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Prevalence, Laboratory Findings and Clinical Characteristics of Campylobacteriosis Agents among Hospitalized Children with Acute Gastroenteritis in Lebanon

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ABSTRACT

Purpose: *Campylobacter* species are currently the most common cause of bacterial gastroenteritis. In Lebanon, *Campylobacter* infection occurrence is underdiagnosed owing to the lack of specific culture and rapid test kits, particularly among children. This study aimed to evaluate the prevalence, laboratory findings, and clinical characteristics of *Campylobacter* infection in hospitalized children with acute gastroenteritis in South Lebanon.
Methods: We conducted a 6-month retrospective cohort study between January and June 2018, including 291 children aged between 1 month and 12 years, who were admitted to a tertiary healthcare center in South Lebanon. The medical files of the patients were reviewed to retrieve the required clinical information, including clinical and laboratory data.
Results: The prevalence of campylobacteriosis agents in pediatric patients with acute gastroenteritis is 12.02%. Patients infected with *Campylobacter* had more severe acute gastroenteritis than *Campylobacter*-negative patients and often presented with high-grade fever, diarrhea episodes more than six times per day, diarrhea lasting for more than five days, and dehydration. Indeed, children with high-grade fever (≥38.5°C) were five times more likely to test positive for *Campylobacter* than those with low-grade fever. In addition, the results showed a higher Vesikari score for the majority of *Campylobacter*-positive patients with severe acute gastroenteritis compared to a moderate profile for *Campylobacter*-negative patients.
Conclusion: The present study findings highlight that *Campylobacter* infection is frequent among children with acute gastroenteritis. Therefore, the detection of *Campylobacter* should be carried out for the diagnosis of human gastroenteritis in Lebanon, along with the detection of routine enteropathogens.

Keywords: Campylobacteriosis; *Campylobacter*; Gastroenteritis; Children; Diarrhea; Lebanon

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INTRODUCTION

Campylobacteriosis-causing agents are the most common cause of bacterial gastroenteritis in developed countries [1], with an incidence rate of 64.8 per 100,000 population in Europe [2]. According to the United States (US) Centers for Disease Control and Prevention, it is estimated that around 1.3 million cases of campylobacteriosis are found every year in the US alone, with an annual economic loss of up to $ 6.8 billion [3]. Campylobacteriosis is an intestinal infection caused by *Campylobacter* species [4] and presents with common gastrointestinal symptoms, including abdominal pain, diarrhea, fever, nausea, and vomiting. This infection can be accompanied by clinical manifestations that are fatal in children, the elderly, and immunocompromised individuals [5].

Campylobacteriosis in humans usually occurs after ingestion of water or food contaminated with bacteria, especially from poultry, which represents the highest percentage (50–70%) of cases [6]. Once ingested, *Campylobacter* prompts a toxin-releasing inflammatory response in the colon, accompanied by infiltration of the intestinal mucosa with neutrophils and lymphocytes [7]. Clinical signs of the disease range from mild self-limiting enteritis with watery diarrhea to bloody mucoidal diarrhea in cases of severe acute campylobacteriosis [8]. Diagnosing this disease usually requires direct stool culture on *Campylobacter* specific media or polymerase chain reaction (PCR)-based methods [9]. Currently, since no vaccination is available to protect against this enteropathogen (as in *Rotavirus*), the only way to hinder the spread of campylobacteriosis is via hygiene and biosecurity [10].

Campylobacter species are spiral, microaerobic, and gram-negative bacteria belonging to the 16S rRNA superfamily VI. The genus includes 17 species and six subspecies, although most are thought to be nonpathogenic [11]. *Campylobacter* species are sometimes isolated from symptomatic patients and healthy individuals, with *Campylobacter jejuni* being the most commonly isolated species among children in the US [12]. In Lebanon, *Campylobacter* infection in both children and adults is underestimated owing to the lack of specific culture and rapid test kits. In addition, the extensive use of antibiotics in Lebanon increases the occurrence of resistant bacteria [13]. To the best of our knowledge, few studies have been published in Lebanon on the prevalence, laboratory findings, and clinical characteristics of campylobacteriosis in children. In our study, we aimed to evaluate these parameters in hospitalized children with acute gastroenteritis in South Lebanon.

MATERIALS AND METHODS

Ethics approval and consent to participate

All experimental protocols followed in this study were approved by the ethics committee of the Sheikh Ragheb Harb University Hospital (SRHUH) (Approval No. AER2017). Ethical clearance was obtained as per the norms and in accordance with relevant guidelines and regulations of the tertiary healthcare center. Recruitment was performed randomly after obtaining written informed consent for participation from the patients' caregivers, in accordance with the Declaration of Helsinki.

Patient selection

During a six-month period, from January to June 2018, we collected clinical, demographic, and laboratory data from hospitalized Lebanese children (aged 1 month to 12 years), admitted to the pediatric department of a tertiary care center located in South Lebanon.

The patients included in this study were hospitalized children with acute gastroenteritis, i.e., vomiting and/or diarrhea. The exclusion criteria were children with chronic diarrhea, immunodeficiency, malnutrition, and multiple malformations, as these might negatively affect the length of hospitalization and severity of the disease, and hence, disrupt the analysis of our results.

Clinical variables

Upon admission, data were collected as follows:
- Demographic data included age, sex, date of admission, status of breastfeeding, type of drinking water, family size of patients, and use of antibiotics prior to hospitalization.
- Clinical data comprised signs and symptoms of acute gastroenteritis, such as fever, diarrhea (frequency and duration), vomiting (frequency and duration); dehydration, and presence of blood and mucus in stool; stool texture (classified based on a combination of clinical experience and scientific evidence based on the Bristol stool chart); duration of hospitalization; and calculation of the index of severity "Vesikari Score" (VSS) [14].
- Laboratory findings included blood levels of white blood cells, red blood cells, hemoglobin, hematocrit, sugars, and C-reactive protein (CRP) and absolute neutrophil count (ANC).

Laboratory methods

Stool samples were freshly acquired from patients and directly analyzed within one hour for the presence of infectious agents. Rotavirus, adenovirus, and *Entamoeba histolytica* kit tests (CerTest; CerTest Biotec, Zaragoza, Spain) were used to detect possible enteropathogens. As an additional diagnostic tool, which is not routinely used in the diagnosis of acute gastroenteritis in hospitals, stool samples were cultured for the detection of *Campylobacter*.

Campylobacter culture

Campylobacter stool cultures were performed on the selective medium "charcoal cefoperazone desoxycholate agar" (Liofilchem, Roseto degli Abruzzi, Italy) containing cefoperazone and amphotericin B (32 mg/L and 10 mg/L, respectively). After sample streaking, the plates were incubated micro-aerobically (6% O_2) at 37°C for 48 hours. Suspect colonies (small, gray, and translucent colonies [12 mm in diameter] with a metallic sheen and butter-like consistency) were identified at the *Campylobacter* genus level using oxidase and catalase tests and the Gram stain technique (gram-negative rods, oxidase positive, and catalase positive).

Sample size and power of the study

Sample size calculations were performed to calculate the prevalence rate based on the following assumptions:
- An effect size-g-of 6%. The value of the constant proportion was set conservatively to 11.1% for the prevalence rate of *Campylobacter* infection in children in the region of North Lebanon [15].
- An alpha, type 1 error, balanced on each side, set to 5%.
- A type 2 error of 20%, yielding a statistical power of 80%.

Using the G*Power 3.1.9.2 software [16], the exact binomial distribution yielded 255 subjects as the minimum required sample size to satisfy the above assumptions.

Statistical analysis

Descriptive statistics were carried out and reported as numbers and percentages for categorical variables, whereas mean and standard deviation (±) were used for continuous variables. Patients' clinical characteristics were tabulated and comparisons between the two groups

(*Campylobacter*-positive vs. *Campylobacter*-negative patients) were made using the Mann–Whitney test for continuous variables. To evaluate whether a significant difference was present between the categorical variables, the chi-squared test was used. We determined the associations between *Campylobacter* cases (yes/no) as the dependent variable and sex, breastfeeding, daycare, fever, and antibiotic use before hospitalization as independent variables using logistic regression analyses. Normality was tested using the Kolmogorov–Smirnov test. Statistical analysis was performed using SPSS Statistics for Windows, Version 22.0 (IBM Co., Armonk, NY, USA). The level of significance was set at $p<0.05$ for all statistical analyses.

RESULTS

Socio-demographic and clinical characteristics of patients

This study included 291 hospitalized children with acute gastroenteritis aged between 1 month and 12 years. Of these, 169 (58%) were male and 122 (42%) were female. The age distribution of our study participants was as follows: 3.43% aged 1–3 months, 19.58% aged 4–11 months, 24.74% aged 12–23 months, 15.46% aged 24–35 months, 10.99% aged 36–47 months, 5.49% aged 48–59 months, and 20.27% aged >60 months, which was equivalent to 5 years of age (**Table 1**).

Examination of the stool specimens revealed that 35 out of 291 samples (12.02%) were positive for *Campylobacter* spp., while the rest were positive for non-*Campylobacter* enteropathogens, including viruses (such as rotavirus and adenovirus), parasites (such as *Entamoeba histolytica*), and other bacteria. The percentages of *Campylobacter*-positive patients among hospitalized males and females were similar (12.4% vs. 11.5%, respectively). With regard to age distribution, the highest rates of infection were observed in the 36–47 month group (25%), with no statistical difference observed between the groups.

The clinical characteristics of the patients are summarized in **Table 2**. Our results revealed statistically significant differences between the study groups with respect to fever,

Table 1. Socio-demographic characteristics of patients

Demographic	Campylobacteriosis		Total gastroenteritis	*p*-value
	Yes	No		
Age (mo)				0.067
1–3	2 (5.71)	8 (3.13)	10	
4–11	8 (22.86)	49 (19.14)	57	
12–23	4 (11.43)	68 (26.56)	72	
24–35	2 (5.71)	43 (16.79)	45	
36–47	8 (22.86)	24 (9.38)	32	
48–59	2 (5.71)	14 (5.47)	16	
≥60	9 (25.72)	50 (19.53)	59	
Sex				0.806
Female	14 (40.00)	108 (42.19)	122	
Male	21 (60.00)	148 (57.81)	169	
Breastfeeding*				0.798
No	3 (60.00)	52 (54.17)	55	
Yes	2 (40.00)	44 (45.83)	46	
Daycare*				0.102
No	1 (20.00)	55 (57.29)	56	
Yes	4 (80.00)	41 (42.71)	45	
Mean family size	4.20±0.45	4.43±1.19	4.42	0.927

Values are presented as number (%) or mean±standard deviation.
*Missing data.

stool texture, diarrhea duration, and dehydration, among other variables. Interestingly, *Campylobacter*-positive patients presenting with high-grade fever (≥38.5℃) comprised a significantly higher percentage (52.38%) than those with low-grade fever (<38.5℃) (17.86%), and *Campylobacter*-negative patients mainly had low-grade fever (*p*=0.001). Regarding stool texture, the majority of the Campylobacter-positive patients had mucoidal (56.52%) diarrhea, whereas most of the *Campylobacter*-negative patients had watery diarrhea (50.66%) (*p*<0.001). Furthermore, the results showed that a significantly higher percentage of *Campylobacter*-positive patients passed stool >6 times per day (22.22%) or 4–5 times a day (66.67%). Whereas, 38.66% and 54.64% of *Campylobacter*-negative patients passed stool 1–3 times or 4–5 times a day, respectively (*p*=0.002). Almost 25.93% of patients in the *Campylobacter*-positive group experienced diarrhea for ≥5 days, while barely 2.58% of patients in the *Campylobacter*-

Table 2. Clinical characteristics of patients

Clinical characteristic	Campylobacteriosis		Total gastroenteritis	*p*-value
	Yes	No		
Stool texture*,†				<0.001
Mucoidal	13 (56.52)	40 (26.32)	53	
Watery	0 (0.00)	77 (50.66)	77	
Bloody	8 (34.78)	32 (21.05)	40	
Semi-solid	0 (0.00)	3 (1.97)	3	
Bloody mucoidal	2 (8.70)	0 (0.00)	2	
Fever*				0.001
Low-grade fever (<38.5℃)	5 (17.86)	110 (52.38)	115	
High-grade fever (≥38.5℃)	23 (82.14)	100 (47.62)	123	
Abdominal pain*				0.077
No	0 (0.00)	19 (14.39)	19	
Yes	19 (100.00)	113 (85.61)	132	
Diarrhea				0.456
No	0 (0.00)	4 (1.56)	4	
Yes	35 (100.00)	252 (98.44)	287	
Diarrhea episodes per day*				0.002
1–3	3 (11.11)	75 (38.66)	78	
4–5	18 (66.67)	106 (54.64)	124	
≥6	6 (22.22)	13 (6.70)	19	
Diarrhea duration (d)*				<0.001
1–4	20 (74.07)	189 (97.42)	209	
≥5	7 (25.93)	5 (2.58)	12	
Vomiting				0.767
No	15 (42.86)	103 (40.23)	118	
Yes	20 (57.14)	153 (59.77)	173	
Vomiting episodes per day*				0.815
0	15 (53.57)	103 (47.69)	118	
1	3 (10.71)	30 (13.89)	33	
2–4	10 (35.72)	83 (38.42)	93	
Vomiting duration (d)*				0.134
0	15 (53.57)	103 (47.68)	118	
1	6 (21.43)	87 (40.28)	93	
2	6 (21.43)	22 (10.19)	28	
≥3	1 (3.57)	4 (1.85)	5	
Severity of dehydration (%)*				0.004
No	13 (38.23)	160 (64.26)	173	
1–5	21 (61.77)	82 (32.93)	103	
≥6	0 (0.00)	7 (2.81)	7	
Antibiotic before hospitalization				0.002
No	34 (97.14)	188 (73.44)	222	
Yes	1 (2.86)	68 (26.56)	69	

Values are presented as number (%).
*Missing data. †Stool texture was classified based on a combination of clinical experience and scientific evidence based on the Bristol stool chart.

Table 3. Clinical and laboratory data of patients

Clinical and laboratory characteristic	Campylobacteriosis		Total gastroenteritis	p-value
	Yes	No		
Frequency (%)	35 (12.0)	256 (88.0)	291	-
Body temperature (°C)	39.23±0.90	38.28±0.99	38.39±1.02	<0.001
Vesikari score	11.37±2.76	9.92±2.50	10.09±2.57	0.004
Duration of hospitalization (hr)	66.31±18.30	64.78±20.83	64.90±20.61	
Laboratory findings				0.885
PLT (×10³ per mm³)	234.00±103.59	283.15±80.34	280.71±81.71	0.343
ANC (×10² per mm³)	51.62±19.71	62.21±16.07	61.41±16.55	0.026
HGB (g/dL)	11.45±0.83	11.48±1.11	11.48±1.09	0.847
BS (mg/dL)	85.00±19.54	92.71±20.73	91.81±20.52	0.426
CRP (mg/L)	33.32±24.01	27.36±28.72	27.83±28.35	0.180
HCO₃ (mEq/L)	17.57±2.07	17.19±2.98	17.24±2.86	0.948
BUN (mg/dL)	9.71±3.19	11.68±5.39	11.47±5.22	0.438
Creatinine (mg/dL)	0.26±0.06	0.99±3.87	0.92±3.65	0.788

Values are presented as number (%) or mean±standard deviation.
PLT: platelets, ANC: absolute neutrophil count, HGB: hemoglobin, BS: blood sugar, CRP: C-reactive protein, BUN: blood urea nitrogen.

negative group showed a significant difference ($p<0.001$). Lastly, it is noteworthy that while dehydration represented a major presenting clinical sign in *Campylobacter*-positive patients (61.77%), the majority of patients did not have dehydration (64.26%) ($p=0.004$).

The laboratory and clinical findings are outlined in **Table 3**. Our results revealed that the mean body temperature of *Campylobacter*-positive patients was significantly higher by almost 1°C than that of *Campylobacter*-negative patients (39.23±0.90 vs. 38.28±0.99; $p<0.001$). As expected, following the analysis of the previously mentioned clinical characteristics (diarrhea, vomiting, dehydration, and body temperature), the VSS showed a severe profile of acute gastroenteritis in *Campylobacter*-positive patients (11.37±2.76) compared to a moderate profile of acute gastroenteritis in *Campylobacter*-negative patients (9.92±2.50) ($p=0.004$).

The severity of gastroenteritis based on the VSS

As shown in **Fig. 1**, no mild gastroenteritis cases were observed in patients infected with *Campylobacter*. In contrast, those patients showed moderate and severe profiles with a higher percentage of severe cases (57.1%) compared to *Campylobacter*-negative patients (41.0%).
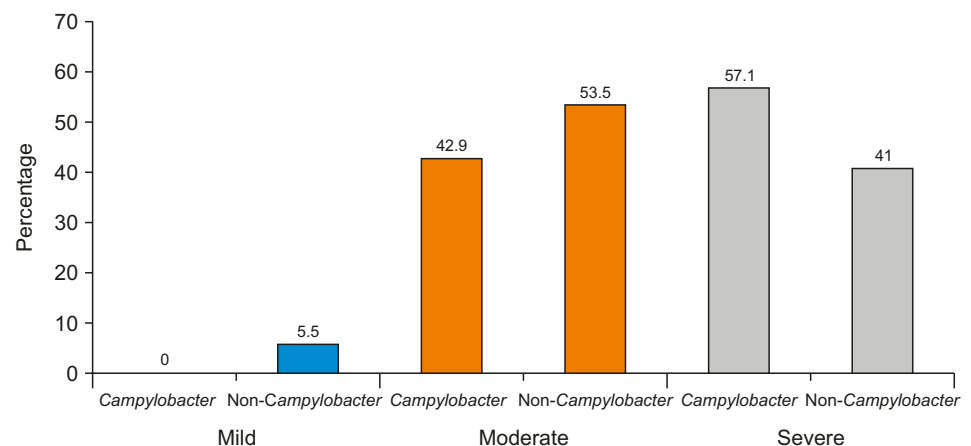

Fig. 1. The severity of gastroenteritis in *Campylobacter*-negative and *Campylobacter*-positive patients based on the Vesikari score system (Severity rating scales: mild, <7; moderate, 7–10; severe, ≥11).

Table 4. Predictors of *Campylobacter* infection among the 291 pediatric patients enrolled in the study

Clinical characteristic	Campylobacteriosis		Unadjusted	
	Yes	No	OR (95% CI)	*p*-value
Sex				
Female	14 (11.5)	108 (88.5)	1	
Male	21 (12.4)	148 (87.6)	1.095 (0.533–2.249)	0.806
Breastfeeding*				
No	3 (5.5)	52 (94.5)	1	
Yes	2 (4.3)	44 (95.7)	0.788 (0.126–4.930)	0.799
Daycare*				
No	1 (1.8)	55 (98.2)	1	
Yes	4 (8.9)	41 (91.1)	5.366 (0.578–49.817)	0.139
Fever*				
Low-grade fever (<38.5°C)	5 (4.3)	110 (95.7)	1	
High-grade fever (≥38.5°C)	23 (18.7)	100 (81.3)	5.060 (1.853–13.814)	0.002
Antibiotic before hospitalization				
No	34 (15.3)	188 (84.7)	1	
Yes	1 (1.4)	68 (98.6)	0.081 (0.011–0.606)	0.014

Values are presented as number (%).
OR: odds ratio, CI: confidence interval.
*Missing data.

Laboratory characteristics of patients

Infectious markers such as ANC and CRP levels were also measured in the patients' peripheral blood (**Table 3**). Interestingly, *Campylobacter*-positive patients were found to have significantly lower ANC than *Campylobacter*-negative patients (*p*=0.026). In contrast, children with campylobacteriosis had higher levels of CRP than *Campylobacter*-negative patients, though the difference was not statistically significant (*p*=0.180). As for other laboratory findings, there were no significant differences between the two groups.

Prevalence and predictors of *Campylobacter* infection

Among the predictors for *Campylobacter* infection shown in **Table 4**, sex, breastfeeding, and daycare appeared to have no significant association with *Campylobacter* culture positivity. In contrast, children with high-grade fever (≥38.5°C) were 5-folds more likely to be positive for *Campylobacter* than those with low-grade fever (odds ratio [OR]=5; confidence interval [CI]=1.8–13.8; *p*=0.002). In addition, children who had been treated with antibiotics prior to hospitalization were less likely to be tested positive for *Campylobacter* (OR=0.08; CI=0.01–0.6; *p*=0.014) compared to non-treated children.

DISCUSSION

Acute gastroenteritis is the second major cause of pediatric morbidity and mortality following acute lower respiratory tract infections in Lebanon and other developing countries [17]. Identifying specific enteropathogens responsible for acute gastroenteritis is essential for the application of appropriate and suitable clinical measures to manage the disease. It has been shown that infection with *Campylobacter* species is the leading cause of common bacterial gastroenteritis [4]. This study aimed to investigate *Campylobacter* infection over a period of 6 months in hospitalized children in a rural area of South Lebanon and to examine the relationship between campylobacteriosis, its severity, and routine laboratory tests. To the best of our knowledge, this study is one of the few to investigate the prevalence and occurrence of campylobacteriosis in Lebanon [13,15,18], and the first study conducted on children in South Lebanon.

We previously reported causative enteropathogens for acute gastroenteritis that are routinely tested in children [17,19,20], and showed that *E. histolytica*, rotavirus, and adenovirus are the main agents. The present study shows an additional diagnostic tool for the detection of *Campylobacter*, which is not routinely performed in gastroenteritis cases in Lebanese hospitals. The prevalence of campylobacteriosis in pediatric patients with acute gastroenteritis enrolled in our study was 12.02%, which was very close to that reported by Dabboussi et al. [15] in 2012 (11.1%), who evaluated the frequency of *Campylobacter* infection in children in the region of North Lebanon. Moreover, our results are similar to those reported in other countries, such as Papua New Guinea (Oceania) [21] and Kolkata, India [22], where the prevalence of *Campylobacter* among hospitalized children with gastroenteritis was 12% and 10%, respectively. Our findings were also similar to those of previous studies, such as those in Mexico (15.7%) and China (14.9%) [3].

Since *Campylobacter* is recognized as an essential foodborne pathogen associated with human gastroenteritis, mainly from contaminated poultry, our findings could be supported by several studies previously conducted in Lebanon and revealed a high degree of contamination in broiler chickens collected from Lebanese slaughterhouses [13,23-25].

Among the *Campylobacter*-positive patients, there were more male (60%) than female (40%), which was in agreement with the results reported by Sadiq et al. [26] in 2019; the study reported that the prevalence of *C. jejuni* was statistically higher in men than in women [26]. Moreover, our results revealed a more severe status of acute gastroenteritis in patients infected with *Campylobacter* than in those infected with other enteropathogens. This severe profile was characterized by a higher percentage of patients with high-grade fever, diarrhea episodes occurring more than six times per day, diarrhea lasting for more than five days, and dehydration. In addition, our results revealed that the mean body temperature of *Campylobacter*-positive patients was significantly higher by almost 1°C than that of *Campylobacter*-negative patients. Although this increase might not seem clinically relevant, it is important to report it as it could define the severity of high-grade fever. Notably, a high proportion of *Campylobacter*-negative patients tested positive for viral infection (adenovirus and rotavirus). In accordance, Shim et al. [27] reported that bacterial gastroenteritis is associated with more episodes of diarrhea and longer-lasting diarrhea than common viral infections. Consequently, our data resulted in a higher VSS score for *Campylobacter*-positive patients, showing a severe profile of acute gastroenteritis compared to a moderate profile for *Campylobacter*-negative patients, which is consistent with our previous results on rotavirus and adenovirus cases showing a lower severity of acute gastroenteritis in children when compared to other enteropathogens [17].

Infectious markers, such as the ANC and CRP levels, were also measured using the patients' peripheral blood. Although previous data have shown that the accumulation of neutrophils is part of the classical response of the innate immune system to campylobacteriosis [28], our findings demonstrated that the ANC was significantly lower in *Campylobacter*-positive patients. This could be explained by the fact that *Campylobacter*-negative patients were, as mentioned above, tested positive for other enteropathogens, namely rotavirus and adenovirus, that might have been more invasive, leading to a more pronounced acute inflammation. Our results also showed that children with campylobacteriosis presented with higher levels of CRP compared to *Campylobacter*-negative patients; however, the difference was not significant. The significance of the statistical tests was more pronounced with the analysis of the VSS parameters discussed above, compared to ANC and CRP level analysis,

which is in agreement with previous findings showing an advantage of the VSS over CRP and ANC in the detection of bacterial acute gastroenteritis [27].

Our study had several limitations. First, it was conducted within a period of six months, hence it lacked the seasonality parameter that had been previously shown to affect acute gastroenteritis cases when evaluated over a complete year [13,17]. Second, the antibiogram test was not performed to evaluate the susceptibility of *Campylobacter* strains to commonly prescribed antibiotics. Third, identification tests were not performed to discriminate between *Campylobacter* species. This could have been achieved by a standardized hippurate hydrolysis test and PCR technique. Fourth, some data were missing from the medical records of the patients (marked using asterisks in the corresponding tables). Fifth, the percentage of other enteropathogens causing non-*Campylobacter* gastroenteritis was not identified. Our group is currently conducting another study on a larger cohort of pediatric patients covering a one-year period to assess the non-*Campylobacter* group separately and more thoroughly to identify viral, bacterial, and parasitic infections causing gastroenteritis in this subgroup.

In conclusion, the present study highlights that *Campylobacter* infection is frequent among children with acute gastroenteritis. We believe that the detection of *Campylobacter* should be tested for the diagnosis of human gastroenteritis in Lebanon, along with routine enteropathogens.

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