PREVALENCE AND ANTIBIOTIC RESISTANCE PATTERNS OF CAMPYLOBACTER SPECIES ISOLATED FROM DIFFERENT SOURCES IN EGYPT

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ABSTRACT

Campylobacter food poisoning is understated in developing countries. To determine the occurrence and antimicrobial resistance of this pathogen in Zagazig City, Sharkia Governorate, Egypt, a total of 286 samples from chicken (195), cattle (47) and human (44) were collected. Bacteriological examination of the collected samples revealed high prevalence rates of Campylobacter species from human stool, chicken and raw milk samples (90.91%, 86.15% and 82.98%, respectively). C. jejuni was recognized as the most frequently recovered species (63.29%). There were no significant effects on the prevalence of Campylobacter spp. based on the sample type (p = 0.54). Thirty eight campylobacter isolates were confirmed molecularly using PCR amplification of 23S rRNA gene. Furthermore, PCR targeting mapA and ccaE genes were then applied for the confirmation of C. jejuni and C. coli isolates, respectively. The antibiotic resistance results showed that all isolates (100%) were resistant to ampicillin, amoxicillin and erythromycin. On the other hand, the lowest resistance rates were detected against amikacin, impenem and cefoxitin (28.74%, 32.39% and 47.77, respectively). In total, 207 (83.81%) campylobacter isolates were MDR, while 38 isolates (15.38%) and 2 chicken C. jejuni isolates (0.81%) were XDR and PDR, respectively. One hundred and forty five antimicrobial resistance profiles were generated with an MAR index of 0.45 or greater. Our results suggested that the presence of MDR campylobacters in chicken and raw milk, specifically to erythromycin and/or ciprofloxacin aggravates the human health alarm and emphasizes the necessity to educate the consumers about the safeness and the goodness of such foods.

Keywords: Campylobacter spp., Prevalence, Multiple drug resistance, mapA, ccaE

INTRODUCTION

Thermophilic Campylobacter species, mainly Campylobacter jejuni (C. jejuni) and Campylobacter coli (C. coli), have been considered as major causes of acute bacterial gastroenteritis globally. They are microaerophilic microorganisms growing best in an atmosphere containing approximately 10% CO₂ and 5% O₂ at 41.5 °C (Humphrey et al., 2007). Majority of human illness are attributed to C. jejuni (80-85%), while the residual cases are credited with C. coli (Narvaez-Bravo et al., 2017). Campylobacter species colonize the intestine of many farm animals (Silva et al., 2011). The predominant reservoir of these pathogens is chicken and it carries them with no clinical signs (Sahin et al., 2002). This bacterium is transmitted to humans via contaminated undercooked foods of animal origin, especially undercooked chicken meat and unpasteurized dairy products (Gharbi et al., 2018). With a low infective dose, Campylobacter species cause a gastrointestinal infection known as campylobacteriosis. Campylobacteriosis is an important zoonotic illness with a universal distribution (Thomrongsuwannakij et al., 2017). In the most recent decades, there was an increase in the number of human infection cases in developing and industrialized countries with 96 million cases of acute gastroenteritis and 21 thousand deaths yearly worldwide (WHO, 2015; Abdulreesh et al., 2017). Actually, diarrheal disease has a great importance particularly in developing economies, where children < 2 years may die when infected with these microorganisms (Saeed et al., 2015). Campylobacteriosis is characterized by inflammatory diarrhea (Humphrey et al., 2007). In some cases, more severe complications like arthritis, septicaemia and Guillain-Barré syndrome (GBS) can also occur when infection caused by either C. jejuni or C. coli (García-Sanchez et al., 2018). Human campylobacteriosis is usually self-limiting. However, immunocompromised patients and young age with severe infections may need antimicrobial medications (Sharp et al., 2016). Erythromycin is generally the first drug of choice, but fluoroquinolones and on a smaller scale tetracycline constitutes other options (Szczepanska et al., 2017).

Campylobacters become more resistant to antimicrobials and many strains developed the multi-drug resistant (MDR) pattern to multiple medicaments (Bouhamed et al., 2018). Multi-drug resistant campylobacter, particularly against quinolones and erythromycin, has increased globally and this has triggered worldwide alarms, as they are the main antibiotics used for the therapy of campylobacteriosis (Zhou et al., 2016). Contaminated foods with MDR campylobacter strains harbor a significant hazard to the public health (Szczepanska et al., 2017).

As the incidence of the campylobacter infections has increased, there is an urgent need to take measures to identify the source of the incrimented bacterium (Eberle and Kiess, 2012). In Egypt, several authors reported relatively high prevalence rate of Campylobacter spp. in human; 27.5% (Abushahba et al., 2018), 9.37% (Sainato et al., 2018) and 6% (Ghoneim et al., 2020). At species level, C. jejuni and C. coli were previously recorded with prevalence rates 12.3% and 2.8%, respectively (El-Tras et al., 2015). While, thirteen C. jejuni isolates (17.33%) were distinguished from 75 diarrheic persons (Ghoneim et al., 2020).

To the best of our knowledge, the previously conducted studies in Zagazig (Awadallah et al., 2014; Abd El Tawab et al., 2018; El-Hamid et al., 2019) stated limited data about the contribution of chicken and raw milk as potential sources of MDR C. jejuni and C. coli infections in humans. Therefore, the aim of the present investigation was to detect Campylobacter species, particularly C. jejuni and C. coli in chicken and raw milk as well as human samples using conventional and molecular tools in addition to identifying the antimicrobial resistance profiles of the recovered isolates against 10 different antimicrobial classes.
MATERIALS AND METHODS

Sampling

The study was conducted from March 2017 to September 2019. A total of 286 samples were collected from various sources; chickens (n = 195), cattle (n = 47) and human (n = 44) in Zagazig City, Sharkia Governorate, Egypt. The samples collected from broiler chickens included cloacal swabs (n = 75), breast meat (n = 40), caecal parts (n = 40) and liver (n = 40) were collected from retail outlets, while raw milk samples were collected from retail shops. Moreover, human stool samples were taken from gastroenteritis patients from clinical laboratories. Samples were collected in a sterile Bolton enrichment broth (Oxoid, UK) and transported in an ice box within 3 h to the laboratory for bacteriological analysis. The animal study was endorsed by the committee of Animal Welfare and Research Ethics, Faculty of Veterinary Medicine, Zagazig University. Regarding the human samples, it was approved by the research ethical committee of Faculty of Medicine, Zagazig University and the work was conducted in compliance with the Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans. Written informed permissions were taken from patients taking part in the research study after a complete explanation of the aim of the study.

Isolation and identification of thermophilic Campylobacter species

For isolation of Campylobacter species, the collected samples in Bolton enrichment broth were incubated at 41.5°C for 24 h in the culture vessel with headspace less than 1 cm and firmly capped lids. After enrichment, a loopful of the broth was streaked onto modified Cefoperazone Charcoal Deoxycholate agar, mCCDA (Oxoid, UK) prepared from Campylocatter Blood-Selective Agar Base CM0739 and CCDCA Selective Supplement SR155 (Oxoid, UK). The plate's incubation was done at 41.5°C in darkness for 48 h under microaerophilic conditions (5% O2, 10% CO2 and 85% N2) using CampyGen sachets (Oxoid, UK) (ISO, 2006). The presumptive identification of isolates as C. jejuni and C. coli was done by biochemical tests including catalase, oxidase, indoxyl acetate and hippurate hydrolysis and susceptibility to cephalothin and nalidixic acid (30 μg /disc, each) (Quinn et al., 1994).

Table 1 Oligonucleotide primers used for PCR amplification of Campylobacter specie genes with their respective annealing temperatures

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Target gene</th>
<th>Oligonucleotide primer sequence (5'-3')</th>
<th>product (bp)</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus Campylobacter</td>
<td>23S rRNA</td>
<td>F' TATACCCGGTAAAGGGCGCTGA</td>
<td>650</td>
<td>55°C</td>
</tr>
<tr>
<td>Campylobacter coli</td>
<td>cesE</td>
<td>R' ATCAATTAAACTTCCAGACCG</td>
<td>462</td>
<td>58°C</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>mapA</td>
<td>F' CTA TTT TAT TTT TTA GGG TCT CTT</td>
<td>589</td>
<td>55°C</td>
</tr>
</tbody>
</table>

Antimicrobial susceptibility testing

Susceptibility of all campylobacter isolates to 22 antimicrobials representing ten different classes was tested using the following antimicrobial discs; β-lactams [ampicillin, AM (10 μg); amoxicillin, AX (25 μg); sulbactam-ampicillin, SAM (20 μg)]; aminoglycosides [streptomycin, S (10 μg); gentamicin, CN (10 μg) and amikacin, AK (30 μg)]; macrodiles [erythromycin, E (15 μg) and azithromycin, AZM (15 μg)]; quinolones [ciprofloxacin, CIP (5 μg) and nalidixic acid, NA (30 μg)]; sulfonamides [trimethoprim-sulfamethaxole, SXT (25 μg)]; phenicols [chloramphenicol, C (30 μg); polypeptides (colistin, CT (10 μg)); oxazolidones [linezolid, LNZ (30 μg)]; lincosamides [clindamycin, DA (2 μg)]; and tetracylines [doxycycline DO (30 μg)] (Oxoid, UK). This test was conducted following the Kirby-Bauer disk diffusion method (Bauer et al., 1966). Each overnight culture of Campylobacter spp. was suspended in sterile normal saline and adjusted to a turbidity of a 0.5 McFarland standard. Each suspension was inoculated with a sterile swab on the entire surface of Mueller Hinton agar plates (Oxoid, UK) supplemented with 5% sheep blood. After drying the plates, the antimicrobial discs were aseptically placed on the plates. After incubation at 41.5°C for 48 h under the microaerophilic condition, the inhibition zones were measured and interpreted on the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2016) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST, 2017). In the cases when the CLSI recommendations were not available for campylobacters, the Enterobacteriaceae CLSI guidelines were followed (CLSI, 2013). The MDR was identified as acquired resistance of a microorganism to at least one antibiotic in three or fewer antimicrobial categories and pan drug resistant (PDR) was identified as resistance of a microorganism to all antibiotics in all antimicrobial categories (Magiorakos et al., 2012).

Multiple antimicrobial resistance indexing

The multiple antimicrobial resistances (MAR) indexing was used to quantify the multi-resistance of campylobacter isolates, as following:

\[ \text{MAR index} = a/b \]

Base CM0739 and CCDCA Selective Supplement SR155 (Oxoid, UK). The plate's incubation was done at 41.5°C in darkness for 48 h under microaerophilic conditions (5% O2, 10% CO2 and 85% N2) using CampyGen sachets (Oxoid, UK) (ISO, 2006). The presumptive identification of isolates as C. jejuni and C. coli was done by biochemical tests including catalase, oxidase, indoxyl acetate and hippurate hydrolysis and susceptibility to cephalothin and nalidixic acid (30 μg /disc, each) (Quinn et al., 1994).

Molecular confirmation of Campylobacter species

A conventional PCR was used for the confirmation of biochemically identified campylobacter isolates. Bacterial DNA was extracted from fresh cultures using QIAamp DNA mini kit (Qiagen, USA) in accordance with the manufacturer's instructions. Oligonucleotide primers (Metabion, Germany) that specifically amplify target Campylobacter spp. genes were used for molecular identification (Table 1). The PCR was performed in a total reaction volume of 25μL using Taq DNA Polymerase Kit (Invitrogen, USA) in accordance with the manufacturer's instructions. The use concentration of each forward and reverse primers was 20 pmol. The thermal profile comprised of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at temperatures specific for each gene (Table 1) for 45 sec and extension at 72°C for 45 sec and finally an extension step at 72°C for 10 min. Reference strains of C. jejuni (NCTC11322) and C. coli (NCTC11366) were considered positive controls ( the reference strains were kindly obtained from Biotechnology unit, Reference laboratory for Veterinary Quality Control on Poultry production, Animal Health Research Institute, Dokki, Giza, Egypt). PCR-grade water without template was served as a negative control.

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Multiple antimicrobial resistance indexing

The multiple antimicrobial resistances (MAR) indexing was used to quantify the multi-resistance of campylobacter isolates, as following:

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Table 2 Prevalence of thermotolerant Campylobacter spp. in the collected samples

<table>
<thead>
<tr>
<th>Sample type (No.)</th>
<th>Total No. of Campylobacter spp. (%)</th>
<th>No. of Campylobacter spp. (C. jejuni, C. coli) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken samples (195)</td>
<td>168 (86.15)</td>
<td>116 (59.49) 52 (26.67)</td>
</tr>
<tr>
<td>Cloacal swabs (75)</td>
<td>70 (93.33)</td>
<td>50 (66.67) 20 (26.67)</td>
</tr>
<tr>
<td>Breast meat (40)</td>
<td>30 (75)</td>
<td>22 (55) 8 (20)</td>
</tr>
<tr>
<td>Cecal parts (40)</td>
<td>36 (90)</td>
<td>24 (60) 12 (30)</td>
</tr>
<tr>
<td>Chicken liver (40)</td>
<td>32 (80)</td>
<td>20 (50) 12 (30)</td>
</tr>
<tr>
<td>Raw milk (47)</td>
<td>39 (82.98)</td>
<td>33 (70.21) 6 (12.77)</td>
</tr>
<tr>
<td>Human stool (44)</td>
<td>40 (90.91)</td>
<td>32 (72.73) 8 (18.18)</td>
</tr>
<tr>
<td>Total (286)</td>
<td>247 (86.36)</td>
<td>181 (63.29) 66 (23.08)</td>
</tr>
</tbody>
</table>

The isolation rates were calculated in relation to the total number of the examined samples.

Molecular confirmation of campylobacter isolates

Thirty eight campylobacter isolates (29 and 9 biochemically suspected C. jejuni and C. coli isolates, respectively) were confirmed by PCR amplification of 23S rRNA gene. All the isolates generated an amplicon of 650 bp size (figure 1). The results showed that all 29 C. jejuni isolates from chicken (n = 19), cattle (n = 5) and human (n = 5) and 9 C. coli isolates from chicken (n = 7), cattle (n = 1) and human (n = 1) those were identified phenotypically were confirmed by PCR amplifications of mapA and ceuE genes with amplicons’ sizes of 589 bp (figure 2A) and 462 bp (figure 2B), respectively.

Analysis of the antimicrobial resistance of 247 campylobacter isolates against the 22 tested antimicrobial agents demonstrated that all isolates (100%) were resistant to ampicillin, amoxicillin and erythromycin. Moreover, high resistance rates were observed against trimethoprim-sulfamethaxole (98.79%), followed by clindamycin (97.17%), cefalothin (96.76%), azithromycin (91.09%) and nalidixic acid (90.28%). Additionally, majority of campylobacter isolates were resistant to azetronam (81.78%), doxycycline (81.38%), amoxicillin-clavulanic acid (80.97%), cefepime (80.57%), cefoperazone (80.16%), colistin (75.71%) and linezolid (73.28%). On the other hand, our results showed that amikacin, imenpen and cefoxitin had the lowest resistance rates against the tested isolates (28.74%, 32.39% and 47.77%, respectively) (Table 3).

Based on Campylobacter species and regardless of the type of the collected samples, our results presented higher resistance rates in C. coli isolates than C. jejuni for the investigated antibiotics except for cefalothin, cefoxitin, imenpen, streptomycin, gentamycin, cipprofloxacin, linezolid, colistin and doxycycline (Figure 3). Statistically, there was a significant effect (P < 0.05) on the resistance prevalence between C. jejuni and C. coli isolates against amoxicillin-clavulanic acid (84.53% and 71.21%), cefepime (83.98% and 71.21%), azithromycin (93.37% and 84.85%), chloramphenicol (83.43% and 71.21%) and colistin (72.38% and 84.85%), respectively. The resistance prevalence in C. jejuni and C. coli isolates was significantly higher for 3 of the tested antimicrobials; azetronam (86.19% and 69.69%) and streptomycin (58.01% and 77.27%), respectively (P < 0.01) and clindamycin (100% and 89.39%), respectively (P < 0.001). Meanwhile, there were no significant differences (P > 0.05) in the resistance of C. jejuni and C. coli isolates against the other antimicrobials; sulbacampicillin, cefalothin, cefoxitin, imenpen, gentamycin, amikacin, cipprofloxacin, nalidixic acid, trimethoprim-sulfamethaxole, linezolid and doxycycline (P values = 0.192, 0.911, 0.48, 0.27, 0.81, 0.99, 0.54, 0.78, .795, 0.59 and 0.22, respectively) (Supplementary table 1).
According to the isolates’ origins, the resistance levels of campylobacter isolates recovered from chicken, cattle and human samples were different (Figure 4). Statistically, there was a significant effect ($P < 0.05$) on the resistance rates between chicken, cattle and human isolates against imipenem (36.9%, 15.38% and 30%) and nalidixic acid (94.05%, 84.62% and 80%), respectively. The difference in rates among chicken, cattle and human isolates were significantly higher for 11 of the tested antimicrobials; cefepime (78.57%, 69.23% and 100%), amikacin, ciprofloxacin, colistin and doxycycline ($P < 0.001$). However, there is no significant effect ($P > 0.05$) on the resistance prevalence between campylobacter isolates from the three sources against the other antibiotics: cephalothin, cefotixin, amikacin, ciprofloxacin, colistin and doxycycline ($P = 0.12, 0.24, 0.9, 0.22, 0.31$ and 0.05, respectively) (Supplementary Table 2). The clustering pattern of Campylobacter isolates were classified by their species and host. The distance among isolates is of the Sorensen type (Figure 5).

Table 3 Frequency of resistance of thermotolerant Campylobacter spp. recovered from different sources

<table>
<thead>
<tr>
<th>Antimicrobial group</th>
<th>Antimicrobial</th>
<th>No. of resistant Campylobacter spp. from different sources (%)</th>
<th>Total (247)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. jejuni (n=116)</td>
<td>C. coli (n=52)</td>
<td>C. jejuni (n=33)</td>
</tr>
<tr>
<td>Beta-lactams</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>116 (100)</td>
<td>52 (100)</td>
<td>33 (100)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>116 (100)</td>
<td>52 (100)</td>
<td>33 (100)</td>
</tr>
<tr>
<td>Sulbactam-ampicillin</td>
<td>64 (55.17)</td>
<td>24 (46.15)</td>
<td>31 (93.94)</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>112 (96.55)</td>
<td>36 (69.23)</td>
<td>20 (60.61)</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>76 (65.52)</td>
<td>40 (76.92)</td>
<td>12 (36.36)</td>
</tr>
<tr>
<td>Gentiamicin</td>
<td>90 (77.59)</td>
<td>38 (73.08)</td>
<td>11 (33.33)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30 (25.86)</td>
<td>12 (23.08)</td>
<td>9 (27.27)</td>
</tr>
<tr>
<td>Macrolides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>116 (100)</td>
<td>52 (100)</td>
<td>33 (100)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>104 (89.66)</td>
<td>42 (80.77)</td>
<td>33 (100)</td>
</tr>
<tr>
<td>Quinolones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>85 (73.28)</td>
<td>41 (78.85)</td>
<td>20 (60.61)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>110 (94.83)</td>
<td>48 (92.31)</td>
<td>30 (90.91)</td>
</tr>
<tr>
<td>Sulphonamides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxole</td>
<td>116 (100)</td>
<td>52 (100)</td>
<td>33 (100)</td>
</tr>
<tr>
<td>Phenics</td>
<td>90 (77.59)</td>
<td>36 (69.23)</td>
<td>33 (100)</td>
</tr>
<tr>
<td>Polypeptides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td>87 (75)</td>
<td>45 (86.54)</td>
<td>24 (72.73)</td>
</tr>
<tr>
<td>Oxazolidone</td>
<td>90 (77.59)</td>
<td>41 (78.85)</td>
<td>15 (45.45)</td>
</tr>
<tr>
<td>Lincosamide</td>
<td>116 (100)</td>
<td>50 (96.15)</td>
<td>33 (100)</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>90 (77.59)</td>
<td>48 (92.31)</td>
<td>22 (66.67)</td>
</tr>
</tbody>
</table>

Figure 4 Prevalence of resistance in campylobacter isolates from different sources against 22 antimicrobial agents
Antimicrobial resistance profiles and multiple antimicrobial resistance indices of campylobacter isolates

The obtainable results showed that in chicken isolates, *C. jejuni* isolates were resistant to 7 (10.35%), 8 (21.55%), 9 (35.34%) and 10 classes (12.5%) of the tested antimicrobial agents while, *C. coli* isolates were resistant to 7 (5.77%), 8 (19.23%), 9 (30.77%) and 10 classes (44.23%) of the investigated antimicrobial agents. Besides, in cattle isolates, *C. jejuni* isolates were resistant to 7 (18.18%), 8 (30.30 %), 9 (33.33%) and 10 classes (18.18 %) and in *C. coli* isolates were resistant to 7 (16.67%), 8 and 9 (33.33%, each) and 10 classes (16.67%) of the tested antimicrobial agents. Additionally, in human isolates, *C. jejuni* isolates were resistant to 8 (28.13%), 9 (40.63%) and 10 classes (31.25%) of the antimicrobial agents and in *C. coli* isolates were resistant to 7 (50%), 9 (37.5%) and 10 classes (12.5%) of the antimicrobial agents (Table 4).

In total, the prevalence of resistance to seven and eight antimicrobial classes was higher in cattle campylobacter isolates (17.95% and 30.77%, respectively) than chicken and human campylobacter ones. Meanwhile, the resistance to nine antimicrobial classes was the highest in human isolates (40%) and that to ten classes was the highest in chicken isolates (36.31%) (Table 4).

Interestingly, it was noticed that 207 (83.81%) isolates of campylobacter were MDR (resistant to 3-8 antimicrobial classes). Additionally, our results identified 38 XDR isolates (15.38%); 10 (25%), 26 (15.5%) and 2 (5.1%) were recovered from human, chicken and cattle samples, respectively. It is important to note the presence of 2 (0.81%) chicken *C. jejuni* those were PDR (Table 4).

Estimating the MAR indices for campylobacter isolates from different sources revealed that all tested chicken isolates presented an MAR index of 0.45 or greater. Moreover, human isolates had an index of 0.59 or greater and cattle isolates revealed an index of 0.63 or greater indicating a high risk source of contamination, where the antibiotics were often used.

### DISCUSSION

Human campylobacteriosis is caused primarily due to consuming chicken meat, raw milk and inadequately pasteurized milk. The presence of campylobacter pathogens in food animals is particularly worrying for human health and controlling them has a significant implication on health of the public. The current study includes more completed and updated information about the frequency and the antimicrobial resistance of *C. jejuni* and *C. coli* from different sources in Zagazig.

Our results demonstrated that the occurrence of *Campylobacter* spp. in samples obtained from broilers was 86.15% (168 campylobacter isolates out of 195 samples). These results are consistent with previous reports in Poland (87.2%) (Wieczorek et al., 2012) and Algeria (85%) (Messad et al., 2014). Moreover, higher prevalence rates of *Campylobacter* spp. were reported in Italy (100%) (Giacomelli et al., 2014) and Algeria (96%) (Guessoum et al., 2016). On the other hand, low prevalence rates of avian *Campylobacter* spp. were previously documented in Harare (60.2%) (Simango, 2013) and India (44.9%) (Vaishnavi, 2015). In Egypt, in Giza and Cairo Governorates, 7 out of 360 chicken cloacal
swabs collected from different households, farms, and shops were positive for Campylobacter spp. (1.9 %) (Ghoneim et al., 2020). In Assuit Governorate, 24% of 104 chicken carcasses from two slaughterhouses contained Campylobacter spp. (Abushalha et al., 2018).

In regard to Campylobacter spp. distribution, C. jejuni was the most common isolated distribution is comparable to other previous reports in Ireland, 68.9% and 32.4% and Austria, 65.1% and 33.3% for C. jejuni and C. coli, respectively (EFSA, 2010). A recent studies also documented that C. jejuni was more often isolated from chicken in Tunisia (Gharbi et al., 2018) and Egypt (Ghoneim et al., 2020). Most recently, we could identify the importance of the prevalence of Campylobacter spp. in the among the isolates of chicken origin (Nobile et al., 2013). Generally, the variations in Campylobacter sp. isolation rates between different studies could be attributed to different reasons such as the type of examined samples, location, climatic factors, hygienic measures and isolation as well as techniques of identification (Jorgensen et al., 2011; Chatur et al., 2014).

Herein, Campylobacter spp. was isolated from 82.98% of raw milk samples, which is higher than levels obtained by El-Kholo et al., (13%) (El-Kholo et al., 2016) and El-Zamkan and Hameed (22%) (El-Zamkan and Hameed, 2016) in Egypt. Khashoma et al., (13.4%) in Tanzania (Khashoma et al., 2016) and Andrajezska et al., (11.8%) in Poland (Andrajezska et al., 2019). C. jejuni were identified in the current study from 70.21 % of the examined raw milk samples. However results obtained by Hussain et al. in Pakistan, showed that the incidence rate of C. jejuni was 92.4% (Hussain et al., 2007).

The more frequent second source of campylobacteriosis is raw milk (Kashoma et al., 2016). Conventional PCR has been increased, so consumers need to be aware of the danger related to consumption of unpasteurized milk (Mie et al., 2017). The high occurrence of Campylobacter spp. in raw milk in the current study could be due to environmental contamination of milk during or after milking with infected animal wastes or from contaminated equipment or hands of workers and storage practices (Saad et al., 2007).

The occurrence of Campylobacter spp. in human stool samples was 90.91%. This result was higher than those reported Giza (16.7%) (Hassanain, 2011) and Assuit Governates (27.5%) (Abushalha et al., 2018) in Egypt. Also higher than in other countries: Ethiopia (72.7%) (Ewetu and Mihret, 2010), Nigeria (62.7%) (Gwimi et al., 2015), Ghana (20.3%) (Karikari et al., 2017) and Poland (9.6%) (Szczezepanska et al., 2017). The C. jejuni were identified in the current study from 72.73 % of the examined human stool samples. This result was nearly similar to 69.3% and 76.9% reported in Romania (Sorokin et al., 2007) and Egypt (Ab El Tawab et al., 2018), respectively. The high occurrence of Campylobacter spp. from human stool samples in our study could be ascribed to incorporation of stool samples mainly obtained from individuals with diarrhea rather than suspicion of campylobacteriosis in population in general.

In the last few years, antimicrobial resistance in foodborne microorganisms involving campylobacters is considered one of the vitally important problems on public health (Wieczorek and Osek, 2013; EFSA, 2013). In clinical setting, macrolides (i.e erythromycin), fluoroquinolones (i.e ciprofloxacin) and tetracycline are widely used in the treatment of campylobacter infections because of their availability and low cost.

Some reports have demonstrated a slow increase in the resistance rate of campylobacters to macrolides which are considered the first drugs of choice for treatment of campylobacteriosis, especially in paediatric patients (Kurinčič et al., 2007; Wieczorek and Osek, 2013).
In 2017, this study, all campylobacter isolates from chicken, human and cattle were resistant to erythromycin (100% each). This is in accordance with a recent report in Egypt, where 100% of campylobacter isolates from chicken were resistant to erythromycin (Ab El Tawab et al., 2018). On the other hand, lower resistance rates to this drug were recorded for campylobacter isolates from milk samples in Iran (7.69%) (Rahimi et al., 2013) and from human in China (21.8%) (Pan et al., 2016). Laday et al. reported that exposing campylobacters to macrolides for a long duration results in their resistance (Laday et al., 2007).

Our research demonstrated a high level of ciprofloxacin resistance among campylobacter isolates from chicken, human and cattle (75%, 75% and 61.54%, respectively). Similarly, a higher resistance rate (99.2%) was also reported for campylobacter isolates from chicken in Tunisia (Gharbi et al., 2018). Using quinolones in veterinary fields leads to the emergency of ciprofloxacin resistance among campylobacters isolated from human and chicken (Engberg et al., 2004; Gupta et al., 2004).

However, small number of campylobacter isolates was resistant to quinolones in some countries such as Australia, in which quinolones were not allowed to be used in chicken production (Sharpe et al., 2016). Due to the increased resistance to fluoroquinolones and quinolones, these agents became ineffective in the treatment of Campylobacter spp. There were also high resistance rates to doxycycline in our campylobacter isolates from human, chicken and cattle (90%, 82.14% and 69.23% respectively). This is in accordance with a previous report carried out on campylobacter isolates from raw milk (71.43 %) in Poland (Wysok et al., 2011). Due to the broad spectrum of activity and the low cost of tetracycline, they have been widely used in the prophylaxis treatment of human and animal infections and as feed supplements for chicken. These have resulted in the emergence of high resistant bacteria (Hassanain, 2011). Alarmingly, the increased resistance of campylobacter isolates to antimicrobials, particularly erythromycin, (fluoro) quinolones and tetracycline can result in failure in the treatment resulting in higher illness and death rates in humans (Zhu et al., 2006).

The high resistance of campylobacter isolates to the antimicrobials in the present study might due to the widespread and the uncontrolled use of these agents in veterinary medicine as growth promoters or in human and animal treatments. This gives a reflection about the extent of using these antimicrobials in Egypt and therefore proposes a challenge to the management of campylobacter infections.

 Nowadays, the emergence of MDR campylobacter isolates is becoming a growing challenge as it can impair the effective therapy of campylobacter infections. In the current study, there were XDR and PDR campylobacter isolates especially to the antimicrobials those are used in the treatment of campylobacter infections leading to more difficulties in controlling these infections. It is important to note that this study was performed on raw milk samples, which had an MAR index greater than 0.45, which indicates a high frequency of antibiotics usage in Egypt.

This worrisome resistance rates were also recorded for campylobacter isolates from chicken in many countries like Italy (100%) (Fraquzea et al., 2014), Algeria (100%) (Messet et al., 2014) and Pakistan (90.4%) (Nisar et al., 2017). The appearance of MDR can be attributed to the attainment of several resistance determinants in the same DNA molecule or individual determinants such as multiple drug pumps (Ley, 2002). The CmeABC (multi-drug efflux pump) has been concerned with the campylobacter resistance mechanisms to macrolides, fluoroquinolones and tetracyclines (Ventola, 2015).

In order to confirm the identification and discrimination of C. jejuni and C. coli using the conventional PCR results, we were not able to include the prevailing Campylobacter spp. from the first drugs of choice for antimicrobials. Indeed, an ongoing project will be taken into consideration in future studies. However, including control positive and negative compensated, to some extent, this limitation. Other shortcoming is that we depended on phenotypic approach to define the resistance to antimicrobials. Indeed, an ongoing project that involves this point on the same isolates is in plane for future publication. In addition, we were not able to include other sample types from “Cattle” (i.e. other than milk) due to unavailability and lack of access to the sampled animals.

CONCLUSION

Our data demonstrated that the relatively high isolation rate of campylobacters from chicken, raw milk and human stool samples in addition to the development of MDR strains to multiple antimicrobial classes, especially to macrolides, quinolones and tetracycline are alarming situations with potential serious consequences to the health of human. Therefore, there is a need to reduce using these agents and to implement specific control procedures to decrease contamination levels by campylobacters to prevent resistant campylobacter strains from emerging and spreading.

REFERENCES


