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 ccdE 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, imipenem 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 safety and the goodness of such foods.

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

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-Braavo 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 (Thomrongsuwanakij 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; Abulreesh 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, septicemia 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 medications (Boughamed 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 incrimated 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 (17.3%) 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 ºC 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 Campylocacter Blood-Selective Agar Base CM0739 and CCDA 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).

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); amoxicillin-clavulanic acid, AMC (30 μg); cephalothin, CF (30 μg); ceftoxin, FOX (10 μg); cepfime, FEP (30 μg); impenem, IMP (10 μg) and azetromin, ATM (30 μg)], aminoglycosides [streptomycin, S (10 μg); gentamicin, CN (10 μg) and amikacin, AK (30 μg)], macrolides [erythromycin, E (15 μg) and azithromycin, AZM (15 μg)], quinolones [ciprofloxacin, CIP (5 μg) and nalidixic acid, NA (30 μg)], sulphonamides [trimethoprim, SMT (25 μg); sulfamethoxazole, SFX (25 μg); sulfamethaxole, sulfamethazine, SME (25 μg); phenicols [chloramphenicol, C (30 μg)]; polypeptides [colistine, CT (10 μg); oxazolidones [linezolid, LNZ (30 μg)]; lincosamides [clindamycin, DA (2 μg)]; tetracyclines [doxycycline DO (30 μg)]] (Oxoid, UK). The 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 CLSI recommendations were not available for campylobacters, the Enterobacteriaceae CLSI guidelines were tracked (CLSI, 2013). The MDR was identified as resistance of single bacterium to all antibiotics except two 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).

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

<table>
<thead>
<tr>
<th>Genus/Campylobacter spp.</th>
<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>Campylobacter jejuni</td>
<td>mapA</td>
<td>F’ CTA TTT TAT TTT TGA GTO CTT GF</td>
<td>589</td>
<td>55 ºC</td>
<td></td>
</tr>
<tr>
<td>Campylobacter coli</td>
<td>ceul</td>
<td>F’ AAT TGA AAA TTT CTC CAA CTA TG</td>
<td>462</td>
<td>58 ºC</td>
<td></td>
</tr>
<tr>
<td>Genus Campylobacter</td>
<td></td>
<td></td>
<td>R’ ATCAATTTAACCTTCGAGACC</td>
<td>650</td>
<td>55 ºC</td>
</tr>
</tbody>
</table>

RESULTS

Prevalence of thermophilic Campylobacter spp. in the examined samples

According to the phenotypic identification, Campylobacter spp. were isolated from 247 out of 286 of the examined samples (86.36%); 90.91% from human stool, 86.15% from chicken samples and 82.98% from raw milk (Table 2). Of note, each investigated sample contained only one identified campylobacter isolate. The results demonstrated a high isolation rate of chicken Campylobacter spp. from cloacal swabs (93.33%), followed by caecal parts (90%), chicken liver (80%) and breast meat (75%). Identification of campylobacters to the species level showed that C. jejuni was found to be the predominant species with an overall prevalence rate of 63.29%, while that of C. coli was 23.08%. The highest isolation rate of C. jejuni was detected in human stool (72.73%), followed by raw milk (70.21%). Meanwhile, the highest isolation rate of C. coli was observed in chicken (26.67%) (Table 2). Statistically, there were no significant effects on the prevalence of Campylobacter spp. based on the sample type (p = 0.54) and on the prevalence of both C. jejuni and C. coli from different sample types (p = 0.08).

Multiple antimicrobial resistance indexing

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

Where, “a” represents the number of antimicrobials to which the microorganism was resistant and “b” represents the total number of antimicrobials to which the microorganism was tested (Krumperman, 1983).

Statistical analysis

The results were analyzed using the SPSS v18.0 software (SPSS Inc., Chicago, IL, USA). The occurrence of Campylobacter spp. in different sources and variations in antimicrobial susceptibility between C. jejuni and C. coli were evaluated using chi square test. Fisher’s exact test was applied for smaller number of samples. To visualize the clustering pattern among campylobacter isolates from various hosts, non-metric multidimensional scaling was generated based on sorensen distance using the PC-O RD software.
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 amplification of mapA and ceeE genes with amplicons' sizes of 589 bp (Figure 2A) and 462 bp (Figure 2B), respectively.

Figure 1 PCR amplification products of 23S rRNA gene of Campylobacter spp. (650 bp). Lane L: 100 bp DNA ladder ‘Marker’, lanes 1-38: positive campylobacter isolates from poultry (lanes 1-26), cattle (lanes 27-32) and human (lanes 33-38) origins, lane P: positive control, lane N: negative control.

Figure 2 PCR amplification products of mapA gene specific for C. jejuni (589 bp) (A) and ceeE gene specific for C. coli (462 bp) (B). Lanes 1-19: C. jejuni from poultry, lanes 20-24: C. jejuni from cattle, lanes 25-29: C. jejuni from human, lanes 30-36: C. coli from poultry, lane 37: C. coli from cattle, lane 38: C. coli from human, lane L: 100 bp DNA ladder ‘Marker’, lane P: positive control, lane N: negative control.

Antimicrobial susceptibility profile of the recovered Campylobacter spp. from different sources

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%), cefuroxime (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%), chloramphenicol (80.16%), colistin (75.71%) and linezolid (73.28%). On the other hand, our results showed that amikacin, impenem 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. jejuni isolates than C. coli for the investigated antibiotics except for cephalexin, cefotaxim, impenem, streptomycin, gentamycin, cipefloxacin, 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; sulbactam-ampicillin, cephalexin, cefoxitin, impenem, gentamycin, amikacin, ciprofloxacin, 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, 0.795, 0.59 and 0.22, respectively) (Supplementary table 1).

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. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. jejuni</td>
<td>C. coli</td>
</tr>
<tr>
<td>Chicken samples (195)</td>
<td>168 (86.15)</td>
<td>116 (59.49)</td>
</tr>
<tr>
<td>Cloacal swabs (75)</td>
<td>70 (93.33)</td>
<td>50 (66.67)</td>
</tr>
<tr>
<td>Breast meat (40)</td>
<td>30 (75)</td>
<td>22 (55)</td>
</tr>
<tr>
<td>Cecal parts (40)</td>
<td>36 (90)</td>
<td>24 (60)</td>
</tr>
<tr>
<td>Chicken liver (40)</td>
<td>32 (80)</td>
<td>20 (50)</td>
</tr>
<tr>
<td>Raw milk (47)</td>
<td>39 (82.98)</td>
<td>33 (70.21)</td>
</tr>
<tr>
<td>Human stool (44)</td>
<td>40 (90.91)</td>
<td>32 (72.73)</td>
</tr>
<tr>
<td>Total (286)</td>
<td>247 (86.36)</td>
<td>181 (63.29)</td>
</tr>
</tbody>
</table>

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

Figure 3 Prevalence of Campylobacter spp. resistance against 22 antimicrobial agents
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; cefepine (78.57%, 69.23% and 100%), streptomycin (69.05%, 38.46% and 62.5%), trimethoprim-sulfamethaxole (100%, 100% and 92.5%) and clindamycin (98.81%, 100% and 87.5%), respectively ($P < 0.01$) and sulbactam-ampicillin (52.38%, 92.31% and 50%), amoxicillin-clavulanic acid (88.09%, 61.54% and 70%), azetronin (81.55%, 100% and 65%), gentamycin (76.19%, 35.89% and 72.5%), azithromycin (86.9%, 100% and 100%), chloramphenicol (75%, 100% and 82.5%) and linezolid (77.98%, 46.15% and 80%), respectively ($P < 0.001$). 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, cefoxitin, 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>Chicken (168)</th>
<th>C. coli (n=52)</th>
<th>C. jejuni (n=33)</th>
<th>C. jejuni (n=6)</th>
<th>Human (40)</th>
<th>Total (247)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. jejuni (n=116)</td>
<td>C. coli (n=33)</td>
<td>C. coli (n=6)</td>
<td>C. jejuni (n=32)</td>
<td>C. coli (n=8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>116 (100)</td>
<td>52 (100)</td>
<td>33 (100)</td>
<td>6 (100)</td>
<td>32 (100)</td>
<td>8 (100)</td>
<td>247 (100)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>116 (100)</td>
<td>52 (100)</td>
<td>33 (100)</td>
<td>6 (100)</td>
<td>32 (100)</td>
<td>8 (100)</td>
<td>247 (100)</td>
</tr>
<tr>
<td>Sulbactam-ampicillin</td>
<td>64 (55.17)</td>
<td>24 (46.15)</td>
<td>31 (93.94)</td>
<td>5 (83.33)</td>
<td>15 (66.67)</td>
<td>5 (62.5)</td>
<td>144 (58.29)</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>112 (96.55)</td>
<td>36 (69.23)</td>
<td>20 (60.61)</td>
<td>4 (66.67)</td>
<td>21 (65.63)</td>
<td>7 (87.5)</td>
<td>200 (80.97)</td>
</tr>
<tr>
<td>Cefalothin</td>
<td>111 (95.69)</td>
<td>52 (100)</td>
<td>32 (96.97)</td>
<td>4 (66.67)</td>
<td>32 (100)</td>
<td>8 (100)</td>
<td>239 (96.76)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>54 (46.55)</td>
<td>22 (42.31)</td>
<td>14 (42.42)</td>
<td>4 (66.67)</td>
<td>16 (50)</td>
<td>8 (100)</td>
<td>118 (47.77)</td>
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<tr>
<td>Cefepime</td>
<td>98 (84.48)</td>
<td>34 (65.38)</td>
<td>22 (66.67)</td>
<td>5 (83.33)</td>
<td>32 (100)</td>
<td>8 (100)</td>
<td>199 (80.57)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>41 (35.34)</td>
<td>21 (40.38)</td>
<td>5 (15.15)</td>
<td>1 (16.67)</td>
<td>9 (28.13)</td>
<td>3 (37.5)</td>
<td>80 (32.39)</td>
</tr>
<tr>
<td>Azetronin</td>
<td>101 (87.07)</td>
<td>36 (69.23)</td>
<td>33 (100)</td>
<td>6 (100)</td>
<td>22 (68.75)</td>
<td>4 (50)</td>
<td>202 (81.78)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>76 (65.52)</td>
<td>40 (76.92)</td>
<td>12 (36.36)</td>
<td>3 (50)</td>
<td>17 (53.13)</td>
<td>8 (100)</td>
<td>156 (63.16)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>90 (77.59)</td>
<td>38 (73.08)</td>
<td>11 (33.33)</td>
<td>3 (50)</td>
<td>21 (65.63)</td>
<td>8 (100)</td>
<td>171 (69.23)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30 (25.86)</td>
<td>12 (23.08)</td>
<td>9 (27.27)</td>
<td>3 (50)</td>
<td>13 (40.63)</td>
<td>4 (50)</td>
<td>71 (28.74)</td>
</tr>
<tr>
<td>erythromycin</td>
<td>116 (100)</td>
<td>52 (100)</td>
<td>33 (100)</td>
<td>6 (100)</td>
<td>32 (100)</td>
<td>8 (100)</td>
<td>247 (100)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>104 (89.66)</td>
<td>42 (80.77)</td>
<td>33 (100)</td>
<td>6 (100)</td>
<td>32 (100)</td>
<td>8 (100)</td>
<td>225 (91.09)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>85 (73.28)</td>
<td>41 (78.85)</td>
<td>20 (60.61)</td>
<td>4 (66.67)</td>
<td>25 (78.13)</td>
<td>5 (62.5)</td>
<td>180 (72.87)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>110 (94.83)</td>
<td>48 (92.31)</td>
<td>30 (90.91)</td>
<td>3 (50)</td>
<td>24 (75)</td>
<td>8 (100)</td>
<td>223 (90.28)</td>
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<tr>
<td>Trimethoprim-sulfamethaxole</td>
<td>116 (100)</td>
<td>52 (100)</td>
<td>33 (100)</td>
<td>6 (100)</td>
<td>30 (93.75)</td>
<td>8 (100)</td>
<td>244 (98.79)</td>
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<td>Chloramphenicol</td>
<td>90 (77.59)</td>
<td>36 (69.23)</td>
<td>33 (100)</td>
<td>6 (100)</td>
<td>28 (87.5)</td>
<td>5 (62.5)</td>
<td>198 (80.16)</td>
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<td>Colistin</td>
<td>87 (75)</td>
<td>45 (86.64)</td>
<td>24 (72.73)</td>
<td>3 (50)</td>
<td>20 (62.5)</td>
<td>8 (100)</td>
<td>187 (75.71)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>90 (77.59)</td>
<td>41 (78.85)</td>
<td>15 (45.45)</td>
<td>3 (50)</td>
<td>26 (81.25)</td>
<td>6 (75)</td>
<td>181 (73.28)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>116 (100)</td>
<td>50 (96.15)</td>
<td>33 (100)</td>
<td>6 (100)</td>
<td>32 (100)</td>
<td>3 (37.5)</td>
<td>240 (97.17)</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>90 (77.59)</td>
<td>48 (92.31)</td>
<td>22 (66.67)</td>
<td>5 (83.33)</td>
<td>32 (100)</td>
<td>4 (50)</td>
<td>201 (81.38)</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 (16.67%), 8 (33.33%, each) and 10 classes (18.18%) of the investigated 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 updated information about the frequency and the antimicrobial resistance of C. jejuni and C. coli from different sources in Zagazig.
swabs collected from different households, farms and shops were positive for Campylobacter spp. (1.9%) (Ghoneim et al., 2020). In Assiut Governorate, 24% of 104 chicken carcasses from two slaughterhouses contained Campylobacter spp. (Abushahba et al., 2018).

In regard to the 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) and it is considered the predominant species of the prevailing Campylobacter spp. among the isolates of chicken origin (Nobile et al., 2013). Generally, the variations in Campylobacter spp. isolation rates between different studies could be attributed to different reasons such as the type of examined samples, location, climate factors, hygienic measures and isolation and 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-Kholy et al., (13%) (El-Kholy et al., 2016) and El-Zamkan and Hameed (22%) (El-Zamkan and Hameed, 2016) in Egypt. Kashoma et al., (13.4%) in Tanzania (Kashoma et al., 2016) and Andrzejewska et al., (11.8%) in Poland (Andrzejewska 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). The increasing organically processed milk 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 contamination of some of the utensils, inventory 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%) (Abushahba et al., 2018) in Egypt. Also higher than in other countries; Ethiopia (72.7%) (Ewetu and Milret, 2010), Nigeria (62.7%) (Gwimi et al., 2015), Ghana (20.3%) (Kariari et al., 2017) and Poland (9.6%) (Szczepanska et al., 2017). 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 (Abd El Tawab et al., 2018), respectively. The significantly high percentage 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 inspection of campylobacteriosis in population in general.

In the last few years, antimicrobial resistance in foodborne microorganisms involving campylobacters is considered one of the vital important problems on public health (Wiezorek and Osik, 2013; EFSA, 2015). 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 campylobacteriosis which are considered the first drugs of choice for treatment of campylobacteriosis, especially in paediatric patients (Kurinčič et al., 2018).

In the current study might be due to the widespread use of tetracycline are widely used in the treatment of campylobacter infections because of their availability and low cost. Also, due to the high prevalence of the fluoroquinolones spectrum of activity and the low cost of tetracyclines, they have been widely used in the prophylaxis therapy of human and animal infections and as feed supplements for chickens. 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 be 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 by using multiple drug spores for the diagnosis of MDR campylobacter isolates in the current study, there was 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%) (Frazqueza et al., 2014), Algeria (100%) (Messad 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 (Levy, 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 (Nobile et al., 2013). The same results were reported in Egypt (Abushahba et al., 2018). The present work the conventional PCR results confirmed the identification of thirty eight campylobacter isolates (29 and 9 phenotypically suspected C. jejuni and C. coli isolates, respectively). Accordingly, the conventional culture methods and biochemical reactions were 100% in accordance with the results of PCR for identification and differentiation of C. jejuni and C. coli. The same results were reported in Egypt (Girgis et al., 2014).

This study is limited by some factors, mainly related to lack of fund: first, the dependence on PCR for characterizing the bacterial species could be subjected to lack of specificity, and other techniques (e.g. MALDI-TOF) is recommended and 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 place 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 tetracyclines are alarming situations with potential serious consequences to the health of human. Therefore, there is a need to reduce using antimicrobial agents and to implement specific control procedures to decrease contamination levels by campylobacters to prevent resistant campylobacter strains from emerging and spreading.

REFERENCES


