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ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER JEJUNI* FROM POULTRY MEAT IN LOCAL MARKETS OF IRAQ

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Abstract

Antimicrobial resistance is a serious public health problem worldwide. The consequence of antimicrobial-resistant *Campylobacter* for public health is due to the propensity of bacteria to rapidly acquire and propagate the resistance gene. Thus, creating mode for the emergence of new and very pathogenic clones resulting to difficulty in treatment with antimicrobials. So, the appearance of new and very pathogenic clones with the consequent difficulty in the treatment with antimicrobials. Therefore, this study aimed to determine the antimicrobial resistance (ARP) models, the multi-resistant models (MDR) and the multiple antibiotic resistance index (MAR index) of *Campylobacter jejuni* (*C. jejuni*) isolated from meat of local and imported chicken sold in local markets. Twenty (20) *C. jejuni* recovered from poultry meat comprising local chicken meat isolates (n = 10) and imported chicken meat isolates (n = 10), were experienced against five antimicrobials called tetracycline (TET), Erythromycin (ERY),gentamicin (GEN), nalidixic acid (NA), Enrofloxacin (ENF) using the disc diffusion test. The ARP of *C. jejuni* isolates exhibited four Antibiotypes. These isolates were originate resistant to one or more antimicrobials by which 85% of them exhibited MDR resistance to two or more experienced antimicrobials. Multidrug resistant model TET ERY is the most prevalent model with prevalence of 45% among the experienced isolates. Ninety-five percent of the isolates had MAR index 0.8 and below. Antimicrobial resistance in *Campylobacter* not only increases the risk of treatment failure in both humans and animals, but also spreads antimicrobial resistance genes. Therefore, the presence of *Campylobacter* in meat could be a potential source of human infections and environmental contamination.

Keywords **:** Antimicrobial resistance, Baghdad province, *Campylobacter jejuni,* chicken meat.

Introduction

Campylobacter is considered the leading cause of gastroenteritis in humans responsible for approximately 166 million cases of diarrhea and 37,600 deaths per year worldwide (Oh *et al*., 2018). The species *C. jejuni* and *C. coli* are associated with the majority of human cases (Mikulić *et al*., 2016). The main risk factors associated with human campylobacteriosis are the consumption of raw poultry or the poor management of raw or undercooked poultry meat, particularly chicken meat, responsible for more than 80% of all human cases (Bahrndorff, *et al*., 2013; Tang *et al*., 2016). The incidence of human campylobacteriosis is increasing worldwide, as is the number of antimicrobial resistant isolates to drugs used in human therapy (Moore *et al*., 2006). Although new antimicrobials have been developed, bacteria have been reported to keep pace and adapt defense mechanisms against these antimicrobials, resulting in the development of resistance to new antimicrobials (Tillotson & Nicolette, 2013).

Campylobacter becomes more resistant to antibiotics and some of them have formed MDR (Mansouri *et al*., 2012). The MDR *Campylobacter*, particularly against quinolones and ERY, has increased internationally and has created global concerns (Ge *et al*., 2013), which could have serious consequences for public health (Iovine, 2013). It was believed that the resistant *Campylobacter* was naturally harder than the sensitive strain (Chai *et al*., 2008). Some authors consider edible meat as the main reservoir of antibiotic-resistant genes in pathogenic bacteria, while others are the chief challenging reason due to the nonsense use of antibiotics in humans (Kurincic *et al*., 2005). The increase in the use of antimicrobial agents in livestock and poultry has raised concerns about the continued increase in the incidence of foodborne diseases and the resistance of foodborne pathogens to drugs during the last decade (Mc Nulty *et al*., 2016), which indicates a possible risk for the client when these pathogenic bacteria are zoonotic like *Campylobacter* (Taylor, 2012).

Chicken meat is favorite by buyers locally because of its appropriate nutritional possessions and its content in all the essential amino acids needed by humans and due to lack of data on the prevalence of resistant *Campylobacters* in chicken meat, this study was undertaken to inspect the ARP of *C. jejuni* as well as to investigate the MAR index of these isolates to link the emergence of resistance in retail chicken meat with management practices.

Materials and Methods

Ethical Approval

Meat samples were obtained from the markets, so there is no need for such approval.

Bacterial Strains and Growth Conditions

A total of 20 *C. jejuni* isolated from poultry meat comprising local chicken meat isolates (n = 10) and imported chicken meat isolates (n = 10), were obtained from previous study (Ghaffoori, 2017). The isolates were collected and identified as *C. jejuni* using biochemical test and confirmed by Polymerase Chain Reaction (PCR) as previously described (Ghaffoori, 2017), before they were stored in glycerin at -18°C. All isolates were thawed in at 4°C overnight, then sub cultured on modified Charcoal Deoxycholate agar (mCCDA) (Oxoid, CM739) without supplement ,the plates were raised in an anaerobic jar (Oxoid, AG25) under microaerophilic condition (O2 5 %, CO2 10 %, N2 85 %) using the Oxoid Campy Gen ™ atmosphere packs (Oxoid, CN0025A) at 42°C for 24 h.

Antibiogram of *C. jejuni*

The technique of diffusion of the agar disk according to (Quinn *et al*., 2004) was adopted to determine ARP in isolates of *C. jejuni*. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2013). A direct method of colony suspension was used in which the inoculum was prepared producing a direct broth of selected isolated colonies from a 24-h agar plate (mCCDA without supplement). This approach has been recommended to check demanding organisms such as *Campylobacter* (Quinn *et al*., 2004). Sterile cotton swabs were used to evenly distribute the inoculum on Mueller-Hinton agar plates (Oxoid, CM0337) supplemented with 5% horse blood (SR0048C). Five antibiotic discs were selected and placed on the surface of the agar to check their susceptibility to *C. jejuni*. Certain antibiotics were NA (30 µg), ENF (10 µg), ERY (15 µg), TET (30 µg) and GEN (10 µg). The plates were incubated under microaerophilic condition at 42°C for 24 h.

Multiple Antibiotic Resistance (MAR index)

The MAR index of isolates was detected as the proportion between the numeral of multiple antibiotics to which the recovered isolates are resistant to the numeral of multiple antibiotics to which the specific isolates are exposed (Kanaan & Abdulwahid, 2019).

Statistics

Data analysis were done using MedCalc Software bvba version 18 (BE, USA). Descriptive statistics such as proportion, mean and standard deviation were used. Two samples Chi square between percents was used to compare significance between percentages, t-test between means with a 5% significant levels was used to compare (mean ± SD) for the selected antibiotics (https:// www.medcalc.org/).

Results

In this study higher rates of resistance were observed toward TET and ERY up to 90%, whereas low rates of resistance were recognized to ENF and GEN up to 30% (Table 1). Statistically there are no significant differences ($P > 0.05$) in the levels of resistance by chicken meat source were found against the selected antimicrobials ($\chi^2 = 0.765$, $p = 0.3819$), ($\chi^2 = 0.394$, $p = 0.5302$), ($\chi^2 = 0.394$, $p = 0.5302$) and ($\chi^2 = 0.000$, $p = 1.000$) for (ENF, ERY, TET and GEN) respectively (Table 1). Moreover, according to sample type and among the resistance strains there was significant difference ($P<0.05$) between the Mean ± SD for the inhibition zones for the selected antibiotics only seen with GEN ($t = -2.881$, $p = 0.010$) (Table 2).

The prevalence of resistant, intermediate and sensitive *C. jejuni* isolates against the selected antimicrobials were ranging from (0-90%), (0-10%) and (5-100%), respectively (Fig. 1). But, with regard the origin of samples, our results revealed higher prevalence of resistance in *C. jejuni* recovered from local chicken meat than those recovered from imported chicken meat against the selected antimicrobials (Fig. 2).

The ARP and MAR index of *C. jejuni* isolates were surveyed and the results are presented in (Table 3). The obtainable results exhibited that 19 (95%) of the tested isolates displayed resistance to one or more antimicrobials. Also, ARP of *C. jejuni* isolated from chicken meat produced six Antibiotypes were detected in four Antibiogroups according to the number of antimicrobials that each isolate was resistant. The most common ARP is TET ERY that had been detected in 45% of the experienced isolates (Table 3). Additionally, prevalence of *C.jejuni* recorded MAR index 0.2, 0.2, 0.4,0.6,0.6 and 0.8 were 5%, 5%, 45%, 10%, 20% and 10%, respectively (Table 3).

Discussion

It is well documented that resistant *Campylobacter* has been detected in animal species and in the food chain. The presence of antibiotic-resistant *Campylobacter* in birds can lead to their presence in poultry carcasses and their products, endangering human health (Otto, 2011; Migura *et al.*, 2014).

The results (Table 1) and (Fig. 1) showed that a high percentage of the tested *C. jejuni* isolates were resistant to ERY and TET up to 90%, followed by ENF and GEN up to 30%. While all *C. jejuni* isolates were sensitive to ND. Additionally, our results revealed higher prevalence of resistance in *C. jejuni* recovered from local chicken meat than *C. jejuni* recovered from imported chicken meat against the selected antimicrobials (Fig. 2).

The high percentage of antibiotic resistance found in *C. jejuni* isolates could be attributed to the abuse and misuse of antibiotics in poultry production, particularly in food, as well as due to indiscriminate use (Nguyen *et al.*, 2016). The increased resistance to TET could be linked to the fact that it has been widely used in the prophylaxis and therapy of animal infections and as food additives for livestock and poultry, these selective pressures led to the emergence of resistant organisms (Hassanain, 2011). In addition, there is evidence to suggest that TET persisted longer in the environment than other antibiotics, which may be crucial in keeping the level of TET resistance at a high level (Kanaan& Khashan, 2018). Macrolides (such as spiramycin) have been the most used agents to promote growth in poultry production (Kanaan& Khashan, 2018), this use could help explain the selection of resistance to ERY in isolates of *C. jejuni*. Cross resistance has been observed among all macrolides in all previously reported cases (Reina *et al*., 1994). In addition, antimicrobial resistant bacteria colonize the intestines of broilers, such as *Enterococci* spp. they were multi-resistant to several antibiotics that possibly transferred resistance to *Campylobacter* to TET and ERY. Therefore, broilers may be exposed to these environmentally resistant bacteria (Kanaan, 2018).

Fluoroquinolones resistance among *C. jejuni* isolates could be linked to veterinary usage of fluoroquinolones (sarafloxacin and ENF) to combat respiratory infection due to *Escherichia coli* and as prophylaxis in poultry production (Kanaan, & Al-Isawi, 2019). The occurrence of GEN resistance in *C. jejuni* isolates from animals could be linked to the use of apramycin (aminoglycoside, structurally related with gentamycin) for veterinary cure (Kanaan, & Al-Isawi, 2019).

The results of this study were consistent with the results obtained by Kurincic *et al*. (2005) to assess the prevalence of resistance in *Campylobacter* spp. recovered from retail poultry meat in Slovenia against ampicillin (AMP), amoxicillin/clavulanic acid, ciprofloxacin (CIP), ERY, GEN, pefloxacin and TET, they found that 61.8% of the isolates analyzed expressed resistance to at least one of every seven antimicrobials analyzed. In addition, a high CIP resistance rate was found among *C. jejuni* isolates (58.2%) and pefloxacin, ERY and TET resistance rates were 58.2,

49.1 and 12.7% respectively. Also, Hassanain *et al.*(2011) studied *C. jejuni* ARP recovered from clinically ill humans and fecal poultry feces in Egypt against AMP, streptomycin (STR), chloramphenicol (CHL), ERY and TET, they found that isolates of *C. jejuni* from birds showed 64.71% of resistance to AMP, STR and CHL and 58.82% to ERY and TET. While the model of human resistance to *C. jejuni* isolates was 87.5% with AMP, 75% with STR and TET, 62.5% with ERY and 50% with CHL. Wieczorek *et al.* (2012) in Poland studied the antimicrobial resistance of *Campylobacter* recovered from poultry meat to CIP, ERY, TET, GEN and STR, and found that the highest resistance rate was found with fluoroquinolones, with 88.1% of the isolates were resistant to CIP and 49.2% were also resistant to TET. In addition, 0.6% of *C. jejuni* isolates showed resistance to STR, while the percentage of ERY resistant isolates did not exceed 1% and none of the isolates was resistant to GEN. Another study was conducted by Ge *et al*. (2013), who scrutinized the antimicrobial susceptibilities of 378 *Campylobacter* isolates of poultry meat in the United States to CHL, CIP, doxycycline, ERY, GEN and TET, and found resistance to TET it was the most commonly documented in the isolation of poultry by up to 82%, followed by resistance to doxycycline (77%), ERY (54%) and CIP (35%). On the other hand, none of the isolates was resistant to GEN. A study conducted in Iraq by Kanaan and Abdulwahid (2019), showed that a high percentage of *Campylobacter* experienced isolates recovered from poultry products showed resistance to TET and ERY with a prevalence (85.2% and 72.2%) respectively. While the resistance against GEN is low with prevalence rate of (29.6%) which in accordance with our results.

The lower resistance to antimicrobials among *C. jejuni* poultry isolates in these studies compared to our results was probably due to the restrictive use of antibiotics in poultry production (Saenz *et al*., 2000; Saleha, 2002). Differences in antibiotic resistance frequencies have been observed in *Campylobacter* strains according to the origin of the strains and the alleged history of antibiotic use in the hosts (Thakur *et al*., 2009).

Our results (Table 2) demonstrated that according to sample type and among the resistance strains there was significant difference ($P<0.05$) between the Mean ± SD for the inhibition zones for the selected antibiotics only seen with GEN. This could be due to that this antibiotic considered as a magical drug to treat severe systemic infections with *Campylobacters* (Engberg *et al*., 2006).

The results (Table 3) revealed that 95% of *C. jejuni* isolates were resistant to one or more antimicrobial agents by which the MDR (TET ERY) is the more MDR model that has been detected in 45% of the experience isolates. Multiple drug resistance was defined as an isolate that exhibitions resistance to two or more antimicrobials simultaneously (Levy, 2002). The appearance of MDR may reflect the acquisition of different resistance determinants in the same DNA molecule or individual determinants, such as multi-drug pumps, which specify the outflow activity against different antimicrobial agents (Engberg *et al*.,2001). The mechanisms of genetic resistance could be chromosomal or plasmid-transmitted and represent a combination of endogenous and acquired genes (Furtula *et al*., 2010).

Multiple drug resistance was earlier detected in *Campylobacters* from poultry meat. Wieczorek *et al*. (2012) in Poland, inspected ARP of *C. jejuni* recovered from meat, in which *C. jejuni* isolates were screened against CIP, ERY, TET, GEN and STR, they found that the vast majority of the tested isolates exhibited resistance to two or more classes of antibiotics and among them 10 *C. jejuni* isolates showed resistance to three different classes of antimicrobials (CIP, STR, TET); (CIP, STR, TET), 53 isolates were resistant to two antimicrobials (CIP, STR); (CIP, TET), 48 isolates were resistant to CIP, while 11 isolates were sensitive for all antimicrobial agents. Additionally, Nguyen *et al*. (2016), in their study investigated antimicrobial susceptibility of *C. jejuni* isolates from small scale and backyard chickens in Kenya against: CHL, ERY, CIP, GEN, STR and TET, they found that MDR to two or more classes of antibiotics was found among 19 *C. jejuni* isolates , in which one isolate was resistant to six antimicrobial agents (TET, CIP, ERY, GEN, STR, CHL), five isolates were resistant to five antimicrobial agents (TET, CIP, ERY, STR, CHL) ; (TET, CIP, ERY, GEN, STR); (TET, CIP, GEN, STR, CHL), four isolates were resistant to four antimicrobial agents (TET, CIP, ERY, STR); (TET, CIP, ERY, CHL); (CIP, ERY, STR, CHL), three isolates were resistant to three antimicrobial agents (TET, CIP, ERY); (TET, CIP, STR), two isolates were resistant to two antimicrobial agents (TET, CIP); (CIP, STR). Also, Kanaan and Khashan (2018), in Iraq found that 10% of the chicken isolates of *Campylobacter* were resistant to six antimicrobial agents (ND NOR TET ERY GEN CIP], 16.7% of the isolates were resistant to five antimicrobial agents (ND NOR TET ERY GEN) , (NOR TET ERY GEN CIP), 30% of the isolates were resistant to three antimicrobial agents (NOR TET ERY), 10% of the isolates were resistant to two antimicrobial agents (TET ERY). The revealing of CIP, ERY, and GEN resistant *C. jejuni* isolates in poultry is alarm since these antibiotics are used universally in cure of human infections with *Campylobacters* (Taylor, 2012). The public health issue of resistance in *Campylobacter* has global dimensions because of ever-increasing worldwide occupation and travel (Engberg *et al*., 2001).

The consequences of our study (Table 3) suggested the existence of differences in the breeding practices used in the period of poultry production. This clarifies the differences in the MAR index between *Campylobacter* isolates found in poultry meat. Since a high proportions of the antimicrobials administered through food or water are not completely absorbed in the intestines of birds plus up to 90% of the direct amount of medications in particular can be defecated in feces, raw waste can be a vital resource antimicrobial residues when used as fertilizer (Furtula *et al*., 2010). Therefore, a high MAR index would indicate that these isolates were recovered from meat from the high dangers of contaminating animal waste (Tang *et al*., 2016). And when these produces were imported from several countries and from dissimilar origins, therefore, diverse improvement practices could be applied that postulate the differences in the MAR index that fluctuates from (0.2-0.8) to the farmers of these countries.

Conclusion

Our results established that the most experienced isolates offered resistance to ERY, TET and/or ENF with greater emergence of resistance against GEN. And since contaminated poultry can explain most human infections with *Campylobacter*, this information is alarming when you realize that these antibiotics are considered drugs of first choice for human infections. Our results predicted that poultry production could be the source of a serious public

health problem through the spread of antibiotic resistance. These results highlight the need to further explore the mechanism of antimicrobial resistance acquisition and the role of virulent genes in the pathogenesis of the disease in order to ensure effective prevention and control of the spread of resistant strains from farm to the table to augment public defenses in contradiction of infections with *Campylobacter*.

Authors' Contributions

The laboratory work involved in this study, organize, writing and revising the manuscript was attained by MHGK. Analysis of data and interpretation of the results was attained by FAM. All researchers have read and approved the final version of the manuscript.

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Conflict of Interest

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

Table 1 : Analysis of antimicrobial sensitivity data of *C. jejuni* isolates from chicken meat based on the sample source.

Antibiotics	No. of resistant isolates based on sample sources (%)		Total percentage (%)	χ^2	*p. value*
	Imported	Local			
Enrofloxacin	2 (10)	4(20)	6 (30)	0.765	0.3819 (NS)
Tetracycline	8 (40)	10 (50)	18 (90)	0.394	0.5302 (NS)
Erythromycin	8 (40)	10 (50)	18 (90)	0.394	0.5302 (NS)
Gentamycin	2 (10)	2 (10)	4 (20)	0.000	1.000 (NS)

NS= non-significant

Table 2 : Analysis the effect of sample source on the resistance in *C. jejuni* against the selected antibiotics.

Antimicrobials	Source of cattle meat				t-statistic	*p. value*
	Imported		Local			
	Inhibition zone (mm)	Mean ± SD	Inhibition zone (mm)	Mean ± SD		
Nalidixic acid	19-26	21.8 ± 2.5	19-25	21.8 ± 2.4	0.000	1.0000 NS
Enrofloxacin	0-34	22.3 ± 11.9	0-24	16.8 ± 7.8	-1.222	0.2373 NS
Tetracycline	0-23	4.5 ± 9.0	0-6	0.6 ± 1.8	-1.344	0.1957 NS
Erythromycin	0-30	8.3± 9.0	0-13	6.5 ± 4.99	-0.553	0.5870 NS
Gentamycin	11-30	20.8 ± 6.5	6-17	14.2 ± 3.2	-2.881	0.0100 S

S= significant, NS= non-significant

Table 3 : Antibiogram and MRA index of *C. jejuni* isolates from local and imported chicken meat

Antibiotypes	No. of antimicrobial resistance determinants	No. of isolates based on sample type (%)		Antibiogroups	MDI
		Local	Imported		
ENF TET ERY GEN	4	1 (5)	1 (5)	1 A	0.8
ENF TET ERY	3	3 (15)	1 (5)	2 A	0.6
TET ERY GEN	3	1 (5)	1 (5)	2 B	0.6
TET ERY	2	5 (25)	4 (20)	3 A	0.4
TET	1	0 (0)	1 (5)	4 A	0.2
ERY	1	0 (0)	1 (5)	4 B	0.2
Sensitive	-	0 (0)	1 (5)	-	-

MDI= multiple antibiotic resistance index.

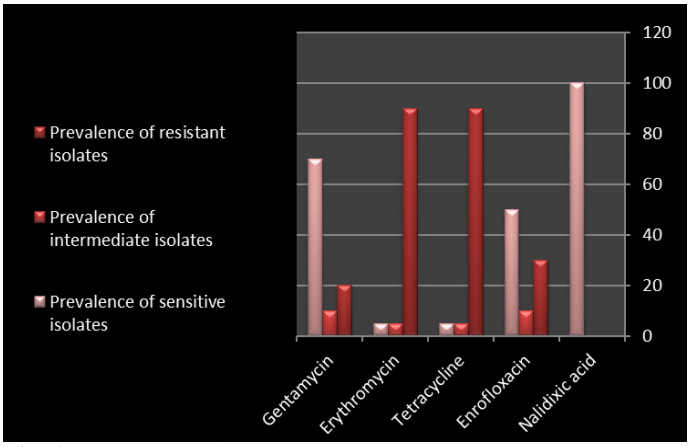

Fig. 1 : Prevalence of antimicrobial resistance in *C. jejuni* recovered from chicken meat.

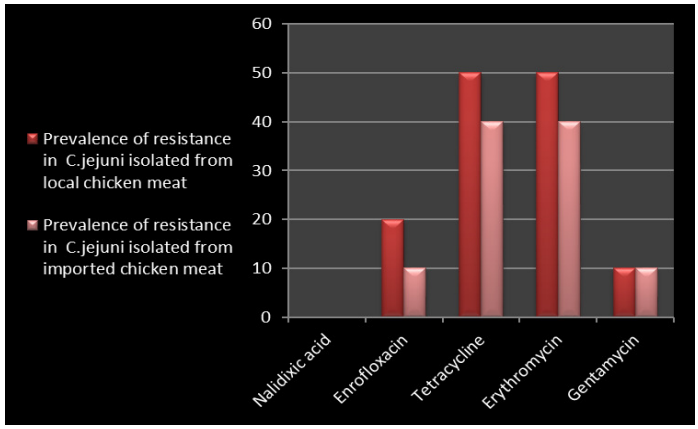

Fig. 2 : Prevalence of resistance in *C. jejuni* isolates based on origin of samples

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