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Prevalence of *Campylobacter jejuni* In Chicken Meat Marketed In Baghdad Province

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Abstract

Campylobacter are the main cause of human bacterial intestinal disease identified worldwide. Over 90% of cases are caused by *C. jejuni* and about 5% of cases are caused by *C. coli*. Mishandling of raw chicken carcasses and consumption of undercooked chicken meat are the major risk factors for human campylobacteriosis. Despite the control measures for reducing cross contamination, the detection of *Campylobacter* in carcasses after chilling/freezing remains very high. The aim of this study was to determine the prevalence of *C. jejuni* in chicken meat sold inBaghdad province. A total of eight live chickens and 20 frozen whole carcass chickens, purchased two per week during a 12-week period(from January 2016 to march 2016), from different markets in Baghdad regions, were examined for the presence of *C. jejuni*. The results showed that 100% of the live chickens were positive for *C. jejuni*, while carcasses isolation rates ranged from 0-100%. Bacteriological, biochemical, and microscopic tests were used in the isolation and identification of the isolates as *C. jejuni.* The results revealed that, this method was highly specific, and can be easily used for reliable and rapid identification. In addition to this , confirmation of *C. jejuni* isolates was performed using multiplex PCR technique .

Keywords: *Campylobacter*, *C. jejuni*, chicken meat, Baghdad province.

Introduction

Chicken meat can easily be contaminated during process steps by pathogenic and harmful bacteria that are present in chicken's interior organs, skin surface and feather. Contamination is mostly seen at steps like scalding, plucking, and evisceration at which contamination with feathers/skin and fecal content due to rupture of internal organs from the birds will occur(Sequeira *et al.*, 2013 ; Goddard *et al.*, 2014).Among these bacteria, *Campylobacter* has been recognized as a most commonly encountered pathogen in chicken products (Nguyen *et al.*, 2016). Thermophilic *Campylobacters* have become the most frequent cause of bacterial gastroenteritis in humans worldwide (WHO, 2012). The most commonly species detected associated with campylobacteriosis are *C. jejuni*, followed by *C. coli* (Whiley *et al* ., 2013; EFSA/ECDC , 2014) . *Campylobacter jejuni* is the most important species from the public health stand point which responsible for more than 90 % of all the confirmed human cases (Gilliss *et al*., 2013; Tang *et al* ., 2016).Contaminated raw or undercooked chicken meat is considered to be the predominant source of foodborne infection in humans worldwide(Sahin *et al* ., 2015 ; CDC, 2015) , some estimates suggest that up to 80 % of all cases of human campylobacteriosis are due to consumption of chicken meat worldwide (Hermans *et al* ., 2012 ; Bahrndorff *et al*., 2013). In addition to this, multiple other sources exist, including

consumption of unpasteurized milk or dairy products, and unprocessed water can be considered (Epps *et al* ., 2013).

In Iraq , chicken meat is considered the most popular meat item in many communities and due to the lacking of data regarding the prevalence of *C.jejuni* in chicken meat , this study was conducted to investigate the prevalence of *C.jejuni* in chicken meat marketed in Baghdad province.

Materials and Methods

1. Collection and processing of samples:-

A. Collection and processing of live chickens:

Eight chickens were collected on a weekly basis from four different regions of Baghdad province , two regions in Al-Rusafa (Baghdad Aljadeeda, Al-Kurrada) and two regions in Al-Kargkh (Abu Ghraib , Al-Dawoodi). The chickens were transferred immediately to the laboratory. Chickens were killed by neck dislocation and the caeca cut aseptically with sterile scissor from the remaining part of the intestines , then the caeca were aseptically opened by cutting the end with sterile scissor and squeezing out the contents to be processed (about 2 gm) in to a universal bottle each contained 10 ml Preston broth, the contents were homogenized by vortex mixer then pre-enriched for 4 hours at 37°C in an anaerobe jar (2.5 L) under microaerophilic condition using Oxoid CampyGen^{TM} atmosphere packs then enriched at 42°C for 24 hours. Lids on the bottles were loosened to allow rapid equilibration of their contents with the gas jar atmosphere (FDA/BAM , 2001 ; Boxall ,2005).After enrichment, one loopful of the medium was streaked onto Preston selective agar [Campy agar base(Oxoid, CM 0689B) supplemented with antibiotics (Oxoid, SR 0204E) and *Campylobacter* growth supplement (Oxoid, SR 0232E)]; and onto Blood agar and parallel streaks were made using sterile loops to obtain discrete colonies . Then , all plates were incubated in an anaerobic jarunder microaerophilic condition at 42°C for 24 - 48 hours. Preston agar and Blood agar positive colonies typical of *Campylobacters* growth were sub cultured onto Preston agar without supplement for biochemical characterization.

B. Collection and processing of broiler chicken carcasses (BCC) :

Ten different regions of Baghdad province were chosen, five in Al- Rusafa (Al-Kurrada , Al-Masbah, Baghdad Aljadeeda , Zayona , Al-Habibia) and five in Al-Kargkh (Al-Hurriya , Al-Mansour , Al-Khadra , Al-Ghazaliya, Al-Jamiaa). Two frozen (BCC) ⁄region were collected from different supermarkets and retailer shops , with an average weight of 1200-1500 gm /carcass . Whole chicken carcasses (WCC) were packed separately in sterile polyethylene bag and transferred to the laboratory in an ice box within 2-3 hours . The whole chicken carcasses (WCC) were divided into two halves. Half chicken carcass was thawed in a refrigerator at 4°C overnight , then rinsed in 200 ml buffer peptone water (BPW) by shaking for 1 minute in a sterile polyethylene bag and processed within 3 hours. The bag was tilted and the carcass was hold back to let the rinse liquid flow to one corner, then the bottom back corner was sanitized with 70% ethanol and rinsed with sterile water . The corner was aseptically cut ,then 25 ml of the carcass rinse was placed by a disposable syringe in a sterile bottle containing 100 ml of Preston broth and pre-enriched for 4 hours at 37°C in an anaerobe jarunder microaerophilic condition , then enriched at 42°C for 24 hours. Lids on the bottles were loosened to allow rapid equilibration of their contents with the gas jar atmosphere (Chon *et al* ., 2012). After enrichment, one loopful of the medium was streaked onto Preston selective agar [Campy agar base(Oxoid,CM 0689B) supplemented with antibiotics (Oxoid, SR 0204E) and *Campylobacter* growth supplement (Oxoid, SR 0232E)] ; and onto Blood agar and parallel streaks were made using sterile loops to obtain discrete colonies . All plates were incubated in an anaerobic jar under microaerophilic condition at 42°C for 24 - 48 hours. Preston agar and Blood agar showing positive colonies typical of *Campylobacters* growth were sub cultured onto Preston agar without supplement for biochemical characterization. A generous amount of pure culture was removed from agar plate to a bijoux bottles containing 3 ml of nutrient broth with 20% (v/v)of pure medical glycerin then incubated in an anaerobic jar under microaerophilic condition at 37°C for 24 hours then all isolates were stored at -18°C (Donnison , 2003).

2. Isolation and characterization of *C.jejuni* :

Colonies isolated from live chickens and carcass rinse samples with morphological characteristic of *Campylobacters* on Preston agar (round to irregular with smooth edges, somemay have translucent white growth to spreading, film like transparent growth, some colonies appear tan or slightly pink)and Blood agar (Translucent moist colonies)were further confirmed as *Campylobacters* by subjected to microscopic and biochemical identification using

microscopic examination of morphology and motility, catalase test, oxidase test, hydrogen sulphide (H2S) production, microaerobic growth at different temperatures and hippurate hydrolysis test . Further biochemical investigation were performed for the differentiation of *Campylobacter* isolates from other Gram-negative organisms using O.B.I.S. Campy which is a rapid colorimetric test for the detection of L-alanyl amino peptidase and incorporates a Gram-lysis test to demonstrate Gram status .

3. Confirmation of *C. jejuni* isolates using multiplex PCR technique:

To confirm the identification of the *Campylobacter* isolates to species level a multiplex PCR assay based on the method described by Wang *et al*., (2002) was used.

3.1. Sample preparation

1)The isolated and biochemically confirmed *C. jejuni* isolates which were preserved in glycerin at -18°C were thawed in a refrigerator at 4°C overnight. The isolates were subcultured on Preston agar without supplement,and the plates were incubated in an anaerobic jar under microaerophiliccondition at 42°C for 24 hours.

2) Five well-isolated colonies from each isolate were picked up and inoculated intoBijoux bottles containing 3 ml of Lauryl tryptose broth ,then incubated in an anaerobic jar under microaerophiliccondition at 37°C for 24 hours and sent to the laboratory for PCR confirmation .

3.2. Extraction and purification of genomic DNA

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

3.3. DNA primers

The five pairs of primers were designed to identify the genes *hipO* from *C. jejuni* ; *glyA* from *C. coli*,*C. lari*, and *C. Upsaliensis* and *sap* B*2* from *C. fetus*subs. *Fetus* . The primers sequences used in the multiplex PCR were presented in Table 1.

Table 1. Primers sequences used in the multiplex PCR assay

Primer	Size (in bp)	Sequence (5'–3')	Target gene
CJF	650	ACTTCTTTATTGCTTGCTGC	*C. jejuni hipO*
CJR		GCCACAACAAGTAAAGAAGC	
CCF	126	GTAAAACCAAAGCTTATCGTG	*C. coli glyA*
CCR		TCCAGCAATGTGTGCAATG	
CLF	251	TAGAGAGATAGCAAAAGAGA	*C. lari glyA*
CLR		TACACATAATAATCCCACCC	
CUF	204	AATTGAAACTCTTGCTATCC	*C.upsaliensis glyA*
CUR		TCATACATTTTACCCGAGCT	
CFF	435	GCAAATATAAATGTAAGCGGAGAG	*C. fetus sap B2*
CFR		TGCAGCGGCCCCACCTAT	

3.4.Multiplex PCR conditions:

A multiplex PCR amplification was performed in 25 µl volumes containing 200 µM deoxynucleoside triphosphate; 2.5 µl of 10 × PCR reaction buffer (500 mM Tris-HCl [pH 8.3] , 100 mM KCl, and 50 mM $[NH4]_2SO4$); 20 mM MgCl2 ; primers [0.5 µM *C. jejuni* and*C.lari* primers ; 1 µM *C.coli* and *C. fetus* primers, 2 µM *C.upsaliensis* primers] ; nucleotide stock [1.5 µl of dNTP- Mix (10mM)] ; 1.25 U of Fast Start *Taq* DNA polymerase (Promega, USA), and 2.5 µl of whole-cell template DNA . The volume was adjusted with sterile distilled water to give 25 µl (Wang *et al*., 2002).

3. 5. PCR Cycle Programs

DNA amplification was carried out in a Perkin-Elmer thermocycler system using an initial denaturation step 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 minutes (Wang *et al*., 2002).

3. 6.Detection of *Campylobacters.*

The final PCR products were electrophoresed using 1.5% agarose gel at 10 V for 90 minutes. Bands were visualized with UV transilluminator (Alpha Imager HP, Alpha Innotech, CA, USA), after staining with ethidium bromide for analyzing the result. A 100-bp DNA ladder (NL1405, Vivantis, USA) was used as a DNA molecular size marker (Wang *et al*., 2002). Following agarose gel electrophoresis, a multiplex PCR assay produced fivebands from a mixture of DNA containing each of the five *Campylobacter* spp. The bands of(126 , 204,251, 435 , 650) bp for (*C. coli,C.upsaliensis ,C.lari , C. fetus , C. jejuni hipO*) respectively.

Results and Discussion

1.Isolationpercentage of *C. jejuni* from live chickens collected from different regions of Baghdad province.

A total of eight chickens were collected from four different regions (two birds/ region) of Baghdad province . Two from Al-Rusafa (Baghdad Aljadeeda , Al-Kurrda) and two from Al-Kargkh (Abu Ghraib, Al- Dawoodi) to determine the presence of *C. jejuni* in live chickens (Table 2). The results showed that all samples were positive for *C. jejun*i with an isolation percentage of 100 % in live chickens .

Table 2. Isolation percentage of *C.jejuni* from live chickens collected from different regions of Baghdad province.

Regions	No. of samples tested	Total No. +ve /Total samples tested	Isolation percentage
Abu Ghraib	2 Abu Ghraib2	2/2	100%
Al- Dawoodi	2 Al- Dawoodi2	2/2	100%
Baghdad Aljadeeda	2 Baghdad Aljadeeda 2	2/2	100%
Al-Kurrda	2 Al-Kurrda 2	2/2	100%

These results probably reflect insufficient on farm biosecurity measures which lead to widespread of *C. jejuni* in the environment and poor or insufficient hygiene barriers which lead to the transfer of organisms by farm workers via their clothes and boots or via crates and vehicles . As well as, contamination from previous flock and exposure to potential source of the bacterium such as other animals on the farm, insects, rodents, litter andfecal contamination of chicken feed and water may occurred (Schroeder, 2012 ; Toth *et al*., 2013).Additionally , coprophagic behavior of chickens increase the risk of colonization, especially since once a flock has become *Campylobacter*-positive, 80-100% of fecal samples have tested positive and most birds were colonized within a week after *Campylobacter* were first detected (Bull *et al*., 2006 ; Potturi-Venkata *et al*., 2007) .

The results of this study were in agreement with the results obtained by Zeenathul , (1994) in Malaysia and Taylor, (2012) in the USA who reported prevalence rates for *C. jejuni* in live chickens of 97.1% and 98.7% ,respectively. Another study was carried out by Bodi , (2014) who found that among the 36 flocks in Valencia region in Spain , 84.2% were positive for *Campylobacter* spp. and*C. jejuni* was detected as the most predominant species isolated in 55.3% of the positive cloacal samples , the possible reasons for lower isolation rate than obtained by current study could be attributed to good hygiene control of the catching equipment and personnel at these farms , sampling time (climatic factor)and method of isolation. A direct correlation of the various studies may be difficult because of the wide variety of sampling procedures and isolation methods that are used. In addition, farm management practices, geographical and seasonal variations are other factors likely to contribute to differences seen between studies. High level of biosecurity on the farm and improved disease prevention measures and hygiene may lead to a lower prevalence of *Campylobacters*.

2. Isolation percentage of *C. jejuni* from imported and local broiler chicken carcasses (BCC) collected from different regions of Baghdad province.

A total of 20BCC were collected from 10 different regions (two samples/ region) of Baghdad province. Fivefrom Al- Rusafa (Al-Kurrada , Al-Masbah, Baghdad Aljadeeda , Zayona , Al-Habibia) andfivefrom Al-Kargkh (Al-Hurriya , Al-Mansour , Al-Khadra , Al-Ghazaliya , Al-Jamiaa) to determine the isolation percentageof *C. jejuni* fromBCC (Table 3). The results showed that the highest isolation percentage (100%) from the imported CC were recorded in Zayonaand Al-Kurrada followed by 50% from Al-Masbah ,Baghdad Aljadeeda ,Al-Hurriya ,Al-Mansour, Al-KhadraandAl-Ghazaliya, and no isolations were made in Al-Habibia . While the isolation percentage from the local BCC (Al-Jamiaa) was (100%) .

Table 3. Isolation percentage of *C. jejuni* from imported and local broiler chicken carcasses (BCC) collected from different regions of Baghdad province.

Source	Regions	No. of samples tested	Total No.+ve / Total samples	Isolation percentage
Imported	Zayona	2	2/2	100%
	Al-Kurrda	2	2/2	100%
	Al-Masbah	2	1/2	50%
	Baghdad Aljadeeda	2	1/2	50%
	Al-Hurriya	2	1/2	50%
	Al-Mansour	2	1/2	50%
	Al-Khadra	2	1/2	50%
	Al-Ghazaliya	2	1/2	50%
	Al-Habibia	2	0/2	0%
Local	Al-Jamiaa	2	2/2	100%

The differences in isolation percentage could be attributed to several factors such asdifferencein regions, the source that BCC come from (imported and local) , climatic factor , storage conditions , performance of slaughter operations, hygienic measures , the initial bacterial count and genetic differences between isolates . Furthermore, The higher isolation percentage obtained by this study might be attributed to pre-enrichment carcass rinse fluid in enrichment selective broth , this could potentially improve recovery of sub-lethally damaged cells and may be particularly relevant for frozen samples (Jorgensen *et al* ., 2002) . 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 findings could be supported by Pearson *et al.*, (1996) and Sampers *et al.*, (2008) who reported that the higher the initial bacterial count, the higher is the number of survivals after exposure to a chilling and freezing temperature stress. Moreover, genetic differences between strains of *C. jejuni* have been described, so it is expected that the resistance of *C.jejuni* to temperature stress could be strain related (Martinez-Rodriguez and Mackey, 2005; Oyarzabal *et al.*, 2010).

Contamination of chicken carcasses occurred mainly during de feathering and evisceration as a result of high levels of *Campylobacter* spp. on their feathers, skin and intestine, de feathering and scalding the carcasses opens up follicles, giving *Campylobacter* a place to hide from further methods of cleaning (Jacobs-Reitsma ,2000).Chicken skin provides suitable microenvironment for the survival of *Campylobacter* spp. due to accumulation of water which increases the surface area available for bacterial contamination , and even under frozen conditions or storage at 4˚C, *Campylobacter* spp. are able to persist in the carcass (Scherer *et al*., 2006). Survival of *Campylobacters* in water could be enhanced at low temperatures, particularly at 4°C (Thomas *et al.*, 2002) , and may be revert from viable but non culturable (VBNC) to pathogenic state in water (Federighi *et al*., 1999) and since the temperature of chillers is 4°C this could be potentially support survival of *Campylobacters* in water and increase the chances for contamination of carcasses.

The results were in agreement with the results obtained by Jorgensen *et al* .,(2002) who found that the prevalence of *Campylobacter* spp. in chilled and raw frozen chickens purchased from a variety of retail outlets located around Exeter and Preston in England was (83%) in which *C. jejuni* accounting for (98%) of the isolated strains from these samples . Additionally, Bodi , (2014) found that 100% of the neck skin samples analyzed at various stages of processing in the slaughterhouse in the Valencia region in Spain were positive for *Campylobacter* spp. and *C. jejuni* was the most common serotype detected, isolated in 69.0% of the skin samples . Furthermore , the results of this study revealed that the isolation percentage of *C. jejuni* from BCC were lower than in live chickens and this might be related to the freeze-damaged cells encountered on frozen carcasses. The ability of *Campylobacter* spp. to survive in food during storage represents a riskfor human health due to the ability of the organism toproduce infection with low infectious dose (Lori *et al.*, 2007). Proper hygienic conditions while processing poultry meat can reduce the load of *Campylobacter* on the meat surfaces.

3.Isolation and identification of *C. jejuni* isolates isolated from live chickens and broiler chicken carcasses (imported and local) collected from different regions of Baghdad province.

The isolation and identification of the strains (Table 2 and 3) were done according to food microbiological protocols (FDA / BAM, 2001 ; ISO,2006; OIE, 2016).The results(Table 4) showed that,20 out of 28 isolates were identified as *C. jejuni*. Eight were isolated from live chickens (one isolate/sample) and 12*C. jejuni* isolates from 20 samples of imported and local BCC[Zayona (2) , Al-Kurrda (2) , Al-Masbah (1) , Baghdad Aljadeeda (1), Al-Hurriya(1) , Al-Mansour(1) , Al-Khadra(1) , Al-Ghazaliya (1) and Al-Jamiaa(2)] . Table 4 summarizes the bacteriological, biochemical, and microscopic tests used in the isolation and identification of the isolates as *C. jejuni*. The isolates (*C. jejuni*) showed characteristic colonies on Preston selective agar and on blood agar , motile , gram-negative curved rod , catalase positive , oxidase positive , hippurate hydrolysis positive and when grown at differentincubationtemperatures, they were positive at 43°C ,while negative at 25°C . All isolates were negative for H2S production in iron medium. Moreover, these isolates when passed on Oxoid Biochemical Identification system(OBIS) they were negative for gram lysis state and negative for the acquisition of a-alanyle amino peptidase.

Table 4. Isolation and identification of *C.jejuni* from live chickens and broiler chicken carcasses (BCC/imported and local) collected from different regions of Baghdad province.

Source	Regions	Growth on		Motility	Gram stain	Catalase	Oxidase	Hippurate hydrolysis	Growth at		H_2S in iron medium	OBIS		Total
		Preston agar	Blood agar						25°C	43°C		Gram state	a-alanyle amino peptidase	
Live	Abu Ghraib (2)	+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	8
		+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
	Dawoodi (2)	+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
		+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
	Baghdad Aljadeea (2)	+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
		+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
	Kurrda (2)	+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
		+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
BCC local	Jamiaa (2)	+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	12
		+ve	+ve	+ve	G-ve	-ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
BCC imported	Zayona (2)	+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
		+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
	Kurrda (2)	+ve	+ve	+ve	G-ve	-ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
		+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
	Masbah (1)	+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
	Baghdad Aljadeea (1)	+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
	Hurriya (1)	+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
	Mansour (1)	+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
	Khadra (1)	+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
	Ghazalia (1)	+ve	+ve	+ve	G-ve	+ve	+ve	+ve	-ve	+ve	-ve	G-ve	-ve	
Total	20													

(FDA / BAM, 2001 ; ISO, 2006; OIE, 2016; with modification by the authors)

The results of this study showed superior selectivity of Preston agar (PA) for isolation of *Campylobacters* with inhibition the competing bacterial growth. The high performance of PA may be related to the combination of antibiotics present and to addition of blood and *Campylobacter* growth supplement (sodium pyruvate, sodium metabisulphite and ferrous sulphate) to improve the quenching of toxic oxygen derivatives (Bolton *et al*., 1984). It is difficult to predict how contaminated the food sample is, but this combination of antibiotics appears to be a good compromise for the isolation of *Campylobacter* spp. from poultry meat . This could be supported by the results obtained by Chon *et al*., (2012) who found that the number of agar plates that showed positive results for *Campylobacters* was significantly higher with the use of PA than with the use of modified Charcoal Cefoperazone Deoxycholate agar (mCCDA)and Campy-Cefex agar (CCA) when compare the performance of the three selective media used for detecting *Campylobacters* in carcass-rinse samples in Korea , and attributed to that mCCDA and CCA contain only one antibiotic, cefoperazone while PA contains four selective antibiotic agents . Furthermore, fewer PA plates showed contamination than the other media plates, indicating the superior selectivity of PA . Preston agar is used with antimicrobics for the selective isolation of *C. jejuni*and*C. coli* (Acumedia , 2010) . Conventional biochemical tests for discrimination between *C. jejuni*and*C. coli* rely mainly on hippurate hydrolysis which is the only phenotypic test for differentiating the two species (Waino *et al*., 2003) .

The results of this study were in agreement with Jorgensen *et al* ., (2002) who isolated and identified *Campylobacter* spp. from carcass-rinse or carcass-rinse plus whole skin samples and neck-skin in UK based on typical colony morphology on Charcoal Cefoperazone Deoxycholate agar(CCDA) and confirmed by testing for growth on blood agar in aerobic and microaerobic atmospheres at 37 °C for 48 h, oxidase activity and examination of cell morphology using Gram-staining and microscopy by which the *Campylobacter* isolates (425) comprised *C. jejuni* (98%) and*C. coli* (2%) . Another study was conducted by Salihu *et al*., (2009) in Sokoto / Nigeria, who relied on several bacteriological , biochemical and microscopic tests for the isolation and identification of *Campylobacter* spp. isolated from raw poultry samples in which typical colonies morphology on mCCDA - Preston agar were presumptively identified as *Campylobacter* by Gram negative curved rods, oxidase positive . Identification and biotyping of the isolates were performed based on indoxyl-acetate hydrolysis , resistance to nalidixic acid and cephelotin , rapid H2S test in a semisolid medium supplemented with *Campylobacter* growth supplement. Characterization the isolates to species level was done based on hippurrate hydrolysis test and DNase test by which *C. jejuni* comprised (49.9%) of the 681 and*C. coli* (22.9%) of raw poultry samples .

The results of this study are partially consistent with the results of Cean *et al* ., (2013) who concluded that *Campylobacter* spp. was successfully isolated from cloaca and cecum content of broilers in Western part of Romania , and confirmed as thermotolerant *Campylobacter* based on minimum tests required for confirmation (colonies appearance , traditional Gram stain and oxidase test) . These findings highlight the importance of accurate identification and optimal culturing procedures of *C. jejuni* from live chickens and broiler chicken carcasses for epidemiological studies of campylobacteriosis , and which may be of value to the industry if a logistic scheduling process is incorporated to reduce the chances of contamination of free flock during processing (Potturi-Venkata *et al* ., 2007).

4.Confirmationof *C. jejuni* isolates isolated from live chickens and broiler chicken carcasses using multiplex PCR technique.

All of the 20 *C. jejuni* isolates isolated from live chickens and BCC (imported and local) from different regions of Baghdad province according to standard bacteriological and biochemical tests were subjected to multiplex PCR technique (Table 5) . The multiplex PCR assay showed that ,all isolates were produced one band of (650) bp for (*C. jejuni hipO*) and confirmed as *C. jejuni* (Table 5and Fig. 1).

Table 5.Confirmation of *C.jejuni* isolates isolated from live chickens and broiler chicken carcasses(BCC) using multiplex PCR technique.

Source	Regions	No. of samples tested	No. of +ve samples	Target genes					
				C. jejuni hipO	*C. coli glyA*	*C. lari glyA*	*C.upsaliensis glyA*	*C.fetus sapB2*	Size (in bp)
Live chickens	Abu Ghraib	2	2	+ve	–	–	–	–	650
	Al-Dawoodi	2	2	+ve	–	–	–	–	650
	Baghdad Aljadeeda	2	2	+ve	–	–	–	–	650
	Al-Kurrda	2	2	+ve	–	–	–	–	650
BCC local	Al-Jamiaa	2	2	+ve	–	–	–	–	650
BCC imported	Zayona	2	2	+ve	–	–	–	–	650
	Al-Kurrda	2	1	+ve	–	–	–	–	650
	Al-Masbah	2	1	+ve	–	–	–	–	650
	Baghdad Aljadeeda	2	1	+ve	–	–	–	–	650
	Al-Hurriya	2	1	+ve	–	–	–	–	650
	Al-Mansour	2	1	+ve	–	–	–	–	650
	Al-Khadra	2	1	+ve	–	–	–	–	650
	Al-Ghazaliya	2	1	+ve	–	–	–	–	650
	Al-Habibia	2	0	–	–	–	–	–	

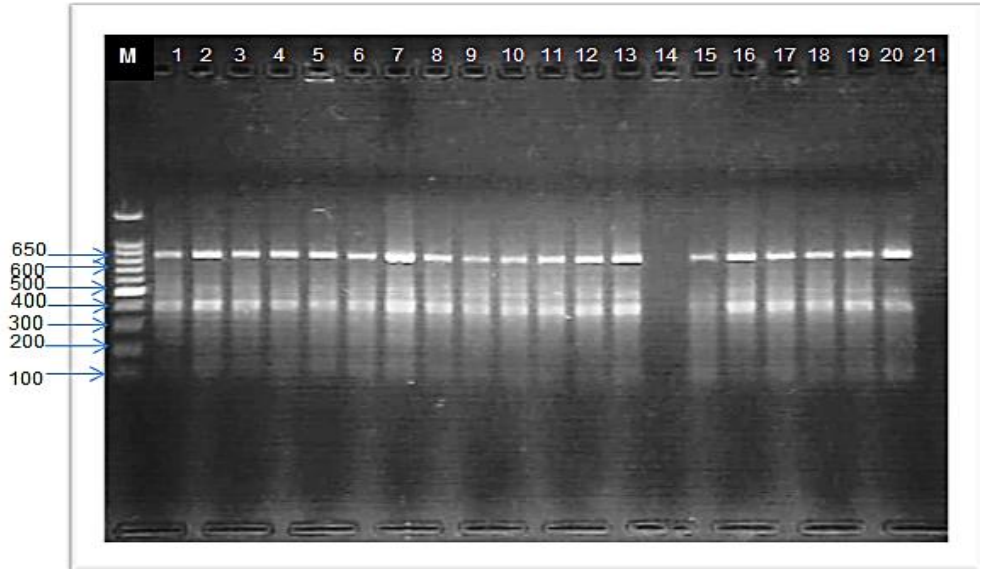

Figure 1. Gel electrophoresis of the multiplex PCR. Lanes 1- 20 , 650 bp target of *hipO* of *C.jejuni* ; lane 21 NC, Negative Control, lane M, 100 bp DNA molecular size marker.

The results of this study were inagreement with the results obtained by Al Amri *et al* ., (2007) who evaluated the multiplex PCR protocol based on the detection of *CadF* (genus specific), and *hipO* (*C. jejuni*) and *asp* (*C. coli*) genes, using 114 stool specimens from patients with *Campylobacter* enteritis and chickens (54 of human and 60 of chickens) and found that , all specimens were positive for *Campylobacters* in which 70 (61.4 %) were identified as *C. jejuni*, 35 (30.7 %) as *C. coli* and 9 (7.9 %) as a mixed infection/colonization with both species. Another study was carried out by Mäkelä, (2011) to identify the *Campylobacter* isolates isolated from broiler chickens to species level using a multiplex PCR assay based on the detection of *hipO* from *C. jejuni* ; *glyA* from *C. coli*and the internal control 23S rRNA, and found that out of the 33 isolates 31 were identified as *C. jejuni* (94%) and two were identified as *C. coli* (6%). As well as found that all of the 121biochemically identified *Campylobacter* isolates isolated from 11 different sampling sites at turkey processing plant were identified as *C. jejuni* with the multiplex PCR method. Also a study was conducted by Shams *et al* ., (2017) to differentiate between *C. jejuni* and *C. coli* species using a set of specific primers for *asp*, *hipO*, and *CadF* genes in a multiplex PCR assay , found that ,according to the culture results, the number of *Campylobacter* positive cases of children with bacterial systemic and gastrointestinal symptoms was 35, in which 33 cases (94 %) were infected by *C. jejuni*, and two cases (6 %) were infected by *C. coli*. Additionally, they concluded that the designed multiplex PCR assay in their study was a sensitive and specific tool which could be useful for the rapid and simultaneous detection and differentiation of *C. jejuni* and *C. coli* species in clinical settings.

Conclusion

From the data obtained from this study, can be concluded that the live chickens and chicken carcasses samples collected from different regions of Baghdad province (Al-Rusafa andAl-Kargkh) were highly contaminated with *C. jejuni* during the study period.The methods of isolation and identification of *C. jejuni* adopted by this study were simple to performed, highly specific, and can be easily used in laboratories for reliable and rapid identification of *C. jejuni* in live chicken and carcasses samples. Using PCR technique, *C.jejuni* isolates isolated from chicken meat showed relatedness to live chickens isolates, which confirm the results suggested by the biochemical tests used in this study.

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