., & Klawe, J. J. (2015). Trends in the occurrence and characteristics of *Campylobacter jejuni* and *Campylobacter coli* isolates from poultry meat in Northern Poland. *Food Control*, 51, 190-194. http://dx.doi.org/10.1016/j.foodcont.2014.11.014.

Barakat, A. M. A., Sobhy, M. M., El Fadaly, H. A. A., Rabie, N., Khalifa, N., Hassan, E., Kotb, M. H. R., Amin Girh, Z. M. S., Sedeek, D. M., & Zaki, M. S. (2015). Zoonotic hazards of campylobacteriosis in some areas in Egypt. *Life Science Journal*, 12(7), 9-14. http://dx.doi.org/10.7537/marslsj120715.02.

Berrang, M. E., Buhr, R. J., Cason, J. A., & Dickens, J. A. (2001). Broiler carcass contamination with *Campylobacter* from feces during defeathering. *Journal of Food Protection*, 64(12), 2063-2066. http://dx.doi.org/10.4315/0362-028X-64.12.2063. PMid:11770639.

Boukraa, L., Messier, S., & Robinson, Y. (1991). Isolation of *Campylobacter* from livers of broiler chickens with and without necrotic hepatitis lesions. *Avian Diseases*, 35(4), 714-717. http://dx.doi.org/10.2307/1591600. PMid:1786003.

Chatur, Y. A. (2014). *Molecular characterization, typing and antibiotic resistance pattern of Campylobacter spp. isolated from animal and human sources*. Anand Agricultural University, Anand. Retrieved from http://krishikosh.egranth.ac.in/handle/1/76299

Di Giannatale, E., Di Serafino, G., Zilli, K., Alessiani, A., Sacchini, L., Garofolo, G., Aprea, G., & Marotta, F. (2014). Characterization of antimicrobial resistance patterns and detection of virulence genes in *Campylobacter* isolates in Italy. *Sensors*, 14(2), 3308-3322. http://dx.doi.org/10.3390/s140203308. PMid:24556669.

Doyle, M. P., & Erickson, M. C. (2006). Reducing the carriage of foodborne pathogens in livestock and poultry. *Poultry Science*, 85(6), 960-973. http://dx.doi.org/10.1093/ps/85.6.960. PMid:16776463.

Edwards, D., Milne, L., Morrow, K., Sheridan, P., Verlander, N., Mulla, R., Richardson, J. F., Pender, A., Lilley, M., & Reacher, M. (2014). Campylobacteriosis outbreak associated with consumption of undercooked chicken liver pâté in the East of England, September 2011: identification of a dose-response risk. *Epidemiology and Infection*, 142(2), 352-357. http://dx.doi.org/10.1017/S0950268813001222. PMid:23711104.

El Fadaly, H., Barakat, A., Ahmed, S., Abd El-Razik, K. A., Omara, S., Ezzat, E., & Zaki, M. S. (2016). Zoonotic concern of *Campylobacter jejuni* in raw and ready-to-eat barbeque chickens along with Egyptian handlers and consumers via molecular and immunofluorescent characterization. *Der Pharma Chemica*, 8(2), 392-397. Retrieved from http://derpharmachemica.com/archive.html

Elgabry, E. A., Barakat, A. M., Abd El-Razik, K. A., Abuelnaga, A. S., & El Fadaly, H. A. (2016). Incidence of zoonotic *Campylobacter jejuni* in fast meal meat, grill chickens and symptomatic Egyptians. *African Journal of Microbiological Research*, 10(18), 608-615. http://dx.doi.org/10.5897/AJMR2016.8014.

Elmali, M., & Can, H. Y. (2019). Antimicrobial susceptibility and virulence-associated genes in *Campylobacter* isolates from milk and wastewater in Hatay, Turkey. *Ciência Rural*, 49(5), 1-8. http://dx.doi.org/10.1590/0103-8478cr20180227.

Elsayed, M., Tarabees, R., Shehata, A., Harb, O., & Sabry, A. (2019). Virulence repertoire and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from some poultry farms in Menoufia governorate, Egypt. *Pakistan Veterinary Journal*, 39(2), 261-265. http://dx.doi.org/10.29261/pakvetj/2019.009.

El-Tras, W. F., Holt, H. R., Tayel, A. A., & El-Kady, N. N. (2015). *Campylobacter* infections in children exposed to infected backyard poultry in Egypt. *Epidemiology and Infection*, 143(2), 308-315. http://dx.doi.org/10.1017/S095026881400096X. PMid:24774694.

EMBL's European Bioinformatics Institute – EMBL-EBI. (2020). *MUSCLE*. Retrieved from https://www.ebi.ac.uk/Tools/msa/muscle/

European Food Safety Authority – EFSA. (2010). Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008. Part A: *Campylobacter* and *Salmonella* prevalence estimates. *EFSA Journal*, 8(3). http://dx.doi.org/10.2903/j.efsa.2010.1503.

Eurosurveillance Editorial Team. (2012). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. *Eurosurveillance*, 17(10), 1. Retrieved from http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20113

Fonseca, B. B., Soncini, R. A., Gimarães, A. R., & Rossi, D. A. (2006). *Campylobacter* sp in eggs from cloacal swab positive breeder hens. *Brazilian Journal of Microbiology*, 37(4), 573-575. http://dx.doi.org/10.1590/S1517-83822006000400032.

Frasao, B. S., Marin, V. A., & Conte-Junior, C. A. (2017). Molecular detection, typing, and quantification of *Campylobacter* spp. in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety*, 16(4), 721-734. http://dx.doi.org/10.1111/1541-4337.12274.

Frost, J. A., Oza, A. N., Thwaites, R. T., & Rowe, B. (1998). Serotyping scheme for *Campylobacter jejuni* and *Campylobacter coli* based on direct agglutination of heat-stable antigens. *Journal of Clinical Microbiology*, 36(2), 335-339. http://dx.doi.org/10.1128/JCM.36.2.335-339.1998. PMid:9466737.

Ge, Z., Xue, S., Jianmei, Z., Yuehua, L., Juan, W., Xiumei, H., Zhina, Q., Yudong, W., Shigan, Y., & Junwei, W. (2016). Isolation, identification, and characterization of foodborne pathogens isolated from egg internal contents in China. *Journal of Food Protection*, 79(12), 2107-2112. http://dx.doi.org/10.4315/0362-028X.JFP-16-168. PMid:28221968.

Girgis, S. A., Rashad, S. S., Othman, H. B., Bassim, H. H., Kassem, N. N., & El-Sayed, F. M. (2014). Multiplex PCR for identification and differentiation of *Campylobacter* species and their antimicrobial susceptibility pattern in Egyptian patients. *International Journal of Current Microbiology and Applied Sciences*, 3(4), 861-875.

Hafez, A., Younis, G., El-Shorbagy, M., & Awad, A. (2018). Prevalence, cytotoxicity and antibiotic susceptibility of *Campylobacter* species recovered from retail chicken meat in Mansoura, Egypt. *African Journal of Microbiological Research*, 12(22), 501-507. http://dx.doi.org/10.5897/AJMR2018.8865.

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

Hedawey, K. A. A., & Youssef, A. S. (2014). Incidence of *Campylobacter* species in laying hens and table egg in Sohag governorate. *Assiut Veterinary Medical Journal*, 60(141), 120-124.

Jokinen, C., Edge, T. A., Ho, S., Koning, W., Laing, C., Mauro, W., Medeiros, D., Miller, J., Robertson, W., Taboada, E., Thomas, J. E., Topp, E., Ziebell, K., & Gannon, V. P. (2011). Molecular subtypes of *Campylobacter* spp., *Salmonella enterica*, and *Escherichia coli* O157:H7 isolated from faecal and surface water samples in the Oldman River watershed, Alberta, Canada. *Water Research*, 45(3), 1247-1257. http://dx.doi.org/10.1016/j.watres.2010.10.001. PMid:20971491.

Jones, D. R., Anderson, K. E., & Guard, J. Y. (2012). Prevalence of *coliforms, Salmonella, Listeria*, and *Campylobacter* associated with eggs and the environment of conventional cage and free-range egg production. *Poultry Science*, 91(5), 1195-1202. http://dx.doi.org/10.3382/ps.2011-01795. PMid:22499879.

Kalantar, M., Soltan Dallal, M.-M., Fallah, F., & Yektaei, F. (2017). Monitoring the virulence genes in *Campylobacter coli* strains isolated from chicken meat in Tehran, Iran. *Infection Epidemiology and Microbiology*, 3(1), 12-15. http://dx.doi.org/10.18869/modares.iem.3.1.12.

Khalifa, N. O., Afify, J. S., & Rabie, N. S. (2013). Zoonotic and molecular characterizations of *Campylobacter jejuni* and *Campylobacter coli* isolated from beef cattle and children. *Global Veterinaria*, 11(5), 585-591. http://dx.doi.org/10.5829/idosi.gv.2013.11.5.818.

Konkel, M. E., Gray, S. A., Kim, B. J., Garvis, S. G., & Yoon, J. (1999). Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the *cadF* virulence gene and its product. *Journal of Clinical Microbiology*, 37(3), 510-517. http://dx.doi.org/10.1128/JCM.37.3.510-517.1999. PMid:9986804.

Li, L., Mendis, N., Trigui, H., Oliver, J. D., & Faucher, S. P. (2014). The importance of the viable but non-culturable state in human bacterial pathogens. *Frontiers in Microbiology*, 5, 258. http://dx.doi.org/10.3389/fmicb.2014.00258. PMid:24917854.

Miller, R. S., Miller, W. G., Behringer, M., Hariharan, H., Matthew, V., & Oyarzabal, O. A. (2010). DNA identification and characterization of *Campylobacter jejuni* and *Campylobacter coli* isolated from caecal

samples of chickens in Grenada. *Journal of Applied Microbiology*, 108(3), 1041-1049. http://dx.doi.org/10.1111/j.1365-2672.2009.04507.x. PMid:19735321.

Modirrousta, S., Shapouri, R., Rezasoltani, S., & Molaabaszadeh, H. (2016). Prevalence of *Campylobacter* spp. and their Common Serotypes in 330 Cases of Red-meat, Chicken-meat and Egg-shell in Zanjan City, Iran. *Infection Epidemiology and Microbiology*, 2(1), 8-10. http://dx.doi.org/10.18869/modares.iem.2.1.8.

Neill, S. D., Campbell, J. N., & O'Brien, J. J. (1985). Egg penetration By *Campylobacter Jejuni. Avian Pathology*, 14(3), 313-320. http://dx.doi.org/10.1080/03079458508436233. PMid:18766924.

Omara, S. T., Fadaly, H. A. E., & Barakat, A. M. A. (2015). Public health hazard of zoonotic *Campylobacter jejuni* reference to Egyptian regional and seasonal variations. *Research Journal of Microbiology*, 10(8), 343-354. http://dx.doi.org/10.3923/jm.2015.343.354.

Parisi, A., Lanzilotta, S. G., Addante, N., Normanno, G., Di Modugno, G., Dambrosio, A., & Montagna, C. O. (2007). Prevalence, molecular characterization and antimicrobial resistance of thermophilic *Campylobacter* isolates from cattle, hens, broilers and broiler meat in South-eastern Italy. *Veterinary Research Communications*, 31(1), 113-123. http://dx.doi.org/10.1007/s11259-006-3404-3. PMid:17180449.

Penner, J. L. (1988). The genus *Campylobacter*: a decade of progress. *Clinical Microbiology Reviews*, 1(2), 157-172. http://dx.doi.org/10.1128/CMR.1.2.157. PMid:3069194.

Persson, S., & Olsen, K. E. P. (2005). Multiplex PCR for identification of *Campylobacter coli* and *Campylobacter jejuni* from pure cultures and directly on stool samples. *Journal of Medical Microbiology*, 54(11), 1043-1047. http://dx.doi.org/10.1099/jmm.0.46203-0. PMid:16192435.

Rao, M. R., Naficy, A. B., Savarino, S. J., Abu-Elyazeed, R., Wierzba, T. F., Peruski, I., Abdel-Messih, I., Frenck, R., & Clemens, J. D. (2001). Pathogenicity and convalescent excretion of *Campylobacter* in rural Egyptian children. *American Journal of Epidemiology*, 154(2), 166-173. http://dx.doi.org/10.1093/aje/154.2.166. PMid:11447051.

Rouby, S., Abdel-Latef, G., & Abdel Aziz, S. (2019). Bacteriological and molecular identification of thermophilic *Campylobacters* of animal and human origins in Beni-Suef governorate. *Journal of Advanced Veterinary Research*, 9(3), 102-106.

Sainato, R., ElGendy, A., Kuroiwa, J., Poly, F., Riddle, M. S., Guerry, P., & Porter, C. K. (2018). Epidemiology of *Campylobacter* infections among children in Egypt. *The American Journal of Tropical Medicine and Hygiene*, 98(2), 581-585. http://dx.doi.org/10.4269/ajtmh.17-0469. PMid:29260646.

Samad, A., Abbas, F., Ahmed, Z., Akbar, A., Naeem, M., Sadiq, M. B., Ali, I., Saima, Roomeela, Bugti, F. S., & Achakzai, S. K. (2019). Prevalence, antimicrobial susceptibility, and virulence of *Campylobacter jejuni* isolated from chicken meat. *Journal of Food Safety*, 39(2), e12600. http://dx.doi.org/10.1111/jfs.12600.

Shane, S. M., Gifford, D. H., & Yogasundram, K. (1986). *Campylobacter jejuni* contamination of eggs. *Veterinary Research Communications*, 10(6), 487-492. http://dx.doi.org/10.1007/BF02214012. PMid:3798738.

Sheppard, S. K., Dallas, J. F., Strachan, N. J., Macrae, M., McCarthy, D. N., Wilson, D. J., Gormley, F. J., Falush, D., Ogden, I. D., Maiden, M. C., & Forbes, K. J. (2009). *Campylobacter* genotyping to determine the source of human infection. *Clinical Infectious Diseases*, 48(8), 1072-1078. http://dx.doi.org/10.1086/597402.

Shin, E., & Lee, Y. (2009). Comparison of three different methods for *campylobacter* isolation from porcine intestines. *Journal of Microbiology and Biotechnology*, 19(7), 647-650. PMid:19652510.

Stojanov, I., Orlić, D., Stojanović, D., & Ratajac, R. (2007). Importance of *Campylobacter* spp. in laying hens. *Luce* Ştiinłifice *Medical Veterinary*, XL, 177-181.

Taylor, E. V., Herman, K. M., Ailes, E. C., Fitzgerald, C., Yoder, J. S., Mahon, B. E., & Tauxe, R. V. (2013). Common source outbreaks of *Campylobacter* infection in the USA, 1997-2008. *Epidemiology and Infection*, 141(5), 987-996. http://dx.doi.org/10.1017/S0950268812001744. PMid:22892294.

Vandamme, P., Gevers, D., & Debruyne, L. (2008). Taxonomy of the Family *Campylobacteraceae*. In I. Nachamkin, C. M. Szymanski & M. J. Blaser (Eds.), *Campylobacter* (3rd ed., pp. 3-25). Washington: American Society for Microbiology.

Verwoerd, D. J. (2000). Ostrich diseases. *Revue Scientifique et Technique*, 19(2), 638-661. http://dx.doi.org/10.20506/rst.19.2.1235. PMid:10935285.

Vinueza-Burgos, C., Wautier, M., Martiny, D., Cisneros, M., Van Damme, I., & De Zutter, L. (2017). Prevalence, antimicrobial resistance and genetic diversity of *Campylobacter coli* and *Campylobacter jejuni* in Ecuadorian broilers at slaughter age. *Poultry Science*, 96(7), 2366-2374. http://dx.doi.org/10.3382/ps/pew487. PMid:28339716.

Wieczorek, K., Szewczyk, R., & Osek, J. (2012). Prevalence, antimicrobial resistance, and molecular characterization of *Campylobacter jejuni* and *C. coli* isolated from retail raw meat in Poland. *Veterinarni Medicina*, 57(2), 293-299. http://dx.doi.org/10.17221/6016-VETMED.

Younis, G., Awad, A., & Khairy, M. (2018). Molecular characterization and virulence of *Campylobacter jejuni* isolated from broiler chickens. *International Journal of Poultry Science*, 17(10), 499-506. http://dx.doi.org/10.3923/ijps.2018.499.506.

Youseef, A. G., Ibrahim, A., Sayed, A. S., & Sobhy, M. M. (2017). Occurrence of *Campylobacter* species in chickens by multiplex polymerase chain reaction. *Journal of Veterinary Medicine*, 63(152), 66-72.