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Prevalence of multidrug resistant thermotolerant species of *Campylobacter* in Retail Frozen Chicken meat in Baghdad Province

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ABSTRACT

Macrolides and fluoroquinolones are considered first-line drugs that have been used in the treatment of human *Campylobacter* diseases. Application of antimicrobials for this reason and in animal production has led to the emergence of resistant *Campylobacter* spp. resulted in protection of these pathogens against selected antimicrobials. And since infected chicken meat can represent the majority of cases of human campylobacteriosis, the application of antibiotics in the animal production may reduce the effective therapeutic life of these clinically lifesaving human antibiotics. The objective of this study was to investigate the prevalence of multidrug -resistant *Campylobacter* spp. in retail chicken meat . A total of 40 samples were purchased five per week for 8 weeks [from August 2017 to September 2017] from different markets in the Baghdad regions, were examined for the presence of *Campylobacter* spp. The results showed that the percentage of positive samples for *Campylobacter* spp. was [75%] of which *C. jejuni* and *C. coli* included [25% and 50%], respectively. Isolation, identification and confirmation of the isolates were performed on the basis of standard bacteriological , biochemical and multiplex PCR protocols. In this study, 30 isolates [10 *C. jejuni* and *20 C. coli*] were analyzed for sensitivity to six different antibiotics [ciprofloxacin, erythromycin, gentamicin, nalidixic acid, norfloxacin and tetracycline] by disc diffusion test . Resistance to tetracycline and erythromycin was the most common [86.7%] followed by resistance to norfloxacin [56.7%], gentamicin [26.7%], nalidixic acid [23.3%] and ciprofloxacin [13, 3%]. *Campylobacter coli* showed higher rates of resistance to all tested antimicrobials compared to *C. jejuni* . In addition, the results also revealed that MDR was predominant in *C. coli* [75%] than in isolates of *C. jejuni* [50%] and that the MDR pattern [Nor T E] was the most prevalent among *Campylobacter* spp. with a percentage of [30%].

Keywords: Multidrug -resistant, Thermotolerant *Campylobacter*, Chicken meat, Baghdad province.

1. INTRODUCTION

Campylobacter is a dominant bacterial cause of acute food-borne gastroenteritis in humans, which represents an unrelenting global public health problem in Europe and many other countries [1]. Species of *C. jejuni* and *C. coli* are associated with most human cases [2]. The incidence of human campylobacteriosis is increasing worldwide, as well as the amount of resistant isolates to antimicrobials used in human therapy [3]. The main risk factors associated with human campylobacteriosis are the consumption of raw poultry or the poor handling of raw or undercooked poultry meat, particularly chicken meat, responsible for more than 80% of all human cases [4, 5, 6]. In addition, the ingestion of unpasteurized milk or milk products and untreated water can be considered another vehicle of transmission and a risk factor to cause campylobacteriosis in humans [7]. *Campylobacter* becomes more resistant to antibiotics and some of it have formed multiple drug resistance [8]. Multidrug -resistant *Campylobacter*, especially against quinolones

and erythromycin, has been increased internationally and created worldwide concerns [9], which could have serious potential public health consequences [10]. It was believed that the resistant *Campylobacter* was biologically stronger than the susceptible strain [11]. Some authors consider edible meat as the main reservoir for resistant genes in pathogenic bacteria to antibiotics, while others as a principal problematic cause due to the foolish use of antibiotics in man [12]. The usage of antimicrobial agents in livestock and poultry can result in a bacterial population resistant to drugs, which signifies a possible risk to the customer when these pathogenic bacteria are zoonotic such as *Campylobacter* [13]. In Iraq, chicken meat is considered the most popular meat item in many communities and due to lack of data on the prevalence of resistant *Campylobacter* spp. in chicken meat, this study was undertaken to investigate the prevalence of multidrug - resistant *Campylobacter* spp. in retail chicken meat sold in the province of Baghdad.

2. MATERIALS AND METHODS

2.1. Collection and processing of samples:

Ten different regions of Baghdad province were chosen, five in Al- Rusafa [Al-Arassat, Al-Aadhamiya, Al-Ghadeer , Al-Baladiyat , Al-Ameen] and five in Al-Kargkh [Al-Yarmouk , Al-Saydiyah , Al-Adl , Al-Mansour , Al-Hurriya]. Four frozen chicken thighs / region were collected from various supermarkets and seller shops . The samples were packed unconnectedly in sterile polyethylene bag and transmitted to the laboratory in an ice box in 3 hours . Chicken thigh was defrosted in a refrigerator overnight , then rinsed in 100 ml buffer peptone water [BPW] by stirring for 3 minutes in a sterile polyethylene bag and processed in 3 hours. The bag was slanted and the sample was hold to allow the rinse liquid to flow into a corner, then the lower corner of the back was sterilized with 70% ethanol and rinsed with sterile water. The angle was aseptically cut, then 25 ml of the rinse sample was placed in a sterile bottle containing 100 ml of Preston broth with the following formulation used: [Nutrient broth No. 2 (Oxoid, CM0067); *Campylobacter* selective antibiotics (Oxoid, SR0204E); *Campylobacter* growth supplement (Oxoid, SR 0232E); Laked horse blood (SR0048C)] , the rinsing of the sample was previously enriched for 4 hours at 37 ° C in an anaerobic jar [Oxoid, AG25] under microaerophilic conditions using the Oxoid Campy Gen TM atmosphere packs [Oxoid, CN0025A] , subsequently enriched at 42 ° C for 24 hours [14,15,16]. After enrichment, two loopful of the medium was streaked onto plates of modified Charcoal Deoxycholate agar [Oxoid, CM 739] supplemented with CCDA antibiotic supplement [Oxoid, SR155] , a similar streaks were made using sterile loop to obtain separate colonies . All plates were incubated in an anaerobic jar under microaerophilic condition at 42°C for 24 - 48 hours. The plates showing positive colonies typical of *Campylobacter* growth were sub cultured one or further times onto same medium without supplement until monocultures were obtained for biochemical identification. The *Campylobacter* isolates were preserved in a double strength Nutrient broth [Oxoid ,CM0001] with 20% [v/v] of pure medical glycerin at -18°C [17].

2.2. Isolation and identification of *Campylobacter* spp. :

Colonies exhibiting typical morphological characteristic of *Campylobacter* on mCCDA [greyish, flat and moistened, with a tendency to spread] were identified presumptively as *Campylobacter* spp. through standard biochemical tests using microscopic examination of morphology and motility , catalase test, oxidase test, microaerobic growth at different temperatures and hippurate hydrolysis test . For the identification of thermotolerant spp. of *Campylobacter*, Oxoid Dry Spot *Campylobacter* kit [Oxoid , DR0150] was used which is a latex agglutination test for the detection of specific *Campylobacter* cell surface antigens.

2.3. Confirmation of *Campylobacter* isolates using multiplex PCR technique:

Campylobacter isolates were identified at the species level using the multiple PCR test depend on the technique designated by Wang *et al*. [18].

2.3.1. Extraction and purification of genomic DNA:

Bacterial DNA was extracted and purified from pure cultures grown in Lauryl tryptose broth [Oxoid, CM0451] using the Wizard® Genomic DNA Purification Kit [USA], following the manufacturer's instructions for the fast DNA extraction protocol.

2.3.2 DNA primers:

The two pairs of primers were designed to identify the genes *hipO* & *glyA* from *C. jejuni* & *C. coli*, respectively. The primers sequences were designed based on the method described by Wang *et al*. [18]. Primers were CJF [5'-ACT TCT TTATTG CTT GCT GC- 3'] and CJR [5'-GCC ACA ACA AGT AAA GAA GC-3'] for *C.jejuni* [size 650 bp], CCF [5'-GTA AAA CCA AAG CTT ATC GTG-3'] and CCR [5'-TCC AGC AAT GTG TGC AAT G-3'] for *C. coli* [size 126 bp], [18].

2.3.3 Multiplex PCR conditions and cycle programs:

A multiplex PCR amplification was done in 25 µl volumes containing 2.5 µl of whole-cell template DNA ; µM 2.5 µl of 10 × PCR reaction buffer [500 mM Tris-HCl (pH 8.3) , 100 mM KCl, and 50 mM (NH4) 2 SO4 , 20 mM MgCl2] ; primers (0.5 µM *C. jejuni* , 1 µM *C.coli*) ; nucleotide stock [1.5 µl of dNTP- Mix (10mM)] ; 1.25 U of Fast Start *Taq* DNA polymerase [Promega, USA] . The volume was adjusted with sterile distilled water to give 25 µl [18]. The PCRs were implemented in a Perkin-Elmer thermal cycler system using an initial denaturation phase at 95 ° C for 6 min. followed by 30 cycles of amplification [denaturation at 95 ° C for 0.5 min, annealing at 59 ° C for 0.5 min. and extension at 72 ° C for 0.5 min.], ending with a final extension at 72 ° C for 7 min. [18]. The final PCR products were electrophoresed using 1.5% agarose gel at 10 V for 90 minutes. The gel was photographed with UV transilluminator [Alpha Imager HP, Alpha Innotech, CA,

USA], After coloring with ethidium bromide to analyze the result. A 100 bp DNA ladder [NL1405, Vivantis, USA] was used as molecular DNA marker [18]. After agarose gel electrophoresis, a multiplex PCR assay yielded two bands of a DNA mixture containing each of the two *Campylobacter* spp. The bands of [126 & 650] bp for [*C. coli glyA & C. jejuni hipO*], respectively.

2.4. Antibiotic Resistance Patterns of *C. jejuni* and *C. coli* :

The agar disk diffusion technique according to Quinn *et al*. [19] was adopted to determine the antibiotics resistance patterns [ARP] in *C. jejuni* and *C. coli* isolates. The results were interpreted according to the CLSI Clinical and Laboratory Standards Institute [20]. A direct colony suspension method was used in which the inoculum was prepared by making a direct broth of isolated colonies selected from a 24 hours agar plate [mCCDA without supplement]. This approach was recommended for testing the fastidious organisms such as *Campylobacter* [19]. Sterile cotton swabs were used to spread evenly the inoculum on to Mueller-Hinton agar plates [Oxoid , CM0337] supplemented with horse blood [SR0048C]. Six antibiotic discs were selected and placed on the agar surface to test on its susceptibility to *C. jejuni* and *C. coli*. The certain antibiotics were nalidixic acid [30 µg], norfloxacin [10 µg], erythromycin [15 µg], tetracycline [30 µg], gentamycin [10 µg], ciprofloxacin [5 µg]. The plates were incubated under microaerophilic condition at 42°C for 24 hours.

3. RESULTS AND DISCUSSION

3.1 Isolation percentage of thermotolerant spp. of *Campylobacter* from retail chicken meat in Baghdad province.

A total of 40 frozen chicken thighs were collected from 10 different regions [four samples / region] of Baghdad province. Five from Al- Rusafa [Al- Arassat , Al-Aadhamiya , Al-Ghadeer, Al-Baladiyat , Al-Ameen] and five from Al-Kargkh [Al- Yarmouk , Al- Saydiyah , Al- Adl , Al- Mansour , Al-Hurriya] to determine the isolation percentage of thermotolerant spp. of *Campylobacter* from retail chicken meat [Table 1]. The results showed that the highest isolation percentage [100%] were noted in Al-Baladiyat , Al-Ameen, Al-Saydiyah and Al- Adl followed by [75%] from Al-Ghadeer, Al- Yarmouk and Al-Hurriya then [50%] from Al- Arassat and Al-Aadhamiya , while the lowest isolation percentage [25%] was from Al- Mansour.

Table 1: Isolation percentage of thermotolerant spp. of *Campylobacter* from chicken meat retailed in Baghdad province.

Regions	No. of samples tested	Total No.+ve / Total samples tested	Isolation percentage
Al-Arassat	4	2/4	50%
Al-Aadhamiya	4	2/4	50%
Al-Ghadeer	4	3/4	75%
Al-Baladiyat	4	4/4	100%
Al-Ameen	4	4/4	100%
Al-Yarmouk	4	3/4	75%
Al-Saydiyah	4	4/4	100%
Al-Adl	4	4/4	100%
Al-Mansour	4	1/4	25%
Al-Hurriya	4	3/4	75%

The dissimilarities in isolation percentage could be attributed to numerous reasons such as the difference in the areas, the source from which the chicken thighs come [their origin], the climatic influence, the conditions of preservation , the execution of the slaughter actions and the hygienic procedures . In addition, the highest isolation rate obtained from the existing study might be related to use of a selective enrichment broth for the pre-enrichment sample rinse fluid, this could increase the recovery of sub-lethal cells and could be particularly appropriate for frozen samples [21]. As well as the higher isolation percentage might be reflect the highest initial bacterial count and genetic differences between isolates which lead to survival of *Campylobacter spp.* in food during storage. These results could be emphasized by the results of Pearson *et al.* [22] and Sampers *et al.* [23] who stated that the greater the preliminary number of bacterial cells lead to greater survivors after contact with stress by cooling and freezing temperature. Moreover, the genetic differences among *Campylobacter* spp. have been described, so it has been predicted that resistance to thermal stress among *Campylobacter* spp. could be strain related [24, 25]. Contamination of chicken carcasses and subsequently chicken meat occurred mainly during defeathering and evisceration, defeathering and scalding the carcasses opens up follicles, giving *Campylobacter* a place to hide from extra approaches of cleaning [26]. The skin of the chicken offers appropriate environment to persistence of *Campylobacter* spp. as buildup of water rises the surface region obtainable for contamination, and even under conditions of freezing or storage at 4 ° C, *Campylobacter* spp. are capable to survive in the carcass [27]. Persistence of *Campylobacter* spp. in water could be improved at low temperatures, in particular at 4 °C, and they could be restored from viable but non culturable [VBNC] to the pathogenic

state [28], and since the temperature of the chillers is 4°C this could be potentially maintenance the existence of *Campylobacter* spp. in water and increase the possibility of contamination of the meat. In addition, the formation of biofilms can influence the persistence of *Campylobacter* spp. by prolonging the survival time in water since bacteria are protected from chlorine in a biofilm [29].

The results of this study were in agreement with the results obtained by Jorgensen *et al*. [21] in England and Bodi, [30] in Spain , who found that the prevalence of *Campylobacter* spp. were [83% and 100%] in chilled chickens and neck skin samples, respectively. The lower isolation percentage [25%] obtained by current study could be attributed to low initial bacterial load which might be due to good hygiene control at the slaughterhouses facilities . Furthermore , this could be further explained by the findings reached by Bodi , [30] who attributed the lower isolation rate of *Campylobacter* spp. to that the bacteria might enter in the state of VBNC after exposure to dissimilar stress influences which can happen during the processing and storing cells. The capability of *Campylobacter* spp. for persistence in meat during storing poses a threat to customer safety due to their capability to yield infections with low infectious doses [31]. Suitable hygienic situations during the processing of chicken meat capable of reducing the load of *Campylobacter* spp. on the surfaces of the meat.

3.2 Isolation percentage of *C.jejuni* and *C.coli* from retail chicken meat in Baghdad province.

The isolation percentages of *C.jejuni* and *C.coli* from retail chicken meat in Baghdad province were listed in Table 2. The results demonstrated that 30 out of 40 samples were positive for *Campylobacter* spp., by which, *C. jejuni* and *C. coli* were identified in 25% and 50% , respectively . Furthermore , the results also showed that the isolation percentages of *C.jejuni* ranged from [0% - 50%] , while isolation percentages of *C.coli* ranged from [0% - 75%] . *Campylobacter jejuni* and *C. coli* were isolated from [Al- Arassat , Al-Aadhamiya , Al-Ghadeer, Al-Baladiyat , Al-Ameen , Al- Yarmouk , Al-Saydiyah , Al- Adl , Al- Mansour , Al-Hurriya] with the isolation percentages were given in Table 2.

Table 2: Isolation percentage of *C.jejuni* and *C.coli* from retail chicken meat in Baghdad province.

Regions	No. of samples tested	Total No.+ve / total samples tested	Total No.+ve (*C.jejuni*) / total samples tested	Isolation percentage (*C.jejuni*)	Total No.+ve (*C.coli*) / total samples tested	Isolation percentage (*C.coli*)
Al-Arassat	4	2/4	1/4	25%	1/4	25%
Al-Aadhamiya	4	2/4	1/4	25%	1/4	25%
Al-Ghadeer	4	3/4	0/4	0%	3/4	75%
Al-Baladiyat	4	4/4	1/4	25%	3/4	75%
Al-Ameen	4	4/4	2/4	50%	2/4	50%
Al-Yarmouk	4	3/4	1/4	25%	2/4	50%
Al-Saydiyah	4	4/4	1/4	25%	3/4	75%
Al-Adl	4	4/4	1/4	25%	3/4	75%
Al-Mansour	4	1/4	1/4	25%	0/4	0%
Al-Hurriya	4	3/4	1/4	25%	2/4	50%
Total	40	30/40	10/40	25%	20/40	50%

It is clear from the results of this study that *C. coli* predominates *C. jejuni* , this is in contrast with other results reported that *C. jejuni* as the most predominant species, the results of this study were agreed with Kurincic *et al.* [12] in Slovenia; Henry *et al.*[32] in Reunion island ; Anderson *et al*. [33] & Marinou *et al.* [34] in Greece ; Wieczorek *et al.*[35] in Poland , who reported that the comparable isolation rates of *C. coli* versus *C. jejuni* were [53% versus 47%] ;[55.5% versus 31.4%]; [6.6% versus 55.5%] & [57.5% versus 0%]; [58.9% versus 41.1%] as respectively, as well as they attributed the highest isolation rates of *C. coli* to the differences in the geographical regions in which the studies were piloted. The greater colonization of broilers with *C. coli* could be attributed to the administration of β-lactam antibiotics to farm chickens and to the type of ration as suggested by the results obtained by Marinou *et al.* [34] in France. Additionally, Preston enrichment broth used in current study possibly owned selective effects which favor *C. coli* isolation [12]. In addition, some authors have suggested that *C. coli* could be more resistant than *C. jejuni* to unfavorable environmental conditions [36], and since the isolates were recovered from frozen meat so this could explain the differences observed in the isolation rates between both species which lead to higher isolation rate of *C. coli* due to their resistant to critical conditions related to the frozen meat. Despite this difference, it is important to consider as an absolute priority that the two species are considered important agents of diarrheal disease and that contaminated chicken meat is documented as the main source of outbreaks and sporadic cases of campylobacteriosis [37].

3.3 Confirmation of *C.jejuni* and *C.coli* isolated from retail chicken meat using multiplex PCR technique.
All of the 30 *C. jejuni* and *C. coli* isolated from retail chicken meat samples according to standard bacteriological and biochemical tests were subjected to multiplex PCR technique [Table 3]. The multiplex PCR assay showed that 10 isolates were produced one band of [650] bp for [*C. jejuni hipO*] so they confirmed as *C. jejuni*, and 20 isolates were produced one band of [126] bp for [*C. coli glyA*] so they confirmed as *C. coli* [Table 3 and Fig.1 & Fig.2].

Table 3: Confirmation of *C.jejuni* and *C.coli* isolated from retail chicken meat using multiplex PCR technique.

Regions	No. of samples tested	No. of +ve samples	Target genes			
			C.jejuni hip O	Size in (bp)	*C.coli gly A*	Size in (bp)
Al-Arassat	4	2	+ve (1)	650	+ve (1)	126
Al-Aadhamiya	4	2	+ve (1)	650	+ve (1)	126
Al-Ghadeer	4	3	_	_	+ve (3)	126
Al-Baladiyat	4	4	+ve (1)	650	+ve (3)	126
Al-Ameen	4	4	+ve (2)	650	+ve (2)	126
Al-Yarmouk	4	3	+ve (1)	650	+ve (2)	126
Al-Saydiyah	4	4	+ve (1)	650	+ve (3)	126
Al-Adl	4	4	+ve (1)	650	+ve (3)	126
Al-Mansour	4	1	+ve (1)	650	_	_
Al-Hurriya	4	3	+ve (1)	650	+ve (2)	126

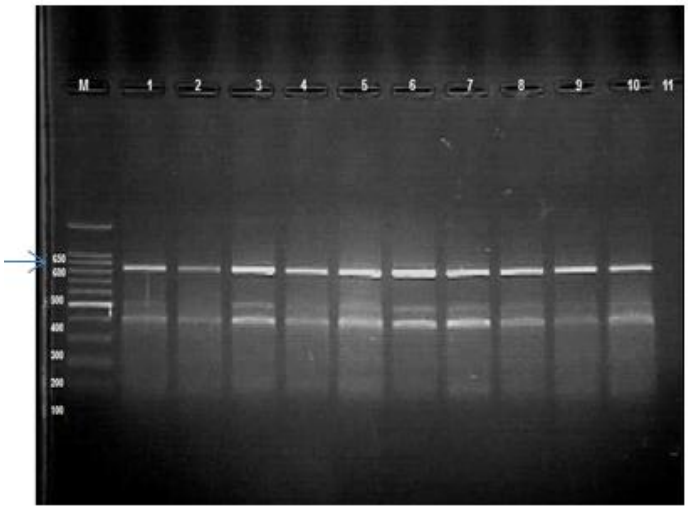

Figure 1: Gel electrophoresis of the multiplex PCR . Lanes 1- 10, 650 bp target of *hipO* of *C.jejuni*; lane 11, NC, Negative Control; lane M, 100 bp DNA molecular size marker.

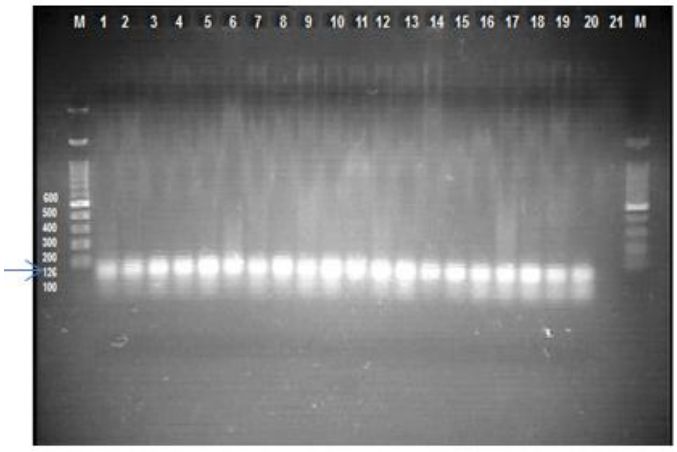

Figure 2: Gel electrophoresis of the multiplex PCR. Lanes 1- 20, 126 bp target of *glyA* of *C. coli* ; lane 21, NC, Negative Control; lane M, 100 bp DNA molecular size marker.

The results of this study agreed with Al Amri *et al* . [38] who evaluated the multiplex PCR protocol for the identification of *Campylobacter* spp. isolated from human cases with *Campylobacter* enteritis and chickens [54 of human and 60 of chickens] to species level based on the detection of *CadF* [genus specific], and *hipO* [*C. jejuni*] and *asp* [*C. coli*] genes, and they found that the multiplex PCR assay designed in their study was a precise tool that could be valuable for the rapid and concurrent detection and confirmation of *Campylobacter* at the species level. Another study was carried out by Abd and Al-Nasrawi, [39] identified the *Campylobacter* spp. isolated from freshly slaughtered and frozen poultry samples to species level using PCR assay in province of Al-Muthanna in Iraq by amplification the specific *hipO* gene in chicken tissue samples and found that the chicken products in retail markets were highly contaminated with *C.jejuni* also they concluded that this assay is suitable for screening poultry products and identification of this pathogen.

3.4 Antibiotic Resistance Patterns of *C. jejuni* and *C. coli*:

A total of 30 thermotolerant spp. of *Campylobacter* isolates comprised [(10 *C. jejuni*) and (20 *C. coli*)] isolated from retail chicken meat were screened against six antimicrobial agents to determine the antibiotic resistance patterns [ARP] of these isolates . The results [Table 4] showed that a high percentage [86.7%] of the tested isolates exhibited resistance to tetracycline [T] 30 µg and to erythromycin [E] 15 µg by which *C. jejuni* and *C. coli* isolates displayed resistance up to 70% and 95% , respectively , followed by norfloxacin [Nor] 10 µg [56.7%] by which *C. jejuni* and *C. coli* isolates presented resistance around [40% and 65%], respectively , then to gentamycin [Gm] 10 µg and to nalidixic acid [ND] 30 µg [26.7% and 23.3%], respectively, by which *C. jejuni* and *C. coli* isolates displayed resistance [10% and 35%], respectively to gentamycin. While resistance to nalidixic acid [ND] was observed only among *C. coli* isolates with resistance percentage of [23.3%]. Furthermore, several isolates were resistant to ciprofloxacin [Cip] 5 µg [13.3%] by means of which the isolates of *C. jejuni* and *C. coli* had a resistance [10% and 15%], respectively. The results of this study also revealed that a large percentage of resistance was found in *C. coli* than in *C. jejuni* for all examined antimicrobials.

Table 4: Antimicrobial resistance percentage of thermotolerant spp. of *Campylobacter* isolated from chicken meat retailed in Baghdad province.

Antimicrobials	Percentage of antimicrobial resistance					
	C.jejuni n/N	(%)	*C.coli* n/N	(%)	Total n/N	Total (%)
Nalidixic acid	0/10	0%	7/20	35%	7/30	23.3%
Norfloxacin	4/10	40%	13/20	65%	17/30	56.7%
Erythromycin	7/10	70%	19/20	95%	26/30	86.7%
Tetracycline	7/10	70%	19/20	95%	26/30	86.7%
Gentamycin	1/10	10%	7/20	35%	8/30	26.7%
Ciprofloxacin	1/10	10%	3/20	15%	4/30	13.3%

n/N = The number of resistant isolates /the number of experienced isolates

The high resistance rate found between *Campylobacter* spp. could be attributed to the abuse and misuse of antimicrobial drugs in chicken production, mainly in feed , and because of being use massively [40]. Antibiotics are widely used in livestock as growth enhancements and to prevent infections [41]. The increased resistance to tetracycline could be attributed to the fact that it has been widely used in the prophylaxis and treatment of human and animal infections and as feed additives for livestock and poultry, these selective pressures have led to the emergence of resistant organisms [42]. In addition, there is a suggestion to indicate that the persistence of tetracycline in the environment is longer than that of other antibiotics which may be critical for maintaining resistance to tetracycline at a high level [43]. Macrolides [such as spiramycin] were the most commonly used agents as growth promoter in chicken production [44], this use could help explain the selection of erythromycin resistance in *Campylobacter* isolates. Microorganisms resistant to a given antimicrobial can also resist other antimicrobials that share a mechanism of action "cross-resistance" and this phenomenon exists mainly among chemically closely related agents, "cross-resistance" among all macrolides was observed in all previously cases [45]. In addition, antimicrobial resistant bacteria colonize the intestine of broilers such as *Enterococci* spp. were multi-resistant to various antibiotics, such as bacitracin, ciprofloxacin, erythromycin, streptomycin and tetracycline, which can transfer resistance to *Campylobacter* spp. so that broilers can be exposed to such environmentally resistant bacteria [46]. On the other hand, most antimicrobial agents administered through feed or water are not completely absorbed in the chicken intestine and up to 90% of the administered dose of some antimicrobials can be excreted in the faeces, so the untreated litter can be an

important source of antimicrobial residues when used as fertilizer [47]. These residues could also contribute to the selection of resistant bacteria to antibiotics [48]. Resistance to fluoroquinolones among *Campylobacter* isolates could be attributed to the veterinary practice of fluoroquinolones (sarafloxacin and enrofloxacin) to combat respiratory diseases caused by *E. coli* and for prophylaxis in chicken production [11]. Sarafloxacin and enrofloxacin-resistant *Campylobacter* spp. are also resistant to norfloxacin, ciprofloxacin, nalidixic acid and other fluoroquinolones used in human medicine [6]. The onset of resistance to gentamicin in *Campylobacter* spp. may be related to the use of apramycin (aminoglycoside, structurally related to gentamicin) for veterinary treatment [49].

The results of this study were consistent with the results obtained by Ge *et al*.[50] who investigated the antimicrobial susceptibilities of *Campylobacter* spp. isolated from poultry meat to seven antimicrobial agents , they established that the onset of resistance to tetracycline was [82%], followed by resistance to doxycycline [77%], erythromycin [54%], nalidixic acid [41%] and ciprofloxacin [35%]. On the other hand, they reported that all isolates tested were susceptible to gentamicin. In addition, they found that the antimicrobial resistance of the *Campylobacter* isolates varied according to the species of the organism and *C. coli* isolates showed higher resistance rates to all tested antimicrobials compared to *C. jejuni*. Another study was conducted by Kurincic *et al.* [12] to evaluate the prevalence of resistance to antimicrobials among *Campylobacter* spp. in chicken meat in Slovenia against eight antimicrobials, they reported that the onset of resistance was much higher in *C. coli* [75.9%] than in *C. jejuni* [38.5%] and the resistance to pefloxacin, nalidixic acid, erythromycin and tetracycline were [58.2, 49.1, 14.5 and 12.7%], respectively, in which a higher percentage of resistance to tetracycline and erythromycin was found in *C. coli* [20, 7% and 17.2%] compared to *C jejuni* [3.8% and 11.5%], respectively. As well as the resistance to ciprofloxacin was higher in *C. coli* [75.9 %] than in *C. jejuni* [38.5 %] . The results of this study were partially consistent with Wieczorek *et al.* [35] who studied the antimicrobial resistance of *Campylobacter* spp. isolated from retail poultry meat in a commercial market in Poland to several antimicrobial agents, and found that there was no significant difference in the susceptibility to these drugs among the isolates of *C. coli* and *C. jejuni*. In addition, 88.1% [91% for *C. jejuni* and 86.1% for *C. coli*] and 86.8% [89.3% for *C. jejuni* and 85% for *C. coli*] of the isolates were resistant to ciprofloxacin and nalidixic acid , respectively. In addition to discovering that most of the isolates were resistant to tetracycline, the percentage of *C. coli* [63.3%] was higher than that of *C. jejuni* [49.2%]. Whereas resistance to streptomycin was observed between different strains with which more strains of *C. coli* [31.1%] compared to *C. jejuni* [10.6%] showed this property. The lower antimicrobial resistance among the *Campylobacter* isolates in these studies compared to the results obtained from the current study was probably due to the restrictive use of antibiotics in the production of chickens [51]. The frequencies of resistance to antibiotics observed in *Campylobacter* strains varied according to the origin of the strains and the presumed history of antibiotic use of the hosts [49].

The results [Table 5] revealed that 26 [86.6%] out of 30 *Campylobacter* isolates were resistant to one or more antimicrobial agents ; by which 10% of the isolates [15% of *C. coli* isolates] were resistant to six antimicrobial agents [ND Nor T E Gm Cip] ; 16.7% of the isolates [10% of *C. jejuni* and 20% of *C. coli* isolates] were resistant to five antimicrobial agents [ND Nor T E Gm] , [Nor T E Gm Cip] ; 30% of the isolates [30% of *C. jejuni* and 30% of *C. coli* isolates] were resistant to three antimicrobial agents [Nor T E] ; 10% of the isolates [10% of *C. jejuni* and 10% of *C. coli* isolates] were resistant to two antimicrobial agents [T E] and 20% of the isolates [20% of *C. jejuni* and 20% of *C. coli* isolates] were resistant to one antimicrobial agent [T]; [E]. Moreover, the results also revealed that 13.3% of the isolates [30% of *C. jejuni* and 5% of *C. coli* isolates] were sensitive to all experienced antimicrobials [Table 5] . Additionally , the results also revealed that MDR was more common in *C. coli* [75%] than in *C. jejuni* [50%] isolates recovered from chicken meat , in which 30% of *C. jejuni* isolates showed [Nor T E] MDR pattern which was the most prevalent , followed by [Nor T E Gm Cip] & [T E] patterns with a percentage of [10% and 10%], respectively. However , among *C. coli* isolates the MDR pattern [Nor T E] was the most common [30%], followed by [ND Nor T E Gm] with a percentage of [20%], [ND Nor T E Gm Cip] with a percentage of [15%] and then the [T E] pattern with a percentage of [10%], [Table 5].

Multidrug resistance [MDR] was defined as an isolate that shows resistance to two or more antimicrobials at the same time [52]. The appearance of MDR may reflect the acquisition of different resistance determinants in the same DNA molecule or individual determinants, such as multiple drug pumps, which specify the efflux activity against various antimicrobial agents [53]. The mechanisms of genetic resistance could be chromosomal or plasmid-borne and represent a combination of endogenous and acquired genes. In general, antibiotic resistance mechanisms such as modification of the antibiotic by aminoglycoside-modifying enzymes, enzymatic inactivation of the antibiotic by β-lactamase and modified DNA-gyrase lens, 23R mutations in the rRNA genes were included for aminoglycosides, beta -lactams, fluoroquinolones, macrolides and tetracyclines, respectively [10]. The multi-drug efflux pump for CmeABC has been implicated in the *Campylobacter* resistance mechanisms to tetracyclines, fluoroquinolones, macrolides and beta-lactams [41]. The results of this study were consistent with the results obtained by Thakur *et al.* [52] who analyzed the antimicrobial sensitivity of the *Campylobacter* isolates recovered from retail meat in North Carolina against four antimicrobials: erythromycin [ERY], ciprofloxacin [CIP],

gentamicin [GEN] and doxycycline [Dox]. They found that MDR was more common in *C. coli* than in *C. jejuni* from all meat sources, with Ery-Dox [11.2%] being the dominant phenotype observed among overall *C. coli* isolates whereas among *C. jejuni* isolates the Cip -Dox MDR pattern [8.7%] was the most predominant. Another study conducted by Wieczorek *et al.* [35] to investigate the antibiotic resistance profiles of *Campylobacter* spp. recovered from meat in a retail market in Poland against different antibiotics including ciprofloxacin [CIP], erythromycin [ERY], tetracycline [TET], gentamicin [GEN], streptomycin [STR] and nalidixic acid [Nal], and they reported that [88, 1%] of the *Campylobacter* strains were resistant to one or more antibiotics by means of which resistance was found to two or more classes of antibiotics [60.9%] of *Campylobacter* spp. and the most common pattern of resistance observed between *C. jejuni* and *C. coli* was (Cip Nal Tet) , where 114 (37.7%) of the isolates were identified.

Table 5 . Antimicrobial resistance patterns (ARP) of thermotolerant spp. of *Campylobacter* isolated from retail chicken meat in Baghdad province.

ARP	No. of *C.jejuni* isolates	% n/N (n/10)	No. of *C.coli* isolates	% n/N (n/20)	Total No. of isolates	No. of antimicrobial resistance factors	% Total (n/30)
(ND Nor T E Gm Cip)	_	0%	3	15%	3	6	10%
(ND Nor T E Gm) ; (Nor T E Gm Cip)	1	10%	4	20%	5	5	16.7%
(Nor T E)	3	30%	6	30%	9	3	30%
(T E)	1	10%	2	10%	3	2	10%
(T) ; (E)	2	20%	4	20%	6	1	20%
- -	3	30%	1	5%	4	Sensitive	13.3%
5							
Total	10	100%	20	100%	30		100%

ND = nalidixic acid , Nor = norfloxacin , E = erythromycin , T= tetracycline, Gm = gentamycin , Cip = ciprofloxacin.
n/N = The number of resistant isolates /the number of experienced isolates.

The detection of resistance to CIP, ERY and GEN among *Campylobacter* spp. in poultry is a cause for concern because these antibiotics are commonly used in the treatment of human campylobacteriosis [13]. The problem of resistance to public health in *Campylobacter* spp. has global dimensions due to constantly increasing international trade and travel [54].

4. CONCLUSION

From the data obtained from this study, it can be concluded that the majority [75%] of chicken samples sold in different regions of the province of Baghdad have been contaminated with the thermotolerant *Campylobacter* spp. during the study period. With respect to antimicrobial resistance, [86.6%] of the evaluated isolates expressed resistance to one out of six antimicrobials experienced. Additionally, five ARPs were recorded to one or more antimicrobial agents [multidrug-resistance], by which MDR was observed between [50% & 75%] of the isolated *C. jejuni* and *C. coli*, respectively. And since the infected chicken meat may account for most cases of human campylobacteriosis, this widespread of multidrug-resistance is a public health concerns when these lifesaving antimicrobials are used for treatment of the patients. These results call attention to the need to control the prevalence and the phenomenon of multidrug -resistance of *Campylobacter* isolates from the food chain.

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