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Article

Differential Virulence Gene Profiling and Expression of *Campylobacter* Species from Chicken and Human Fecal Samples in Egypt

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Abstract: *Campylobacter* is considered to be the most leading bacterial cause of human gastroenteritis worldwide. Consumption of undercooked or contaminated chicken food is the main source of human campylobacteriosis. Although *Campylobacter* is a leading cause of gastroenteritis, data on the comparative virulence gene profiles of *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) from chicken and human sources in Egypt remain scarce. This study aimed to characterize the virulence genes profiles of both *C. jejuni* and *C. coli* isolated from chicken and human fecal samples in Ismailia governorate, Egypt. A total of 20 isolates of each species were screened for 15 virulence genes. All isolates carried *virB11*, *iam*, *racR*, and *tetO*. In chicken isolates, the prevalence of additional virulence genes was higher, with *pldA*, *dnaJ*, *flaA*, *cdtB*, *ciaB*, and *wlaN* detected in 100%, 100%, 100%, 80%, 60%, and 0% of *C. jejuni* isolates and 100%, 100%, 60%, 60%, 40%, and 20% of *C. coli* isolates, respectively. In contrast, human isolates showed a markedly lower prevalence, with *dnaJ*, *flaA*, and *cdtB* detected in 20% of *C. jejuni* and 40% of *C. coli* isolates, while *pldA*, *ciaB*, and *wlaN* were absent in all human isolates. qPCR revealed significantly higher expression levels of *dnaJ*, *virB11*, *flaA*, and *iam* in chicken isolates compared to human isolates, with mean fold changes of 11.5, 7.16, 5.39, and 3.72 for *C. jejuni*, and 8.34, 5.21, 2.84, and 2.5 for *C. coli*, respectively. Differential expression of *racR*, *cdtB*, and *tetO* was not significant. Ganglioside mimicry genes (*Cst11*, *wlaN*, *Waac*, *ggt*, and *cgtB*) were absent in all human isolates. These findings underscore the significant variability in virulence gene profiles between chicken and human *Campylobacter* isolates and highlight the importance of molecular characterization in risk assessment and epidemiological surveillance of *Campylobacter* infections.

Keywords: *Campylobacter*; *C. jejuni* and *C. coli*; Virulence genes; Gene expression; chicken; Human

1. Introduction

Campylobacter is one of the most important four causes for global diarrheal diseases with an estimated 96 million cases and 37,600 deaths annually, predominantly in developing countries [1]. In

developing countries, campylobacteriosis in children is frequent and sometimes resulting in death [2]. Mainly *C. jejuni* and *C. Coli* are well-recognized causes of human campylobacteriosis with symptoms ranging from mild watery diarrhea to serious neuropathies as Guillain-Barré syndrome [3].

Campylobacter spp. are gram-negative, microaerophilic bacteria characterized by their spiral shape and motility, which is facilitated by a polar flagellum. These organisms thrive at higher temperatures (42°C), making avian hosts, particularly poultry, an ideal reservoir for *C. jejuni* and *C. coli*. Notably, these bacteria colonize poultry asymptotically, unlike in humans, where they cause disease [4]. Poultry is considered to be the main source for human campylobacteriosis [5]. Chicken, in contrast to human, scarcely develop pathological lesions [6]. The high body temperature of poultry species provides an optimal environment for the growth of thermophilic *Campylobacter* species, particularly *C. jejuni* and *C. coli* which make poultry constitute the main source of human campylobacteriosis [7].

Despite the significant burden of disease, the molecular basis of *Campylobacter* pathogenicity remains incompletely understood. However, several virulence factors have been identified based on in vitro and in vivo studies. For example, *flaA*, which encodes flagellin and *cadF*, which encodes a protein that interacts with a host extracellular matrix protein fibronectin are required for *Campylobacter* adherence to and/ or colonization of the host cell surface [8]. Other genes such as *ciaB*, *pldA*, and genes of the pVir plasmid are involved in host cell invasion [9]

Virulence genes like *cdtA*, *cdtB*, and *cdtC* are responsible for the expression of *Campylobacter* cytolethal distending toxin and eventually cell death. Lipo-oligosaccharides associated genes; *cgtB*, *waaC*, and *wlaN*, responsible for β -1,3 galactosyltransferase production, invasion associated genes; *ciaB*, *pldA*, and *virB11*, and antibiotic resistance genes; *tetO*, *tetA* and *gyrB* were investigated by [10]. Systematic surveillance and molecular characterization are essential for understanding epidemiological patterns and designing effective interventions. This study aims to explore the genetic diversity, virulence gene profiles, and differential gene expression in *Campylobacter* isolates from chicken and human fecal samples, with a focus on the comparative molecular mechanisms underlying zoonotic transmission.

2. Materials and Methods

2.1. Sample Preparation and Bacterial Isolation

A total of 200 Chicken broilers and human fecal samples (100 of each) were collected as follow; 100 Chicken ceca from ten different poultry commercial slaughterhouses and 100 human stool samples from medical laboratories in Ismailia governorate during 2018. All samples were collected in sterile polyethylene cubs and transported in an ice box with refrigerants to be examined within a few hours for isolation of *Campylobacter* species at the Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP), Ismailia branch. All samples were prepared as follows; from Chicken, fecal samples were obtained by removal of cecal wall using sterile scissors and taking one gram of cecal contents. One gram from each sample was added to 9 ml Thioglycolate broth (1/10 dilution) to form a fecal suspension. For Human samples, one gram from each fecal sample was added to 9 ml Thioglycolate broth (1/10 dilution) to form a fecal suspension. All samples were examined by passive filtration method [11].

Approximately 0.1 ml from the freshly prepared fecal suspension was carefully and aseptically layered onto a 0.45 μ m cellulose nitrate filter, which has been previously placed on top of a freshly prepared non-selective blood agar plate. Care must be taken not to allow the inoculum to spill over the edge of the filter. The bacteria were allowed to migrate through the filter for 30–45 min. at 37°C or at room temperature. The filter was aseptically removed, and then the fluid that passed through the filter was streaked on the blood agar plate using a sterile bacteriological loop and then incubated micro-aerobically at 42°C for 48 hrs.

2.2. Molecular Confirmation and Identification of Campylobacter Virulence Genes by PCR

A total of 20 *Campylobacter* isolates were selected for molecular detection of 15 different virulence genes, comprising 10 isolates from chicken samples (5 *C. jejuni* and 5 *C. coli*) and 10 isolates from human samples (5 *C. jejuni* and 5 *C. coli*). DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN, Germany) following the manufacturer’s instructions.

Oligonucleotide primers were supplied from Metabion (Germany). Primers sequences and cycling conditions for confirmation and typing of *Campylobacter* spp. were performed as reported previously [12] [13]. The target genes included: amplification of *flaA* and *csrA* genes responsible for adhesion [14] and [15], *virB11*, *ciaB*, *pldA*, *iam* genes responsible for invasion [9];[14];[16], *cdtB* gene responsible for cytotoxicity [9], *dnaJ* and *racR* genes responsible for *Campylobacter* resistance to stress conditions [17] and [9], *waaC*, *wlaN*, *cstII*, *cgtB* and *Ggt* genes responsible for *Campylobacter* ganglioside Mimicry [15] [18], and *tetO* gene responsible for tetracycline resistance [19]. For all assays, Emerald Amp GT PCR master mix (Takara, Japan) was used. PCR products were separated by electrophoresis using a Gelpilot 100 bp DNA Ladder (Qiagen, Germany) and visualized with a gel documentation system. Images of the gels were captured, and data were analyzed using dedicated software to confirm the presence of the targeted genes.

2.3. Gene Expression of 7 Campylobacter Virulence Genes in Chicken Samples Versus Human Samples

The expression levels of seven virulence genes (*dnaJ*, *virB11*, *flaA*, *iam*, *racR*, *cdtB* and *tetO*) in *C. jejuni* and *C. coli* were analyzed in isolates from chicken and human samples, with 23S rRNA serving as the reference housekeeping gene. Total RNA was extracted using the RNeasy Mini Kit (QIAGEN, Germany) following the manufacturer’s protocol. Complementary DNA (cDNA) synthesis was performed using RevertAid Reverse Transcriptase (Thermo Fisher, 200 U/μL). qPCR was conducted using the Quantitect SYBR Green PCR Kit (QIAGEN). Specific oligonucleotide primers for each target gene were synthesized by Metabion (Germany). Cycling conditions for qPCR are detailed in Table (1). Amplifications were performed on the Stratagene MX3005P Real-Time PCR System, a fully integrated platform for quantitative PCR detection and data analysis.

Table 1. Cycling conditions for SYBR green real time PCR.

Gene	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)			References
			Secondary denaturation	Annealing (Optics on)	Extension	Secondary denaturation	Annealing	Final denaturation	
23S rRNA	50°C 30 min.	94°C 15 min.	94°C 15 sec.	55°C 30 sec.	72°C 30 sec.	94°C 1 min.	55°C 1 min.	94°C 1 min.	[12]
cdtB	50°C 30 min.	94°C 15 min.	94°C 15 sec.	51°C 30 sec.	72°C 30 sec.	94°C 1 min.	51°C 1 min.	94°C 1 min.	[21]
dnaJ	50°C 30 min.	94°C 15 min.	94°C 15 sec.	42°C 30 sec.	72°C 30 sec.	94°C 1 min.	42°C 1 min.	94°C 1 min.	[17]
flaA	50°C 30 min.	94°C 15 min.	94°C 15 sec.	55°C 30 sec.	72°C 30 sec.	94°C 1 min.	55°C 1 min.	94°C 1 min.	[14]
racR	50°C 30 min.	94°C 15 min.	94°C 15 sec.	45°C 30 sec.	72°C 30 sec.	94°C 1 min.	45°C 1 min.	94°C 1 min.	[22]
VirB11	50°C 30 min.	94°C 15 min.	94°C 15 sec.	53°C 30 sec.	72°C 30 sec.	94°C 1 min.	53°C 1 min.	94°C 1 min.	[9]

<i>Iam</i>	50°C 30 min.	94°C 15 min.	94°C 15 sec.	50°C 30 sec.	72°C 30 sec.	94°C 1 min.	50°C 1 min.	94°C 1 min.	[23]
<i>tetO</i>	50°C 30 min.	94°C 15 min.	94°C 15 sec.	55°C 30 sec.	72°C 30 sec.	94°C 1 min.	55°C 1 min.	94°C 1 min.	[16]

2.4. Analysis of the SYBR Green RT-PCR for Gene Expression

Amplification curves and cycle threshold (C_T) values were generated using the Stratagene MX3005P software. To estimate the variation of gene expression on the RNA of the different samples, the C_T of each sample was compared with that of the control group according to the “ΔΔC_T” method stated previously [20] using the following ratio: (2^{-ΔΔC_T}). Whereas ΔΔC_T = ΔC_T reference (23srRNA) – ΔC_T target (for each gene).

ΔC_T target (for each gene) = C_T control (Human) – C_T treatment (chicken)

ΔC_T reference (23srRNA) = C_T control (Human) – C_T treatment (chicken).

2.5. Ethical Statement

All procedures involving the collection of human fecal samples were conducted with prior informed consent from participants, ensuring anonymity and confidentiality, in compliance with ethical guidelines and institutional approval.

3. Results

3.1. Prevalence of Campylobacter Virulence Genes and tetO Gene

The prevalence of virulence genes in both chicken and human samples could be categorized into; Group no. 1, genes that were detected in 100% of tested chicken and human samples (*virB11*, *iam*, *racR* and *tetO*), Group no. 2, genes that were detected in chicken and human samples with different ratios (*dnaJ*, *flaA* and *cdtB*), Group no. 3, genes that were detected in chicken samples and not in human samples (*pldA*, *ciaB* and *wlan*), and Group no. 4, genes that were not detected in any of tested samples neither chicken nor human (*Cst11*, *csrA*, *Waac*, *ggt* and *cgtB*) Table (2)

For chicken samples, *pldA* and *dnaJ* genes were detected in all *C. jejuni* and *C. coli* samples, *flaA* gene was detected in all *C. jejuni* and in 60% of *C. coli* samples. *cdtB* gene was detected in 80% and 60% of *C. jejuni* and *C. coli* samples; respectively, and *ciaB* gene was detected in 60% and 40% of *C. jejuni* and *C. coli* samples; respectively. *Wlan* gene was detected in 20% of *C. coli* and not detected from any of *C. jejuni* samples. For human samples, *dnaJ*, *flaA*, and *cdtB* were detected in 20% *C. jejuni* and 40% of *C. coli* samples, while *pldA*, *ciaB*, and *wlan* were not detected in any sample from human source as shown in table (2) & Figure (1).

Table 2. Results of Campylobacter species virulence genes and tetO gene in human and chicken fecal Samples.

Virulence gene	Human		Chicken	
	<i>C. jejuni</i> (%)	<i>C. Coli</i> (%)	<i>C. jejuni</i> (%)	<i>C. Coli</i> (%)
<i>flaA</i>	20	40	60	100
<i>csrA</i>	0	0	0	0
<i>virB11</i>	100	100	100	100
<i>iam</i>	100	100	100	100
<i>pldA</i>	0	0	100	100
<i>ciaB</i>	0	0	60	40
<i>cdtB</i>	20	40	80	60
<i>dnaJ</i>	20	40	100	100

<i>racR</i>	100	100	100	100
<i>wlaN</i>	0	0	0	20
<i>waaC</i>	0	0	0	0
<i>cstII</i>	0	0	0	0
<i>cgtB</i>	0	0	0	0
<i>ggt</i>	0	0	0	0
<i>tetO</i>	100	100	100	100

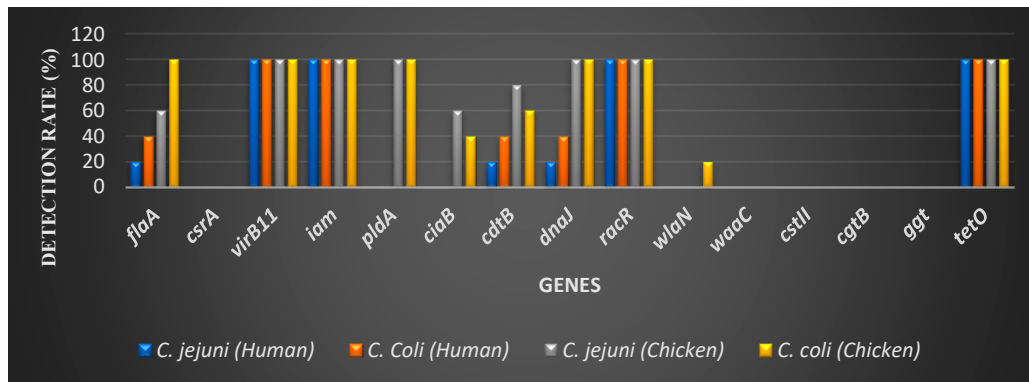


Figure 1. Detection rate (%) of *Campylobacter* virulence genes and *tetO* gene in human and chicken fecal samples.

3.2. Expression of 7 *Campylobacter* Virulence Genes in Chicken Samples Versus Human Samples

The expression of seven virulence genes from *C. jejuni* and *C. coli* isolates obtained from chicken and human samples is presented in Table (3). The data revealed significant differences in the expression of four out of the seven tested virulence genes between the chicken and human groups (control). The mean fold changes for these four genes were as follows: *dnaJ* (11.5), *virB11* (7.16), *flaA* (5.39), and *iam* (3.72) in *C. jejuni*, and *dnaJ* (8.34), *virB11* (5.21), *flaA* (2.84), and *iam* (2.5) in *C. coli*. In contrast, the expression of *tetO*, *racR*, and *cdtB* did not show significant differences between the chicken and human groups for either *C. jejuni* or *C. coli*.

Table 3. Results of 7 virulence genes expressions in *C. jejuni* and *C. coli* in chicken versus human group (control).

gene \ source	<i>C. jejuni</i>		<i>C. coli</i>	
	Human Group (control)	Chicken Group	Human Group (control)	Chicken Group
<i>flaA</i>	1 ^b	5.39 ^a	1 ^b	2.84 ^a
<i>VirB11</i>	1 ^b	7.16 ^a	1 ^b	5.21 ^a
<i>iam</i>	1 ^b	3.72 ^a	1 ^b	2.5 ^a
<i>cdtB</i>	1 ^a	0.7 ^a	1 ^a	0.8 ^a
<i>dnaJ</i>	1 ^b	11.5 ^a	1 ^b	8.34 ^a
<i>racR</i>	1 ^a	0.81 ^a	1 ^a	0.86 ^a
<i>tetO</i>	1 ^a	1.06 ^a	1 ^a	1.1 ^a

4. Discussion

Poultry especially chicken comprises the main source of human campylobacteriosis. *Campylobacter* are able to colonize the caecum of chicken in extremely high numbers of up to 10^9 CFU/g of fecal matter, even though this pathogen is present in such high quantities, the chicken rarely exhibits symptoms of disease [24]. Variable virulence genes detection in *Campylobacter* species isolated from chicken and human fecal samples has been reported [25]. Ref. [26] emphasized that the molecular profiling of *Campylobacter* spp. can enhance microbial risk assessment by providing insights into the genetic relatedness of strains implicated in foodborne outbreaks.

In this study, *flaA*, which is the main gene responsible for *Campylobacter* motility and involved in adhesion and colonization, was detected in rate of 20% and 40% in human *C. jejuni* and *C. coli* and of 60% and 100% in chicken *C. jejuni* and *C. coli* respectively. This is in agreement with a previous study that detected *flaA* gene in 20% of human *C. jejuni* isolates in Indonesia [27]. On the other hand, ***flaA* gene was found in 100% of *C. jejuni* and *C. coli* isolates** [28]. Our study revealed higher expression of *flaA* gene in chicken *C. jejuni* group (5.39) and *C. coli* (2.84) than in human (control groups). These data agreed with Stintzi who revealed upregulated between 1.5- to 2-fold at 5 and 10 min after a temperature increase from 37 to 42°C in cluster C which contains *flaA* gene [29]. These data suggested higher flagellar biosynthesis and subsequently colonization of *Campylobacter* species in chicken than in human gastrointestinal tract.

The global regulator, *CsrA* (Carbon starvation regulator) gene, has been well characterized in several bacterial genera and is known to regulate several independent pathways via a post-transcriptional mechanism, but remains relatively uncharacterized in the genus *Campylobacter*. Previously, reported data illustrating the requirement for *CsrA* in several virulence-related phenotypes of *C. jejuni* strain 81-176, indicating that the *Csr* pathway is important for *Campylobacter* pathogenesis [30]. The *csrA* mutant exhibits changes in several virulence-related properties, including oxidative stress resistance, motility, adherence, and invasion. In this study we couldn't detect *CsrA* gene in any of examined human or chicken fecal samples using conventional PCR, while another group have been detected *CsrA* gene in 100% of examined *C. jejuni* human origin isolates and 87.3% of *C. jejuni* chicken meat origin isolates [15]. Many of virulence genetic factors connected with *Campylobacter* invasiveness are placed on the *pVir* plasmid for example, *virB11* gene that encodes the IV secretory system protein [31].

In this study, results revealed that all *C. jejuni* and *C. coli* samples were positive for *virB11* gene. These results are higher than that detected by Wiczorek and Osek who found *virB11* in (32.7%) of *C. jejuni* and (92.9%) form *C. coli* isolates from poultry carcasses while detection rate from poultry fecal samples was 65% and 40% for *C. jejuni* and *C. coli*; respectively [32]. Another study examined *VirB11* gene in *C. jejuni* and *C. coli* from different sources and found that (13.6% and 9.1%) of chicken, (41.7% and 0%) of pigs, (14.3% and 100%) of Dogs, and (16.7% and 0%) of children samples were positive for *VirB11* gene in *C. jejuni* and *C. coli*; respectively [33].

In contrary, *virB11* gene was not detected in any of the examined 37 *C. jejuni* and 8 *C. coli* isolates from poultry and poultry by-products [34]. The role of the protein encoded by the *virB11* gene in the colonization and invasion of eukaryotic cells by *Campylobacter* spp. has not been elucidated [33]. 100% detection of *virB11* gene indicates the important role of the *pVir* plasmid and its virulence factors in *Campylobacter* colonization and invasiveness in chicken and human. Higher expression of *virB11* gene in chicken than human indicates higher colonization of *Campylobacter* species in chicken than human gastrointestinal tract. Data reported revealed higher expression of *virB11* gene in chicken *C. jejuni* group (7.16) and *C. coli* (5.21) than human samples (control groups). These data comply with the higher colonization of *Campylobacter* species in chicken than human gastrointestinal tract.

The *ciaB* gene plays a significant role because of the secretion of a *CiaB* 73 kDa protein which is important for the invasion of epithelial cells as well as the colonization of intestines of avian species, and *CiaB* proteins are also secreted in the presence of poultry serum and mucus [35]. In this study, the *ciaB* gene was detected in 60% and 40% in *C. jejuni* and *C. coli* samples; respectively, from chicken origin while this gene was not detected in any of human samples neither *C. jejuni* nor *C. coli*.

Previously, *CiaB* gene was detected in 47% *C. jejuni* and in 10% *C. coli*; respectively, from chicken fecal samples and in 45% *C. jejuni* and in 43% of *C. coli*; respectively, from human clinical samples [25]. On the other side, *CiaB* gene was detected in all *C. jejuni* isolates from human and chicken fecal samples [36].

The invasion-associated marker (*iam*) gene is one of most important factors responsible for *Campylobacter* invasion of host cell and it was detected in 85% of invasive strains and 20% of non-invasive strains [37]. In this study we detect *iam* gene in 100% of *C. jejuni* and *C. coli* from chicken and human fecal samples. This is similar to what was observed before in Canada, where the *iam* gene was detected in 92.31% of human clinical samples [35]. 100% detection of invasion-associated marker *iam* gene indicates its important role in *Campylobacter* invasion of host cells and its higher expression in chicken than human indicates higher colonization of *Campylobacter* species in chicken than human gastrointestinal tract.

The *pldA* gene is also related to cell invasion and is responsible for the synthesis of an outer membrane phospholipase that is important for cecal colonization [25]. Interestingly, the distribution of *pldA* gene in this study is dissimilar among the two hosts investigated; in chicken, *C. jejuni* and *C. coli* showed 100% positive, while in human no positive sample was found. In South Africa, they detected *pldA* gene in 57% and 100% of chicken fecal *C. jejuni* and *C. coli* isolates; respectively, while human fecal samples showed lower detection with a rate of 49% and 57% for *C. jejuni* and *C. coli*, respectively [25]. Another group reported an increase from 88% to 100% in the presence of the *pldA* gene in *C. jejuni* isolates from chicken with age of broilers as a major contributing factor [9]. In this study, detection of *cdtB* gene is dissimilar among the two hosts; *Campylobacter* species with detection rate of 20% and 40% in human *C. jejuni* and *C. coli*; respectively was observed. While detection rate of 80% and 60% in chicken *C. jejuni* and *C. coli*; respectively was obtained. These data aligns partially with a group who detected *cdtB* in 20% and 14% in human *C. jejuni* and *C. coli*; respectively and 53% and 60% in chicken *C. jejuni* and *C. coli*; respectively [25]. In contrast, other studies detected *cdtB* in 100% of human clinical and chicken fecal *C. jejuni* and *C. coli* samples [9,15].

The study elucidates that, *dnaJ* gene was detected in 100% of chicken *C. jejuni* and *C. coli*, while in human samples, detection was 20% and 40% of *C. jejuni* and *C. coli* samples; respectively. Results of chicken samples were in accordance with Datta et al. who detected *dnaJ* in 100% of all chicken fecal samples examined, while found a difference in human samples with a detection rate of 98% [9]. On the other hand, our results in human samples was closely similar to those who detected *dnaJ* gene in 46% and 50% of human *C. jejuni* and *C. coli* samples; respectively, although there are some differences in results of *dnaJ* from chicken samples which came in a rate of 69% and 70% for *C. jejuni* and *C. coli*; respectively [25]. Others detected *dnaJ* gene in 100% of human *C. jejuni* and *C. coli* [28]. Higher expression of *dnaJ* gene in chicken *C. jejuni* group (11.5) and *C. coli* (8.34) than human isolates (control groups) was observed in our study. These results represent a great role of *dnaJ* in *Campylobacter* colonization in chicken and its high environmental persistence, although it has been assumed to be delicate bacteria. Coherently, Stintzi confirmed the upregulation of *dnaJ* upon temperature stress and demonstrated the temperature-responsive regulation of many other heat shock proteins [29].

Herein, results reported that *racR* gene was detected in 100% of human and chicken *C. jejuni* and *C. coli* samples and these data confirmed the importance of *racR* gene in *C. jejuni* and *C. coli* colonization. Likewise, *racR* gene was detected in 98.2 and 100% of *C. jejuni* isolates from human and chicken; respectively [9]. This also partially agree with findings by [35] who reported *racR* in 94.2% of *C. jejuni* from human clinical samples. The consistent detection of *racR* underscores its critical role in *Campylobacter* colonization and environmental adaptation. In our study, we couldn't detect any of LOS associated genes in any of the examined human or chicken fecal samples using conventional PCR except *wlaN* gene that found in 20% of chicken *C. coli*. *wlaN* gene was detected in 25%, 23.8%, and 4.7% of *C. jejuni* isolates from human, poultry meat, and chicken fecal; respectively [9]. While *cst-II* and *ggt* genes were detected in 83.6% and 32.7% of 55 examined *C. jejuni* human origin isolates and in 40% and 5.5% of 55 *C. jejuni* broiler chicken meat origin isolates in Chile [15].

Tetracycline resistance in *Campylobacter* spp. is primarily mediated by a ribosomal protection protein (*tetO*), which is transferred as a plasmid-encoded gene [34]. Our data showed that all human and chicken *C. jejuni* and *C. coli* tested samples were positive for *tetO* gene. These results agreed with Hassanain who reported that the rate of tetracycline resistance in *C. jejuni* isolates from human was 75% [38]. These data indicated that *tetO* is widespread among *Campylobacter* spp. probably due to conjugative plasmids. Lower results of tetracycline resistance with a rate of 52.5% and 27.1% of human and poultry isolated *C. jejuni*; respectively, and 34.8% and 56.7% of human and poultry isolated *C. coli*; respectively were reported [39]. 100% detection of *tetO* indicates high tetracyclines resistance of *Campylobacter* in both chicken and human.

5. Conclusions

These results revealed higher colonization of *Campylobacter* species in chicken than human gastrointestinal tract and suggest its zoonotic transmission from broiler chicken to human. There are significant differences in the expression of *dnaJ*, *virB11*, *flaA*, and *iam* genes of both *C. jejuni* and *C. coli* in chicken group versus human (control group). While the expression difference of *tetO*, *racR*, and *cdtB* was not significant in human and chicken groups for *C. jejuni* nor *C. coli*. Further studies are needed to explore other antibiotic resistance. The variable detection of different virulence genes underscores the genetic diversity of *Campylobacter* spp., warranting larger studies to assess its prevalence in Egypt and emphasizing the importance of molecular characterization of different virulence genes in epidemiological evaluation and risk assessment of *Campylobacter* infection. The *wlaN* gene, detected in 20% of *C. coli* chicken samples, was the only ganglioside mimicry (GM) gene found, while *Cst11*, *csrA*, *Waac*, *ggt*, and *cgtB* were absent in both chicken and human samples. These findings highlight the need for further studies on GM genes and their role in *Campylobacter* pathogenesis.

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