Antimicrobial Resistance and Antibiogram of Thermotolerant Campylobacter Recovered from Poultry Meat in Baghdad Markets/Iraq

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Abstract

Antimicrobial resistance is a critical public health issue that affects people all over the world. Because bacteria have a proclivity for rapidly acquiring and propagating the resistance gene, antimicrobial-resistant Campylobacter has a negative impact on public health. As a result, the creation of new and highly pathogenic clones is facilitated, making antimicrobial treatment more challenging. In order to determine the antimicrobial resistance (ARP) models, multi-resistant models (MDR), and multidrug resistance index (MAR index) of Campylobacter species isolated from poultry meat sold in Baghdad markets, this study was conducted. Using the disc diffusion test, 30 Campylobacter strains from chicken meat, including C. jejuni (n = 10) and C. coli (n = 20), were exposed to tetracycline (TET), erythromycin (ERY), gentamicin (GEN), nalidixic acid (NA), ciprofloxacin (CIP), and norfloxacin (NOR). The ARP of Campylobacter isolates revealed up to five Antibiotypes for those two species. Those isolates revealed resistance to one or more antimicrobials, and 67 percent of them had MDR resistance to two or more experienced antimicrobials. Model NOR T E is the most common MDR, having a prevalence of 30% among experienced isolates. MAR index 1 and lower was found in 87 percent of the isolates. Antibiotic resistance in Campylobacter raises the probability of treatment failure in humans and animals, as well as the propagation of antimicrobial resistance genes. As a result, the presence of Campylobacter in meat could pose a risk of human infection as well as pollution of the environment.

Keywords: Antimicrobial resistance, antibiogram, Campylobacter, poultry meat
1. Introduction

*Campylobacter* is the most common cause of human gastroenteritis, accounting for around 166 million cases of diarrhea and 37,600 fatalities per year (1). The majority of human cases are caused by the bacteria *C. jejuni* and *C. coli* (2). Eating raw or undercooked poultry meat, particularly chicken meat, causes human campylobacteriosis, which accounts for more than 80% of all human cases (3, 4). The frequency of antimicrobial resistant isolates to medications used in human therapy is increasing worldwide, as is the incidence of human campylobacteriosis (5). Despite the development of novel antimicrobials, bacteria have been found to stay up with and modify defensive mechanisms against them, resulting in antimicrobial resistance (6). Antibiotic resistance increases in *Campylobacter*, and some strains have developed MDR (7). Internationally, MDR *Campylobacter*, notably against quinolones and ERY, has grown, causing global worry (8) that linked to major worldwide health effects (9). It was supposed that the resistant bacteria was naturally harder than the sensitive ones (10). Various experts believe that the central pool of antibiotic-resistant determinants in pathogens is edible meat, while some of them believe that the fundamental challenge is the indiscriminate use of antibiotics in humans (11). The increased use of antimicrobial agents in livestock and poultry over the last decade has raised concerns about the continued rise in the incidence of foodborne diseases and drug resistance among foodborne pathogens (12), indicating a potential risk for the buyer when those pathogens are zoonotic like *Campylobacter* (13). Buyers in the Iraq choose poultry meat because it has the right nutritional properties and contains all of the key amino acids that people require. And because of a paucity of documents on the occurrence of this phenomenon in *Campylobacters* related to poultry meat, this research carried out to examine the ARP of *Campylobacters* as well as the MAR index of these isolates in order to connect the evolution of resistance in retail chicken meat with management techniques.

2. Methodology

2.1. Ethical Approval

There was no requirement for such approval because the meat samples were collected from the marketplaces.
2.2. Bacterial Strains and Growth Conditions

A total of 30 Campylobacter strains were recovered from poultry flesh in a prior investigation, including C. jejuni strains (n = 10) and C. coli strains (n = 20). Before being preserved in glycerin at -18°C, all strains were identified using biochemical tests and validated at the species level by Polymerase Chain Reaction (PCR) as previously described (14). The strains were thawed overnight at 4°C, then sub cultured on modified Charcoal Deoxycholate agar (mCCDA) (Oxoid, CM739) without supplement. The plates were raised at 42°C for 24 h in an anaerobic jar (Oxoid, AG25) under microaerophilic situations (O2 5%, CO2 10%, N2 85%) using Oxoid Campy Gen™ atmosphere.

2.3. Antibiogram of Campylobacters

To assess ARP in Campylobacter isolates, a disk diffusion agar based on Quinn et al. (15) approach was used. The Clinical and Laboratory Standards Institute was used to interpret the results (16). The inoculum was generated as a direct broth of certain colonies from a 24-h agar plate (mCCDA without enhancement) using a direct method of colony suspension. This approach has been suggested for checking difficult-to-control pathogens like Campylobacter (15). The inoculum was equally distributed on Mueller-Hinton agar plates (Oxoid, CM0337) enhanced with 5% horse blood using sterile cotton swabs (SR0048C). Discs of antibiotic positioned on the surface of agar to test susceptibility of bacteria to nalidixic acid (30 g), norfloxacin (10 g), erythromycin (15 g), tetracycline (30 g), gentamycin (10 g), and ciprofloxacin (5 g). The plates incubated at 42°C for 24 h in a microaerophilic environment.

2.4. Multiple Antibiotic Resistance (MAR index)

The proportion between the number of multiple antibiotics to which the recovered isolates are resistant and the number of multiple antibiotics to which the individual isolates are exposed was defined as the MAR index of isolates (17).

2.5. Statistics

MedCalc Software bvba version 18 (BE, USA) was used to analyze the data. The proportion was utilized as a descriptive statistic. To compare significance between percentages for the selected antibiotics, two samples Chi square between percents were employed (https://www.medcalc.org/).

3. Results
In this investigation, increased rates of resistance to TET and ERY were observed up to 95%, while low rates of resistance to CIP were observed up to 15% (Table 1). Only with ND ($\chi^2=4.413$, p=0.0357) did data analysis demonstrate that there were significant differences in levels of resistance by organism type (Table 1). Our findings revealed that _C. coli_ strains have a higher rate of resistance to all antimicrobials than _C. jejuni_ strains (Table 1).

The ARP and MAR index of _C. jejuni_ have been investigated, and the outcomes are provided in Table 2. According to the findings, 7 (70 percent) of the tested strains were resistant to one or more antimicrobials. In addition, based to the amount of antimicrobials to which each strain was resistant, ARP of _C. jejuni_ created five Antibiotypes that were discovered in four Antibiogroups. The most prevalent ARP is NOR T E, which was discovered in 30% of the tested strains (Table 2). Furthermore, the prevalence of _C. jejuni_ was 10%, 10%, 10%, 10%, 30%, and 10%, respectively, according to the MAR index 0.2, 0.2, 0.3, 0.5, and 0.83. (Table 2).

_C. coli_ strains' ARP and MAR indexes were also examined, with the results published in (Table 3). According to the findings, 19 (95 percent) of the tested strains were resistant to one or more antimicrobials. In addition, based to the amount of antimicrobials to which each strain was resistant, ARP of _C. coli_ created seven Antibiotypes that were discovered in five Antibiogroups. The most prevalent ARP is NOR T E, which was discovered in 30% of the tested strains (Table 3). Furthermore, the prevalence of _C. coli_ with a MAR index of 0.16, 0.16, 0.33, 0.5, 0.83, 0.83, and 1 was 10%, 10%, 10%, 30%, 5%, 15%, and 15%, respectively (Table 3).

4. Discussion

Resistant bacteria have been found in animal species and across the food chain, as has been thoroughly documented. The existence of antibiotic-resistant bacteria in birds can lead to their presence in chicken carcasses and products, posing a health risk to humans (18,19).

According to the findings (Table 1), a substantial percentage of the tested isolates were resistant to ERY and TET (up to 95%), followed by NOR (up to 65%). Furthermore, our findings revealed that _C. coli_ strains recovered from chicken flesh had a higher prevalence of antibiotic resistance than _C. jejuni_ strains (Table 1 & Fig 1).
The high rate of antibiotic resistance reported in *Campylobacter* isolates could be ascribed to antibiotic abuse and overuse in poultry production, notably in food, as well as indiscriminate use of antibiotics (20). TET resistance may be connected to its widespread usage in the prevention and treatment of animal diseases, as well as food additions for animals. These selective burdens resulted in the creation of this phenomenon (21). Macrolides (such as spiramycin) have been the most commonly utilized drugs to enhance growth in chicken production (22), which could explain why isolates of *C. jejuni* have developed resistance to ERY. Antimicrobial resistant bacteria, such as *Enterococci* spp., colonize the intestines of broilers and are multi-resistant to various antibiotics, perhaps transferring resistance to *Campylobacter* toward TET and ERY. As a result, broilers may be exposed to these environmentally resistant germs (24).

Resistance to fluoroquinolones in *Campylobacters* could be linked to the use of fluoroquinolones (sarafloxacin and ENF) in veterinary medicine to treat *E. coli* respiratory infections and as a preventive in chicken production (25). The use of apramycin in veterinary medicine could be linked to the development of GEN resistance (25).

Our findings were similar to those obtained by Ge *et al.* (8), Kurincic *et al.* (11), and Hassanain *et al.* (21). In contrast, Wieczorek *et al.* (26) from Poland investigated the prevalence of resistance in *Campylobacter* recovered from poultry meat to CIP, ERY, TET, GEN, and STR discovered that fluoroquinolones had the highest resistance rate, with 88.1 percent of the isolates resistant to CIP and 49.2 percent resistant to TET. Furthermore, 0.6 percent of *C. jejuni* isolates were STR resistant, whereas the number of ERY resistant isolates was less than 1%, and none of the isolates were GEN resistant.

The reduced resistance among *Campylobacter* poultry isolates in those investigations likened to our findings was most likely owing to the use of antibiotics in poultry production being restricted (27, 28). Antibiotic resistance rates in *Campylobacter* strains have been found to differ depending on the strain's origin and the hosts' reported history of antibiotic use (29).

According to the amount of antimicrobials to which each strain was resistant, ARP of *C. jejuni* produced five antibiotypes that were discovered in four Antibiogroups (Table 2). According to the obtained data (Table 3), *C. coli* ARP
produced seven antibiotypes that were discovered in five Antibiogroups based on the number of antimicrobials to which each strain was resistant.

Individual determinants that control antimicrobial outflow activity, such as multi-drug pumps, might cause MDR to emerge as a consequence of the acquisition of many resistance determinants in the same DNA molecule or as a result of individual determinants that control antimicrobial outflow activity (30). It's possible that genetic resistance mechanisms are chromosomal or plasmid-based, and that they reflect a mix of endogenous and acquired genes (31).

Several researchers had previously discovered multiple drug resistance in Campylobacters from poultry meat (17, 20, 26). The discovery of CIP, ERY, and GEN resistant Campylobacter isolates in poultry is concerning because these antibiotics are widely used to treat human Campylobacter infections (13). And because the ever-increasing global occupation and travel, the public health concern of Campylobacter resistance has global ramifications (31).

Our findings (Table 3) indicated that there were changes in the breeding practices used during the time of poultry production. This explains why the MAR index of Campylobacters identified in retail poultry differs. Raw excrement can be a valuable source of antimicrobial residues when used as fertilizer because a considerable proportion of antimicrobials provided through diet or water are not completely absorbed in the intestines and up to 90% of the direct amount of drugs can be excreted in feces (31, 32). As a result, a high MAR score would imply that these isolates were obtained from meat due to the high risk of raw waste pollution (4). And, because these foodstuffs were purchased from a variety of nations with different origins, different improvement procedures might be used to explain the discrepancies in the MAR index, which ranges from 0.16 to 1, to the farmers in these countries.

4. Conclusion

Our findings revealed that the more experienced isolates had resistance to ERY, TET, and/or NOR, as well as a higher rate of resistance to GEN. And, given that infected poultry is responsible for the majority of human Campylobacter illnesses, this finding is concerning, especially given that those medications are regarded first-line treatments for human contagious. Our findings suggested that poultry farming could be a major public health issue due to the spread of antibiotic resistance. These findings highlight the need for more research into antimicrobial resistance acquisition.
mechanisms and the role of virulent genes in disease pathogenesis to ensure effective prevention and control of resistant strains from farm to table to supplement public defenses against Campylobacter infections.

**Authors’ Contributions**

MHGK completed the laboratory work for this study, as well as organizing, writing, and reviewing the manuscript. AJO and FAM were in charge of data analysis and interpretation of the outcomes. The final version of the manuscript has been read and approved by all of the researchers.

**Acknowledgments**

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**Conflict of Interest**

The researchers pronounce they do not have any conflict of interest.

**References**


Table 1: Analysis of antimicrobial sensitivity data of Campylobacter isolated from poultry meat based on the species of the organisms.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>No. of resistant isolates based on species of organisms (%)</th>
<th>$\chi^2$</th>
<th>$p.\ value$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. jejuni n/10 (%)</td>
<td>C. coli n/20 (%)</td>
<td></td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>0 (0)</td>
<td>7 (35)</td>
<td>4.413</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>4 (40)</td>
<td>13 (65)</td>
<td>1.640</td>
</tr>
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</table>
Table 2: Antibiogram and MRA index of *Campylobacter jejuni* isolated from poultry meat.

<table>
<thead>
<tr>
<th>Antibiotypes</th>
<th>No. of antimicrobial resistance determinants</th>
<th>No. of <em>C. jejuni</em> isolates (%)</th>
<th>Antibiogroups</th>
<th>MDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOR T E GM CIP</td>
<td>5</td>
<td>1 (10)</td>
<td>1 A</td>
<td>0.83</td>
</tr>
<tr>
<td>NOR T E</td>
<td>3</td>
<td>3 (30)</td>
<td>2 A</td>
<td>0.5</td>
</tr>
<tr>
<td>T E</td>
<td>2</td>
<td>1 (10)</td>
<td>3 A</td>
<td>0.3</td>
</tr>
<tr>
<td>T</td>
<td>1</td>
<td>1 (10)</td>
<td>4 A</td>
<td>0.2</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>1 (10)</td>
<td>4 B</td>
<td>0.2</td>
</tr>
<tr>
<td>Sensitive</td>
<td>--</td>
<td>3 (30)</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

*Campylobacter jejuni* = *C. jejuni*; *Campylobacter coli* = *C. coli*; MDI = multiple antibiotic resistance index.

Table 3: Antibiogram and MRA index of *Campylobacter coli* isolated from poultry meat.

<table>
<thead>
<tr>
<th>Antibiotypes</th>
<th>No. of antimicrobial resistance determinants</th>
<th>No. of <em>C. coli</em> isolates (%)</th>
<th>Antibiogroups</th>
<th>MDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND NOR T E GM CIP</td>
<td>6</td>
<td>3 (15)</td>
<td>1 A</td>
<td>1</td>
</tr>
<tr>
<td>ND NOR T E GM</td>
<td>5</td>
<td>3 (15)</td>
<td>2 A</td>
<td>0.83</td>
</tr>
</tbody>
</table>

*Campylobacter jejuni* = *C. jejuni*; *Campylobacter coli* = *C. coli*; MDI = multiple antibiotic resistance index.
<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>NOR T E GM</th>
<th>CIP</th>
<th>1 (5)</th>
<th>2 B</th>
<th>0.83</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOR T E</td>
<td>3</td>
<td>6</td>
<td>3 A</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>T E</td>
<td>2</td>
<td>2</td>
<td>4 A</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>1</td>
<td>2</td>
<td>5 A</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>2</td>
<td>5 B</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Sensitive</td>
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<td>--</td>
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<td></td>
</tr>
</tbody>
</table>

*Campylobacter jejuni=C. jejuni; Campylobacter coli=C. coli* MDI= multiple antibiotic resistance index.

Figure 1: Prevalence of antimicrobial resistance in *Campylobacter* species isolated from poultry meat