

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 biotyping of 30 biochemically identified and confirmed *Campylobacter* isolates (10 *C. jejuni* & 20 *C. coli*) recovered from chicken meat samples which were collected from different regions of Baghdad province using Lior biotyping 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. coli* isolates exhibited high prevalence of biotype I (80% & 75%), in respectively compared to biotype II (20% & 25%), in respectively. And since *C. jejuni* and *C. coli* biotype I prevailed in humans, while biotype II was more common in animals, so the results support the role played by these products as probable vehicles to transmit infections 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* is a zoonosis transmitted by food in most cases and the most common zoonosis in the majority of industrialized countries (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* every year, it is imperative that steps be taken to identify the source of the bacterium. Typing systems help identify strains isolated from human cases 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 differentiation is essential to both clinicians and epidemiologists to better understand the pathophysiology and epidemiology of these 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 were isolated from chicken meat sold 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 assay and preserved in glycerin at -18°C as

described previously (9), were thawed in a refrigerator at 4°C overnight, then the isolates were subcultured on Preston agar without supplement (Oxoid, CM 0689B), the plates were incubated in an anaerobic jar (Oxoid, AG25) under microaerophilic condition using Oxoid Campy GenTM atmosphere packs (Oxoid, CN0025A) at 42°C for 48 hours. All isolates were grown at 43°C and not at 25°C under microaerophilic conditions, and did not grow aerobically at 43°C or microaerobically 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* isolates recovered from retail 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	<i>C. jejuni</i>				<i>C. coli</i>		<i>C. laridis</i>	
	Biotype				Biotype		Biotype	
	I	II	III	IV	I	II	I	II
Hippurate hydrolysis	+	+	+	+	-	-	-	-
Rapid H ₂ S test	-	-	+	+	-	-	+	+
DNA hydrolysis	-	+	-	+	-	+	-	+

(Lior, 1984)

3. Results and Discussion

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

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

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 test whereas 66.7% of the isolates (0% of *C. jejuni* & 100% of *C. coli*) were negative. On the other hand, for rapid H₂S assessment 100% of the isolates (100% of *C. jejuni* & 100% of *C. coli*) were negative. Additionally,

the results also showed that 76.7% of the isolates (80% of *C. jejuni* & 75% of *C. coli*) were negative for DNA hydrolysis test, while 23.33% of the isolates (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* isolates recovered from retail chicken meat in Baghdad province

No. of samples tested according to regions	Total No.+ve	Total No.+ve (<i>C. jejuni</i>)	Total No.+ve (<i>C. coli</i>)	Hippurate hydrolysis		Rapid H ₂ S		DNA Hydrolysis		ND Resistance	
				+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Al-Arassat (4)	2	1	1	1	1	-	2	-	2	-	2
Al-Aadhmiya (4)	2	1	1	1	1	-	2	1	1	-	2
Al-Ghadeer (4)	3	0	3	0	3	-	3	-	3	3	-
Al-Baladiyah (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 biotyping thermotolerant *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 & Hirschfeld, (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 from retail chicken meat in Baghdad province.

No. of samples tested according to regions	Total No.+ve	<i>C. jejuni</i> biotypes				<i>C. coli</i> biotypes		<i>C. lariidis</i> biotypes	
		I	II	III	IV	I	II	I	II
Al-Arassat (4)	2	+(1)	-	-	-	+(1)	-	-	-
Al-Aadhmiya (4)	2	-	+(1)	-	-	+(1)	-	-	-
Al-Ghadeer (4)	3	-	-	-	-	+(3)	-	-	-
Al-Baladiyah (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

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 dairy cattle in South of Chile and found that within dairy cattle, *C. jejuni* were showed highest prevalence of biotype 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, Eberle and 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 meat collected from different regions of Baghdad province were showed high prevalence of biotype I (80% & 75%) for *C. jejuni* and *C. coli* , in respectively compared to biotype II (20% & 25%) for *C. jejuni* and *C. coli*, in respectively. And since *C. jejuni* and *C. coli* biotype I prevailed in humans , while biotype II was more common in animals , so our findings support 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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