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Research Article

Molecular Epidemiology and Antimicrobial Resistance of *Campylobacter coli* from Caeca and Carcass of Poultry in Lebanon

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Abstract

Campylobacter is the primary bacterial cause of human intestinal infections worldwide. Species identification of fifty one *Campylobacter* positive isolates collected at two slaughterhouses in Lebanon was done using the 16 S rRNA sequencing. Antimicrobial susceptibility testing (AST) was performed using a wide range of β lactam agents and tetracycline. Polymerase chain reactions (PCR) detection of the bla_{OXA-61} and *tet*(O) genes in resistant and susceptible isolates to ampicillin and tetracycline respectively was determined. Genomic diversity of the isolates was assessed using Random Amplified Polymorphic DNA (RAPD). Sequencing analysis revealed that all tested isolates were *Campylobacter coli*. AST showed resistance in the isolates to cephalothin and aztreonam (100%), cefamandole and cefoxitin (98%), tetracycline (94%), ampicillin (49%), amoxicillin (47%), piperacillin (45%), carbenicillin (37%), ticarcillin (20%), ceftazidime (18%), cefotaxime (8%) and amoxicillin-clavulanic acid (2%). All ampicillin-resistant isolates and 84% of the ampicillin-sensitive ones carried the bla_{OXA-61} gene. All tetracycline-resistant isolates were positive for the *tet*(O) gene with 98% encoded on plasmids. RAPD analysis revealed nine distinct clusters with a minimum percentage of 43.5% genomic similarity. This study emphasizes the importance of surveillance in identifying and controlling commonly circulating food borne pathogens.

Keywords: Campylobacter; Antimicrobial Resistance; Sequencing; Random Amplified Polymorphic DNA (RAPD); Lebanon; Poultry

Abbreviations

AST: Antimicrobial Susceptibility Testing; BLAST: Basic Local Alignment Search Tool; *C. coli: Campylobacter coli*; *C. jejuni: Campylobacter jejuni*; Exo: Exonuclease I; fla typing: Flagellin Typing; LARI: Lebanese Agriculture Research Institute; MoPH: Ministry of Public Health; PCR: Polymerase Chain Reactions; PFGE: Pulsed-Field Gel Electrophoresis; RAPD: Random Amplified Polymorphic DNA; RFLP: Restriction Fragment Length Polymorphism; SAP: Shrimp Alkaline Phosphatase; UPGMA: Unweighted Pair Group Method Using Arithmetic Averages

Introduction

Campylobacter is the primary cause of human intestinal infections of bacterial origin worldwide (EFSA, 2013). *Campylobacter* spp. are commonly isolated from the intestinal tract of domestic and wild animals such as pigs, cows, poultry, dogs, cats, hamsters, and others (Lehtopolku, 2011). Presently, 17 species and six subspecies of *Campylobacter* have been identified; *C. jejuni* and *C. coli* being the most commonly reported species in human diseases (WHO, 2011). These species can be differentiated using a wide range of conventional methods such as growth temperature, cephalothin and nalidixic acid susceptibility, hippurate hydrolysis, and biochemical tests. In addition, alternative methods of differentiation include API Campy, mass spectrometry, and molecular methods such as Polymerase chain reaction (PCR) assays or sequencing using the 16S rRNA gene (Martiny *et al.,* 2011).

Campylobacter species are usually susceptible to a wide range of antimicrobial agents such as macrolides, fluoroquinolones, tetracycline, and aminoglycosides making them the drug of choice for severe cases of human infections (Lehtopolku, 2011). Nonetheless, resistance to antimicrobial agents has been emerging especially to fluoroquinolones such as ciprofloxacin and macrolides such as erythromycin (Bae et al., 2005; Lehtopolku, 2011; Vlieghe, *et al.,* 2008). A significant rise in resistance to tetracycline was also observed in *Campylobacter* isolates from fowl, broiler meat, pigs and cattle (EFSA, 2012). This resistance is encoded mostly by a chromosomal or plasmid borne gene, *tet*(O). The plasmid borne gene is widespread and has a high rate of conjugation making tetracycline resistance common (Taylor, 1988). Although ampicillin is not considered to be the drug of choice for *Campylobacter* infections, a large number of *Campylobacter* strains produce β-lactamases and these strains have been shown to be more resistant to amoxicil-

lin, ampicillin, and ticarcillin than β-lactamases negative strains. This is due to the presence of a gene carried by the *Campylobacter* chromosome called bla_{OXA-61} which encodes an amino acid putative periplasmic class D β-lactamase, Cj0299 (Griggs *et al.,* 2009; Parkhill *et al.*, 2000).

The diversity within *Campylobacter* spp. can be detected using a wide range of phenotypic typing such as phage typing and genotypic methods namely Pulsed-Field Gel Electrophoresis (PFGE), Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), flagellin typing (fla typing), and ribotyping (Wassenaar and Newell, 2000 ; Nielsen *et al*., 2000). The genotypic methods usually have a higher discriminatory power and are more efficient in detecting outbreaks (Nielsen *et al*., 2000).

In Lebanon, *Campylobacter* can be under-detected since it is not part of routine stool culture. Additionally, very few studies have been conducted on *Campylobacter* spp. isolated from animals and humans. A study by Talhouk., *et al.* (1998) showed that out of 281 diarrheic stool specimens, 150 caeca, and 31 chicken carcasses collected in Lebanon, 0.7% of human stool specimens, 22.7% of the ceca samples and 9.7% of the chicken carcasses were Campylobacter positive. Hence, due to scarcity of studies in Lebanon on *Campylobacter*, the aims of this study were to: assess the prevalence of *Campylobacter* spp. in chicken carcass and caeca collected at two slaughterhouses in Lebanon, identify the isolates to the species level, evaluate the antimicrobial resistance to a number of β-lactam agents and tetracycline, and determine genomic variation.

Materials and Methods

Bacterial isolates

Campylobacter isolates were obtained from the Lebanese Agriculture Research Institute (LARI) through the Ministry of Public Health (MoPH). The samples were collected at two slaughterhouses that receive broiler meat from different owners and were of two types: chicken caeca samples taken during the evisceration process and whole poultry carcass. Twenty-six batches of chickens were collected and tested for the presence of *Campylobacter* spp.; each comprising one carcass taken at the end of the processing line and several caecal samples randomly collected from 5 to 10 birds. Detection of *Campylobacter* was performed according to the ISO 10272: 2006 standard.

DNA extraction

Genomic and plasmid borne DNA were extracted using a Qiamp DNA mini kit (Qiagen, Hilden, Germany) and QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany) respectively according to the manufacturer's instructions.

Species Identification

Polymerase Chain reaction (PCR): The primers used in the amplification were 27Forward and 519Reverse for the 16S rRNA gene according to Lane., *et al.* (1991) and Turner., *et al.* (1999) respectively (Sigma Aldrich, Missouri, United States). Each reaction assay (20 µl) contained the following: 500 µM dNTPs, × 1 Taq buffer, 2.5 mM $MgCl_2$, 0.4 µM of each primer, 0.1 U of Taq polymerase, and genomic DNA with a minimal concentration of 20 ng. The cycling conditions were as follows: initial denaturation at 95°C for 12 minutes, 30 cycles of: 94°C for 30 seconds, 53°C for 30 seconds and 72°C for 1 minute, and a final extension cycle at 72°C for 10 minutes. The amplicons were subsequently purified using a combination of Exonuclease (Exo) I and Shrimp Alkaline Phosphatase (SAP) (Thermoscientific, Ulm, Germany): 1 µl of SAP enzyme and 0.5 µl of Exonuclease I (Exo) enzyme added to 6 µl of PCR product. Consequently, the purified amplicons (502bp) were analyzed on a 1.5% agarose gel run at 120V for 45 minutes.

Sequencing: Sequencing analysis was done using the Big Dye Terminator 2.0 kit (Applied Biosystems, California, United States) according to the manufacturer's instruction. Species identification of the *Campylobacter* isolates was determined using the Basic Local Alignment Search Tool (BLAST).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed following the Clinical and Laboratory Standards Institute guidelines (CLSI) using the Kirby-Bauer method on Mueller Hinton II agar plates with 5% blood (CLSI, 2009). The antibiotics used were: (Oxoid, Hampshire, England) ampicillin, amoxicillin, carbenicillin, ticarcillin, piperacillin, amoxicillin with clavulanic acid, ceftazidime, cephalothin, cefamandole, cefotaxime, aztreonam, cefoxitin, and tetracycline. The *Campylobacter jejuni* ATCC 29428 strain was used as a quality control.

Detection of resistance encoding genes

PCR assay: The Taq PCR Master Mix kit (Qiagen, Hilden, Germany) was used to prepare the reaction mix according to the manufacturer's guidelines. A final reaction mix (50 µl) consisted of buffer solution, 4 mM $MgCl_2$, a 0.4 mM of each dNTPs, and 0.05 u/µL Taq DNA polymerase, and genomic or plasmid borne DNA. The presence of the tet(O) (in both genomic and plasmid borne DNA) and blaOXA-61 genes, were detected using previously described primers [8], (Griggs *et al.,* 2009; Pratt and Korolik, 2005). Cycling conditions of the tet(O) gene consisted of an initial denaturation at 94°C for 4 minutes, followed by 30 cycles of: 94°C for 30 seconds, 60˚C for 1 minute and 72°C for 2 minutes, and a final extension at 72°C for 5 minutes. Whereas, the conditions of the bla_{OXA61} gene were as follow: an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, 56˚C for 45 seconds and 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. Consequently, Amplicons were detected on a 1% agarose gel run at 120V for 45 minutes. The band sizes of the bla_{OXA61} and the *tet*(O) genes were 281 bp and 559 bp respectively.

Random Amplified Polymorphic DNA (RAPD)

RAPD analysis was carried out using the Ready-To-Go RAPD Analysis Beads Kit (GE, Amersham Place, United Kingdom) as per the manufacturer's instructions. The RAPD analysis primer 2 was provided by the kit and the cycling parameters were: 1 cycles of 95°C for 5 minutes, followed by 45 cycles of 95°C for 1 minute, 36°C for 1 minute and 72°C for 2 minutes. The resultant products were detected by gel electrophoresis, on a 2% agarose gel run for 3 hours at 150V. The dendrograms were generated using the UPGMA method (unweighted pair group method using arithmetic averages) with the BIONUMERICS software.

Results

Among the samples submitted to analysis, 13 of 26 carcasses (50%) and 38 of 104 caeca (37%) were *Campylobacter* positive (n: 51); a total percentage of 39% positive samples. Moreover, five batches were *Campylobacter* positive in both the carcass and caeca samples corresponding to a specific batch. Sequence analysis revealed that all tested *Campylobacter* isolates were *C. coli*.

Antibiotic resistance

All isolates were resistant to cephalothin and aztreonam and 98% showed resistance to cefamandole and cefoxitin. In addition, 94% of the isolates were resistant to tetracycline. About half of the isolates were resistant to ampicillin and a range of 37 - 47% was resistant to amoxicillin, piperacillin, and carbenicillin. Twenty percent of the isolates were resistant to ticarcillin. The percentage of the isolates that was resistant to cefotaxime and ceftazidime was eight percent and 18% respectively. Moreover, only two percent of the isolates were resistant to amoxicillin-clavulanic acid. A higher percentage of the isolates within the carcass samples than within the caeca samples were resistant to the antimicrobial agents used except for cefamandole and tetracycline. Table 1 shows the total resistance of the isolates to the antimicrobial agents including resistance among the carcass and caeca isolates.

Classes of antibiotic	Subclass of antibiotic	Antibiotic	Percentage of resistant *Campylobacter* (%)	Source (%)	
				Carcass	Caeca
Penicillin	Aminopenicillin	Ampicillin	49	62	45
		Amoxicillin	47	69	40
	Ureidopenicillin	Piperacillin	45	62	40
	Carboxypenicillin	Carbenicillin	37	62	29
		Ticarcillin	20	23	18
β-Lactam/β-lactamase inhibitor combinations		Amoxicillin and clavulanic acid	2	8	0
Cephems	First generation Cephalosporin	Cephalothin	100	100	100
	Second generation Cephalosporin	Cefamandole	98	92	100
	Third generation Cephalosporin	Cefotaxime	8	15	5
		Ceftazidime	18	31	13
	Cephamycin	Cefoxitin	98	100	97
Monobactam		Aztreonam	100	100	100
Tetracyclines			94	92	95

Table 1: Total resistance of Campylobacter isolates to the antimicrobial agents including resistance among the carcass and caeca isolates.

All isolates were resistant to four or more antimicrobial agents. Among the isolates received, 43% were resistant to five antimicrobial agents. The antimicrobial resistance pattern in these isolates was identical. Additionally, 29% and 16% of the isolates were resistant to nine and ten antimicrobial agents respectively. Among these isolates, a high percentage exhibited a specific resistance pattern to the antimicrobial agents. Resistance to four and seven antimicrobial agents was observed in four percent (each) of the isolates; the patterns of resistance were different. Additionally, two percent of isolates showed resistance to six and eleven antimicrobial agents (each). Moreover, *Campylobacter* isolates identified in carcass and caeca samples or several caeca samples from the same individual animal did not always have similar resistance profile (Table 2b). Table 2a shows the antimicrobial resistance patterns of the isolates. Table 2b shows the resistance profile of the caeca and carcass isolates obtained from the same individual chicken samples.

Number of Antibiotics to which isolates are resistant	Number of isolates that are resistant (RAPD cluster)	Pattern	Source	
			CBcass (RAPD cluster)	Caeca (RAPD cluster)
4	1 (Type H)	Pattern 1: KF, MA, ATM, FOX,	-	1
	1 (Type H)	Pattern 2: KF, MA, ATM, TET	-	1
5	22 (12 Type H, 5 Type F, 1 Type C, 1Type A, 3 Type D)	KF, MA, ATM, FOX, TET.	4 (1 Type H, 1 Type C, 1Type A, 1 Type D)	18 (11 Type H, 5 Type F, 2 Type D)
6	1 (Type F)	Pattern 1: AMP, KF, MA, ATM, FOX, TET	-	1
7	1 (Type H)	Pattern 1: AMP, TIC, KF, MA, ATM, FOX, TET	-	1
	1 (Type H)	Pattern 2: AMP, CB, KF, MA, ATM, FOX, TET	-	1

9	11 (2 Type F, 4 Type C, 3Type G, 1 Type I)	Pattern 1: AMP, AMX, CB, PIP, KF, MA, ATM, FOX, and TET	3 (1Type G, 1 Type I)	8 (2 Type F, 4 Type C, 2 Type G)
	1 (Type B)	Pattern 2: AMX, CB, PIP, KF, MA, CTX, CAZ, ATM, FOX	-	1
	1 (Type H)	Pattern 3: AMP, AMX, AMC, CB, KF, MA, ATM, FOX, and TET	1	-
	1 (Type H)	Pattern 4: AMP, AMX, CB PIP, TIC, KF, ATM, FOX, and TET	1	-
	1 (Type H)	Pattern 5: AMP, AMX, PIP, TIC, KF, MA, ATM, FOX, and TET	-	1
10	5 (1Type A, 2 Type E, 1 Type I)	Pattern 1: AMP, AMX, PIP, TIC, KF, MA, CAZ, ATM, FOX, and TET	1 (Type A)	4 (2 Type E, 1 Type I)
	1 (Type A)	Pattern 2: AMP, AMX, CB PIP, TIC, KF, MA, CAZ, ATM, FOX	1	-
	1 (Type A)	Pattern 3: AMX, CB, PIP, KF, MA, CTX, CAZ, ATM, FOX, and TET	1	-
	1 (Type H)	Pattern 4: AMP, AMX, CB PIP, TIC, KF, MA, ATM, FOX, and TET	-	1
11	1 (Type B)	AMP, AMX, CB, PIP, KF, MA, CTX, CAZ, ATM, FOX, and TET.	1	-

Table 2a: The number of Campylobacter isolates, sub-grouped to caeca and carcass, resistant to four or more antimicrobial agents and the patterns of resistance of each. (The numbers in the brackets represent the number of isolates in each cluster). AMP: Ampicillin, AMX: Amoxicillin; CB: Carbenicillin; PIP: Piperacillin; KF: Cephalothin; MA: Cefamandole; CTX: Cefotaxime; CAZ: Ceftazidime; ATM: Aztreonam; FOX: Cefoxitin; TET: Tetracycline; TIC: Ticarcillin; AMC: Amoxicillin and clavulanic acid.

Isolates from the same chicken sample		**Number of Antimicrobial agents to which the isolates were resistant to/Pattern***	
Caeca	Carcass	Caeca	Carcass
1	1	9 Pattern 2	10 Pattern 3
1	1	9 Pattern 1	9 Pattern 1
4	1	10 Pattern 1	10 Pattern 1
8	1	7 isolates: resistant to 9 antibiotics Pattern 1, 1 isolate: resistant to 5 antibiotics Pattern 1	9 Pattern 1
8	1	7 isolates: resistant to 5 antibiotics Pattern 1, 1 isolate: resistant to 6 antibiotics Pattern 1	5 Pattern 1
2	0	7 Pattern1 and 2	-
8	0	5 Pattern 1	-
4	0	2 isolate: resistant to 5 antibiotics Pattern 1, 2 isolate: resistant to 4 antibiotics Pattern 1and 2	-

Table 2b: Comparison of the resistance profile of caeca and carcass Campylobacter isolates obtained from the same chicken sample. *Refer to patterns in 2a. (Each row represents a different animal).

Detection of the resistance encoding gene

PCR amplification of the bla_{OXA-61} gene showed that 92% (n: 47) of the *Campylobacter* isolates were positive for the gene. Additionally, all ampicillin resistant *Campylobacter* isolates carried the bla_{OXA-61} gene. However, 84% (n: 22) of the ampicillin sensitive isolates were found positive for the bla_{OXA-61} gene. A higher proportion of the caeca isolates (95%; n: 36) showed the presence of the bla_{OXA-61} gene than carcass isolates (85%; n: 11).

PCR results showed that 94% (n: 48) of the isolates harbored the tet(O) gene. All tetracycline resistant Campylobacter were positive for the tet(O) gene; 98% (n: 50) were located on plasmids. Only one carcass isolate had the gene located on the chromosome. On the other hand, the tetracycline sensitive isolates were negative for the tet(O) gene. Moreover, a similar percentage of carcass and caeca isolates showed the presence of this gene (92%; n: 12 and 95%; n: 36 respectively).

RAPD

RAPD analysis revealed the presence of nine distinct clusters namely A, B, C, D, E, F, G, H, I. The incidence of these clusters were 8%, 4%, 10%, 6%, 4%, 16%, 6%, 39%, and 4% respectively. The most common RAPD type, H, contained 20 isolates which were 55.1% genomically related; 17 isolates were from caeca and three were from carcass. These isolates were resistant to a range of antimicrobial agents (Table 2a). Two sets of samples which included three (caecal samples) and two isolates (one caeca and one carcass samples) were clonal (Figure 1).

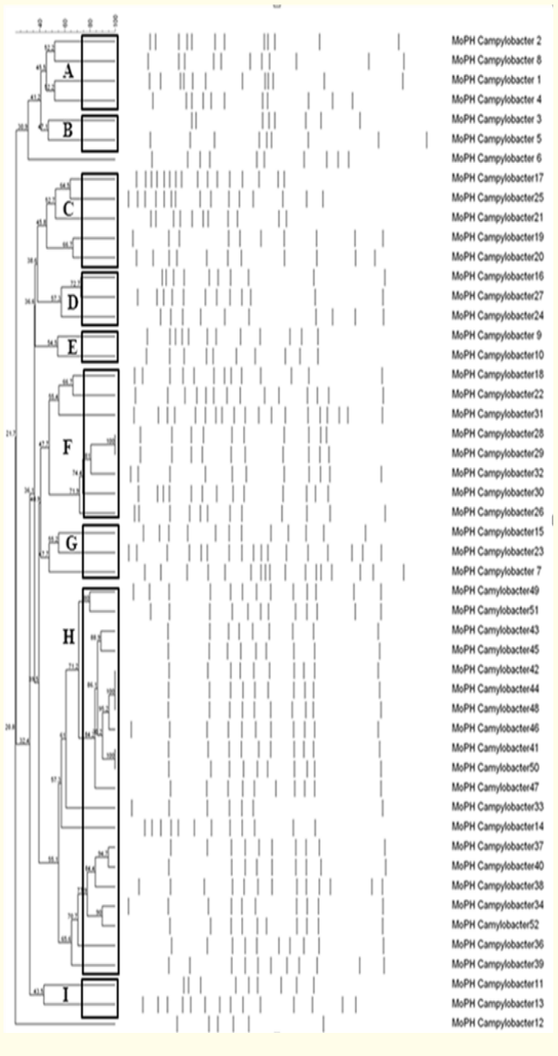

Figure 1: Dendrogram obtained from RAPD analysis of the 51 Campylobacter isolates.

RAPD Type F included eight isolates having 47.7% genomic similarity, all of which were derived from caeca. The isolates exhibited three different patterns of antimicrobial resistance (Table 2a). Within this cluster, two isolates were genetically identical (Figure 1). The RAPD Type C had a genomic relatedness of 45.8% and included five isolates; four isolates were from caeca and one was from carcass. The majority of these isolates showed resistance to a specific pattern of antimicrobial agents (Table 2a). RAPD Type A included four isolates which were all isolated from carcass and had a genomic similarity of 45.5%. The isolates exhibited a variety of antimicrobial resistance pattern (Table 2a).

RAPD Type D and G had a genomic relatedness of 57.3% and 47.7% respectively. They both contained three isolates; two derived from caeca and one from carcass. The isolates in each cluster showed a single antimicrobial resistance pattern (Table 2a).

RAPD Type B, E, and I included two isolates each having a genomic similarity of 47.1%, 54.5%, and 43.5% respectively. The isolates of type B and I were derived from both caeca and carcass, however that of type E was derived from caeca only. These isolates showed different antimicrobial resistance patterns (Table 2a). Figure 1 shows the dendrogram obtained by RAPD analysis.

Discussion

Many studies conducted in a number of countries have shown that a significant portion of chicken was contaminated with Campylobacter spp. (Dufrenne *et al*., 2001; Meldrum *et al.,* 2004; Uyttendaele et al. 1999; Wilson, 2002). Our study showed that the percentage of *Campylobacter* spp. isolated from poultry was considerable; a higher percentage was isolated from carcass than from caeca samples. Caeca are commonly used for the detection of *Campylobacter* in poultry; however the carcass can be contaminated with the bacteria (Allen *et al.*, 2007; World organization for animal health, 2008). Moreover, the amount of *Campylobacter* spp. present on the surface of carcass might change during the processing procedure before getting the final product such as during: scalding, defeathering, evisceration, washing, and air or water immersion chilling (Guerin *et al.*, 2010). The cecum of the chicken harbors a large number of *Campylobacter* species and when this raptures, the bacteria could spread to the carcass (Silva *et al.*, 2011). This could explain why the percentage of *Campylobacter* in carcass was higher than that in caeca in our study.

Although phenotypic methods for the identification of *Campylobacter* species are available, these techniques lack standardization and have a limited discriminatory power. A rapid and effective method to identify *Campylobacters* to the species level is 16S rRNA sequence analysis (Gorkiewicz *et al.*, 2003). In our study, all species of the *Campylobacter* isolates were identified as *C. coli.* In addition, the same species was found in carcass and caeca isolates. Several studies have shown that *C. jejuni* is the most common species found in poultry while *C. coli* is the predominant species isolated from pigs. However, *C. coli* species can still be found in chicken isolates, even if not as much as *C. jejuni* (FDA, 2010; Reich *et al*., 2008; Saenz et al. 2000; World organization for animal health, 2008). Moreover, a study by Talhouk., *et al*. (1998) is in accordance with our study in which *C. coli* was isolated more than *C. jejuni* in chicken carcass and caeca. Similarly, *C. coli* were identified more commonly than was *C. jejuni* from broiler chickens in Italy (Pezzotti *et al*., 2003), turkey breast specimens from Washington, (Zhao *et al*., 2001), and commercial chicken livers in Chile (Fernández and Pisón, 1996). On another note, a study done by Allen., *et al*. (2007) showed that the same species found in the caecal contents was also isolated on the carcass which is in line with our study.

A large number of the isolates (38/51) showed a single pattern of resistance to a panel of antimicrobial agents. Additionally, isolates from caecal and carcass samples from the same individual animals did not always show the same resistant pattern. Environmental stress during processing procedures such as storage conditions may have played a role in the selection of resistance profiles in these isolates. *Campylobacters* are usually considered to be resistant to β-lactam drugs including penicillins and first and second generation cephalosporin. Additionally, they show moderate resistance to third generation cephalosporin such as ceftazidime and cefotaxime; however these drugs have been rarely used clinically (Allos and Blaser, n.d; Van der Auwera and Scorneaux, 1985). A study done Dohne., *et al.* (2012) showed that *C. coli* exhibited moderate resistance to ampicillin. Moreover, Griggs., *et al.* (2009) showed that the majority of *C. coli* isolates from poultry in the United Kingdom (UK) exhibited resistance to a wide range of antimicrobial agents belonging to pencillins and cephems class (a number of these antimicrobial agents were used in our study). Our study is in-line with some of the results of these studies.

There is a strong correlation between resistance to ampicillin and β-lactam drugs and the presence of the bla_{OXA-61} gene. Our study showed that all ampicillin resistant isolates and 84% of ampicillin susceptible isolates harbored the bla_{OXA-61} gene. Various studies carried out in Brazil and the UK indicated that a very high percentage of ampicillin resistant *Campylobacter* isolates from poultry products carried the bla_{OXA-61} gene (Griggs *et al*., 2009; Sierra-Arguello *et al*., 2015). One of the studies also showed that 1) 59% of ampicillin susceptible isolates carried the bla_{OXA-61} gene and 2) the inactivation of this gene resulted in greater susceptibility to several β-lactam drugs. The presence of β-lactamase genes is not always linked with resistance to β-lactams and might have a function other than mediating resistance in *Campylobacters*, explaining why a wide number of ampicillin susceptible isolates carried the bla_{OXA-61} gene (Griggs *et al.*, 2009).

Tetracycline has been used in the treatment of Campylobacteriosis, however, the trend of resistance is on the rise (Moore *et al.,* 2005; Rahimi *et al.*, 2010). Our study showed that: a very high percentage of samples were resistant to tetracycline, all tetracycline-resistant isolates were positive for the *tet*(O) gene with majority encoded on plasmids, and tetracycline sensitive isolates did not carry the *tet*(O) gene. A report by NARMS indicated that a moderate percentage (~42-60%) of *C. coli* isolates from chicken over a period of a decade were resistant to tetracycline (FDA, 2010). Although resistance to tetracycline in *Campylobacter* is common, its use in animal farms might increase the pool of multidrug resistant strains (Lee *et al.*, 1994). A study done in Kuwait showed that 88% of tetracycline resistant *Campylobacter* isolates carried the gene and 56% of these isolates had the gene located on plasmids (Albert *et al*., 2009). The location of this gene on conjugative plasmids or chromosomal elements is the main cause for the wide distribution and the rapid transfer of the *tet*(O) gene without antimicrobial selection pressure in *Campylobacter* isolates (Avrain *et al.*, 2004).

RAPD as a subtyping tool for *Campylobacter* spp. has a high discrimination power and good reproducibility and typability (Madden et al., 1996; Nielsen et al., 2000; Wassenaar and Newell, 2000). Although PFGE and RFLP are among the best genotyping methods, they are labor intensive. Therefore, RAPD was chosen in this study for its simplicity and rapidity. Isolates 28 and 29 belonging to cluster F, isolates 42, 44, 48, and isolates 41 and 50, all belonging to cluster H, were identical at the genomic level. Additionally, several isolates in clusters H and I showed close genomic similarity (> 85%). This might indicate that they are closely related epidemiologically and are consistent with a single genetic event such as mutation, insertion, or deletion of DNA (Tenevor *et al*., 2005). RAPD analysis also showed a wide diversity among the isolates with high genomic heterogeneity. This observation points out to the presence of different types among different animals which is consistent with several studies (Madden *et al*., 1996; Weijtens *et al.*, 1993). Our results also show that carcass and caeca isolates or two caeca isolates from the same individual animals did not necessarily have the same genotype. This is in contrast with the observation that individual animals are colonized by a single isolate at every sampling site (Madden *et al*., 1996). Moreover isolates within most clusters had a predominant source, caeca or carcass (all clusters except B and I) and exhibited a prevalent antimicrobial pattern (all except A, B, I).

Conclusion

In conclusion, the study provides an insight about the high prevalence of antimicrobial resistance, detection of their resistance encoding genes, and the significant degree of genomic diversity of *Campylobacter coli*, in two slaughterhouses present in the Lebanese market. The study 1) emphasizes the need for further investigations to determine the genomic link between *Campylobacter* spp. detected in animals and humans 2) recommends improved surveillance and stresses the need for implementing strict guidelines and regulations and better inspection procedures during food processing.

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