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, 8 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.

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; Nyachuga, 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 chlorotetracycline 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
associated with the \textit{tet} (O) gene, which is carried on transmissible plasmids (Taylor and Courvalin, 1988).

In Egypt, genotyping confirmation of environmental \textit{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 \textit{Campylobacter} spp. among chickens, water, and humans. Antibiotic susceptibility and the tetracycline resistance gene \textit{tet} (O) were also investigated to determine the resistance pattern of \textit{Campylobacter} isolates recovered from different sources. In addition, the \textit{tet} (O) genes were sequenced to trace the potential source of such genes among \textit{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**

\textit{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 and Campy Gen 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 \textit{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 were purified using the 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 \textit{tet} (O) gene in the selected isolates of \textit{C. jejuni} and \textit{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
Online First Article

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

<table>
<thead>
<tr>
<th>Target agent &amp; genes</th>
<th>Primer sequence (5’-3’)</th>
<th>Amplified Length (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em> 23S rRNA</td>
<td>TATACCGGTAAGGAGTGCTGGAG (F) ATCAATTAACCTTCCGACGACCG (R)</td>
<td>650</td>
<td>Wang et al., 2002</td>
</tr>
<tr>
<td><em>C. jejuni</em> mapA</td>
<td>CTATTTATTTTTGATGCTGTTG (F) GCTTTATTTTCATTGTTTATTA (R)</td>
<td>589</td>
<td>Eunju and Lee, 2009</td>
</tr>
<tr>
<td><em>C. coli</em> CeuE</td>
<td>AAT TGA AAA TTG CTC CAA CTA TG (F) TGA TTT TAT TAT TTG TAG CAG CG (R)</td>
<td>462</td>
<td>Eunju and Lee, 2009</td>
</tr>
<tr>
<td><em>Campylobacter</em> tet O</td>
<td>GGCGTTTTGTTTATGTGCG (F) ATGGACAACCCCGACGAGC (R)</td>
<td>559</td>
<td>Gibreel et al., 2004</td>
</tr>
</tbody>
</table>

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.**

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>Sample number</th>
<th><em>Campylobacter</em> isolates</th>
<th>Positive (%)</th>
<th><em>C. jejuni</em> (%)</th>
<th><em>C. coli</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>50</td>
<td></td>
<td>3 (6.0)</td>
<td>2 (66.7)</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Chicken</td>
<td>360</td>
<td></td>
<td>7 (1.9)</td>
<td>4 (57.1)</td>
<td>3 (42.9)</td>
</tr>
<tr>
<td>Water</td>
<td>15</td>
<td></td>
<td>1 (6.7)</td>
<td>1 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>425</td>
<td></td>
<td>11 (2.6)</td>
<td>7 (63.6)</td>
<td>4 (36.4)</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Classes of antibiotics</th>
<th>Antibiotic concentration (µg)</th>
<th>Resistant strains</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>C. jejuni</em> (n - 7)</td>
<td><em>C. coli</em> (n - 4)</td>
</tr>
<tr>
<td>Nalidixic acid (30)</td>
<td>6 (85.7%)</td>
<td>3 (71.4%)</td>
<td>9 (81.8%)</td>
</tr>
<tr>
<td>Ciprofloxacin (5)</td>
<td>4 (57.1%)</td>
<td>2 (42.9%)</td>
<td>6 (54.5%)</td>
</tr>
<tr>
<td>Gentamycin (10)</td>
<td>1 (14.3%)</td>
<td>2 (28.6%)</td>
<td>3 (27.3%)</td>
</tr>
<tr>
<td>Erythromycin (15)</td>
<td>3 (42.8%)</td>
<td>3 (57.1%)</td>
<td>6 (54.5%)</td>
</tr>
<tr>
<td>Ampicillin (10)</td>
<td>1 (57.1%)</td>
<td>2 (28.6%)</td>
<td>3 (27.3%)</td>
</tr>
<tr>
<td>Tetracycline (30)</td>
<td>5 (71.4%)</td>
<td>3 (57.1%)</td>
<td>8 (72.7%)</td>
</tr>
<tr>
<td>Ceftriaxone (30)</td>
<td>1 (57.1%)</td>
<td>1 (42.9%)</td>
<td>2 (18.2%)</td>
</tr>
<tr>
<td>Chloramphenicol (30)</td>
<td>3 (42.8%)</td>
<td>1 (28.6%)</td>
<td>4 (36.4%)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>MAR index</th>
<th><em>Campylobacter</em> spp.</th>
<th>Isolate source</th>
<th>Antibiotic resistant profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td><em>C. jejuni</em></td>
<td>Human</td>
<td>NA, TE</td>
</tr>
<tr>
<td>0.3</td>
<td><em>C. jejuni</em></td>
<td>Human</td>
<td>CIP, C</td>
</tr>
<tr>
<td>0.4</td>
<td><em>C. coli</em></td>
<td>Human</td>
<td>E, CR, C</td>
</tr>
<tr>
<td>0.6</td>
<td><em>C. coli</em></td>
<td>Cloacal swab</td>
<td>NA, CIP, CN, AMP, TE</td>
</tr>
<tr>
<td>0.5</td>
<td><em>C. coli</em></td>
<td>Cloacal swab</td>
<td>NA, CN, E, TE,</td>
</tr>
<tr>
<td>0.6</td>
<td><em>C. jejuni</em></td>
<td>Cloacal swab</td>
<td>NA, CIP, E, AMP, TE</td>
</tr>
<tr>
<td>0.6</td>
<td><em>C. jejuni</em></td>
<td>Cloacal swab</td>
<td>NA, CIP, E, AMP, C</td>
</tr>
<tr>
<td>0.4</td>
<td><em>C. jejuni</em></td>
<td>Cloacal swab</td>
<td>NA, CR, C</td>
</tr>
<tr>
<td>0.5</td>
<td><em>C. jejuni</em></td>
<td>Cloacal swab</td>
<td>NA, CIP, E, TE</td>
</tr>
<tr>
<td>0.4</td>
<td><em>C. jejuni</em></td>
<td>Water</td>
<td>NA, CN, TE</td>
</tr>
</tbody>
</table>

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.

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

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>No. of positive isolates to <em>tet</em> O gene</th>
<th>No. of strains resistant to tetracycline</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n - 11)</td>
<td>C. coli (n - 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. jejuni (n - 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>50.0 1</td>
<td>66.7 2</td>
<td>NA</td>
</tr>
<tr>
<td>Chicken</td>
<td>66.7 4</td>
<td>85.7 6</td>
<td>1</td>
</tr>
<tr>
<td>Water</td>
<td>100.0 1</td>
<td>100.0 1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>54.5 6</td>
<td>36.4 4</td>
<td>11</td>
</tr>
</tbody>
</table>

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 Marino 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 harbour 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.

ACKNOWLEDGEMENTS

We thank the Zoonoses department, Faculty of Veterinary Medicine, Cairo University for supporting us to complete this work.

Statement of conflict of interest

The Authors declares there is no conflict of interest.

REFERENCES


EFSA, 2012. The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food


Multidrug-Resistant Campylobacter as a Zoonotic Agent


