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Original Article

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. Since 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. This study aimed to determine the antimicrobial resistance pattern (ARP) models, multiple drug resistance (MDR) models, and multiple antibiotic resistance (MAR) index of *Campylobacter* species isolated from poultry meat sold in Baghdad markets, Iraq. By employing 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, Gentamicin, nalidixic acid, ciprofloxacin, and norfloxacin (NOR). The ARP of the *Campylobacter* isolates revealed up to five antibiotypes for two species, which revealed resistance to one or more antimicrobials, and 67% of them had MDR resistance to two or more experienced antimicrobials. The NOR-TET model is the most common MDR, having a prevalence of 30% among experienced isolates. In addition, the MAR index, equal to and lower than one, was found in 87% 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 and 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). Most human cases are caused by *C. jejuni* and *C. coli* bacteria (2). The consumption of raw or undercooked poultry meat, particularly chicken meat, causes human campylobacteriosis, accounting 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 multiple drug resistance (MDR) (7). At a global level, MDR *Campylobacter* has grown notably against quinolones and erythromycin (ERY), causing global worry (8) that is linked to major worldwide health effects (9). It was supposed that the resistant bacteria were naturally harder than the sensitive ones (10). Many experts believe that edible meat is the central pool of antibiotic-resistant determinants in pathogens, while some believe that the indiscriminate use of antibiotics in humans is the fundamental problem (11). Over the last decade, the increased use of antimicrobial agents in livestock and poultry 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 the pathogens are zoonotic, similar to *Campylobacter* (13). Buyers in Iraq choose poultry meat because it possesses suitable nutritional properties and contains all of the key amino acids for humans. Due to a paucity of documents on the occurrence of this phenomenon in *Campylobacters* related to poultry meat, the present study was carried out to examine the antimicrobial resistance pattern (ARP) of *Campylobacters* and the multiple antibiotic resistance (MAR) index of these isolates to connect the evolution of resistance in retail chicken meat with management techniques.

2. Materials and Methods

2.1. Bacterial Strains and Growth Conditions

A total of 30 *Campylobacter* strains, including *C. jejuni* strains (n=10) and *C. coli* strains (n=20), were recovered from poultry flesh in a prior investigation (14). 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 as previously described (14). The strains were thawed overnight at 4°C, then subcultured 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 (O_2 5%, CO_2 10%, N_2 85%) using Oxoid CampyGen™ atmosphere.

2.2. Antibiogram of *Campylobacters*

In order to assess ARP in *Campylobacter* isolates, a disk diffusion agar was used based on Quinn, Carter (15) approach, and the Clinical and Laboratory Standards Institute interpreted 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 colony suspension method. This approach has been suggested for checking difficult-to-control pathogens, such as *Campylobacter* (15). The inoculum was equally distributed on Mueller-Hinton agar plates (Oxoid, CM0337) enhanced with 5% horse blood using sterile cotton swabs (SR0048C). The discs of antibiotics positioned on the surface of agar to test the susceptibility of bacteria to nalidixic acid (30 g), norfloxacin (NOR) (10 g), ERY (15 g), tetracycline (TET) (30 g), gentamycin (10 g), and ciprofloxacin (CIP) (5 g). The plates were incubated at 42°C for 24 h in a microaerophilic environment.

2.3. Multiple Antibiotic Resistance 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, were defined as the MAR index of isolates (17).

2.4. Statistical Analysis

MedCalc software (version 18) (BE, USA) was used to analyze the data, and the proportion was utilized as a descriptive statistic. The chi-square two sample test was applied in order to compare the significance among percentages for the selected antibiotics (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). The data analysis demonstrated significant differences in the resistance levels by organism type, only with nalidixic acid (χ^2=4.413, P=0.0357) (Table 1). The results of the present investigation revealed that *C. coli* strains had a higher rate of resistance to all antimicrobials, compared to *C. jejuni* strains (Table 2).

The ARP and MAR indices of *C. coli* strains were also examined, and the results are presented in (Table 3). According to the findings, 19 (95%) tested strains were resistant to one or more antimicrobials; in addition, based on the amount of antimicrobials to which each strain was resistant, ARP of *C. coli* created seven antibiotypes discovered in five antibiogroups. The NOR-TET is the most prevalent ARP, which was discovered in 30% of the tested strains (Table 3). Furthermore, the prevalence rates of *C. coli* with MAR indices of 0.16, 0.16, 0.33, 0.5, 0.83, 0.83, and 1 were 10%, 10%, 10%, 30%, 5%, 15%, and 15%, respectively (Table 3).

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

Antibiotics	Number of Resistant Isolates Based on the Species of the Organisms (%)		χ^2	*P*-value
	***C. jejuni* n/10 (%)**	***C.coli* n/20 (%)**		
Nalidixic acid	0 (0)	7 (35)	4.413	0.0357 (S)
Norfloxacin	4 (40)	13 (65)	1.640	0.2003 (NS)
Tetracycline	7 (70)	19 (95)	3.486	0.0619 (NS)
Erythromycin	7 (70)	19 (95)	3.486	0.0619 (NS)
Gentamycin	1 (10)	7 (35)	2.060	0.1512 (NS)
Ciprofloxacin	1 (10)	3 (15)	0.139	0.7089 (NS)

Campylobacter jejuni=C. jejuni; *Campylobacter coli=C.coli*; S=Significant; NS=Non-significant

Table 2. Antibiogram and MAR indices of *Campylobacter jejuni* isolated from poultry meat

Antibiotypes	Numbers of antimicrobial resistance determinants	No. of *C. jejuni* isolates (%)	Antibiogroups	MAR Index
NOR-TET, GM, CIP	5	1 (10)	1 A	0.83
NOR-TET	3	3 (30)	2 A	0.50
TET	2	1 (10)	3 A	0.30
T	1	1 (10)	4 A	0.20
E	1	1 (10)	4 B	0.20
Sensitive	--	3 (30)	--	--

Campylobacter jejuni=C. jejuni; *Campylobacter coli=C.coli*; MAR=Multiple antibiotic resistance.

Table 3. Antibiogram and MAR indices of *Campylobacter coli* isolated from poultry meat

Antibiotypes	Numbers of antimicrobial resistance determinants	Numbers of *C.coli* isolates (%)	Antibiogroups	MAR Index
ND, NOR-TET, GM, CIP	6	3 (15)	1 A	1
ND, NOR-TET, GM	5	3 (15)	2 A	0.83
NOR-TET, GM, CIP	5	1 (5)	2 B	0.83
NOR-TET	3	6 (30)	3 A	0.5
TET	2	2 (10)	4 A	0.33
T	1	2 (10)	5 A	0.16
E	1	2 (10)	5 B	0.16
Sensitive	--	1 (5)	--	--

Campylobacter jejuni=C. jejuni; *Campylobacter coli=C.coli,* MAR=Multiple antibiotic resistance.

4. Discussion

As has been thoroughly documented, resistant bacteria have been found in animal species and across the food chain. Moreover, 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, the findings of the present investigation revealed that *C. coli* strains recovered from chicken flesh had a higher prevalence of antibiotic resistance, compared to *C. jejuni* strains (Table 1 and Figure 1).

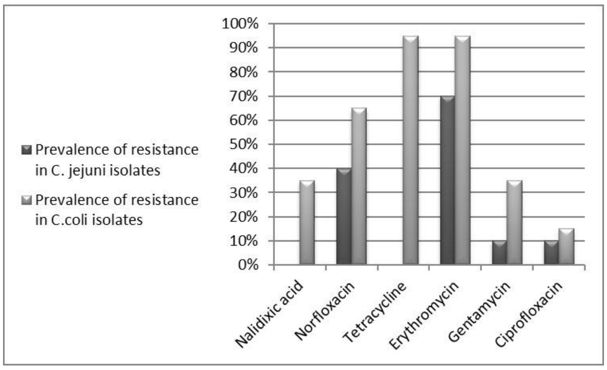

Figure 1. Prevalence of antimicrobial resistance in *Campylobacter* species isolated from poultry meat

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 medications to enhance growth in chicken production (22), which could explain why the isolates of *C. jejuni* have developed resistance to ERY. Some antimicrobial-resistant bacteria, such as *Enterococci spp.*, colonize the intestines of broilers and are multi-resistant to various antibiotics, probably transferring resistance to *Campylobacter* toward TET and ERY; consequently, broilers may be exposed to these environmentally resistant germs (23).

Resistance to fluoroquinolones in *Campylobacters* could be linked to the use of fluoroquinolones (sarafloxacin and enfloxacin) in veterinary medicine to treat *Escherichia coli* respiratory infections and as a preventive in chicken production (24). The use of apramycin in veterinary medicine could be linked to the development of gentamicin (GEN) resistance (24).

The findings of this study were similar to those obtained by Ge, Wang (8), Kurin, Berce (11) and Hassanain (21). In contrast, Wieczorek, Szewczyk (24) investigated the prevalence of resistance in *Campylobacter* recovered from poultry meat to CIP, ERY, TET, GEN, and streptomycin (STR) and discovered that fluoroquinolones had the highest resistance rate, with 88.1% of the isolates resistant to CIP and 49.2% resistant to TET. Furthermore, 0.6% of *C. jejuni* isolates were STR-resistant, whereas the number of ERY-resistant isolates was less than 1%, and none of the isolates was GEN-resistant.

The reduced resistance among *Campylobacter* poultry isolates in the investigations comparable to the findings of the present study was most likely due to the use of antibiotics in poultry production being restricted (25, 26). Moreover, 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 (27).

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

The individual determinants that control antimicrobial outflow activity, such as multidrug pumps, might cause MDR to emerge due to the acquisition of many resistance determinants in the same DNA molecule (28). It is possible to mention that genetic resistance mechanisms are chromosomal or plasmid-based, and they reflect a mix of endogenous and acquired genes (29).

A number of researchers had previously discovered multiple drug resistance in *Campylobacters* from poultry meat (17, 20, 30). The discovery of CIP-, ERY-, and GEN-resistant *Campylobacter* isolates in poultry is concerning because these antibiotics are extensively used to treat human *Campylobacter* infections (13). In addition, due to the ever-increasing global work and travel, the public health concern of *Campylobacter* resistance has global ramifications (29).

The results of this study (Table 3) indicated some changes in the breeding practices used throughout poultry production, which 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 since a considerable proportion of antimicrobials provided through diet or water are not entirely absorbed in the intestines, and up to 90% of the direct amount of medications can be excreted in feces (29, 31). 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 (3). Moreover, since these foodstuffs were purchased from various 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.

5. Conclusion

The results revealed that the more experienced isolates had resistance to ERY, TET, and/or NOR, as well as a higher rate of resistance to GEN. Moreover, since infected poultry is responsible for most human *Campylobacter* illnesses, this result is concerning, especially because the mentioned medications are regarded as first-line treatments for human contagions. Furthermore, the findings suggested that poultry farming could be a significant public health issue due to the spread of antibiotic resistance. These findings highlight the need for more research on antimicrobial resistance acquisition mechanisms and the role of virulent genes in disease pathogenesis to ensure effective prevention and control of resistant strains from farm peoples' tables to supplement public defenses against *Campylobacter* infections.

Authors' Contribution

M. H. G. K. completed the laboratory work for this study and organized, wrote, and reviewed the manuscript. A. J. O. and F. A. M. were in charge of data analysis and interpretation of the outcomes. The final version of the manuscript has been read and approved by all authors.

Ethics

There was no requirement for any approval because the meat samples were collected from the marketplaces.

Conflict of Interest

The authors declare that they have no conflict of interest.

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