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Campylobacter Species Isolated from Chickens in Egypt: Molecular Epidemiology and Antimicrobial Resistance

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ABSTRACT

Campylobacter is one of the most important zoonotic bacterium and the leading cause of human gastroenteritis worldwide. To investigate the occurrence and antimicrobial resistance of this pathogen, a total of 360 chicken cloacal swabs and 15 water samples were gathered from different localities in Giza and Cairo Governorates. An additional 50 stool specimens were collected from individuals in contact with the examined chickens. Eleven *Campylobacter* isolates were recovered through bacteriological examination. *Campylobacter* spp. were identified by polymerase chain reaction (PCR) as *C. jejuni* (63.6 %) and *C. coli* (36.4 %) through the detection of the *Map* A and *Ceu* E genes, respectively. The antibiotic resistance of the *Campylobacter* isolates was determined via the disc diffusion method and was observed most frequently to nalidixic acid (81.8 %), tetracycline (72.7 %), ciprofloxacin (54.5 %), and erythromycin (54.5 %), while low resistance to ceftriaxone (18.2 %) was detected. Among the 11 *Campylobacter* isolates, 9 isolates were multidrug resistant (MDR). The *tet* (O) gene, which is responsible for tetracycline resistance, was detected in only 6 isolates. Phylogenetic analysis of the *tet* (O) gene sequences recovered from the *C. jejuni* isolates revealed that the strains isolated from chickens and drinking water from the same farm were identical. However, the sequence of the *tet* (O) gene from human isolates was highly similar to that from drinking water isolates. Our findings highlight the presence of MDR *Campylobacter* strains in chickens and the role of drinking water as a potential reservoir for tetracycline-resistant isolates. Therefore, regular monitoring of resistance is required, and increased attention should focus on preventing the transmission cycle of such emerging pathogens between different ecosystems to avoid public health hazards.

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Authors' Contribution
NHA, ZSA, EAE and MAS presented the concept. NHA, EAE and MAS wrote the manuscript. ZSA: prepared the samples and applied bacteriological examination and PCR assay. EAE helped in laboratory work.

Key words
***C. jejuni, C. coli*, MDR, *tet* (O), Chicken, Human**

INTRODUCTION

Campylobacter is among the most prevalent causes of human gastroenteritis and is responsible for a significant number of foodborne illnesses and deaths (CDC, 2016; Nyachuba, 2010). Thermophilic *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis* are considered the most important species implicated in foodborne illness (EFSA, 2013).

The majority of *Campylobacter* infections in humans are associated with poor handling practices or the consumption of undercooked chicken (Doorduyn *et al*., 2010). *Campylobacter* infections produce little or no clinical diseases in poultry (Luangtongkum *et al*., 2006). However, the colonization of the intestinal tract of market-age chickens by *Campylobacter* may lead to the heavy contamination of their carcasses in processing plants (Jeffrey *et al*., 2001). Other routes of infection include contact with pets, exposure to farm animals, and the consumption of raw milk, untreated water, and undercooked beef, pork and shellfish (DuPont, 2007).

Campylobacteriosis in humans is usually characterized by self-limiting watery/bloody diarrhoea, abdominal cramps, nausea, and fever. Severe neurological sequelae, bacteraemia, and other extra-intestinal complications develop infrequently (Blaser and Engberg, 2008). Erythromycin (a macrolide) is considered the drug of choice for campylobacteriosis. On the other hand, fluoroquinolone (FQ) (Allos, 2001), tetracycline and gentamicin antibiotics are also frequently used as alternative drugs in cases of *Campylobacter* infection (Blaser, 2008).

Antimicrobial resistance, especially to fluoroquinolone (ciprofloxacin) and macrolides (erythromycin), has emerged in *Campylobacter* (Lehtopolku *et al*., 2011). The use of tetracycline while rearing farm animals has been reviewed in recent years because of its growth-promoting properties (Chopra *et al*., 1992). The addition of a sub-therapeutic dose of chlortetracycline in livestock rations positively affects the rate of growth and feed utilization of young chickens (Stockstad *et al*., 1949). Therefore, a significant increase in tetracycline resistance has been observed in *Campylobacter* isolates recovered from chickens (EFSA, 2012). Such resistance is usually

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associated with the *tet* (O) gene, which is carried on transmissible plasmids (Taylor and Courvalin, 1988).

In Egypt, genotyping confirmation of environmental *Campylobacter* strains, which may contribute to the rapid emergence and dissemination of resistant bacteria and genes among poultry and humans, is lacking. Accordingly, this study was designed to investigate the occurrence of *Campylobacter* spp. among chickens, water, and humans. Antibiotic susceptibility and the tetracycline resistance gene *tet* (O) were also investigated to determine the resistance pattern of *Campylobacter* isolates recovered from different sources. In addition, the *tet* (O) genes were sequenced to trace the potential source of such genes among *Campylobacter* isolates from chickens, water and humans.

MATERIALS AND METHODS

Sample collection

A total of 360 cloacal swab samples were collected from chickens from randomly selected farms (n=200), households (n=60) and poultry shops (n=100) located in El-Giza and Cairo Governorates. Fifty human stool specimens were gathered from housewives rearing poultry (n=20), workers at poultry farms (n=20), and chicken handlers at poultry shops (n=10). Additionally, 15 water samples were obtained from water tanks in farms, households and poultry shops (5 from each) at the same localities of the stool sample collections.

Bacteriological examination

Campylobacter detection and identification were performed according to the ISO 10272 (2006) standard. In brief, samples were taken with sterile swabs, and the swabs were transferred to tubes containing Cary Blair transport medium (Oxoid CM0519) and subsequently inoculated into tubes containing 9 ml of sterile selective enrichment thioglycolate broth. Water samples were prepared according to the standard procedure of the American Public Health Association (APHA, 1981). The enrichment broth tubes were incubated at 37 °C for 4 h, followed by incubation for an additional 24-48 h at 42 °C in a microaerophilic condition using anaerobic jars and Campy Gen generating kits (Oxoid CN0025 and CN0035). A loopful from each of the previously incubated enrichment broth tubes was streaked over mCCDA agar (Oxoid CM0739) supplemented with CCDA and then incubated at 42 °C for 48 h under the same conditions (ISO10272-1, 2006).

Molecular identification and detection of the tet (O) resistance-encoding gene

Genomic DNA was extracted from pure suspected colonies using the boiling method according to Sheedy *et al*. (2004), and the extracted DNA was stored at -20 °C until use.

Primers specific for *Campylobacter* spp. and antibiotic resistance genes are summarized in Table I. The amplification reaction was performed according to Wang *et al*. (2002). Each reaction assay (25 µl) contained 6 µl of template DNA from each isolate, 12.5 µl of Hot Star Taq Master Mix (Thermo Scientific), 1 µl of each primer (20 pmol), and 4.5 µl of PCR-grade water. The cycling conditions were as follows: initial denaturation at 94 °C for 5 min, 35 cycles each consisting of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s, and a final extension step at 72 °C for 10 min. Then, PCR products (15 µl each) were electrophoresed on agarose gel (1.5 %) and visualized under ultraviolet light.

Antimicrobial susceptibility screening

Confirmed isolates were screened using the agar disc diffusion technique according to Finegold and Martin (1982) for susceptibility to nalidixic acid (NA, 30 µg), ciprofloxacin (CIP, 5 µg), gentamycin (CN, 10 µg), erythromycin (E, 15 µg), ampicillin (AMP, 10 µg), tetracycline (TE, 30 µg), ceftriaxone (CR, 30 µg) and chloramphenicol (C, 30 µg). The results of the tested antibiotics were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2014).

MAR index

The multiple antibiotic resistance (MAR) indices of the isolates were determined as a/b, where ‘a’ represents the number of multiple antibiotics to which the particular isolates were resistant, and ‘b’ represents the number of multiple antibiotics to which the particular isolates were exposed (Kamperman., 1983).

Sequencing and phylogenetic analysis

The amplicons of the *tet* (O) gene in the selected isolates of *C. jejuni* and *C. coli* were purified using the GeneJET PCR Purification Kit (Thermo) according to the manufacturer’s instructions and then sequenced at the Animal Health Research Institute (AHRI) in Dokki, El- Giza. The sequencing step was conducted with Big Dye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems). The obtained nucleotide sequences were determined by Basic Local Alignment Search Tool (BLAST) analysis and were compared with the sequences available in GenBank using NCBI.

To assess the relatedness of our gene sequences recovered from human, chicken and drinking water isolates, these sequences were downloaded and imported into Bio Edit version 7.0.1.4 for multiple alignments using

Table I. Primers used for *Campylobacter species* identification and tetracycline resistant gene (*tet O)* of *Campylobacter* isolates.

Target agent & genes	Primer sequence (5'-3')	Amplified Length (bp)	Reference
Campylobacter 23S rRNA	TATACCGGTAAGGAGTGCTGGAG (F)	650	Wang *et al*., 2002
	ATCAATTAACCTTCGAGCACCG (R)		
C. jejuni mapA	CTATTTTATTTTTGAGTGCTTGTG (F)	589	Eunju and Lee, 2009
	GCTTTATTTGCCATTTGTTTTATTA(R)		
C. coli CeuE	AAT TGA AAA TTG CTC CAA CTA TG(F)	462	Eunju and Lee, 2009
	TGA TTT TAT TAT TTG TAG CAG CG(R)		
Campylobacter tet O	GGCGTTTTGTTTATGTGCG(F)	559	Gibreel *et al*., 2004
	ATGGACAACCCGACAGAAGC(R)		

the Clustal W program. The sequence identity matrix and MEGA6 software version (6.06) were used for construction of the phylogenetic tree by the neighbour-joining method (Fig. 1).

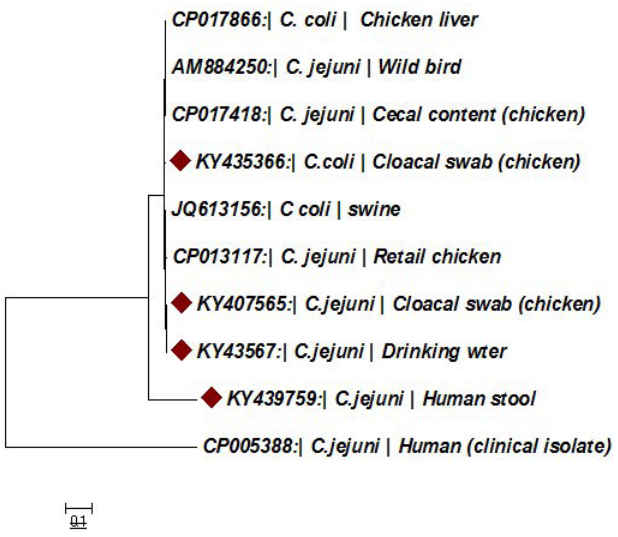

Fig. 1. Phylogenetic tree analysis of *tet* (O) gene sequences of *campylobacter* isolate recovered from different sources (chicken, drinking water and human). The accession numbers for our study sequences and related sequences retrieved from GenBank were shown. The tree was generated based on the neighbour-joining method.

GenBank accession numbers

The nucleotide sequences of the *tet* (O) genes recovered from both *C. jejuni* and *C. coli* isolates in this study were deposited in GenBank under the following accession numbers: KY435367 (water sample, *C. jejuni*), KY407565 (cloacal swab, *C. jejuni*), KY435366 (cloacal swab, *C. coli*), and KY439759 (human stool, *C. jejuni*).

RESULTS

A total of 425 samples were analysed for the presence of *Campylobacter* spp. using bacteriological examination associated with PCR confirmation. Three of 50 human stool specimens (6.0 %), 7 of 360 chicken cloacal swabs (1.9 %) and only one of 15 water samples (6.7 %) from different households, farms and shops in Giza and Cairo Governorates were *Campylobacter* positive. *C. jejuni* was the most frequently isolated species among the positive isolates, representing 63.6 % (Table II).

Table II. Occurrence of *Campylobacter* spp. in the examined samples.

Sample origin	Sample number	*Campylobacter* isolates		
		Positive (%)	*C. jejuni* (%)	*C. coli* (%)
Human	50	3 (6.0)	2 (66.7)	1 (33.3)
Chicken	360	7 (1.9)	4 (57.1)	3 (42.9)
Water	15	1 (6.7)	1 (100.0)	0 (0.0)
Total	425	11 (2.6)	7 (63.6)	4 (36.4)

To identify the antimicrobial susceptibility of *Campylobacter* spp., all isolates (11) were screened against 8 antibiotics that are frequently used as growth promoters or treatments for chickens in Egypt. The highest resistance rates were identified towards nalidixic acid (81.8 %), tetracycline (72.7 %), ciprofloxacin (54.5 %) and erythromycin (54.5 %). A lower frequency (18.2 %) of resistance to ceftriaxone was observed (Table III).

The MAR indices of the isolated *Campylobacter* spp. indicated that each isolate was resistant to at least two antibiotics used in the current study. Among the isolates, only 2 *C. jejuni* strains isolated from humans were resistant to two antibiotics, with a MAR index of 0.3. The other 9 isolates (81.8 %) were resistant to three or more

Table III. Antimicrobial-resistant *C. jejuni and C. coli* isolates recovered from chicken, water and human specimens.

Classes of antibiotics	Antibiotic concentration (µg)	Resistant strains		Total
		C. jejuni (n - 7)	*C. coli* (n – 4)	
	Nalidixic acid (30)	6 (85.7%)	3 (71.4%)	9 (81.8%)
Quinolones	Ciprofloxacin (5)	4 (57.1%)	2 (42.9%)	6 (54.5%)
Aminoglycosides	Gentamycin (10)	1(14.3%)	2 (28.6%)	3 (27.3%)
Macrolides	Erythromycin (15)	3 (42.8%)	3 (57.1%)	6 (54.5%)
Penicillins	Ampicillin (10)	1 (57.1%)	2(28.6%)	3 (27.3%)
Tetracyclines	Tetracycline (30)	5 (71.4%)	3 (57.1%)	8 (72.7%)
Cephalsporins (3rd generation)	Ceftriaxone (30)	1 (57.1%)	1 (42.9%)	2 (18.2%)
Quinolones	Chloramphenicol (30)	3 (42.8%)	1 (28.6%)	4 (36.4%)

antimicrobial agents. More multidrug-resistant (MDR) isolates were identified from chickens than from water and humans, and the isolates from chickens had MAR indices between 0.4 and 0.6 (Table IV).

All isolates were subjected to PCR to screen for the *tet* (O) gene, which contributes to tetracycline resistance. The genetic analysis revealed a 54.5 % (6/11) overall occurrence of the *tet* (O) gene among the examined isolates. Only 6 isolates (75 %) harboured *tet* (O) genes among 8 tetracycline-resistant isolates, whereas *tet* (O) was detected in 36.4 % of *C. jejuni* isolates (4/11) and 18.2 % of *C. coli* isolates (2/11) (Table V).

Table IV. Multiple antibiotic resistance (MAR) index of *Campylobacter spp.* from human, chicken and water samples.

MAR index	*Campylobacter* spp.	Isolate source	Antibiotic resistant profile
0.3	*C.jejuni*	Human	NA, TE
0.3	*C.jejuni*	Human	CIP,C
0.4	*C.coli*	Human	E, CR, C
0.6	*C.coli*	Cloacal swab	NA, CIP, CN, AMP, TE
0.5	*C.coli*	Cloacal swab	NA, CN, E, TE,
0.6	*C.coli*	Cloacal swab	NA, CIP, E, AMP, TE
0.6	*C.jejuni*	Cloacal swab	NA, CIP, E, AMP, TE
0.6	*C.jejuni*	Cloacal swab	NA, CIP, E, TE, C
0.4	*C.jejuni*	Cloacal swab	NA, CR, C
0.5	*C.jejuni*	Cloacal swab	NA, CIP, E, TE
0.4	*C.jejuni*	water	NA, CN, TE

NA: 30 µg nalidixic acid; CIP: 5µg ciprofloxacin; CN: 10 µg gentamycin; E: 15 µg erythromycin; AMP: 10 µg ampicillin; TE: 30µg tetracycline; CR: 30 µg ceftriaxone and C: 30 µg chloramphenicol.

The identity of ≤ 99% to *tet* (O) gene sequences of the *Campylobacter* spp. strains, indicating that the set of primers described here exclusively amplified the 559 bp fragment of the target gene. Phylogenetic analysis of the *tet* (O) gene sequences recovered from the *C. jejuni* isolates revealed that the chicken and drinking water strains isolated from the same farm were identical. However, the sequences of the *tet* (O) genes from human isolates were highly similar to those from drinking water isolates. However, there were also similarities between the study sequences and the sequences retrieved from GenBank, as shown in Figure 1.

Table V. Detection of *tet* O gene in *Campylobacter* species isolated from different sources.

No. of positive isolates to tet O gene						No. of strains resistant to tetracycline		No. of isolates	Source of isolates
Total (n - 11)		*C. coli* (n - 4)		*C. jejuni* (n - 7)					
%	No.	%	No.	%	No.	%	NO.		
50.0	1	0.0	0	50.0	1	66.7	2	3	Human
66.7	4	33.3	2	33.3	2	85.7	6	7	Chicken
100.0	1	0.0	0	100.0	1	100.0	1	1	Water
54.5	6	18.2	2	36.4	4	100.0	11	11	Total

DISCUSSION

Campylobacter has now emerged as one of the leading causes of foodborne illness in humans around the world. Many studies have reported that there has been a rise in the incidence of campylobacteriosis globally in the past decade (Kaakoush *et al.*, 2015). *Campylobacter* is found mostly in chicken meat; therefore, chicken meat and poultry farms play a key role in the epidemiology of human

infection (Zhang *et al*., 2018). In the current study, the low contamination frequency of *Campylobacter* spp. detected in chicken cloacal swab samples is in agreement with Marinuo *et al*. (2012), who recorded a 1.48 % isolation rate of *Campylobacter* spp. in poultry farm samples. In addition, an EFSA report (2010) recorded a relatively low prevalence of *Campylobacter* in broiler flocks in Norway (3.2 %) and Finland (3.9 %). In contrast, Rajagunalan *et al*. (2014) in India and Bardon *et al*. (2008) in the Czech Republic reported *Campylobacter* prevalence rates of 76.32 % and 50.0 %, respectively. These variations may be attributed to geographical location, breeding methods and season-related differences (Daskalov and Maramski, 2012).

Since *Campylobacter* is a zoonotic pathogen and can often be recovered from asymptomatic individuals, the current study investigated the occurrence of this pathogen in workers with close contact to chickens regardless of gastrointestinal symptoms. The occurrence of *Campylobacter* spp. in humans was nearly similar to that recorded by Pazzaglia *et al*. (1993) and Zaghloul *et al*. (2012), who reported that *Campylobacter* spp. were identified in 6.4 and 6.6 % of human stool samples in Alexandria and Cairo Governorates in Egypt, respectively.

The most prevalent species recorded in our study was *C. jejuni* (63.6 %), and a similar result was also recorded in previous studies in chickens (Agunos *et al*., 2014; Sahin *et al*., 2015). This finding is in line with many studies reporting that *C. jejuni* is commonly found in the gastrointestinal tract of broiler chickens and wild birds, while *C. coli* is typically prevalent in other animals (Dasti *et al*., 2010; Epps *et al*., 2013).

C. jejuni was isolated from only one water sample from tanks used to provide drinking water to chickens in the present study. Similarly, Shimaa *et al*. (2015) recorded *Campylobacter* in 12.8 % of the examined water in chicken farms in Egypt. Moreover, a high prevalence (30 %) of *Campylobacter* spp. contamination was recorded by Barakat *et al*. (2015) in tap water. This finding may be attributed to the poor recovery of such pathogens from environmental samples using selective culture methods due to the formation of viable but non-culturable (VBNC) cells. Although a low level of contamination with this pathogen was detected in the drinking water samples in our study, this finding is indicative of a recent contamination of water tanks that were opened with faecal droppings of chickens or wild birds during the rearing period (Friedman *et al*., 2000). This result indicated the potential role of drinking water in poultry farms in the process of *Campylobacter* colonization in chickens. Moreover, several studies have indicated the role of the natural environment (soil and water) in the transmission of campylobacteriosis, either directly to humans or indirectly via farm animals, especially poultry (Bronowski *et al*., 2014). Hence, the use of poor-quality drinking water in poultry farms poses a public health threat.

The overall prevalence of antimicrobial agents to which a *Campylobacter* isolate was resistant ranged from 18.2 % to 81.8 %. In the current study, a high resistance to fluoroquinolones (nalidixic acid), tetracycline, quinolones (ciprofloxacin) and erythromycin was observed. Meanwhile, the isolates were susceptible to ceftriaxone (third-generation cephalosporin), gentamicin, ampicillin, and chloramphenicol. Similar findings have also been reported by Raeisi *et al*. (2017), who reported that poultry *Campylobacter* isolates were resistant to ciprofloxacin, nalidixic acid and tetracycline. On the other hand, Szczepanska *et al*. (2017) reported that in Poland, *Campylobacter* spp. were resistant to ciprofloxacin and tetracycline but were susceptible to erythromycin. The expected similarities and differences among our findings and those reported in previous studies may be attributed to the frequency of antibiotic usage in animal husbandry practices and human therapy (Zhao *et al*., 2010).

Resistance rates to tetracycline and quinolones vary worldwide, and the high prevalence of *Campylobacter* spp. that are resistant to these drug classes has increased and become increasingly worrisome in recent years (Mackiw *et al*., 2012). Alarmingly, the increased resistance of *Campylobacter* to antimicrobials, particularly tetracycline, erythromycin, and (fluoro)quinolones, is associated with a reduced response to therapy, leading to higher morbidity and mortality rates in humans (Zhu *et al*., 2006).

In summary, both human and chicken isolates are generally resistant to nalidixic acid, ciprofloxacin, tetracycline and erythromycin. This might be due to the improper use of these antibiotics in veterinary and human medicine. Therefore, the present findings suggest that antibiotics used for humans should not be used in poultry. Furthermore, the relatively high percentages of resistance to most antimicrobial agents screened in this study could be explained by the widespread and uncontrolled use of these agents as growth promoters or in animal treatment, reflect the extent to which these antibiotics are used in Egypt and pose a challenge to the management of *Campylobacter* infections.

Currently, multidrug resistance is becoming an increasing problem in *Campylobacter* isolates because it can compromise the effective treatment of campylobacteriosis. Nine *Campylobacter* spp. evaluated in the current study were resistant to at least 3 antimicrobial groups. Thus, they are characterized as MDR isolates; high levels of resistance were observed among the isolates from chicken cloacal swabs and water samples. This is in agreement with Said *et al*. (2010), who suggested that a higher prevalence

of MDR strains has been reported from animal and meat isolates than from human isolates. Importantly, emerging MDR *Campylobacter* poses a great threat to the poultry industry and to humans because resistance genes could be transmitted between different hosts.

MAR indexing is a useful tool to identify ecological contamination. All *Campylobacter* spp. in this study had a MAR index greater than 0.3, which indicates a high frequency of antibiotic usage in poultry in Egypt. Moreover, these isolates are considered to originate from animals that have a high potential for contamination (Marian *et al*., 2012), subsequently exacerbating the public health concern associated with *Campylobacter* infections.

To investigate the molecular basis of tetracycline resistance in *Campylobacter* isolates, the presence of the tetracycline resistance gene *tet* (O) was estimated. The current study showed that not all tetracycline-resistant isolates harboured the *tet* (O) gene, and this finding was also observed by Obeng *et al*. (2012), who recorded a low correlation between tetracycline resistance and the presence of the *tet* (O) gene. Although high levels of phenotypic resistance to tetracycline were attributed to the presence of the *tet* (O) gene (Wieczorek *et al*., 2013), nonspecific efflux systems, such as the CmeABC multidrug efflux pump, may also play a role in decreasing the susceptibility to such antibiotics (Lovine, 2013).

Recently, gene sequencing has been considered a novel genotyping method with promising potential for the detection of epidemiological relationships and the diversity of genes recovered from different sources. In this study, the phylogenetic analysis of the isolate sequences demonstrated that chicken (KY407565, KY435366), drinking water (KY435367) and human (KY439759) isolate sequences show relationships with 5 sequences retrieved from GenBank, including retail chicken (CP013117), chicken liver (CP017866), chicken caecal content (CP017418), swine (JQ613156) and wild bird (AM884250), as they were found in the same cluster. From this cluster, the studied *tet* (O) sequences from chicken (KY407565) and drinking water (KY435367) isolates sampled from the same farm were closely related to each other, which reflects the environmental origin of the *tet* (O) gene in such chickens. This scenario highlights the potential role of contaminated drinking water in the transmission of tetracycline-resistant *C. jejuni* to chickens (Trigui *et al*., 2015)

The phylogenetic tree also demonstrates that the human *tet* (O) gene sequence in our study (KY439759) is more similar to the drinking water sequence (KY435367) than the chicken sequence (KY407565). Therefore, *Campylobacter* survival in water is critical for transmission to humans through the consumption of contaminated drinking water and for transmission from one animal reservoir to another (Bronowski *et al*., 2014). The results of our study augment this concept and underscore drinking water as a potential reservoir of resistance genes since they are recipients of bacteria from different sources (Kim *et al*., 2010), including livestock manure coming from neighbouring farms (Clark *et al*., 2003) and/or sewage (O'Reilly *et al*., 2007), and may play a pivotal role in the transmission of *Campylobacter* infection for humans and chickens. Importantly, the isolation of closely related *tet* (O) genes from *C. jejuni* strains in chickens, humans and drinking water at the same farm reflects the epidemiological relationship between them. Thus, increased attention should focus on preventing the transmission cycle of such pathogens between different ecosystems to avoid public health threats.

On the other hand, our study of the *tet* (O) gene sequence of *C. coli* recovered from chickens (KY435366) shows a relationship with the *tet* (O) gene sequence of *C. jejuni* recovered from the caecal content of chickens in the USA (CP017418), which suggests evidence of horizontal gene transfer (HGT) of genetic elements between *C. coli* and *C. jejuni*, especially in the intestinal tract of chickens (Avrain *et al*., 2004). Foodborne pathogens can acquire a variety of resistance genes from the reservoir of commensal bacteria in animals' intestines (Salyers *et al*., 1995). Hence, *Campylobacter* spp. may acquire the *tet* (O) gene from commensal bacteria found in the intestinal tract of chickens, especially after oral administration of tetracycline (Fairchild *et al*., 2005). These pathways of resistance gene acquisition indicate that tetracycline resistance genes can be transmitted between bacteria and humans and between animals and different ecosystems.

In conclusion, this study demonstrated that *C. jejuni* strains were more common than *C. coli* in chicken, human and drinking water isolates at the same farm. Although a low percentage of *Campylobacter* was detected in the examined samples, the identification of high levels of antimicrobial resistance and MDR isolates make this issue even more serious. Most chicken *Campylobacter* isolates were MDR, particularly to antibiotics that are often used as first-line treatments. The closely related *tet* (O) genes from the *C. jejuni* strains in chickens, humans and drinking water were from the same farm. Thus, increased attention should focus on preventing the transmission cycle of such pathogens between different ecosystems to avoid public health threats and on the need to decrease the generation of tetracycline-resistant *Campylobacter* spp. through cautious use of tetracycline in poultry production. Our results emphasize the need for more frequent monitoring of the prevalence and antimicrobial resistance of *Campylobacter* to provide support for actions directed at

reducing this pathogen in the food chain. In addition, we suggest further molecular studies on efflux system genes as an important tool to reduce the antimicrobial resistance and colonization of *Campylobacter* in animals raised for food purposes in Egypt.

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Statement of conflict of interest

The Authors declares there is no conflict of interset.

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