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Biotyping and Resistotyping of *Campylobacter* Isolates Recovered from Frozen Chicken Meat Sold in Baghdad Province

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Abstract: ***Campylobacteriosis caused principally by Campylobacter jejuni(C.jejuni)and Campylobacter coli(C. coli)is among the main causes of bacterial gastroenteritis worldwide.This study was conducted to investigate the biotypingof30biochemically identified and confirmedCampylobacter isolates (10 C.jejuni & 20 C. coli) recovered from chicken meat samples which were collected from different regions of Baghdad province using Liorbiotyping technique.The results showed that, two distinct biotypes were recognized among C. jejuni&C.coli isolates , these biotypes were biotype (I& II). In addition , C. jejuni&C.coliisolatesexhibited high prevalence of biotype I (80% & 75%) , in respectively compared to biotype II (20% & 25%), in respectively.And since C. jejuni and C. coli biotypeI prevailed in humans, while biotype II was more common in animals, sothe results support the role played bythese products as probablevehicles to transmitinfections to customers.***

Keywords: Biotyping, *Campylobacter*, Chicken meat ,Baghdad province

1. Introduction

The thermotolerant species of *Campylobacter* namely *C. jejuni* and *C.coli*are considered as the most common cause of intestinal infection all over the world (1 , 2). Campylobacteriosis isa zoonosis transmitted by food in most cases andthe most common zoonosis in the majority of industrializedcountries (3).The food vehicle associated with the majority of the reported campylobacteriosis infections was contaminated poultry meat, contaminated raw or undercooked chicken meats and/or by-products are particularly important to cause food-borne campylobacteriosis in humans (4, 2 ,5). With the increase in the number of cases of campylobacteriosis everyyear, it is imperative that steps be taken to identifythe source of the bacterium. Typing systems help identify strains isolated from humancases and allow for the comparison of those strains at the species and subspecies levels that are found in poultry and on poultry products. Identification of these isolates provides scientists with the ability to study the pathogenesis of infections, detect and investigate outbreaks, and assist with surveillance and prevention of campylobacteriosis in humans (6,7).The necessity for differentiationis essential to both clinicians and epidemiologists tobetter understand the pathophysiology and epidemiology ofthese organisms (8).In Iraq, chicken meat is considered the most popular meat item in many communities,so the existing study was performed to investigate the epidemiology of*Campylobacter* isolates which isolated from chicken meatsold in Baghdad province using biotyping and resistotyping technique.

2. Materials & Methods

2.1. Sample preparation

All the*Campylobacter*isolates (10 *C. jejuni* & 20 *C.coli*) recovered from chicken meat samples in Baghdad province which were biochemically identified , confirmed by multiplex PCR assayand preserved in glycerin at -18°C as described previously (9) , were thawed in a refrigerator at 4°C overnight , then the isolates were sub cultured on Preston agar without supplement (Oxoid, CM 0689B) ,the plates were incubated in an anaerobic jar (Oxoid, AG25) under microaerophilic condition using Oxoid Campy Gen^{TM} atmosphere packs (Oxoid, CN0025A) at 42°C for 48hours. All isolates were grew at 43°C and not at 25°C under microaerophilic conditions, and did not grow aerobically at 43°C or micro aerobically at 25°C, positive for catalase and oxidase tests, did not produce H2S on triple sugar-iron media and displayed resistance to cephalothin.

2.2. Biotyping of *Campylobacter*isolatesrecovered fromretail chicken meat in Baghdad province.

Biotyping was accomplished using Lior biotyping scheme.All isolates were tested for hippurate hydrolysis , rapid H2S test&DNA hydrolysis test (Table 1). These parameters formed the basis for allocation to *C. jejuni*biotype(I, II, III & IV) ; *C.coli*(I & II) and *C. laridis* (I& II) as proposed by Lior,(8) .

Table 1: Biotyping scheme for *C. jejuni*, *C. coli*,& *C. laridis.*

Test	*C. jejuni* Biotype				*C. coli* Biotype		*C. laridis* Biotype	
	I	II	III	IV	I	II	I	II
Hippurate hydrolysis	+	+	+	+	-	-	-	-
Rapid H2S test	-	-	+	+	-	-	+	+
DNA hydrolysis	-	+	-	+	-	+	-	+

(Lior ,1984)

3. Results and Discussion

3.1. Biotyping and resistotyping of *Campylobacter* isolates recovered fromretail chicken meat in Baghdad province

All the*Campylobacter* isolates (10 *C. jejuni*& 20 *C.coli*) were analyzed tohippurate hydrolysis , rapid H2S test&

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DNA hydrolysis test using the method described by Lior (Table 2).The results showed that, 33.3% of the isolates (100% of *C. jejuni*&0% of *C.coli*) were positive for hippurate hydrolysis testwhereas66.7% of the isolates (0% of *C. jejuni*& 100% of *C.coli*)were negative. On the other hand, for rapid H2S assessment100% of the isolates(100% of*C. jejuni*&100% of*C.coli*) were negative. Additionally , the results also showed that76.7% of theisolates(80% of *C. jejuni*& 75%of *C.coli*were negativefor DNA hydrolysis test, while 23.33% of theisolates(20%of *C. jejuni*& 25%of *C.coli*) were positive. Furthermore, the results revealed that (23.3%) of the tested isolates presented resistance to nalidixic acid (Table 2).

Table 2: Hippurate hydrolysis , rapid H2S test& DNA hydrolysis results of *Campylobacter* isolatesrecovered from retailed chicken meat in Baghdad province

No. of samples tested according to regions	Total No.+ve	Total No.+ve (C. *jejuni*)	*Total No.+ve (C. coli)*	Hippurate hydrolysis		*Rapid H2S*		*DNA Hydrolysis*		ND Resistance	
				+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Al-Arassat (4)	2	1	1	1	1	-	2	-	2	-	2
Al-Aadhamiya (4)	2	1	1	1	1	-	2	1	1	-	2
Al-Ghadeer (4)	3	0	3	0	3	-	3	-	3	3	-
Al-Baladiyat (4)	4	1	3	1	3	-	4	-	4	1	3
Al-Ameen (4)	4	2	2	2	2	-	4	3	1	1	3
Al-Yarmouk (4)	3	1	2	1	2	-	3	-	3	-	3
Al-Saydiyah (4)	4	1	3	1	3	-	4	-	4	-	4
Al-Adl (4)	4	1	3	1	3	-	4	1	3	1	3
Al-Mansour (4)	1	1	0	1	0	-	1	-	1	-	1
Al-Hurriya (4)	3	1	2	1	2	-	3	2	1	1	2
Total (40)	30	10/30	20/30	10	20	0	30	7	23	7	23
%		33.3%	66.7%	100%	100%	0%	100%	23.3%	76.7%	23.3%	76.7%

The results of this study were agreed with Nicholson &Patton(10) in Georgia who relied on the scheme of Lior for biotyping 140 genetically identified *Campylobacter* strains.Another study carried out by Salihu *et al*.(11) , who used Lior scheme for biotypingthermotolerant *Campylobacter* isolates recovered from poultry meat samples in Sokoto, Nigeria. Also, Lior scheme was adopted by Adekunle *et al*.(12) for biotyping *Campylobacter* isolates recovered from stool samples of 602 children with diarrheal symptoms and 100 children without diarrhea in Osogbo , Nigeria.On the other hand, Fernández & Hitschfeld, (13), used Lior technique for biotyping *Campylobacter* strains recovered from dairy and beef cattle in Valdivia .

In this study, biotyping and resistotyping of *Campylobacter*isolates(10 *C. jejuni*&20*C.coli*) recovered from chicken meat was investigated (Table 3) .The results showed that, two distinct biotypes were recognized among *C. jejuni*&*C.coli* isolates , these biotypes were biotype(I& II) with the prevalence of (80% & 20%) for*C. jejuni* and (75% & 25%) for *C.coli* , in respectively.

Table 3: Biotyping of *Campylobacter* isolates recovered fromretail chicken meat in Baghdad province.

No. of samples tested according to regions	Total No.+ve	*C.jejuni* biotypes				*C.coli* biotypes		*C. laridis* biotypes	
		I	II	III	IV	I	II	I	II
Al-Arassat (4)	2	+ (1)	_	_	_	+ (1)	_	_	_
Al-Aadhamiya (4)	2	_	+ (1)	_	_	+ (1)	_	_	_
Al-Ghadeer (4)	3	_	_	_	_	+ (3)	_	_	_
Al-Baladiyat (4)	4	+ (1)	_	_	_	+ (3)	_	_	_
Al-Ameen (4)	4	+ (1)	+ (1)	_	_	_	+ (2)	_	_
Al-Yarmouk (4)	3	+ (1)	_	_	_	+ (2)	_	_	_
Al-Saydiyah (4)	4	+ (1)	_	_	_	+ (3)	_	_	_
Al-Adl (4)	4	+ (1)	_	_	_	+ (2)	+ (1)	_	_
Al-Mansour (4)	1	+ (1)	_	_	_	_	_	_	_
Al-Hurriya (4)	3	+ (1)	_	_	_	_	+ (2)	_	_
Total No.+ve/ No. of tested isolates (%)	30	8/10 (80%)	2/10 (20%)			15/20 (75%)	5/20 (25%)	–	

Campylobacter enteritis is caused by the two related species, *Campylobacter jejuni* and *C. coli*, but *C. jejuni* is the more predominant of the two. Animal sources have been the main reservoirs for strains infecting humans Blaser *et al*.(14). Smith, *et al*.(15) has emphasized the need to determine the possible role of non-human sources play in human infections and differentiation for epidemiological purposes of outbreak isolates. In this study, the Lior technique was able to type all the isolates from frozen chicken meat as *C. jejuni*biotype I& II; *C. coli* biotype I & II (Table 3).Furthermore , the results of the current study revealed that the biotype I was predominant among *C. jejuni*&*C. coli* than biotype II . The results of this study were in agreement with Nadeau, *et al*.(16), who found that, biotypes I and II of *C. jejuni* were the most prevalent biotypes in poultry and human isolates. Approximately 20% of human *Campylobacter* isolates were genetically related to genotypes found in poultry. This genetic relationship and the high prevalence of *C. jejuni* biotypes I and II in poultry indicated that *Campylobacter* in broiler production could be a potential source of hazard for

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public health. Also a study conducted by Salihu *et al.*(11) , who found that *C. jejuni* and *C. coli* isolates presented a large prevalence of biotype I (58.8%) and (68.0%) , respectively compared to biotype II (38.0%) and (32.0%), respectively. On the other hand, a study stated by Fernández & Hitschfeld, (13), to establish the prevalence of *C. jejuni* and *C. coli* and their biotypes in beef and dairycattle in South of Chile and found that within dairy cattle, *C. jejuni* were showed highest prevalence ofbiotype I (60.0%) and biotype II making up the rest (40.0%) whereas (50%) of *C.coli* isolates corresponding to biotype I and the rest (50%) to biotype II. Additionally, among *C. jejuni* isolates recovered from beef cattle biotype I was the most frequent (58.6%), followed by biotype II,III& IV with the percentage of (18.6%, 20% & 2.9%), in respectively, while two biotypes described among *C. coli* isolates with biotype I composing (66.7%)& biotype II making up the rest (33.3%).As well as, Eberleand Kiess, (7), suggested that, phenotypic and genotypic typing methods are often used to discriminate between bacteria at the species and subspecies level and are often used to identify pathogenic organisms, such as *C. jejuni* and *C. coli*, and the test described Lior , is still popular and commonly used when biotyping *Campylobacter* spp. These findings highlight the importance of the biotyping scheme for studying the epidemiology of *Campylobacter* infections in human and animals.

3. Conclusions

From the data obtained from this study, it can be concluded that *Campylobacter* isolates (*C. jejuni* and *C. coli*) recovered from chicken meatcollected from different regions of Baghdad province were showed high prevalence ofbiotype I(80% & 75%)for *C. jejuni* and *C. coli* , in respectively compared tobiotype II (20% & 25%)for *C. jejuni* and *C. coli*,inrespectively. And since *C. jejuni* and *C. coli* biotype I prevailed in humans , while biotype II was more common in animals , soour findingssupport the work of others who suggest that birds and animals harbor*Campylobacter* biotypes similar to those found among humans ,thus reaffirming the mode of zoonotic transmission of this illness. Accordingly, these results may be useful in epidemiological investigations of *Campylobacter*illnesses.

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