Original Article

Prevalence, antimicrobial resistance and risk factors for campylobacteriosis in Lebanon

José-Noel Ibrahim[1], Elias Eghnatios[1], Ali El Roz[1], Taher Fardoun[2], Ghassan Ghssein[1]

[1] *Faculty of Public Health, Lebanese German University (LGU), Sahel Alma, Lebanon*
[2] *Mashrek Medical Diagnosis Center, Tyre, Lebanon*

Abstract

Introduction: The rapid increase in *Campylobacter* strains resistant to antibiotics represents a major problem for public health. In Lebanon, campylobacteriosis is underdiagnosed since bacteria detection in stool samples is not performed routinely. This study aims to evaluate the prevalence, sources and routes of transmission, risk factors and antimicrobial susceptibility patterns of *Campylobacter* spp. in Lebanon.

Methodology: Stool samples collected from 1000 Lebanese patients with diarrhea, and 150 meat samples taken from supermarkets and slaughterhouses were subjected to *Campylobacter* detection. Colonies were identified by Gram staining, oxidase and catalase activities. They were then differentiated at the species level by hippurate test and PCR. Susceptibility of *Campylobacter* spp. to antibiotics was studied by the disc diffusion standard method.

Results: *Campylobacter* spp. were detected in 21.5% of stool samples; the main isolated species being *C. jejuni* (83.2%) and *C. coli* (13.9%). The highest *Campylobacter* infection rates were detected among children (41.8%) and during summer (31.6%). Consumption of contaminated meat and salads, and contact with animals represented the major risk factors for campylobacteriosis, with poultry carcasses and bovine cuts identified as the main bacteria reservoirs. Neither demographic determinants nor season had a major effect on the prevalence of campylobacteriosis. Erythromycin was the most active agent against *Campylobacter* spp. A multi-resistance rate was observed in 35.9% of isolates.

Conclusions: Campylobacteriosis is a major public health concern in Lebanon. Bacteria detection in stool culture should be performed routinely to allow an early diagnosis and a better monitoring of the disease and its burden.

Introduction

Campylobacteriosis is a foodborne diarrheal illness caused by bacteria of the genus *Campylobacter*, with most cases caused by *Campylobacter jejuni* and *Campylobacter coli*. The most common clinical symptoms of *Campylobacter* infections include diarrhea, abdominal pain, fever, headache, nausea and/or vomiting [1]. The incidence of campylobacteriosis has gradually increased over the last 10 years in both developed and developing countries [2]. According to the European Food Safety Authority (EFSA), *Campylobacter* spp. are now considered to be the leading cause of bacterial gastroenteritis worldwide with higher occurrence rates of *Campylobacter* infections observed in children under 5 years old [3,4].

Consumption of contaminated and undercooked poultry meat has been considered as the main source of *Campylobacter* infections in humans [1,5,6]. Raw milk

and dairy products [7], contaminated water [8,9], and contact with pets and farm animals [10,11] have been also identified as possible sources for campylobacteriosis, and have contributed to outbreaks of the disease worldwide.

Although most *Campylobacter* infections are mild, self-limiting and usually resolve within a few days without antibiotic treatment, severe gastrointestinal disease can occur particularly in immunocompromised persons. Moreover, postinfectious complications of campylobacteriosis, including Guillain-Barré syndrome and reactive arthritis, can occur in both immunocompromised and immunocompetent persons [12].

When clinical therapy is warranted, erythromycin is considered the drug of choice. Fluoroquinolones have been also frequently used owing to their broad spectrum of activity against enteric pathogens. A rapid increase in the proportion of *Campylobacter* strains resistant to

macrolides and fluoroquinolones has been reported in several countries worldwide, and therefore represents a major concern for public health [1,13,14].

Even though gastroenteritis is one of the leading cause of hospitalization in Lebanese infants under one year, research work on *Campylobacter* human infections are still very limited in Lebanon. Few studies have investigated the prevalence and antimicrobial susceptibility of *Campylobacter* spp. isolated from humans and poultry in Lebanon, and results have shown that *Campylobacter* is rare in diarrheic stools of patients compared to *Salmonella* and *Shigella.* Indeed, *Campylobacter* infection may be significantly underdiagnosed because its search is not part of the routine stool testing, and its culture is demanding and more difficult than that of other bacteria [15–18]. Moreover, these studies highlighted the increased rates of resistance of *Campylobacter* isolates to antibiotics, and emphasized the need for assessing the routes of transmission of campylobacteriosis to humans and animals [15–18]. These preliminary and inconclusive data warrants therefore further investigations to estimate the true prevalence of campylobacteriosis in Lebanon, and to better understand the involvement of *Campylobacter* species in the pathogenesis of gastroenteritis.

This work is carried out to assess the epidemiological and clinical profiles of *Campylobacter* spp. in stool cultures from Lebanese patients with diarrhea. It also aims to determine the antibiotic susceptibility pattern of isolated species, as well as sources of transmission and risk factors contributing to the emergence of the disease in Lebanon.

Methodology
Patients
The study was conducted in eight districts belonging to the North, South and Mount Lebanon Governorates, according to the Declaration of Helsinki and in agreement with standards of the Ethical Committee of Notre Dame University Hospital. Subjects enrolled in the study were Lebanese outpatients who attended hospitals and medicals centers with three or more watery loose stools in 24-hour period at any time of day between September 2016 and August 2017. After obtaining a written informed consent from all participants, a questionnaire was filled to gather relevant information regarding demographic and clinical data, dietary intake, treatment, risk factors, etc. Patients treated with antibiotics and suffering from chronic diseases were excluded from the study.

Samples collection
Stool samples were collected from all patients enrolled in the study. A total of 150 meat samples, commonly consumed by the Lebanese population (poultry meat (n = 62), beef (n = 48), lamb (n = 25) and goat meat (n = 15)), were randomly collected from 62 supermarkets and 88 broiler slaughterhouses located in different rural areas of the various districts explored in the study. Most of these slaughterhouses had good working conditions and practices. Swabs taken aseptically from carcasses and their matching livers and cuts were performed on different working days and hours. Each sample was packed in a sterile jar containing 100 mL of buffered peptone water (Merck, Darmstadt, Germany) and immediately transported to the laboratory in a small refrigerator under 2-5°C and tested on the same day.

Campylobacter culture
The selective agar used for the isolation of *Campylobacter* was a commercially available preparation, charcoal cefoperazone desoxycholate agar (CCDA) (Liofilchem, Teramo, Italy) containing 32 mg/L of cefoperazone and 10 mg/L of amphotericin B. Plates were incubated microaerobically (6% O_2) at 37°C for two days. Suspect colonies (Small, gray and translucent colonies (1-2 mm in diameter) with a metallic sheen and butter-like consistency) were identified to the genus level by positive oxidase and catalase reactions and a typical Gram stain appearance (curved "seagull wing shaped" or spiral shaped small motile Gram-negative rods).

Campylobacter species identification
The discrimination between *Campylobacter* species was based on the standardized hippurate hydrolysis test and PCR (polymerase chain reaction) technique.

Hippurate hydrolysis test
The hippurate hydrolysis activity of *Campylobacter* species was analyzed using the Hippurate Test Kit (Liofilchem, Teramo, Italy) according to the manufacturer's instructions. The hippurate hydrolysis test allows differentiating *C. jejuni* (positive test revealed by the appearance of a deep blue/violet color in 30 minutes) from all other *Campylobacter* species (negative test).

Molecular identification of Campylobacter species
DNA was extracted from agar plates using Insta-Gene1 matrix (Bio-Rad, California, USA) according to

the manufacturer's instructions. DNA extracts were then stored at -20°C until required for PCR screening.

Campylobacter speciation was performed using PCR targeting the 16S rRNA gene for the co-identification of *Campylobacter jejuni* and *Campylobacter coli*. Both species were discriminated by amplifying the *hippuricase* gene found exclusively in *Campylobacter jejuni* and the *aspartokinase* gene found in *Campylobacter coli*, using specific primers. PCR was carried out in a total volume of 50μl, containing 100 ng genomic DNA, 1X Taq DNA polymerase buffer, 2 mM $MgCl_2$, 0.25 mM dNTP (Solis BioDyne, Tartu, Estonia), 100 ng of each primer [19] and 0.02 U of Taq DNA polymerase (Solis BioDyne, Tartu, Estonia). Following initial denaturation at 95°C for 5 minutes, 30 amplification cycles of denaturation (95°C for 1 minute), annealing (annealing temperature for 1 minute), and elongation (72°C for 1 minute) were performed in a thermal cycler Perkin-Elmer 9600. PCR products were then visualized on a 0.8% agarose gel under UV radiation. Primers and annealing temperatures used for the different amplifications as well PCR products are summarized in Table 1.

Isolation and identification of other diarrheal pathogens

Stool samples were cultured in selenite broth and various solid selective media, namely Salmonella-Shigella (SS) agar for the isolation of *Salmonella* spp. and *Shigella* spp., as well as Sorbitol MacConkey agar, blood agar and thiosulfate citrate bile salt cholera medium for the isolation of *Escherichia coli*, *Staphylococcus aureus* and *Vibrio cholera*, respectively. After 24-hours' incubation at 37°C, isolates were stained by the Gram method and identified by the appropriate biochemical tests and API 20E gallery (BioMérieux, Lyon, France). *Clostridium* spp. toxins were detected by ELISA technique (BioMérieux, Lyon, France). The presence of parasites *(Entamoeba histolytica*, *Giardia intestinalis, etc.)* in feces was routinely determined by microscopic examination of stool samples. *Cryptosporidium* spp. and *Cyclospora*

cayetanensis oocysts were detected by modified Ziehl-Neelsen (MZN). Rotavirus and adenovirus were simultaneously detected in stool samples using the chromatographic immunoassay CerTest Rotavirus + Adenovirus one step combo card test (Biotec, Zaragoza, Spain).

Antibiotic susceptibility

Campylobacter isolates were evaluated for susceptibility to 14 antibiotics (Aztreonam (30 μg), erythromycin (15 μg), ampicillin (30 μg), amoxicillin (30 μg), levofloxacin (5 μg), clindamycin (2 μg), tetracycline (30 μg), imipenem (10 μg), clarithromycin (15 μg), metronidazole (5 μg), cefotaxim (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg) and nalidixic acid (30 μg)) on a 5% sheep blood Mueller-Hinton agar by the disc diffusion standard method. Plates were incubated at 37°C for 48 hours in the microaerophilic atmosphere described earlier. Susceptibility categorization for ampicillin, amoxicillin, erythromycin, ciprofloxacin, tetracycline, and clarithromycin was carried out according to EUCAST 2018 recommendations [20]. For the remaining antibiotics, the *Enterobacteriaceae* breakpoints established by the Clinical and Laboratory Standards Institute were applied [21]. *C. jejuni* ATCC 33291 and *C. coli* ATCC 43473 were used as the reference quality control strains.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, Inc, San Diego, CA, USA). Fisher's exact test was used to compare frequencies between groups. The distribution of *Campylobacter* infection was correlated to the monthly average temperature using Pearson correlation test. *P* values less than 0.05 were considered significant.

Results
Demographic profile of participants

During a one year period between September 2016 and August 2017, stool samples were collected from

Table 1. Primers and reactions conditions used for the amplification.

Gene	Primers	Annealing temperature	PCR products
16S rRNA	F : 5'-AAT CTA ATG GCT TAA CCA TTA-3' R : 5'-GTA ACT AGT TTA GTA TTC CGG-3'	58˚C	854 bp
Hippuricase	F : 5'-GAA GAG GGT TTG GGT G-3' R : 5'-AGC TAG CTT CGC ATA ACT TG-3'	66˚C	735 bp
Aspartokinase	F : 5'- GGT ATG ATT TCT ACA AAG CGA G -3' R : 5'- ATA AAA GAC TAT CGT CGC GTG -3'	60˚C	500 bp

F: forward; R: reverse; bp: base pair; *Campylobacter* speciation was performed by PCR targeting the 16S rRNA gene for the co-identification of *C. jejuni* and *C. coli*, the *hippuricase* gene for screening *C. jejuni* and the *aspartokinase* gene for identifying *C. coli*, using specific primers.

1000 Lebanese patients with diarrhea (51.5% males and 48.5% females), resident in rural areas of the regions of North, South and Mount Lebanon. Among the participants, 36.4% were children aged less than 12 years old (5.6 ± 4.1 years old), 9.3% adolescents (13-17 years old; 15.1 ± 1.4 years old), 21.9% adults (18-45 years old; 31.9 ± 8.3 years old), 10.7% belonged to the middle-aged group (46-64 years old; 55.4 ± 5.5 years old) and 21.7% were elderly aged more than 65 years (78.7 ± 9.2 years old).

Prevalence of Campylobacter infection and distribution regarding gender, age and season

Examination of stool specimens (n = 1000) showed that 21.5% of samples were detected as positive for *Campylobacter* spp. *C. jejuni* was the main species, identified in 179 patients (83.2%) followed by *C. coli* found in 30 patients (13.9%). Other *Campylobacter* species counted only for 2.8% of gastroenteritis. Biochemical identification of *C. jejuni* and *C. coli* was further confirmed by PCR analysis as previously described in Table 1.

Other agents detected in patients' stools included rotavirus (6.7%), *Entamoeba histolytica* (6.0%), *Salmonella* Typhi (5.8%), enterohemorrhagic *Escherichia coli* O157:H7 (5.6%), *Candida albicans* (2.6%) and adenovirus (1.2%). *Clostridium difficile*, *Shigella dysenteriae*, *Giardia lamblia* and *Ascaris lumbricoides* accounted for 4.3% of total gastrointestinal infections. Five percent of *Campylobacter* infected patients (n = 11) were co-infected with one of the following pathogens *Salmonella* Typhi, *Escherichia coli*, *Candida albicans*, and *Entamoeba histolytica*.

The percentage of males infected with *Campylobacter* was slightly higher than females (53.5% *vs* 46.5%). As regards age distribution, the

Figure 1. Seasonal distribution of *Campylobacter spp.* isolated in patients' stool between September 2016 and August 2017.
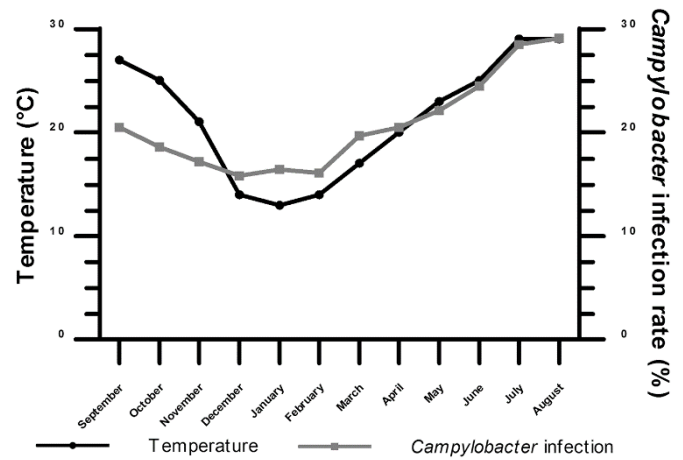
highest rates of *Campylobacter* infection were observed in children (41.8%), followed by the elderly group (21.4%), adults (17.2%), middle-aged people (10.3%) and adolescents (9.3%).

The seasonality of campylobacteriosis was determined in this study. As shown in Figure 1, *Campylobacter* has a clear seasonal pattern, with a large peak seen in the summer, mainly in July and August, while the lowest rates were observed between December and February. In agreement, a significant positive correlation was observed between the frequency of *Campylobacter* infection and the monthly average temperature (r = 0.83; p < 0.0001).

Clinical presentation of campylobacteriosis

Patients infected with campylobacteriosis showed a vast range of clinical symptoms. Most patients (80.0%) had watery diarrhea, while a bloody or bloody-mucous diarrhea was observed in 13% and 7% of cases, respectively. The frequency and period of diarrhea

Table 2. Frequency of *Campylobacter* infection with regard to gender, age and season.

Variables		Negative N (%)	Positive N (%)	*P* value
Gender	Male	400 (77.7)	115 (22.3)	0.54
	Female	385 (79.4)	100 (20.6)	
Age	Children	274 (75.3)	90 (24.7)	0.28
	Adolescents	73 (78.5)	20 (21.5)	
	Adults	182 (83.1)	37 (16.9)	
	Middle-aged	85 (79.5)	22 (20.5)	
	Elderly	171 (78.8)	46 (21.2)	
Season	Fall	82 (82)	18 (18)	0.14
	Winter	154 (83.2)	31 (16.8)	
	Spring	347 (78)	98 (22)	
	Summer	202 (74.3)	68 (25.7)	

N: number of individuals; Fisher's exact test was performed to generate p values for differences in *Campylobacter* spp. isolation rate with regards to gender, age and season.

varied among patients. Interestingly, a high percentage of patients presented an acute severe diarrhea characterized by a passage of stools more than 5 times per day (64.4% of cases) and lasting longer than 48 hours in 61.2% of cases. Other observed clinical symptoms included fever higher than 38.5°C (66.5%), abdominal pain (59.1%), vomiting (49.7%), dehydration (43.7%) and headache (40.9%).

Effect of age, gender and seasonality on campylobacteriosis prevalence

The isolation rate of *Campylobacter* spp. with regards to gender, age and season is shown in Table 2. Frequencies were not significantly different between males and females (p = 0.54) as well as within the age groups (p = 0.28). Moreover, *Campylobacter* infection rates were comparable between seasons (p = 0.14).

Risk factors for campylobacteriosis

The risk factors for campylobacteriosis were also explored in this study. As shown in Table 3, the risk for *Campylobacter* infection increased following consumption of meat, chicken or salad (p < 0.0001) and, to a lesser extent, after contact with animals (p = 0.0068). In contrast, there was no significant correlation between consumption of non-pasteurized milk or non-treated water and the prevalence of campylobacteriosis (p = 0.74; p = 0.26 respectively).

Antimicrobial Susceptibility Pattern of Campylobacter isolates

Table 4 shows the susceptibility pattern of isolated *Campylobacter* spp. to antibiotics. The highest rates of sensitivity were observed for erythromycin, chloramphenicol, clarithromycin and levofloxacin. In contrast, metronidazole was the least active agent against isolates, followed by amoxicillin and ampicillin. Multiple drug resistance to two or more antimicrobial agents was observed in 35.9% of *Campylobacter* isolates.

Reservoirs of Campylobacter

In order to identify the main categories of meat representing the most significant reservoirs of *Campylobacter* in Lebanon, a total of 150 samples of the most commonly consumed raw meats were collected from supermarkets and slaughterhouses in different Lebanese regions. *Campylobacter* spp. were detected in 33 samples (22.0%), among which 31 (93.9%) collected from slaughterhouses and 2 only from supermarkets. *C. jejuni* was identified in 28 (84.8%) samples, while *C. coli* only counted for 15.2% of contaminated samples. Strains were isolated from 45.5% and 33.3% of raw chicken and beef samples, respectively. The lowest rates of contamination with *Campylobacter* were observed in lamb (15.1%) and goat meat (6.1%). *C. jejuni* was the most prevalent in chicken (93.3%), beef (90.1%) and goat meat (100%),

Table 3. Risk factors for campylobacteriosis.

Transmission route	Negative N (%)	Positive N (%)	*P* value
Contact with animals			
Yes	113 (64.9)	61 (35.1)	0.0068[**]
No	472 (75.4)	154 (24.6)	
Consumption of meat			
Yes	224 (54.3)	170 (45.7)	< 0.0001[****]
No	361 (88.9)	45 (11.1)	
Consumption of chicken			
Yes	185 (54.4)	143 (43.6)	< 0.0001[****]
No	400 (84.7)	72 (15.3)	
Consumption of salad			
Yes	229 (64.3)	127 (35.7)	< 0.0001[****]
No	356 (80)	88 (20)	
Consumption of non-pasteurized milk			
Yes	90 (72.0)	35 (28.0)	0.74
No	495 (73.3)	180 (26.6)	
Consumption of non-treated water			
Yes	82 (68.9)	37 (31.1)	0.26
No	503 (73.9)	178 (26.1)	

N: number of individuals; [**] p < 0.01, [****] p < 0.0001 based on Fisher's exact test for differences in *Campylobacter* spp. isolation rate with regards to potential risk factors.

Table 4. Antimicrobial susceptibility pattern of *Campylobacter* spp. isolates.

Antibiotic	*Campylobacter* spp. %			*C. jejuni* %			*C. coli* %		
	R	S	I	R	S	I	R	S	I
Clindamycin	27.3	58.1	14.6	29.3	57.5	13.2	24.4	58.2	17.4
Levofloxacin	17.4	72.7	9.9	14.8	74.4	10.8	16.5	73.5	10.0
Cefotaxime	37.5	44.7	17.8	36.6	45.2	18.3	39.3	42.8	17.9
Imipenem	20.2	66.9	12.9	23.3	68.1	8.6	19.1	68.7	12.2
Metronidazole	66.4	29.9	3.7	64.9	28.3	6.8	68.2	30.1	1.7
Aztreonam	23.4	66.5	10.1	21.8	69.2	9.0	24.1	65.6	10.3
Clarithromycin	9.7	75.5	14.8	8.5	74.8	16.7	9.9	76.2	13.9
Ciprofloxacin	16.2	69.3	14.5	17.8	70.5	11.7	14.9	68.9	16.2
Erythromycin	4.5	91.7	4.8	4.1	90.8	5.1	6.2	92.0	1.8
Ampicillin	32.4	39.3	28.3	30.8	38.7	30.5	31.2	40.1	28.7
Tetracycline	27.1	53.6	19.3	22.9	59.9	17.2	24.6	56.5	18.9
Amoxicillin	35.3	37.7	27.0	34.7	36.5	28.8	36.0	39.6	24.4
Chloramphenicol	5.8	89.9	4.3	6.1	90.1	3.8	6.1	88.8	5.1
Nalidixic acid	17.2	68.5	14.3	17.4	69.7	12.9	15.5	68.6	15.9

R: resistant; S: sensitive; I: intermediate; *Campylobacter* susceptibility testing was determined by the disc diffusion standard method. The diameters of inhibition zones were measured and evaluated according to EUCAST 2018 recommendations for ampicillin, amoxicillin, erythromycin, ciprofloxacin, tetracycline and clarithromycin, and CLSI 2015 interpretive criteria for *Enterobacteriaceae* for the remaining antibiotics.

while *C. coli* was the most frequently isolated species in lamb (60%).

Meat sites that are most likely to be contaminated with *Campylobacter* spp. were investigated by taking random samples of broiler carcasses and their matching livers and cuts. The highest rate of *Campylobacter* contamination in chicken was found in carcasses (46.7%), while in beef, lamb and goat meat, *Campylobacter* was predominant in meat cuts (Table 5). *C. jejuni* was identified in all carcasses (n = 9), as well as in 2 liver samples and 17 meat cuts. In contrast, none of the carcasses showed contamination with *C. coli*. It was mainly identified in cuts of lamb (n = 3) and liver of chicken (n = 1) and beef (n = 1) (Table 5).

Discussion

In agreement with previous studies [4,22,23], *Campylobacter* was the leading cause of gastroenteritis in Lebanon, with *C. jejuni* being the most frequently isolated species followed by *C. coli*. Our study showed a much higher prevalence of *Campylobacter* infection than that observed by Talhouk *et al.* in 1998 (0.7%), as well as to the prevalence reported by Dabboussi *et al.* in 2012 (11.1%) who evaluated the frequency of *Campylobacter* infection in 90 children with diarrhea in Northern Lebanon [15]. These results highlight the remarkable increase of campylobacteriosis rates in Lebanon. Therefore, the detection of *Campylobacter* in patients' stool culture should be part of the routine diagnosis of human gastroenteritis in Lebanon, even if it is difficult, demanding and expensive. Moreover,

Table 5. Distribution of *Campylobacter spp.* isolates per site of isolation.

Meat	Isolation site	*Campylobacter spp.* N (%)	*C. jejuni* N (%)	*C. coli* N (%)
Chicken	Cut	5 (33.3%)	5 (100)	0 (0)
	Liver	3 (20%)	2 (63.7%)	1 (33.3%)
	Carcass	7 (46.7%)	7 (100)	0 (0)
Beef	Cut	9 (81.8%)	9 (100)	0 (0)
	Liver	1 (9.1%)	0 (100)	1 (100)
	Carcass	1 (9.1%)	1 (100)	0 (0)
Lamb	Cut	4 (80%)	1 (25%)	3 (75%)
	Liver	0 (0%)	0 (0)	0 (0)
	Carcass	1 (20%)	1 (100)	0 (0)
Goat meat	Cut	2 (100%)	2 (100)	0 (0)
	Liver	0 (0%)	0 (0)	0 (0)
	Carcass	0 (0%)	0 (0)	0 (0)

N: number of samples; Meat samples were randomly collected at different working days and hours from Lebanese supermarkets and broiler slaughterhouses. Swabs were taken aseptically from carcasses and their matching livers and cuts, and tested for the presence of *Campylobacter* spp.

when compared to reports from other countries, the prevalence was higher to those found in Mexico (15.7%) and China (14.9%), but lower to those obtained in developed countries such as the United States (26.5%), New Zealand (33%) and the Netherlands (71.4%) [4]. This variation might be attributed to differences in surveillance systems and *Campylobacter* detection methods between developed and developing countries, as well as to demographic, geographic and period differences between studies [4,24].

The distribution of *Campylobacter* isolates according to patient's age and gender revealed that *Campylobacter* infections can occur in all age groups, with highest percentages noticed in children and adults, irrespective of the gender. Results reported from previous studies were variable and sometimes contradictory. According to the surveillance study carried out by the National Reference Center for *Campylobacter* and *Helicobacter* in France, children under 5 years of age and people aged over 65 had the highest prevalence of *Campylobacter* infections compared to other groups [25]. In contrast, a case-control study carried out by Friedman *et al.* in 2000 revealed that most populations in industrialized countries showed a bimodal age distribution with the highest incidence seen in young children and young adults [26]. As for developing countries, the infection was mainly limited to children under 2 years of age [27]. The highest campylobacteriosis rates observed among children may be attributed to their relatively naïve immunological system and the high frequency of exposure to *Campylobacter* reservoirs. Another possibility is the lower threshold for medical assessment and testing among children, thus resulting in a higher diagnosis rate in this age group. As for adults and elderly people, high rates of exposure were related to high-risk food consumption patterns and poor hygiene level, respectively. On the other hand, males were overall more affected than females with male-to-female gender ratio of 1.15 to 1. This has shown to be a consistent finding across most populations, and is related to the increased male exposure through high-risk occupations such as farming and meat processing, differences in food and water consumption patterns, and food hygiene practices [28].

The association of *Campylobacter* infection with season was also investigated in the present study. As observed in other developing countries, campylobacteriosis incidence was found to be the highest during the warmest months of the year, namely in July and August [3,29]. These results were further supported by the positive strong correlation between the frequency of *Campylobacter* isolates and the monthly average temperature (r = 0.83; p < 0.0001). The pronounced seasonality observed in our study may be attributed to the increased *Campylobacter* prevalence in various reservoirs and seasonal changes in human behavior that affect exposure [22,30–32].

Several factors can be associated with a higher risk for campylobacteriosis. Consistent with all previous data, the major risk factor identified in the present study was the consumption of meat and salad. Indeed, subjects were 2 to 3 times more likely to develop campylobacteriosis after consuming raw or undercooked food (p < 0.0001). Our results showed that contact with animals is another important source of transmission of *Campylobacter* infection in Lebanon (p = 0.0068). In contrast, consuming raw milk and drinking non-treated water were not statistically associated with culture positivity for *Campylobacter* isolates (p = 0.74 and p = 0.26 respectively). These results can be explained by the high standards of microbiological quality applied to drinking water, as well as by the fact that the majority of the Lebanese population does not consume tap water or non-pasteurized milk. Moreover, the prevalence of *Campylobacter* infection did not significantly differ according to gender, age and seasons (p = 0.54, p = 0.28 and p = 0.11 respectively). These results suggest that, despite the clear seasonal pattern observed in Figure 1, neither the climate nor age and gender can be considered as major determinants for *Campylobacter* infection in Lebanon.

The role of meat as a major source of *Campylobacter* transmission to humans in Lebanon was confirmed in our study by the detection of *Campylobacter* spp. in 22.0% of samples collected from markets and slaughterhouses. Our finding of *C. jejuni* domination over *C. coli* agrees with other studies that establish similar relation between these two species in various types of meat samples [33,34]. The majority of contaminated meat samples (93.9%) were collected from slaughterhouses rather than supermarkets. These findings suggest that transmission to human results from handling and consumption of meat that has been contaminated during slaughter or carcass processing and not during food transport or storage [18,33,35,36]. As expected, the main categories of meat representing the most significant reservoirs of *Campylobacter* in Lebanon were chicken (45.5%), followed by beef (33.3%), lamb (15.1%) and finally goat meat (6.1%). In agreement with results observed by Fadlallah *et al.* in 2018 [16], the highest rate of *Campylobacter* contamination in poultry meat was found in carcasses

(46.7%). In contrast, in beef, lamb and goat meat *Campylobacter* spp. were predominant in the cuts. In fact, the intestinal tract of chicken can harbor a large number of *Campylobacter* spp. During evisceration, the intestinal tract may leak or rupture and the contents are transferred to the skin of the carcass [16,18,35,37]. Moreover, according to the research conducted by Bolton in 2015, *Campylobacter* spp. are particularly sensitive to desiccation. For poultry slaughter, the carcasses are water-chilled, maintaining a wet surface and facilitating the survival of *Campylobacter* spp. [38].

Campylobacter infections are typically self-limited. However, an antibiotic therapy is indicated in the presence of severe complications or a weakened immune system. The failure to administer appropriate antibiotics in the presence of severe complications or a weakened immune system was associated with fatal outcome in bacteremia caused by *Campylobacter* species [39–41]. In this regard, the antimicrobial susceptibility patterns of *Campylobacter* spp. were investigated in the present study. Interestingly, *C. jejuni* and *C. coli* demonstrated comparable susceptibility profiles; a finding that was previously noted by Talhouk *et al.* in 1998 [17]. Despite decades of use, the rate of resistance of *Campylobacter* to erythromycin was quite low. Indeed, due to its low cost, safety use of administration and narrow spectrum of activity, erythromycin is still considered as the optimal drug of choice for the treatment of campylobacteriosis. Chloramphenicol, clarithromycin, levofloxacin, ciprofloxacin, nalidixic acid and imipenem were also among the most effective agents against *Campylobacter* infections. In contrast, the highest rates of resistance were recorded to metronidazole, amoxicillin and ampicillin. The high prevalence of multi-resistant strains observed in our study was previously reported by the European Food Safety Authority [3]. Is it noteworthy to mention that rates of resistance of *Campylobacter* spp. to ampicillin (60.7%), amoxicillin (62.3%) and clindamycin (41.9%) are remarkably higher than those reported by Talhouk *et al.* in 1998 (5%, 23% and 31% respectively) [17]. This may be explained by the uncontrolled use of these antibiotics in human medicine, such as treating infections other than gastroenteritis, self-medication and access without prescription. It can also be attributed to their use in veterinary medicine in order to control, prevent and treat infections, and enhance animal growth.

Conclusion

Campylobacter is the leading cause of gastroenteritis in Lebanon, with *C. jejuni* being the most isolated species from patients' stool culture and meat samples. Even though, the highest infection rates were reported in children and during summer, neither season nor age or gender seems to be major modifying factors for campylobacteriosis in Lebanon. Consumption of contaminated meat and salads resulting from mishandling or cross-contamination remains the principal route of *Campylobacter* transmission to humans. The incidence of *Campylobacter* resistance to erythromycin is still relatively low in the Lebanese population, and thus it should always be regarded as the drug of choice in treatment of campylobacteriosis. In contrast, the highest resistance patterns were observed to the most commonly prescribed antibiotics in human and veterinary medicine. Human campylobacteriosis remains an unresolved public health problem of high importance. Detection of *Campylobacter* spp. in patients' stool culture should be therefore considered as a routine test in the diagnosis of gastroenteritis in order to allow early diagnosis of the disease and better monitoring of antimicrobial resistance among Lebanese patients.

References

1. Silva J, Leite D, Fernandes M, Mena C, Gibbs PA, Teixeira P (2011) Campylobacter spp. as a foodborne pathogen: A review. Front Microbiol 2: 200.
2. Fletcher SM, McLaws M-L, Ellis JT (2013) Prevalence of gastrointestinal pathogens in developed and developing countries: Systematic review and meta-analysis. J Public Health Res 2: 42–53.
3. European Food Safety Authority, European Centre for Disease Prevention and Control (2017) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA J 15: 13–20.
4. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM (2015) Global epidemiology of Campylobacter infection. Clin Microbiol Rev 28: 687–720.
5. Franchin PR, Aidoo KE, Vieira Batista CR (2005) Sources of poultry meat contamination with thermophilic Campylobacter before slaughter. Braz J Microbiol 36: 157–162.
6. Kramer JM, Frost JA, Bolton FJ, Wareing DR (2000) Campylobacter contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. J Food Prot 63: 1654–1659.

7. Peterson MC (2003) *Campylobacter jejuni* enteritis associated with consumption of raw milk. J Environ Health 65: 20–21, 24, 26.

8. Denis M, M Tanguy, Chidaine B, Laisney MJ, Mégraud F, Fravalo P (2011) Description and sources of contamination by Campylobacter spp. of river water destined for human consumption in Brittany, France. Pathol Biol 59: 256–263.

9. Karagiannis I, Sideroglou T, Gkolfinopoulou K, Tsouri A, Lampousaki D, Velonakis EN, Scoulica EV, Mellou K, Panagiotopoulos T, Bonovas S (2010) A waterborne *Campylobacter jejuni* outbreak on a Greek island. Epidemiol Infect 138: 1726–1734.

10. Lengerh A, Moges F, Unakal C, Anagaw B (2013) Prevalence, associated risk factors and antimicrobial susceptibility pattern of Campylobacter species among under five diarrheic children at Gondar University Hospital, Northwest Ethiopia. BMC Pediatr 13: 82.

11. Damborg P, Olsen KEP, Møller Nielsen E, Guardabassi L (2004) Occurrence of *Campylobacter jejuni* in pets living with human patients infected with *C. jejuni*. J Clin Microbiol 42: 1363–1364.

12. Nyati KK, Nyati R (2013) Role of *Campylobacter jejuni* infection in the pathogenesis of Guillain-Barré syndrome: An update. BioMed Res Int 2013: e852195.

13. Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P, Nachamkin I (2001) Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. Emerg Infect Dis 7: 24–34.

14. Wieczorek K, Osek J (2013) Antimicrobial resistance mechanisms among Campylobacter. BioMed Res Int 2013: e340605.

15. Dabboussi F, Alam S, Mallat H, Hlais S, Hamze M (2012) Preliminary study on the prevalence of Campylobacter in childhood diarrhoea in north Lebanon. East Mediterr Health J 18: 1225–1228.

16. M Fadlallah S, El Hajj R, El Hage J, Nasser Z, Ghosn N, Ammar W, M Matar G (2018) Molecular epidemiology and antimicrobial resistance of *Campylobacter coli* from caeca and carcass of poultry in Lebanon. ACTA Sci Microbiol 1: 63–70.

17. Talhouk RS, el-Dana RA, Araj GF, Barbour E, Hashwa F (1998) Prevalence, antimicrobial susceptibility and molecular characterization of Campylobacter isolates recovered from humans and poultry in Lebanon. J Med Liban 46: 310–316.

18. Semaan EH, Dib H, Mrad R, Chami C, Jalkh R (2014) Dynamic of Campylobacter species contamination along a poultry slaughtering chain. Ital J Food Saf 3: 2246.

19. Linton D, Lawson AJ, Owen RJ, Stanley J (1997) PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. J Clin Microbiol 35: 2568–2572.

20. The European Committee on Antimicrobial Susceptibility Testing (2018) EUCAST: Clinical breakpoints. Available: http://www.eucast.org. Accessed 1 July 2018.

21. Clinical and Laboratory standard institute (CLSI) (2007) Performance standards for antimicrobial susceptibility testing, 17th informational supplement. CLSI document M100-S17 (ISBN 1-56238-625-5).

22. Cody AJ, McCarthy NM, Wimalarathna HL, Colles FM, Clark L, Bowler IC, Maiden MC, Dingle KE (2012) A longitudinal 6-year study of the molecular epidemiology of clinical campylobacter isolates in Oxfordshire, United Kingdom. J Clin Microbiol 50: 3193–3201.

23. Baquero F, Barrett JF, Courvalin P, Morrissey I, Piddock L, Novick WJ (1999) Epidemiology and mechanisms of resistance among respiratory tract pathogens. Clin Microbiol Infect 4 Suppl 2: 19-26.

24. Arsenault J, Berke O, Michel P, Ravel A, Gosselin P (2012) Environmental and demographic risk factors for campylobacteriosis: do various geographical scales tell the same story? BMC Infect Dis 12: 318.

25. Gallay A, Bousquet V, Siret V, Prouzet-Mauléon V, Valk Hd, Vaillant V, Simon F, Le Strat Y, Mégraud F, Desenclos JC (2008) Risk factors for acquiring sporadic Campylobacter infection in France: Results from a national case-control study. J Infect Dis 197: 1477–1484.

26. Friedman CR (2000) Epidemiology of *Campylobacter jejuni* *i*nfections in the United States and other industrialized nations. Campylobacter: 121–138.

27. Coker AO, Isokpehi RD, Thomas BN, Amisu KO, Obi CL (2002) Human campylobacteriosis in developing countries. Emerg Infect Dis 8: 237–244.

28. Nichols GL (2005) Fly transmission of Campylobacter. Emerg Infect Dis 11: 361–364.

29. Bessède E, Lehours P, Labadi L, Bakiri S, Mégraud F (2014) Comparison of characteristics of patients infected by *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter fetus*. J Clin Microbiol 52: 328–330.

30. Patrick ME, Christiansen LE, Wainø M, Ethelberg S, Madsen H, Wegener HC (2004) Effects of climate on incidence of Campylobacter spp. in humans and prevalence in broiler flocks in Denmark. Appl Environ Microbiol 70: 7474–7480.

31. Nylen G, Dunstan F, Palmer SR, Andersson Y, Bager F, Cowden J, Feierl G, Galloway Y, Kapperud G, Megraud F, Molbak K, Petersen LR, Ruutu P (2002) The seasonal distribution of campylobacter infection in nine European countries and New Zealand. Epidemiol Infect 128: 383–390.

32. Louis VR, Gillespie IA, O'Brien SJ, Russek-Cohen E, Pearson AD, Colwell RR (2005) Temperature-driven Campylobacter seasonality in England and Wales. Appl Environ Microbiol 71: 85.

33. Alagić D, Smajlović A, Smajlović M, Maksimović Z, Članjak E, Čaklovica K, Tanković S, Veljović E, Ljevaković-Musladin I, Rifatbegović M (2016) Prevalence of Campylobacter contamination in broiler meat. MESO XVIII: 357, 335–361.

34. Kovačić A, Listeš I, Vučica C, Kozačinski L, Tripković I, Siško-Kraljević K (2013) Distribution and genotypic characterization of *Campylobacter jejuni* isolated from poultry in Split and Dalmatia County, Croatia. Zoonoses Public Health 60: 269–276.

35. Rosenquist H, Sommer HM, Nielsen NL, Christensen BB (2006) The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant Campylobacter. Int J Food Microbiol 108: 226–232.

36. Seliwiorstow T, Baré J, Van Damme I, Uyttendaele M, De Zutter L (2015) Campylobacter carcass contamination throughout the slaughter process of Campylobacter-positive broiler batches. Int J Food Microbiol 194: 25–31.

37. Byrd JA, Hargis BM, Corrier DE, Brewer RL, Caldwell DJ, Bailey RH, McReynolds JL, Herron KL, Stanker LH (2002) Fluorescent marker for the detection of crop and upper gastrointestinal leakage in poultry processing plants. Poult Sci 81: 70–74.

38. Bolton DJ (2015) Campylobacter virulence and survival factors. Food Microbiol 48: 99–108.

39. Nielsen H, Hansen KK, Gradel KO, Kristensen B, Ejlertsen T, Østergaard C, Schønheyder HC (2010) Bacteraemia as a result of Campylobacter species: a population-based study of epidemiology and clinical risk factors. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 16: 57–61.
40. Pacanowski J, Lalande V, Lacombe K, Boudraa C, Lesprit P, Legrand P, Trystram D, Kassis N, Arlet G, Mainardi JL, Doucet-Populaire F, Girard PM, Meynard JL, CAMPYL Study Group (2008) Campylobacter bacteremia: clinical features and factors associated with fatal outcome. Clin Infect Dis Off Publ Infect Dis Soc Am 47: 790–796.
41. Fernández-Cruz A, Muñoz P, Mohedano R, Valerio M, Marín M, Alcalá L, Rodriguez-Créixems M, Cercenado E, Bouza E (2010) Campylobacter bacteremia: clinical characteristics, incidence, and outcome over 23 years. Medicine 89: 319–330.

Corresponding author
Jose-Noel Ibrahim; Faculty of Public Health, Lebanese German University (LGU), Sahel Alma, Lebanon; P.O. Box: 206;
Tel.: +961 70 68 31 79;
Fax: + 961 9 93 89 33;
e-mail: jn.ibrahim@lgu.edu.lb

Conflict of interests: No conflict of interests is declared.