The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89 (2), Page 6373- 6381

Detection of Some Virulence and Antibiotic Resistance Genes in *Campylobacter jejuni* isolated from Poultry and Human

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ABSTRACT

Background: *Campylobacter* species is a zoonotic pathogen and one of the most common causes of bacterial foodborne illnesses.

Objective: To investigate the surveillance and differences in antibiotic drug resistance, in addition to tetracycline resistance genes and virulence factors in C. *jejuni* isolated from both some poultry species and humans.

Materials and Methods: A total of 600 samples were collected from poultry species and humans, investigated by bacteriological and biochemical methods, *C.jejuni* were confirmed by *mapA* gene using PCR. Antibiotic resistance was assessed and 108 *C.jejuni* strains were tested for detection of *tetO* and *tetA*, and 6 virulence genes; *flaA*, *virB11*, *cdt*, *cdtA*, *cdtB*, and *cdt*C.

Results: Our results revealed that the occurrence of *C. jejuni* was 23.67%, identified as 20.21 and 37.5 % in the examined poultry and human samples, respectively. The evaluation of phenotypic resistance revealed that *C.jejuni* isolates had high resistance rates to ampicillin, erythromycin, cloxacillin, amoxicillin, azithromycin, and tetracycline 81.69, 79.58, 77.46, 76.76, 76.06 and76.06%, respectively. The results of the molecular technique detected that antimicrobial resistance genes in *C. jejuni* were *tetO* and *tetA* 27.78 and 100%, respectively. All isolates of *C.jejuni* in poultry and humans possessed virulence genes involved in cytotoxin production (*cdt*, *cdtA*, *cdtB*, and *cdtC*). The genes involved in invasion (*virB11*) and Motility, adherence colonization (*flaA*) were also widely dispersed between humans and poultry with the following percentages of 74.07 and 64.81% for *virB11* and *flaA*, respectively.

Conclusion: This study provided an overview of antimicrobial resistance, the presence of tetracycline resistance, and virulence genes of *C.jejuni* isolates in poultry and human, which highlights the possible risk to consumer health in Egypt.

Keywords: *Campylobacter jejuni*, poultry, human, antibiotic resistance, virulence genes, resistance genes.

INTRODUCTION

Campylobacter species are considered the major known cause of foodborne bacterial zoonoses worldwide, with more than 200,000 cases reported each year in the European Union. According to estimates, 400 to 500 million diarrheal cases per year are caused by *Campylobacter* spp., with *C. jejuni* being responsible for about 90% of infections. The most common bacteria that cause gastroenteritis in people worldwide is *C.jejuni* **(1)**.

Campylobacter species are commensal in the gastrointestinal tracts of birds and mammals, and it is mainly spread to people through handling and eating contaminated meat of broiler chickens. Two to three weeks after hatching, *Campylobacter* is often introduced to the production cycle of the broiler, and it quickly spreads throughout the flock [(2)].

Poultry meat and its products, contaminated water consumption or crops, raw or inadequately boiled milk, and contact with animals are the major routes for *Campylobacter* infection. Additionally, handling poultry meat and its products with poor kitchen hygiene is considered a main route of infection. Urinary tract infections, sepsis, or some neuropathies, particularly reactive arthritis, Guillain-Barré syndrome, and Miller-Fisher syndrome, can all be brought on by campylobacters infection complications **(3)**.

Antimicrobial resistance of bacteria has emerged as a result of the wide use of antibiotics to treat infectious diseases, and they now represent a major public health concern on a global scale. Several studies have mentioned this issue in *C. jejuni* strains in recent years. The evolution of the resistance profile for *C. jejuni* is also related to veterinary procedures used to control pathogens in domestic birds and animals. The antibiotics that are released in environments of poultry production can change the characteristics of bacterial biofilms, hinder the development of resistance profiles, or help maintain sessile life forms like *C. jejuni*, which creates mature and highly stable biofilms when exposed to the juice of chicken **(4)**. Additionally frequently exposure to antibiotics in animal husbandry situations, *C. jejuni* frequently inhabits the gastrointestinal tracts of animals used for food, which leads to resistance against clinically significant antibiotics.

The development of Antimicrobial-resistance in *C. jejuni* occurs either by spontaneous point mutations on chromosomes that change the antibiotic target sites or through horizontal gene transfer for the acquisition of antimicrobial resistance genes **(5)**.

Received: 02/07/2022
Accepted: 08/09/2022

Broad-spectrum antibiotics known as tetracyclines are frequently used to treat gram-positive and gram-negative bacteria. These medications disrupt the synthesis of protein by inhibiting aminoacyl-tRNA from binding to the 30s ribosomal subunit, which prevents the growth of susceptible bacteria. Tetracyclines have many benefits, including being widely available, affordable, and having few side effects. As a result, their use as antimicrobial agents to treat infections in both humans and animals has been rising recently and is also regarded as one of the least expensive antibacterial drugs on the market [(6)]. Tetracycline resistance can occur through different mechanisms. In the clinical setting, the production of ribosomal protection proteins and resistance through active efflux pumps are the main mechanisms. Enzymatic degradation, a reduction in permeability of the medication, and target mutation can all contribute to resistance [(7)].

Resistance to tetracycline is controlled by tet genes, which take part in active drug efflux, enzymatic drug modification, or ribosomal protection. A ribosomal protection protein called *tetO*, which is transferred as a plasmid-encoded gene, is the main important mediator of resistance to tetracycline in *Campylobacter* spp. or in a non-self-mobile region of the chromosome, the efflux genes, *tet* A code for an approximately 46-kDa membrane-bound efflux protein for membrane-associated proteins which export tetracycline from the cell [(8)].

The toxin activity encoded via *cdt* gene cluster is composed of three adjacent genes, *cdt*A, *cdt*B, and *cdt*C. The *flaA* gene appears to be extremely conserved between *Campylobacter* isolates and has a higher transcription. Thus, flagella are involved in motility and adhesion to intestinal epithelial cells, chemotaxis, virulence protein secretion, auto-agglutination, producing a microcolony and evasion of the innate immune response. The primary virulence factors involved in *Campylobacter* strains' pathogenesis have been identified by recent molecular studies. *Campylobacter's* ability to adhere, through the expression of the genes *virB11*, *dnaJ*, *cadF*, *racR*, and *pldA*, and invade intestinal epithelial cells, by the expression of the genes *ceuE* and *ciaB*, produce a toxin, through the expression of *cdtA*, *cdtB*, and *cdtC* genes, and survive in cells of the host, are the main virulence factors discovered till now [(9)].

Therefore, this study planned to investigate the surveillance and differences in antibiotic drug resistance, in addition to tetracycline resistance genes and virulence factors in C. *jejuni* isolated from both some poultry species and humans.

MATERIALS AND METHODS

Ethics statement

This study was approved by the Institutional Animals Care and Use Committee, Research Ethics Board, Faculty of Veterinary Medicine, Benha University (No. BUFVTM01-06-22) following animal welfare guidelines.

Study design and sampling

This study was conducted on 480 samples collected from 160 poultry flocks including broilers (n=50), ballade chickens (no=50), pigeons (no=30), and ducks (no=30). Three samples (Cloacal swabs, liver, and Intestinal content) were collected from each bird. Poultry samples were taken from various farms situated in different governorates in the Nile Delta region of Egypt. After poultry slaughtering, the liver and intestine were collected regularly through the process of slaughtering as well as aseptic removal of caecal contents. The samples were placed in sterile plastic bags then transported in ice boxes to the lab at 4° and examined immediately within 24 hours.

Regarding human samples, 120 stool samples were collected (57 samples were from persons suffering from diarrhea and 63 samples were from apparently healthy persons, who were in direct contact with the sampled birds) in clean cups and transferred immediately to an ice box at 4°, for further examination in the laboratory.

Isolation and identification of *Campylobacter* species

Campylobacter culture was done as previously detailed by Ahmed et al. [(10)]. Briefly, all samples were inoculated into thioglycolate broth medium in sterile tubes for 48 hours at 42°, then a loopful from each tube was cultured on modified *Campylobacter* blood-free selective medium (Oxoid, CM0739B, England) with selective supplement (Oxoid, SR0155E, England).

All inoculated plates were incubated under micro-aerophilic conditions (5% O2, 10% CO2, and 85% N2), using an anaerobic jar with *Campylobacter* gas generating kits (Oxoid, BR056A, England) at 42 °C for 48 hours then were shown daily for the characteristic colonies. After that, the suspected colonies were purified for 24 hours on blood agar media with defibrinated sheep blood that contained *Campylobacter* growth supplement.

Direct smears from a culture that had been in existence for three days were examined using a phase contrast microscope to show the characteristic corkscrew-like motion that is unique to *Campylobacter* species. Different biochemical tests were performed as catalase production, oxidase activity, and sodium hippurate hydrolysis.

Antimicrobial Susceptibility Profiles of *Campylobacter jejuni:*

The Minimum Inhibitory Concentration values were determined in vitro by using the broth microdilution test, which was performed with Sensititre TM *Campylobacter* plates – EUCAMP (Trek Diagnostic Systems Ltd., East Grinstead, UK). Thirteen different therapeutically significant antibiotics (Erythromycin, Cloxacillin, Amoxicillin, Tetracycline, Azithromycin, Streptomycin, Enrofloxacin,

Levofloxacin, Trimetho sulpha Nexo, Neomycin, Cefopozon, Ampicillin, and Ceftriaxone) were used to test *Campylobacter* isolates' susceptibility.

Molecular confirmation:

From 108 colonies on blood agar that had typical growth and subculture, genomic bacterial DNA was prepared. DNA extraction was done from samples using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. PCR amplifications were carried out targeting *16S rRNA* gene (S1: ATC TAA TGG CTT AAC CAT TAA AC, S2: GGA CGG TAA CTA GTT TAG TAT).

The isolates were confirmed as *C. jejuni* by PCR assays based on the *mapA* gene (S1: CTATTTTATTTTTGAGTGCTTGTG**,** S2**:** GCTTTATTTGCCATTTGTTTTATTA). The isolates of C.*jejuni* that were resistant to tetracycline antibiotic were tested using PCR for the detection of 2 resistance genes (*tetA* and *tetO*) and the following virulence genes: *flaA*, *virB11*, cdt *cluster*, *cdtA*, *cdtB*, and *cdtC*. Primers used to identify resistance and virulence genes that were provided by Metabion (Germany) are listed in Table 1**.** Primers were utilized in a 25- µl reaction that contained 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. The following conditions were used: 5 min of initial denaturation at 94°C followed by 35 cycles of a repetitive program of 30 s of denaturation at 94°C, 45 s of extension at 72°C and the annealing temperatures and the sequences of primers are shown in Table (1).

Finally, the Final extension was at 72˚C for 10 minutes. Thermal cycler 2720 from Applied Biosystems was used to carry out the reaction. Using gradients of 5V/cm, the PCR products were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature. For gel analysis, 15 µl of the products were loaded in each gel slot.

A gel pilot 100 bp ladder (Qiagen, Gmbh, Germany), gene ruler 100 bp ladder (Fermentas, Germany), and Genedirex 100-3000 bp DNA ladder H3 RTU (Genedirex, Taiwan) were used to detect the fragment sizes. The gel was photographed via a gel documentation system (Alpha Innotech, Biometra) and the data were analyzed using computer software.

Table (1): Primer sequences of resistance and virulence genes of *C. jejuni:*

Detected genes	Primers sequences	Amplified segment (bp)	Annealing temperature
FlaA	TCCAAATCGGCGCAAGTTCA	217	55˚C 30 sec.
	TCAGCCAAAGCTCCAAGTCC		
VirB11	TCTTGTGAGTTGCCTTACCCCTTTT	494	53˚C 40 sec.
	CCTGCGTGTCCTGTGTTATTTACCC		
CdtB	CAGAAAGCAAATGGAGTGTT	620	51˚C 40 sec.
	AGCTAAAAGCGGTGGAGTAT		
TetO	GGCGTTTTGTTTATGTGCG	559	50˚C 40 sec.
	ATGGACAACCCGACAGAAGC		
CdtA	GGAAATTGGATTTGGGGCTATACT	165	42˚C 30 sec.
	ATCAACAAGGATAATGGACAAT		
CdtC	TGGATGATAGCAGGGGATTTTAAC	555	42˚C 40 sec.
	TTGCACATAACCAAAAGGAAG		
Cdt cluster	CTTTATGCATGTTCTTCTAAATTT	2212	42˚C 1 min.
	GTTAAAGGTGGGGTTATAATCATT		
TetA	TTCTCTATATCGGGCGGATCGTGGC	700	54˚C 40 sec.
	CCACCCGAAGCAAGCAGGACCATG		

RESULTS

Occurrence of *Campylobacter* species in the examined samples:

The overall occurrence of *C.jejuni* in this work was 23.67% (142/ 600) by conventional methods and PCR which was detected in a percentage of 20.21% (97/480) in different poultry samples as follows: 20, 18.67, 16.67, and 26.67% in broilers, ballade chickens, pigeons, and ducks, respectively. On the other hand, C.*jejuni* was detected in a percentage of 37.5% (45/120) in human samples, among them 43.86% (25/57) was detected in diarrheic cases, while *C.jejuni* was detected in apparently healthy persons with a percentage of 31.75 %(20/63) **(Table 2).**

Table (2): Occurrence of *C.jejuni* in the examined samples by conventional methods and PCR

Sources of examined samples		**Poultry**				**Human**	
		Broilers	**Ballade chickens**	**Pigeons**	**Ducks**	**Diarrheic**	**Apparently healthy**
No. of examined samples		150	150	90	90	57	63
C. jejuni +ve samples	**No.**	30	28	15	24	25	20
	%	20	18.67	16.67	26.67	43.86	31.75
Total percent		20.21				37.5	
Overall percent		23.67					

Table 3 revealed the occurrence of *C.jejuni* in samples taken from poultry, in broiler chickens the highest occurrence of *C. jejuni* was detected in the cloacal swab (40%) followed by intestinal content (14%) and liver (6%). Also ballade chicken and pigeons harbored *C. jejuni* with the acquisition of cloacal swab (36 26.66%) followed by intestinal content (16 13.33%) and liver (4 10%), while in ducks the highest occurrence of *C.jejuni* was observed in intestinal content (36.66%), followed by liver (20%) and cloacal swab (23.33%).

Table (3): Occurrence of *C.jejuni* in the examined poultry samples

Sources of examined samples	**No. of examined samples**								
	Cloacal swabs			**Intestinal content**			**Liver samples**		
	Total	**NO. of positive**	**%**	**Total**	**NO. of positive**	**%**	**Total**	**NO. of positive**	**%**
Broiler chickens	50	20	40	50	7	14	50	3	6
Ballade chickens	50	18	36	50	8	16	50	2	4
Pigeons	30	8	26.66	30	4	13.33	30	3	10
Ducks	30	7	23.33	30	11	36.66	30	14	8.75
Total	160	53	33.12	160	30	18.75	160	14	8.75

Antimicrobial Susceptibility Profiles of *C.jejuni*:

This study used 13 antimicrobials from various antimicrobial groups to detect the resistance of *C.jejuni* isolates. **Table 4** displays the antimicrobial resistance results for the isolates of *C. jejuni*. All *C.jejuni* isolates (n=142), were examined for their susceptibility to 13 antimicrobial agents. Overall, the majority of the strains were ampicillin-resistant (total 116; 81.69% isolates), erythromycin (total 113; 79.58%), Cloxacillin, and Amoxicillin (77.46% and 76.76 respectively), azithromycin and tetracycline (76.06% for each). The range of other antibiotic resistance rates was 56.34% to 40.85%.

Table (4): The frequency of antibiotic drug resistance in *C.jejuni* isolates

Antibiotic group	Resistance profile	NO.	%
β-Lactam	Ampicillin	116	81.69
	Cloxacillin	110	77.46
	Amoxicillin	109	76.76
Macrolide	Erythromycin	113	79.58
	Azithromycin	108	76.06
Tetracyclines	Tetracycline	108	76.06
Aminoglycosides	Neomycin	80	56.34
	Streptomycin	80	56.34
Fluoroquinolones	Enrofloxacin	80	56.34
	Levofloxacin	78	54.93
Sulfonamides	Tri metho-sulpha	78	54.93
cephalosporin	Cefopozon	61	42.96
	Ceftriaxone	58	40.85

Prevalence of resistance genes and virulence factors:

The prevalence of antimicrobial resistance genes was detected among isolates of *C. jejuni* as follows 27.78 and 100% for *tetO* and *tetA*, respectively. Regarding the virulence genes, all isolates of *Campylobacter jejuni* contained the *cdt*, *cdtA*, *cdtB*, and *cdtC* genes.

Among the remaining genes, *virB11* (74.07%) was the most prevalent gene followed by *flaA* (64.81%) as shown in Table 5.

Concerning human isolates, the prevalence of antimicrobial resistance gene *tetO* was detected as 52.94% (18/34). On the other hand, the *tetA* resistance gene could be detected in all human isolates as shown in **Table 5**.

According to data shown in **Table 5**, of all the investigated isolates of poultry 16.22 % (12/74) were harboring the *tetO* gene which could be detected only in broiler isolates. All poultry isolates contained *tetA,cdt*, *cdtA,cdtB*, and *cdtC* genes.

On the other hand, *virB11* could be detected in 70.27% (52/74) of the poultry isolates including 69.23% (18/26) of broilers, 77.78%(14/18) of ballade chickens, and 100% (20/20) of ducks.

However *flaA* gene was reported as 60.81% (45/74) among poultry isolates and found in 73.08, 61.11, and 75% of broilers, ballade chickens, and ducks, respectively. While *virB11* and *flaA* genes could not be detected in pigeons.

Table (5): Occurrence of resistance and virulence genes in the examined poultry and human samples

Sources of examined samples	No. of C.*jejuni* isolates	Percentages of detected genes															
		Tetracyclines				Cytoxin production								Invasiveness		Motility, adherence & colonization	
		tetO		*tetA*		*cdt*		*cdtA*		*cdtB*		*cdtC*		*virB11*		*flaA*	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Broilers	26	12	46.15	26	100	26	100	26	100	26	100	26	100	18	69.23	19	73.08
Ballade chickens	18	0	0	18	100	18	100	18	100	18	100	18	100	14	77.78	11	61.11
Pigeons	10	0	0	10	100	10	100	10	100	10	100	10	100	0	0	0	0
Ducks	20	0	0	20	100	20	100	20	100	20	100	20	100	20	100	15	75
Total of poultry	74	12	16.22	74	100	74	100	74	100	74	100	74	100	52	70.27	45	60.81
Human	34	18	52.94	34	100	34	100	34	100	34	100	34	100	28	82.35	25	73.53
Overall total	108	30	27.78	108	100	108	100	108	100	108	100	108	100	80	74.07	70	64.81

DISCUSSION

Campylobacter spp. is the leading cause of human bacterial foodborne illnesses now worldwide. *Campylobacter* infections rarely or never result in clinical diseases in poultry. However, carcasses of poultry have been infected frequently in processing plants because of the high incidence of *Campylobacter* in market-age poultry's intestinal tracts and its ability to be transmitted to humans through poultry carcasses [(11)].

Through our study, a total of 23.67% (142/600) of *C.jejuni* were identified from the examined samples by using conventional methods and PCR. Higher percentages of *C.jejuni* were detected by **Olkkola *et al.*** [(12)] On the other hand; a lower percentage (15.17%) of *C.jejuni* was obtained by **Ghoneim *et al.*** [(13)]. *C.jejuni* was isolated from 20.21% (97/480) of the total poultry samples and more frequently from ducks (26.67%) followed by broiler chickens (20%), ballade chickens (18.67%), and pigeons (16.67%). Similarly, **Kim *et al.*** [(14)] have reported that duck meat is more frequently contaminated with *Campylobacter* than raw chicken. While lower percentages (17 and 11.87%) of *C.jejuni* from broiler chickens and laying chickens were isolated, respectively by **Ghoneim *et al.*** [(13)].

This investigation's findings revealed that the cloacal swabs, intestinal contents, and the liver of poultry samples were contaminated at rates of 33.12, 18.57, and 8.75% with *C. jejuni*, respectively. Nearly similar rates were detected by **Ghoneim *et al.*** [(13)] who isolated *C.jejuni* from the intestine of both broiler and laying chickens with percentages of (20 and 17.75%), respectively. On the other hand, **Ghoneim *et al.*** [(13)] could distinguish *C.jejuni* from cloacal swabs and the liver with the following percentages of 15 and 16.66%, respectively. Lower rate (6.6%) of the liver was obtained by **Hafez *et al.*** [(15)], while higher prevalence (52.8%) was detected by **El Fadaly *et al.*** [(16)] in Egypt. Possible explanations for this discrepancy include genetic variation and resistance among thermophilic *Campylobacter species*, which are explained by geographical diversity, as well as variations in the origin of poultry-originated *Campylobacter* isolates between studies.

In this study, the overall Occurrence of *C. jejuni* in human stool samples was 37.5% as identified by using conventional methods and PCR, with percentages of 43.86 and 31.75 % in diarrheic and apparently healthy persons, respectively. This finding was higher than that obtained by **Ghoneim *et al.*** [(13)] at 17.33%, respectively. While higher isolation rate (51.5%) was obtained by **El Fadaly** [(16)].

There is a significant concern that *Campylobacter* spp. which is isolated from both humans and animals is becoming more resistant to antimicrobials. In developing countries such as Egypt, most antimicrobial substances in the human pharmacopeia are used also in the poultry industry [(17)].

Recent research indicates that the resistance to antibiotics of *C. jejuni* increased all over the world. In our study, the highest percentages of *C. jejuni* were resistant to ampicillin (81.69%). This result was higher than that obtained by **Premarathne *et al.*** [(18)] (69.2%). On the other hand, resistance to Macrolides (Erythromycin and Azithromycin) was 79.58% and 76.06% respectively. Azithromycin is one of the additional antibiotics in this group. Lower percentages were detected by the **European Food Safety Authority** [(19)] which reported the rate of macrolide resistance in Malta for *C. jejuni* (10%), but over the next two years, there was a decline to 1.2% and **Premarathne *et al.*** [(18)] detected that 30.8% of the isolates were resistant to erythromycin. Unfortunately, percentages are much higher 88% of *Campylobacter* isolates from commercially raised poultry in South Africa were erythromycin-resistant [(20)]. There is a concern for public health due to *Campylobacter's* high level of resistance to some significant antibiotics. Since *Campylobacter* considers zoonotic bacteria and is exposed to antibiotics used for treating both animal disease and human disease, this complicates the development and transmission of antibiotic-resistant genes [(21)].

Regarding amoxicillin tetracycline resistance 76.76 &76.06% % of *C. jejuni* isolates were resistant to amoxicillin & tetracycline, respectively. Somewhat closely related ratio of 76.9 and 77.4% of tetracycline was obtained by **Premarathne *et al.*** [(18)]. Lower percentages of 58.1 and 48.8% of amoxicillin and tetracycline were mentioned by **Wysok *et al.*** [(22)] respectively. Several researchers have reported lower tetracycline resistance rates for *C. jejuni* isolates recovered in different geographic locations: 13.7–65.8% in European countries, 35.2% in Iran, 1% in Madagaskar, 33.33–89% in Asia and 50–64.44% in North America rate was detected as 37.5% in 2008 [(23)]. The wide use of those antibiotics in both human medicine and animal husbandry may be the cause of the high antibiotic resistance found in this study.

Fluoroquinolones resistance including Enrofloxacin and levofloxacin were 56.34 and 54.93% respectively. Similar to the global trend, fluoroquinolone resistance in *C. jejuni* has also increased over time. More specifically, the quinolone resistance rate was detected with lower percentages of 0% in 1989, 1.01% in 1994, 28% in 1996, and 14% in 2003 [(23)]. While higher percentages were obtained by **Kayman *et al.*** [(23)] of 63.63% in 2007, 69.23% in 2010, and 74.3% in 2013. Also, **Wysok *et al.*** [(22)] detected that resistance toward fluoroquinolones was predicted to occur in 67.4 % of the isolates. An increase in antibiotic-resistant infections has been caused by the overuse of antibiotics in both the human population and animal husbandry. especially with fluoroquinolones, the mixture of careless application of fluoroquinolones in humans and increased fluoroquinolones use particularly within the poultry industry, has aided in the rise in the frequency of fluoroquinolones resistance in animals and humans. However, a major increase was detected by **Cody *et al.*** [(24)] in the number of

ciprofloxacin-resistant *Campylobacter* isolates recovered in Britain between 1991–2009.

Antimicrobial resistance patterns between the isolated *C. jejuni* showed that 56.34% of the isolates were resistant to streptomycin. A lower percentage of 23.1% of streptomycin resistance was obtained by **Premarathne *et al.*** [18]. Ceftriaxone, aminoglycosides resistance was detected at 40.85and 56.34% which is considered lower than those detected by **Wysok *et al.*** [22] (46.5 & 11.6%), respectively. Differences in sample type, the technique of sampling, antibiotic type, and frequency used in animal husbandry practices and human therapy can be used to explain differences and similarities in antibiotic resistance patterns.

High tetracycline resistance is typically linked to the acquired *tetO* gene, which codes for a protective ribosomal protein [25]. This study reported that not all of the phenotypic tetracycline-resistant isolates had the *tetO* gene, only 27.77% were detected among poultry and human samples, similarly, **Dasti *et al.*** [26] indicated that 54% of tetracycline-resistant isolates possessed the plasmid-mediated *tetO* gene. This current study was not investigated the accurate location (chromosome or plasmid) of *tetO* in isolates that were resistant to tetracycline- generally, it is well known that *tetO* in tetracycline-resistant *Campylobacter* is commonly present on a plasmid although it occasionally may be carried in the chromosome [27].

A higher rate of *tetO* gene (100%) was detected by **Gharbi *et al.*** [9]. Because *tetO* gene considers the most frequently reported determinant conferring tetracycline resistance, the high prevalence of *tetO* gene in some studies represents high tetracycline resistance in *Campylobacter* spp. isolates [28]. On the other hand *tetO* and *tetA* genes could be detected in 16.22 and 100 % of *C.jejuni* of poultry isolates. A higher rate of *tetO* gene (74.4%) and a lower rate of *tetA* gene (16.3%) were obtained by **Abdi Hachesoo *et al.*** [11]. poultry strains with a high prevalence of the *tetA* resistance gene can act as a source of this gene and possibly aid in the spread of this gene to other bacteria like *Campylobacter* spp. in the poultry industry [11].

Worldwide, numerous studies have demonstrated that the presence of *Campylobacter*-related diseases is significantly affected by the expression of various genes implicated in motility, epithelial invasion, cell colonization, and toxin production.

In this study, most *Campylobacter* isolates contain associated-virulence genes related to the adherence of the pathogen, colonization, and invasion traits. This was correlated with the results obtained in a prior study, where genes like *flaA*, *virB11*, and *cdt* were frequently present [29].

In this study, the most prevalent virulence genes in tested *C. jejuni* isolates were those responsible for the production of CDT clusters. High prevalence rates of these genes in C. *jejuni* isolated from both humans and poultry were also reported by **Bardoň *et al.*** [30]. In addition, all *Campylobacter* isolates included the *cdtA*, cdtB, and *cdtC* genes required for the production of the CDT toxin, as reported by **Gharbi *et al.*** [9] who reported that *cdtA*, *cdtB*, and *cdtC* genes were present in every *Campylobacter* isolate. On the other hand, a high prevalence rate of 92% of *cdtA*, *cdtB*, and *cdtC* in *C. jejuni* isolates was obtained by **Krutkiewicz *et al.*** [31]. Lower rate of *cdtA* (85%) *cdtB* (80%) and *cdtC* (92.1%) was obtained by **Sierra-Arguello** [32].

Among the remaining genes, the percentages of *flaA* and *virB11* virulence genes isolated from poultry and human samples were 64.81 and 74.07%, respectively. Higher percentages of *flaA* and *virB11* (100% and 94%) in *C. jejuni* were obtained by **Gharbi *et al.*** [9]. A higher percentage of 78.6% of the *flaA* gene for *C. jejuni* isolates which play an important role in flagellar motility was obtained by **Sierra-Arguello** [32] adding to Previous investigations in Brazil have shown that almost 80% of *Campylobacter* spp. isolates from human and animal samples contain *flaA* [33]. However, *the flaA* gene in this study could be detected in 60.81 and 73.53 % of the poultry and human isolates, respectively. Higher isolation rates (98.7 and 100%) could be detected in poultry and human, respectively by **Wieczorek *et al.*** [34]. These variations might be due to the presence of multiple flagellar motility gene mutations, as these genes undergo mutations including gene deletion that develop when *Campylobacter* is exposed to novel selection conditions through in vitro cultivation in a non-host environment exhibited loss of flagellar motility [35].

Moreover, *virB11* was present in a lower percentage of 70.27% in the poultry than in human isolates 82.35%. Similarly, **Wysok *et al.*** [22] detected a higher percentage of *virB11* in human isolates than in poultry. In previous investigations, this marker was sporadically found in human isolates.

CONCLUSION

This study assesses the occurrence of multi-drug resistance and virulence genes among *C. jejuni* strains isolated from poultry and human, in Egypt. It was reported that the prevalence of strains carrying numerous virulence characteristics is noticeably high, and the evolution of antimicrobial resistance among *Campylobacter* isolates is raising major concerns and could pose a significant risk to public health. Our results also recommend the need for surveillance and monitoring systems for *Campylobacter* prevalence and antimicrobial resistance in poultry and food animals. Additionally, the prevalence of numerous *Campylobacter* species that are resistant to antibiotics necessitates limiting the use of antibiotics in animal husbandry, increasing public education and awareness, and application of good veterinary procedures to reduce the likelihood of emerging superbugs.

Authors' contributions: The final manuscript was written by all authors, who all contributed equally.

Competing interest: The authors claim to have no conflicts of interest.

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