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Campylobacter Data in Childhood from a University Hospital

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Authors' contributions

This work was carried out in collaboration between all authors. Author SB conceived of the study and participated in its design and coordination and drafted the manuscript. Author TG participated in the design of the study and collection and acquisition of data. Author DÇ helped to the design of the study, collection and acquisition of data and helped to draft the manuscript. Author GÇE helped to collection and acquisition of data. Author AV involved acquisition of funding, general supervision of the research group, coordination and helped to draft the manuscript. Authors YG and GÇ carried out bacterial culture and molecular analysis. Authors MU and ÇK performed the statistical analysis. Authors ÖK and ZY conceived of the study, participated in its design and helped to draft the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objectives: Due to underdiagnosis because of the technical difficulties plus inadequacy of laboratories, actual incidence of campylobacteriosis may substantially be greater than the reported incidence in many countries including Turkey. The purpose of this study was to evaluate and emphasize the diagnostic methods of campylobacteriosis, and the clinical and laboratory data of children with Campylobacterial gastroenteritis.

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Methods: This study was conducted in Yeditepe University Hospital, Istanbul, Turkey. Clinical (demographical data, symptoms and findings) and laboratory (stool microscopy, rapid antigen tests, culture, and multiplex PCR and blood test results) variables of children with *Campylobacter* infection between January 2010 and October 2012 were evaluated retrospectively from the hospital database.
Results: Out of 1275 stool cultures, *Campylobacter* spp. was detected in 90 of them (7%). The diagnosis was made by positive stool culture (n = 87) and/or multiplex polymerase chain reaction (PCR) test (n = 8, whereas 3 of them were culture negative). The distribution of *Campylobacter* isolates were; *C. jejuni* (85.5%), *C. upsaliensis* (8.9%), *C. coli* (1.1%), and others (4.5%). The presenting symptoms were diarrhea (100%), fever (68.9%), abdominal pain (34.4%), dehydration (27.8%), vomiting (25.5%), bloody diarrhea (5.6%), and convulsion (1%). Hospitalization was required in 25.5% of patients.
Conclusions: Although stool culture is a reference method in diagnosis, the PCR test can be used in culture negative patients with clinical manifestations. Diarrhea, fever, abdominal pain, and vomiting were most commonly encountered symptoms whereas bloody diarrhea and convulsion were rarely seen in campylobacteriosis. Also antibiotherapy and hospitalisation were not commonly required.

Keywords: Campylobacteriosis; child; polymerase chain reaction; stool culture.

1. INTRODUCTION

Campylobacter species (*C.* spp.) are primarily zoonotic, with a variety of animals implicated as reservoirs for human infection. *C.* spp. are common and important causes of acute bacterial gastroenteritis in both children and adults [1-4]. Campylobacterium was the third bacterial agent causing foodborne illness in the USA and *C.* spp. are one of the important causes of acute gastroenteritis among children, adolescents, and adults in both developed and underdeveloped countries [1,2,4]. Definite diagnosis requires isolation of bacterium in culture (i.e. "gold standard") or polymerase chain reaction (PCR). Due to underdiagnosis because of the technical difficulties plus inadequacy of laboratories, actual incidence of campylobacteriosis may substantially be greater than the reported incidence in many countries including Turkey [5-10]. *Campylobacter* species are often identified by phenotypic tests, but due to the weak biochemical activities of these bacteria, the correct definition of *Campylobacter* species may need to be analysed by molecular analysis. Improvements on molecular methods provide facilitation to diagnose many bacteria including *C.* spp. Clinical and epidemiological advantages of molecular methods in diagnosis preclude the disadvantage of high cost [11,12].

The purpose of this study was to evaluate and emphasize the diagnostic methods of campylobacteriosis, and the clinical and laboratory data of children with Campylobacterial gastroenteritis.

2. MATERIALS AND METHODS

This study was ethically approved by local ethical committee of Yeditepe University Hospital (date: 02.06.2009). Clinical (demographical data, symptoms and findings) and laboratory (stool microscopy, rapid antigen tests, culture, and multiplex PCR and blood test results) variables of children with *Campylobacter* infection between January 2010 and October 2012 were evaluated retrospectively from the hospital database. One thousand two hundred and seventy-five patient files were revised. Stool samples were cultured in *Campylobacter*-BAP medium (Salubris, Turkey) and incubated under microaerophilic conditions (CampyGen, Oxoid, UK) at 42℃ for 48 hours. Identification tests for suspected colonies were done according to conventional biochemical tests by Biomerieux API CAMPY test (Biomerieux, France) and some samples were also tested by Seeplex Diarrhae ACE detection PCR kit (Seeplex,Korea). Continuous and categorical variables were calculated as mean ± SD and frequency (%), respectively. Differences between the groups for categorical variables were evaluated with chi-square or Fisher exact test, and a t test for continuous variables with two independent samples. *P* values < .05 were considered statistically significant.

3. RESULTS

Out of 1275 stool cultures, *C.* spp. were detected in 90 of them (7%). The distribution of *Campylobacter* isolates were; *C. jejuni* (85.5%), *C. coli* (5.5%), *C. upsaliensis* (4.5%), and others

(4.5%). *C. jejuni* was the most commonly detected species in all years (*P*=.02) (Table 1). *C. jejuni* was detected in all months, whereas *C. upsaliensis* was detected only in May (n=3) and July (n=1). The seasonal distribution of all cases were mainly in May (22.2%), January (15.6%), March (12.2%), and July (12.2%) in our study (Fig. 1).

The definite diagnosis was made by positive stool culture (n=87) and/or PCR test (n=8, whereas 3 of them were culture negative).

The mean age of children with campylobacteriosis was 67.96 ± 47.9 months, 56 of patients were (62.2%) males and 34 (37.8%) females with a male/female ratio of 1.6. Age distribution of cases was not significant (Table 2).

The main presenting symptoms were diarrhea (100%), fever (68.9%), abdominal pain (34.4%), dehydration (27.8%), vomiting (25.5%), bloody diarrhea (5.6%), and convulsion (1%). There was no difference between symptoms other than fever according to hospitalisation status. Fever was more common in hospitalised patients (*P* = .01) (Table 3). Also fever was most prominent symptom (100%) among patients who had longer hospitalisation time (*P* = .01).

The mean leukocyte, neutrophil count, CRP, and erythrocyte sedimentation rate were 12.976/µL, 8.930/µL, 59.04 mg/L, and 29.65 mm/hour, respectively. Leukocytosis and neutrophilia were detected in 17 (18.9%) and 23 (25.5%) patients, respectively. Hospitalisation was required in 25.5% of the patients (Table 4). Most of the non-hospitalised patients were not on antibiotheraphy and duration of hospitalisation due to *Campylobacter* species and patient age groups were statistically not significant (Tables 4 and 5).

Table 1. Differentiation of *Campylobacter* isolates according to years

***Campylobacter* species**	**n (%)**			**Total (n)**
	2010	2011	2012	
Campylobacter jejuni	42 (93.3)	19 (65.5)	16 (100.0)	77*
Campylobacter coli	1	4	0	5
Campylobacter upsaliensis	0	4 (27.6)	0	4
Other *Campylobacter* spp.	2 (4.4)	2 (6.9)	0	4
Total	45	29	16	90

**: P = .02*

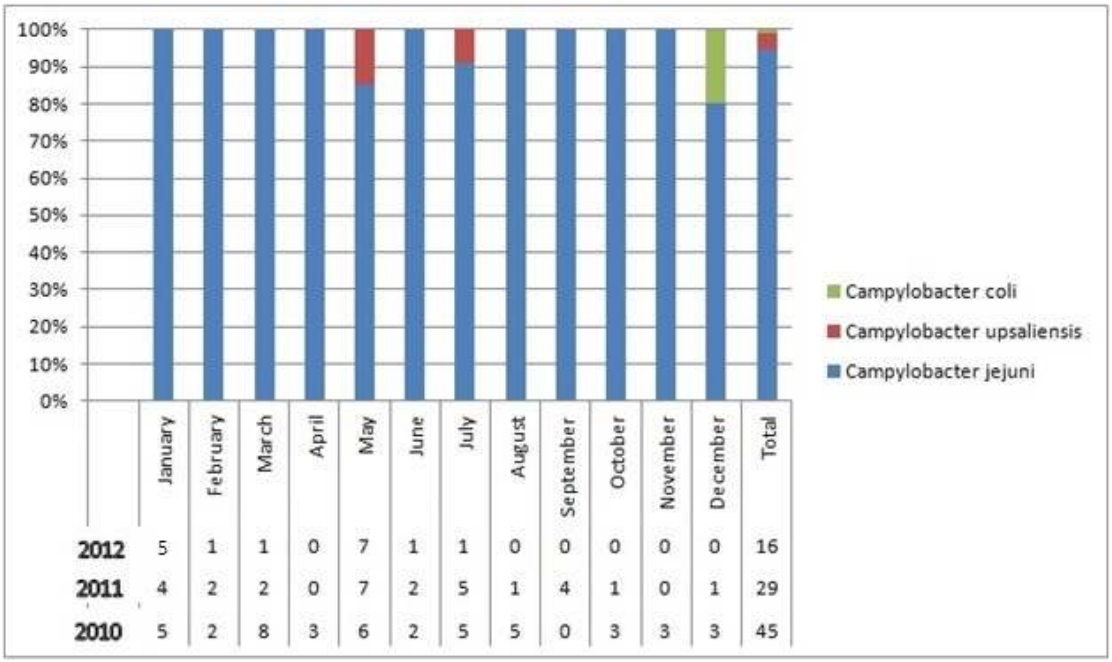

Fig. 1. Seasonal distribution of campylobacteriosis according to months in 2010-2012

Table 2. Age distribution of cases was not statistically different between *Campylobacter* species

	≤ 5 years (n)	> 5 years (n)	Total* (n)
Campylobacter jejuni	44	33	77
Campylobacter upsaliensis	5	3	8
Other *Campylobacter* spp.	2	3	5
Total	51	39	90

**: (Min.- max: 9 – 204 months, mean ± SD: 67.96 ± 47.98)*

Table 3. Distribution of symptoms according to *Campylobacter* species, hospitalisation status of patients, and age groups

Symptoms	Hospitalisation (%)		≤ 5 years n (%)	> 5 years n (%)	Total (n)
	Yes	No			
Vomiting	34.8	22.4	9 (17.6)	14 (35.9)*	23
Abdominal pain	43.5	31.3	13 (25.5)	18 (46.2)†	31
Fever (≥ 38℃)	95.7‡	59.7	31 (60.8)	31 (79.5)	62
Bloody diarrhea	8.7	4.5	5.9	5.1	5
Convulsion	4.3	0	2.0	0	1
Dehydration degree	6		13	12	25
- Mild	17.9	13	9.8	25.6†	15
- Moderate	10.4	13	15.7	5.1	10
Total (n)			51	39	

**: P = .05; †: P = .041; ‡: P = .01*

Table 4. Antibiotic use in patients according to age groups and hospitalisation status

		≤ 5 years n (%)	> 5 years n (%)	Hospitalisation (%)		Total n (%)
				Yes	No	
Antibiotheraphy	Yes	14 (27.5)	19 (48.7)	19 (82.6)	14 (20.9)	33 (36.6)
	No	37 (72.5)	20 (51.3)	4 (17.4)*	53 (79.1)	57 (63.4)
Total		51 (100)	39 (100)	23 (100)	67 (100)	90 (100)

**: P = .000*

Table 5. Duration of hospitalisation according to *Campylobacter* species and age groups

	Not hospitalised (n)	Hospitalised (n)	Duration of hospitalization (n)		
			1 day	2 day	3 day
C. jejuni	50	11	10	0	1
C. upsaliensis	5	3	3	0	0
Other *C.* spp.	3	2	1	0	1
≤ 5 years	30	21	9	8	4
> 5 years	28	11	5	6	0
Total	58	32	14	14	4

4. DISCUSSION

C. spp. were first recognized in humans in 1970 and since then their importance has increased and now they are considered as one of the frequent bacterial causes of acute gastroenteritis [11-13]. Despite high concern for *Campylobacter* infections, they are underestimated due to limited investigations in laboratories in Turkey. For the purpose of receiving further information about etiologic agents of bacterial gastroenteritis, *C.* spp. has been studied at our university microbiology laboratory since 2010.

Campylobacteriosis is a foodborne infection which is acquired from contaminated water, milk, and meat. Also it is a zoonotic infection which is spread by pets and wild animals [2,4,14,15]. *C.* spp. have been isolated in various prevalences according to the geographical location and climate of a country, developmental and socio-economical degree of the people,

water and food hygiene, food preparation methods, and study population [15]. Prevalence of campylobacteriosis was reported as 13/100,000 in developed countries, whereas it is reported as 5% - 20% in developing countries [1,2,4,14]. Isolation rates of *C.* spp. were 6.3% and 15.4% in The Netherlands and Ethiopia, respectively [1,2,4,16,17]. In Turkey, *C. spp.* was reported increasingly in the last 30 years, isolation rates were between 1% and 8.8% in cases with acute gastroenteritis [4,18,19]. *C.* spp. was isolated in 7% of all cases in our study. Kayman et al. [15] have shown that 70.9% of cases with campylobacteriosis were detected in children, *C. jejuni* and *C. coli* were detected in 90.5% and 9.5%, respectively. Lengerh et al. [16] detected the rates of campylobacteriosis as 15.4% among children with acute gastroenteritis in Ethiopia. The isolation rate of *C. spp.* among the etiological agents in childhood acute gastroenteritis was notified as 9.3% from Uganda [11,12].

There are many species of *Campylobacteria* causing diarrhea in children. *C. jejuni*, *C. coli*, *C. fetus*, and *C. lari*. [15,22], *C. jejuni, C. upsaliensis*, and *C. coli* are the species (85%, 8.9%, and 1.1%, respectively) isolated from specimens in our study. We may suggest that the prevalence of gastroenteritis due to *Campylobacter* infection among children in northern Turkey is similar to other countries [20,21]. The reported rates of *C. jejuni* were 85.8% and 76.2%, and *C. coli* were 14.2% and 17.2%, in Iran and France, respectively [22,23]. Lengerh et al. [16] reported the isolated rates of *C.* spp. as *C. jejuni* (90.9%) and *C. coli* (9.1%). Mshana et al. [12] reported the same results from Uganda, *C. jejuni* was the most commonly (80.9%) detected species, others were *C. lari* (9.5%), and *C. coli* (4.8%) in that study. The distribution of *C.* spp. in our study revealed mainly *C. jejuni* (85%), *C. upsaliensis* (8.9%), *C. coli* (1.1%), and others (4.5%).

Campylobacteriosis can occur throughout the year. The seasonal distribution of campylobacteriosis may vary according to geographical area and season [24,25]. Prevalence of campylobacteriosis had shown a peak in the summer months in some literatures [2,4,14,20,26]. Kayman et al. [15] have shown that *C.* spp. were isolated mainly from March to June whereas Hou et al. [3] have shown the peak season of *C. jejuni* to be from May to October in China. Van Hees et al. [16] found a higher percentage in winter (8.2%) than summer (4.3%) in The Netherlands. Although campylobacteriosis was mainly observed in winter in some studies [17,27,28], our cases were mainly distributed in May (22.2%) and in January (15.6%). *C. jejuni* was detected in all months, whereas *C. upsaliensis* was detected only in May and July in our study.

The distribution of *Campylobacter* isolates were; *C. jejuni* (85.5%), *C. upsaliensis* (8.9%), *C. coli* (1.1%), and others (4.5%). The percentages of *C. jejuni* and *C. coli* were reported as the rates of 85.8% and 14.2% in Iran [22], 76.2% and 17.2% in France [23], respectively. In these studies, *C. coli was* detected at higher percentages compared to our study. It may be concluded that for obtaining the precise rates of *C.* spp, PCR method should be used more for the classification [29,30].

The spectrum of acute gastroenteritis ranges from a watery, nonbloody, noninflammatory diarrhea to a severe inflammatory bloody diarrhea, abdominal pain and fever [2,4]. The main presenting symptoms of our patients were diarrhea, fever, abdominal pain, dehydration, vomiting, bloody diarrhea, and convulsion. Yang et al. [28] have found that fever was the most remarkable symptom in patients with campylobacteriosis as in our study (68.9%). It was presented as 63.9% and 40.9% by Yang et al. [28] and Lengerh et al. [16]. Abdominal pain and vomiting were the most common symptoms in the Lengerh's study [16] compared with ours. Although bloody diarrhea was one of the rare symptoms in our patients, it was more commonly (41%) reported from Taiwan [28]. This symptom was notified by Özen et al. [31] in an infant with campylobacteriosis.

The rate of hospitalisation and using antibiotics were low in our patients. Disease severity is more violent in developed countries than in developing countries [2,4,18,19]. The low percentages of bloody diarrhea, abdominal pain, leukocytosis, neutrophilia, hospitalisation, and antibiotherapy demonstrated that noninflammatory diarrhea is mainly presented as a clinical picture in our patients. This may be attributable to the moderate to high income levels of the patients in our study.

5. CONCLUSIONS

Although stool culture is a reference method in diagnosis, the multiplex PCR test may be used in culture negative patients with clinical

manifestations of acute bacterial gastroenteritis including campylobacteriosis. *C. jejuni* was the most common species in patients with campylobacteriosis. Diarrhea, fever, abdominal pain, and vomiting were most commonly encountered symptoms whereas bloody diarrhea and convulsion were rarely seen in campylobacteriosis among patients with moderate to high income levels. Also antibiotherapy and hospitalisation were not commonly required in these patients.

CONSENT

Written informed consent was obtained from all families of the patients of the study.

DISCLAIMER

This study was presented as e-poster in 8th World Congress of the World Society for Pediatric Infectious Diseases (WSPID) European Academy of Paediatric Societies, November 19-22, 2013, Cape Town, South Africa.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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