Prevalence, molecular detection, and virulence gene profiles of Campylobacter species in humans and foods of animal origin

Ashraf M. A. Barakat1, Khaled A. Abd El-Razik2, Hassan A. Eldaly3, Nagwa S. Rabie4, Sabry A. S. Sadek2 and Abdulaziz M. Almuzaini2

1. Department of Zoonotic Diseases, National Research Centre, Dokki, Giza, Egypt; 2. Department of Animal Reproduction, National Research Centre, Dokki, Giza, Egypt; 3. Department of Poultry Diseases, National Research Centre, Dokki, Giza, Egypt; 4. Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia.

Corresponding author: Khaled A. Abd El-Razik, e-mail: khaled707@hotmail.com
Co-authors: AMAB: ashrafbarakat2@hotmail.com, HAE: hassanfadalay67@gmail.com, NSR: nagwasrabie@hotmail.com, SASS: drsabry.nrc@gmail.com, AMA: dr-almuzaini@hotmail.com

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Abstract

Background and Aim: Campylobacteriosis is one of the most well-characterized bacterial foodborne infections worldwide that arise chiefly due to the consumption of foods of animal origin such as poultry, milk, and their products. 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. This study was conducted to determine the prevalence and virulence of Campylobacter isolates from human, chicken, and milk and milk products in Egypt.

Materials and Methods: A total of 1299 samples (547 chicken intestine and liver, 647 milk and milk products, and 105 human stool) were collected and microbiologically investigated, confirmed by multiplex polymerase chain reaction (PCR) targeting the 23S rRNA, hipO, and glyA genes specific for Campylobacter spp., C. jejuni, and Campylobacter Coli, respectively, followed by virulence genes (Campylobacter adhesion to fibronectin F [cadF] and edtB) detection using PCR.

Results: About 38.09%, 37.84%, and 8.5% of human stool, chicken, and milk and milk product samples, respectively, were bacteriologically positive, with a total of 302 Campylobacter isolates. All isolates were molecularly confirmed as Campylobacter spp. (100%) where 285 isolates (94.37%) were identified as C. jejuni and 17 isolates (5.62%) as C. coli. Regarding the virulence pattern, all isolates (100%) carried cadF gene while cytolethal distending toxin B gene was definite in 284/302 isolates (94%), concisely, 282/285 (98.94%) C. jejuni isolates, and in 2/17 (11.76%) C. coli isolates.

Conclusion: The widespread presence of these highly virulent Campylobacter, especially C. jejuni, proofs the urgent need for the implementation of stringent control, public health, and food protection strategies to protect consumers from this zoonotic pathogen. The availability of information about pathogen virulence will enable enhanced local policy drafting by food safety and public health officials.

Keywords: Campylobacter, Egypt, food, human stool, multiplex polymerase chain reaction, virulence genes.

Introduction

Campylobacteriosis is a serious zoonotic gastrointestinal disease worldwide, and most cases are mainly caused by Campylobacter jejuni. Poultry plays an important role in the transmission of campylobacteriosis to humans [1]. C. jejuni colonizes the chicken gut primarily in the cecum and small intestine but also colonizes the liver and spleen [2]. Thus, the intestinal tract of chickens supplies a reservoir of Campylobacter that may spread through fecal material at farms or during processing [3]. Human Campylobacter infection may be due to either the consumption of undercooked meat or the cross-contamination of ready-to-eat food during preparation or storage [4]. Worldwide, C. jejuni is responsible for 85% of foodborne Campylobacter enteritis in humans and is the most frequently isolated Campylobacter species recovered from poultry, while the remaining cases are primarily attributed to Campylobacter coli [5]. Campylobacter species are the main cause of bacterial gastrointestinal disease campylobacteriosis, which causes diarrhea, sometimes dysentery syndrome, and cramps, fever, and pain in developing countries. In particular, C. jejuni and C. coli are accountable for campylobacteriosis [6].

The isolation of campylobacters using the culture method is considered the gold standard for campylobacteriosis disease diagnosis; however, it is time-consuming and laborious because of the fastidious nature of campylobacters [7]. In addition, the differentiation of species using biochemical assays is difficult due to the phylogenetic relatedness of C. jejuni and C. coli species [8]. Thus, molecular-based assays, such as polymerase chain reaction (PCR) and sequencing, can
enable easy, rapid, and specific detection and epidemiological applications [9]. For this purpose, various genes have been used [10].

Several genes have been linked to Campylobacter virulence, but the most important are cytotoxins distending toxin B (cdtB), which disrupts mucosal barriers by causing host cell death, Campylobacter adhesion to fibronectin F (cadF), and the heat survival and stress response proteins htrB and clpP, which are important for survival [10,11]. The disease severity depends on the virulence of the strain and on the host’s immune condition. cadF is one of the reference virulence genes that encode a protein involved in the invasion and adhesion of *C. jejuni* [12], and this gene is present at a high level in *C. jejuni* isolates [13].

Despite the increased recovery of *Campylobacter* as a foodborne pathogen, the specific virulence and pathogenic mechanisms by which microaerophilic *Campylobacter* species cause infection are still poorly understood [14]. The putative virulence factor for adhesion and invasion of epithelial cells, toxin production, and flagellar motility are thought to be important virulence mechanisms [15]. However, different studies have indicated that different virulence markers play a role in the colonization, adherence, and invasion of *Campylobacter* spp. in animals and humans [15].

The aim of this study was to investigate the prevalence of *Campylobacter* spp. using conventional and molecular tools and to determine the virulence gene profile of *Campylobacter* spp. in humans and foods of animal origin in Egypt.

**Materials and Methods**

**Ethical approval**

All aspects of the study were performed in accordance with national ethics regulations and approved by the National Research Ethical Committee, National Research Centre, Giza, Egypt (Ethical Approval no 16220). Written consent was taken from patients before collecting stool samples.

**Sample collection**

A total of 1299 samples (Table-1) from chickens (n=547), milk and milk products (n=647) from various markets, and human stool (n=105) were collected randomly from different governorates in Egypt (Cairo, Giza, Fayoum, and Qalyubia) from January 2018 to December 2018. The human stool specimens were collected at random from people with diarrhea who were admitted to different laboratories, people in contact with backyard chickens and slaughterhouses and from diarrheic children admitted to hospitals for kids in Egypt. Ten grams of each sample (chicken intestine and liver, milk and milk products, and human stool) were collected in a sterile sample collection vial and transferred to the laboratory. All samples were immediately stored at 4°C and processed to isolate campylobacters.

**Isolation and identification of Campylobacter**

A loop of each sample was homogenized in sterile thioglycollate broth (Oxoid). Broth samples were incubated at 42°C for 48 h in a microaerobic atmosphere using an anaerobic jar with CampyGen sachets, which generates 10% CO₂, 5% O₂, and 85% N₂. A loopful of enrichment broth was streaked onto mCCDA plates (Oxoid) and incubated under microaerobic condition at 42°C for 48 h followed by microscopic examination to examine their characteristic motility utilizing phase contrast microscope after staining by Gram’s stain presenting seagull appearance. All isolates were subjected to biochemical tests, such as catalase, oxidase, urease, nitrate reduction, indole acetate hydrolysis, and hippurate hydrolysis tests and susceptibility tests to cephalothin and nalidixic acid by the disk diffusion method [16].

**Molecular characterization of Campylobacter species**

**DNA extraction**

DNA was extracted by the heating and snap chilling method [17]. Two to three colonies of fresh bacterial growth were collected from culture medium, suspended in nuclease-free demonzied water, and heated at 95°C for 10 min. The samples were cooled immediately and centrifuged for 5 min at room temperature. The supernatant was separated and 3 μl was used as the DNA template.

**Confirmation of Campylobacter spp., C. jejuni, and C. coli isolates by multiplex PCR**

A multiplex PCR reaction was used for the confirmation of biochemically identified *Campylobacter* spp. through targeting 23S rRNA specific for *Campylobacter* spp., hipO gene specific for *C. jejuni*, and glyA gene specific for *C. coli* (Table-2) [18-21]. Primers were utilized in a 25 μl reaction containing 12.5 μl of 2× ViRed Taq Master Mixture (Cat. no. CLMM01, Vivantis Technologies, Malaysia), 1 μl of each primer (20 pmol), 5.5 μl of water, and 3 μl of template. Cycling conditions begin with initial

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**Table-1:** Samples collected from different localities in Egypt.

| Site of samples | Number of samples | Chicken samples | Milk and milk products | Human stool |
|-----------------|-------------------|----------------|------------------------|------------|
|                 |                   | Intestine | Liver | Milk | Cheese | Yoghurt |               |
| Giza            | 400               | 165      | 60   | 77   | 40     | 41      | 17           |
| Fayoum          | 319               | 115      | 10   | 65   | 52     | 47      | 30           |
| Cairo           | 266               | 60       | 10   | 65   | 40     | 59      | 32           |
| Qalyubia        | 314               | 80       | 47   | 67   | 48     | 46      | 26           |
| Total           | 1299              | 420      | 127  | 274  | 180    | 193     | 105          |

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denaturation at 95°C for 6 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 59°C for 30 s, and extension at 72°C for 30 s with a single final extension step at 72°C for 10 min. The PCR products were separated by electrophoresis on 1% agarose gel.

**Virulence gene characterization of Campylobacter isolates**

The confirmed isolates of Campylobacter species were characterized for in vitro detection of virulence genes by PCR for two well-known virulence genes encoding the cadF [20] and cdtB genes [14]. The details of the primers for the target virulence genes are described in Table-2. Cycling conditions were as previous with annealing at 45°C for cadF gene and 57°C for cdtB gene.

**Phylogenetic tree construction**

The positive PCR products targeting the cadF gene of two C. jejuni samples (CJ1 and CJ2) were sequenced by MACROGEN Company (Korea) on 3730XL sequencers (Applied Biosystems, USA). The accuracy of the data was confirmed by two-directional sequencing with the forward and reverse primers used in PCR. The nucleotide sequences obtained in this study were analyzed using the programs BioEdit 7.0.4.1 and Muscle (https://www.ebi.ac.uk/Tools/msa/muscle/). The resulting sequences were aligned with the cadF virulence gene of Campylobacter spp. reference sequences (Table-3) using neighbor-joining analysis of the aligned sequences implemented in the program CLC Genomics Workbench 3.

**Results**

**Identification of Campylobacter species**

In this investigation, samples were collected from Giza, Fayoum, Cairo, and Qalyubia in Egypt for the isolation of Campylobacter spp. from chicken, milk, milk products, and human stool. Campylobacter spp. were isolated from 37.84% of chickens, 8.5% of milk and milk product samples, and 38.09% of human stool samples. The confirmed isolates of Campylobacter spp. isolates were molecularly confirmed by the PCR amplification of the four target genes of Campylobacter spp., the hipO gene specific to C. jejuni, and the glyA gene specific to C. coli. All the 302 isolates were confirmed as Campylobacter (Figure-1), of which 94.37% as C. jejuni and 5.62% as C. coli (Figures-2 and 3, Table-4).

In detail, 69.35%, 57.5%, and 63.33% of isolates from chicken, milk and milk product, and human stool samples were confirmed as C. jejuni while 6.76% and 5.62 of chicken and human stools samples as C. coli, respectively (Table-4).

**Virulence determinants**

With regard to the virulence pattern, all isolates carried the virulence associate gene cadF (100%), while cdtB gene was positive in 284 out of the 302 isolates (94%). Briefly, 282 out of 285 (98.94%) of C. jejuni isolates and 2 out of 17 (11.76%) of C. coli isolates were positive for cdtB gene (Figure-4a and b).

**Nucleotide sequence accession numbers**

Two sequences (C. jejuni cadF gene) used in this study (CJ1 and CJ2) have been deposited in the GenBank database under the accession nos. MN103381-MN103382. Phylogenetic analysis confirmed that the two isolates were C. jejuni with

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**Table-2: Primer sets for PCR amplification of the four target genes of Campylobacter species.**

| Target gene                                      | Primer | Primer sequence (5’→3’) | Size (in bp) | References |
|--------------------------------------------------|--------|--------------------------|--------------|------------|
| Campylobacter spp. 23S rRNA                      | CB1    | TATACCGGTAAGGAGTGCTGGAG  | 650          | [19]       |
|                                                   | CB2    | ATCAATTAACC TTCGAGCACC  | 323          | [19]       |
| Campylobacter jejuni hipO                        | CJF    | AACTCTTTAGTGCTGGTCGC     | 126          | [18]       |
|                                                   | CJR    | GCCACAACCAAGTAAGGAAGCG   |              |            |
| Campylobacter coli glyA                          | CCF    | GTAAAACAAGGCTATATCTGT    | 200          |            |
|                                                   | CCR    | TCCAGCAGTGTGCAATG        | 1000         |            |
| Campylobacter adherence gene (cadF)              | cadF   | TTGAGGGTATATTTATGATAT    | 400          | [20]       |
|                                                   | cadR   | CTAATACCTAAGGTTGAAC      |              |            |
| Cytolethal distending toxin subunit B gene (cdtB)| CdB-F  | GTTGGCAACTTGGAAATTTTGGAC | 495          | [21]       |
|                                                   | CdB-R  | GTAAAACTCCCTGCTATCAACCA  |              |            |

PCR=Polymerase chain reaction

**Figure-1:** Amplification of the 23S rRNA gene of Campylobacter spp. positive amplification appeared at 650 bp, lane 1: 100 bp ladder, lane 2: The positive control, lanes 3-7: Positive for Campylobacter spp.
In the phylogenetic tree, all Egyptian isolates formed two separate clusters (Figure-5). Phylogenetic analysis showed that CJ1 (MN103381) and CJ2 (MN103382), which were isolated from the intestine and liver of chickens, respectively, had typical homology with *C. jejuni* isolated from either chicken or human (Figure-5).

### Discussion

In the current study, *Campylobacter* spp. were isolated from 37% to 38.09% of the liver and intestine of chickens, respectively. Similar isolation rate (28.3%) from cloacal swabs was reported in Egypt [22]. Comparable isolation rates (31.9% and 39.2%) were reported in Vietnam [23] and Estonia [24], respectively. Reduced isolation rate (16.83%) was reported by Abushalba *et al.* [25]. The reported prevalence rate of *Campylobacter* spp. is higher in the previous studies than in this study, for instance, 41.2% and 48.5% in Egypt [22,26] and 58% in Brazil [27]. The aforementioned high isolation rates could be attributed to the extensive type of chicken management that increases the exposure of birds to *Campylobacter* infection through insects, rodents, contaminated water, and poor housing hygiene [28]. In addition, high isolation rates of 82.9% in Italy and 51.5% in Nigeria from chicken cloacal swabs, respectively, were reported [29,30], which could be a result of using only conventional methods.

The variation in the isolation rate of *Campylobacter* spp. between different studies could be attributed to the age of the examined chickens [31] and the difference in the sanitation levels, while handling and processing chickens, the type and site of the examined samples, the sampling season, the laboratory methodologies employed for isolation, husbandry

### Table 3: Details of *Campylobacter jejuni* isolates used in the present study and available in GenBank.

| S. No. | Organism          | Strain | Host        | Isolation source | Country | Access. No. |
|--------|-------------------|--------|-------------|------------------|---------|-------------|
| 1      | *Campylobacter jejuni* | CJ1    | Broiler chicken | Intestine        | Egypt   | MN103381    |
| 2      | *Campylobacter jejuni* | CJ2    | Broiler chicken | Liver            | Egypt   | MN103382    |
| 3      | *Campylobacter jejuni subsp. jejuni* | D42a  | Chicken      | Caecum           | USA     | CP007751    |
| 4      | *Campylobacter jejuni* | RM1285 | Chicken      | Breast exudate   | USA     | CP012696    |
| 5      | *Campylobacter jejuni* | YQ2210 | Turkey       | ----             | USA     | CP017859    |
| 6      | *Campylobacter jejuni* | 104    | Chicken      | ----             | Brazil  | CP023343    |
| 7      | *Campylobacter jejuni* | CFSAN032806 | Chicken | Breast          | USA     | CP023543    |
| 8      | *Campylobacter jejuni* | FDAARGOS_421 | Chicken | Carcass         | USA     | CP023866    |
| 9      | *Campylobacter jejuni* | NCTC 12664 | Chicken | ----             | United Kingdom | CP028912 |
| 10     | *Campylobacter jejuni* | FORC_083 | Chicken      | Meat             | South Korea | CP028933 |
| 11     | *Campylobacter jejuni subsp. jejuni* | CLB104 | Chicken      | Liver            | United Kingdom | CP034393 |
| 12     | *Campylobacter jejuni* | 0-0-2425 | Human        | Stool            | Canada  | CP006729    |
| 13     | *Campylobacter jejuni* | CJ074CC443 | Human | ----             | Finland | CP012216    |
| 14     | *Campylobacter jejuni subsp. jejuni* | RM3196 | Human | ----             | South Africa | CP012690 |
| 15     | *Campylobacter jejuni* | FDAARGOS_263 | Human | Stool            | USA     | CP022077    |
| 16     | *Campylobacter jejuni* | FDAARGOS_422 | Human | ----             | USA     | CP023867    |
| 17     | *Campylobacter jejuni subsp. jejuni* | huA17  | Human        | Stool            | Germany | CP028372 |
| 18     | *Campylobacter jejuni subsp. jejuni* | NCTC10983 | Human | Blood            | United Kingdom | LR134511 |

### Table 4: Prevalence of *Campylobacter* genus in the examined samples by conventional and molecular methods.

| Type of samples        | Incidence by conventional method | Incidence of *C. jejuni* by multiplex PCR | Incidence of *C. coli* by multiplex PCR |
|------------------------|----------------------------------|------------------------------------------|---------------------------------------|
|                        | n (%)                           | n (%)                                    | n (%)                                |
| Chickens               | 207/547  37.84                  | 193/207  93.23                           | 14/207  6.76                          |
| Intestine              | 160/420  38.09                  | 158/160  98.75                           | 2/160  1.25                           |
| Liver                  | 47/127   37                      | 35/47   74.66                            | 12/47  25.5                            |
| Milk and milk products | 55/647   8.5                     | 55/55   100                               | 0/55  0                               |
| Raw milk               | 14/274   5.1                     | 14/14   100                               | 0/14  0                               |
| Cheese                 | 14/180   7.77                    | 14/14   100                               | 0/14  0                               |
| Yoghurt                | 27/193   13.98                   | 27/27   100                               | 0/27  0                               |
| Human stool            | 40/105   38.09                   | 37/40   92.5                              | 3/40  7.5                             |
| Total                  | 302/1299 23.24                  | 285/302 94.37                            | 17/302 5.62                           |

*C. jejuni*=*Campylobacter jejuni*, *C. coli*=*Campylobacter coli*, PCR=Polymerase chain reaction
and management, and the production system have the greatest impact on the prevalence rate of campylobacters [32].

Cattle play an important role in human campylobacteriosis. There are cattle-related outbreaks that indicate that raw milk and dairy products are the second most frequent sources of infection. Direct contamination of milk may occur through feces or as a consequence of mastitis [33]. In the current study, Campylobacter spp. was isolated from 8.5% of milk and milk products samples (5.1%, 7.77%, and 13.98% of raw milk, cheese, and yoghurt samples, respectively). The higher percentage of Campylobacter in milk products than in raw milk is explained by the contamination of milk products because of unhygienic conditions during the preparation of milk products. A similar isolation rate of Campylobacter from raw milk (7.2%) was reported in Turkey [1]. Relatively low prevalence rates of 2.91% and 2.32% were reported [17,34], respectively. In contrast, high prevalence rates of Campylobacter in milk at 17.2%, 20%, and 66.8% were reported [35-37]. Here, Campylobacter spp. were isolated from 7.77% and 13.98% of cheese and yoghurt samples, respectively. Similarly, a prevalence rate of 5.0% from cheese was reported by Giacometti et al. [38]. In the contrary, Campylobacter was absent from milk product samples in some reports [17,39]. This emphasizes the importance of milk and dairy products as a potential source of Campylobacter.

In general, Campylobacter is the most common bacterium that induces gastroenteritis in humans globally and can be fatal to young children, geriatric patients, and immunocompromised patients [40]. In the current study, an isolation rate of 38.09% was reported in stool samples from humans. These results were concurrent with the isolation rate (33.33%) detected by Rouby et al. [41] in Egypt but were higher than those (27.5%) detected by Abushahba et al. [25].
in Egypt. The high percentage detected in our study could be attributed to the inclusion of stool samples primarily obtained from gastroenteritis-infected individuals rather than investigating the disease in the general population.

PCR is still rapid, specific, sensitive, and of substantial interest for the recognition and verification of Campylobacter species. Thus, PCR is a dependable substitute for conventional culture [3,42]. Molecular methods were used to confirm the detected Campylobacter species and to differentiate between C. jejuni and C. coli, as the discrimination of C. jejuni and C. coli is considered difficult because it depends only on a single phenotypic test [8]. Therefore, here, multiplex PCR was used to identify the isolated Campylobacter spp. (302 isolates) by targeting the 23S rRNA specific for Campylobacter spp., hipO gene of C. jejuni, and the glyA gene of C. coli. The multiplex PCR results confirmed all the 302 biochemically identified isolates as Campylobacter spp. of which 285 isolates as C. jejuni (94.37%) and 5.62% as C. coli. A total percentage of 69.35%, 57.5%, and 63.33% of isolates from chicken, milk and milk product, and human stool samples were confirmed as C. jejuni while 6.76% and 5.62 of chicken and human stools samples as C. coli, respectively.

One hundred and ninety-three out of 207 Campylobacter isolates (93.23%) recovered from chicken samples were identified as C. jejuni using multiplex PCR. Similar results using PCR, where C. jejuni was the predominant isolate and reached prevalence rates as high as 87.5%, 90%, and 89% in frozen chicken carcasses as reported in Egypt [3], in Great Britain [43], and in Vietnam [23], respectively. The predominance of C. jejuni may be due to its ability to survive high and low temperatures, low pH, and dry conditions [44]. This was contrary to the findings in Egypt [22,26] and in Argentina [45], those reported C. jejuni as the only species isolated and molecularly identified from chickens. Moreover, 14 out of 207 Campylobacter isolates (6.76%) recovered from chicken samples were identified as C. coli using multiplex PCR. Only a few reports are available regarding C. coli as the predominant isolate in Egypt [42] and Greece [46]. This may be due to the type of feed ration because C. jejuni does not frequently colonize birds receiving plant protein-based feed [46].

A total of 55 Campylobacter isolates recovered from milk and milk products were identified as C. jejuni (100%) using multiplex PCR. This was concurrent with the findings in Egypt [17], indicating that this species is distributed widely in the study areas. Similar results reported that C. jejuni was the predominant isolate (85.7%) in Turkey [1].

A total of 37 out of 40 Campylobacter isolates recovered from human stool were identified as C. jejuni (92.5%) and 3 out of 40 isolates as C. coli (7.5%) using multiplex PCR. The isolation rates of C. jejuni that were higher than those of C. coli from Campylobacter-positive stool samples were in relative agreement with that (61.7%) of Sainato et al. [40] and with higher prevalence than those (27.5% and 8.4%) of Abushahba et al. [25] and Omara et al. [47] in Egypt, respectively. The high prevalence observed in our study may be a result of collecting samples from humans originating from villages, where basic hygienic standards and precautions for the contact and handling of live poultry are usually not adopted. This was in contrast with the findings of Rouby et al. [41] who reported that PCR revealed that all isolates were C. jejuni. Moreover, C. coli was the predominant isolate, as high as 1.11% and 3%, as reported in Egypt [25] and in Poland [48], respectively.

The minimal infective dose of C. jejuni is very low, which indicates that C. jejuni is highly virulent, and a very small number of bacterial cells could cause infection in humans. The virulence of Campylobacter species is associated with flagellar motility, adhesion, invasion, and production of cytolethal distending toxins [49]. Several genes have been linked to Campylobacter virulence that might contribute to human infection and colonization of chickens [13]. The most important are cdtB, which disrupts mucosal barriers by causing host cell death, and cadF [26]. The detection of the cadF gene in all C. jejuni and C. coli

**Figure-5:** Phylogenetic relationship of selected strains of Campylobacter jejuni from poultry, milk and milk products, and human; the accession numbers of the isolates used are given.
isolates (100%) from chickens was in agreement with the findings from the previous reports [50,51]; similarly, the results of this study were consistent with the previous results regarding the presence of C. coli from humans [51]. However, a higher percentage was identified in this study than in other reports (41.6% and 8%), among all C. jejuni isolates [1,50]. The high prevalence rate (100%) of the cadF gene in the present study shows that many strains originating from poultry, milk and milk products have potential pathogenic properties toward humans, as reported by Frasao et al. [13] and Kalantar et al. [19].

According to Gonzalez-Hein et al. [52], CdtB cytotoxin subunit is encoded by cdtB gene that plays an essential role in exerting a toxic effect on cells. In the present study, Campylobacter cytotoxic factor (cdtB) was confirmed in 98.94% of C. jejuni isolates and 11.76% of C. coli isolates. The occurrence of cdtB from chicken isolates in this study was higher than that of Abu-Madi et al. [50] and EL-Sayed et al. [26] who reported 6.7% and 0%, respectively, but less than that of Modi et al. [17] that reported the presence of cdtB gene in all isolates from bovine and swine tissue. Our findings confirm the relatively higher prevalence of cdtB gene in C. jejuni in comparison to C. coli. This is in parallel with that of Wysok et al. [53].

**Conclusion**

Our study demonstrated the widespread existence of highly virulent Campylobacter isolates, especially C. jejuni in chicken, milk and milk product, and human, confirming that this species is a serious infection hazard and public health concern. Moreover, this study emphasizes the urgent need for the implementation of stringent control, public health, and food protection strategies to protect consumers from this zoonotic pathogen. The availability of information about pathogen virulence will enable enhanced local policy drafting by food safety and public health officials and will increase the awareness of this medically and economically important pathogen.

**Authors’ Contributions**

AMAB and NSR designed the study, conducted the experiments, and revised the manuscript; HAE and SASS conducted bacterial isolation and identification. KAAE and AMA performed the molecular identification and characterization and wrote the manuscript. All authors read and approved the final manuscript for publication.

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**Competing Interests**

The authors declare that they have no competing interests.

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