International Journal of
Enteric
Pathogens

Int J Enteric Pathog. 2016 August;4(3):e35684 Original Article

http://enterpathog.com

doi 10.15171/ijep.2016.05

Clinical Profile and Epidemiology of *Campylobacter* Associated Diarrhea Among Children in New Delhi, India

Roumi Ghosh[1*], Beena Uppal[2], Prabhav Aggarwal[2], Anita Chakravarti[2], Anand Prakash Dubey[3]

[1]Department of Microbiology, Institute of Post Graduate Medical Education and Research,West Bengal, India
[2]Department of Microbiology, Maulana Azad Medical College, New Delhi, India
[3]Department of Paediatrics, Maulana Azad Medical College, New Delhi, India

***Corresponding Author:**
Roumi Ghosh,
Tel: +91-9831895592;
Email: roumighosh@gmail.com

Published Online May 1, 2016

Keywords: *Campylobacter*, Diarrhea, Polymicrobial infection, Pediatrics

Abstract

Background: *Campylobacter*, a well-known enteropathogen among children shows variable clinical presentations. Age groups and seasonal distribution is dependent on geographical position.

Objectives: To explore clinical manifestations and seasonal variation of *Campylobacter* infection and to study its importance as enteric pathogen among children.

Patients and Methods: Two hundred five children (≤12 years age) having acute diarrhea as cases and 100 children without from diarrhea were taken as control. All the fecal samples were processed for *Campylobacter* species by culture on to modified charcoal cefoperazone deoxycholate agar and Skirrow's Columbia blood agar media. Detection of *Campylobacter* specific antigen in faecal samples was also done by enzyme-immuno assay.

Results: A total of 32 (15.61%) faecal samples of children with diarrhea had positive results for Campylobacter spp. Among them 31.25% cases had polymicrobial infections. Children below 1 year were most commonly (18.96%) affected by the infection. The organism was isolated throughout the year with a higher isolation rates during summer and monsoon months. Watery diarrhea was significantly more common in the *Campylobacter* infected cases.

Conclusions: Application of antigen assay increases detection rate of *Campylobacter* enteritis cases, which was significantly higher than the control group ($P<.05$). Specific clinical profile could not be associated with this infection which, indicates need of microbiological diagnosis of this pathogen for antibiotic therapy.

Received December 22, 2016; **Revised** January 5, 2016; **Accepted** January 10, 2016

Background

Campylobacter species are primarily zoonotic, with a wide variety of wild and domestic animals, especially birds implicated as reservoir. They mainly cause food-borne gastroenteritis following ingestion of chicken, raw milk, untreated water and contact with pets, especially household live chickens. Increasing trend of *Campylobacter* infections have been seen in developed countries for years and it accounts for one of the most common bacterial causes of diarrhea, with an incidence around 10% among diarrhea patients.[1] In recent times there are many reports from developing countries describing *Campylobacter jejuni* and *Campylobacter coli* as important enteropathogen during first 5 years of life with isolation rate ranging from 10% to 46%.[2-5] Though, the epidemiology, clinical presentations and microbial profile are not similar in these two economical world territories.[6] Application of newer, sensitive molecular diagnostic methods beside culture might play role behind the increasing detection rate of this fastidious, microaerophilic organism.[7,8] It is present as colonized gastrointestinal flora and also as asymptomatic carrier commonly in children, but it causes severe gastroenteritis among young children <2 years old, elderly or immunocompromised patients and may require antibiotic therapy. Thus, various virulence markers had been studied to prove its pathogenicity.[9] Clinically, *Campylobacter* infection is indistinguishable from acute gastrointestinal infections produced by other bacterial pathogens. In some patients, the diarrhea is minimal and abdominal cramps and pains are the predominant features; this can lead to a mistaken diagnosis of acute abdomen and unnecessary laparotomy. Fluoroquinolones and macrolides are main parts of treatment used for this infection; however, marked increase in fluoroquinolone resistance and presence of high level azithromycin resistance in *Campylobacter* isolates are becoming threat in In-

dia.[10,11] The most important post-infectious complication of *C. jejuni* infection is the Guillain-Barré syndrome that affects 1–2 persons per 100 000 populations in the United States each year.[6]

Objectives

The present study was designed to investigate clinical manifestations and epidemiology of Campylobacter infection and to study the importance of *C. jejuni* as enteric pathogen among children.

Patients and Methods

The study was conducted with ethical permission in the Department of Microbiology, Maulana Azad Medical College and Department of Pediatrics, LN hospital, New Delhi for 2 consecutive years. The study group included 205 patients aged 12 years or below having acute diarrhea (<14 days duration) admitted in diarrhea ward of the hospital. A total of 100 age and sex matched children without any gastrointestinal complaints were taken as control. After proper counselling, an informed consent was taken from the parents/guardians/person attending the study subject. Detailed personal history, diarrheal episode and associated signs and symptoms were recorded on a pre-designed pro forma.

Exclusion Criteria

Children on antimicrobial therapy were excluded from the study.

Sample Collection and Transport

Stool samples were requested from all patients and controls who fulfilled the inclusion criteria. Proper instructions were given regarding collection of specimen i.e. freshly passed faeces to be collected in a clean, wide mouth, screw capped plastic container and transported to microbiology laboratory within 2 hours of collection. In case of delay of more than two hours, samples were transported in Cary Blair medium/ buffered glycerol saline.

Examination of Sample

The stool specimen was processed as follows:

Culture: All fecal samples were processed for *Campylobacter* species by direct inoculation and after enrichment in BHI broth on modified charcoal cefoperazone deoxycholate agar (CCDA) (Oxoid®) and Skirrow's Columbia blood agar media with *Campylobacter* growth supplement and *Campylobacter* selective supplement (Butzler) (Oxoid®) containing bacitracin (12 500 IU), cycloheximide (25 mg), colistin sulfate (5000 IU), cephazolin sodium (7.5 mg) and novobiocin (2.5 mg). The plates were incubated along with control strain of *C. jejuni* for 48 hours at 42°C under microaerophilic conditions (5% O_2, 5% CO_2, 2% H_2, and 88% N_2 by volume) generated by ANOXOMAT AN2OP system®. Plates were examined after 48 hours and in case of no growth re-examined after 72 hours and then again after 7 days of incubation.[12]

Suspected colonies of *Campylobacter* grown were confirmed by oxidase test, catalase test, hippurate hydrolysis, hydrolysis of indoxyl acetate, growth on 1% glycine and 1.5% NaCl and susceptibility to cefoperazone (30 ug), nalidixic acid (30 ug) and cephalothin (30 ug) as per standard techniques.[13]

All samples were examined by wet mount for the presence of parasites and inoculated on several diagnostic media Such as MacConkey's agar, xylose lysine deoxycholate agar, blood agar and bile salt agar directly and after enrichment in selenite F broth and alkaline peptone water for the isolation of conventional enteropathogens. Characteristic colonies were identified by based on colony characteristics, biochemical reactions and agglutination test with respective antisera.

Detection of *Campylobacter* Antigen in Stool Samples

ProSpecT™ Campylobacter Microplate Assay® (Oxoid Ltd, UK) was used for qualitative detection of *Campylobacter* specific antigen in faecal samples as per manufacturers' instructions.

Statistical Analysis

All data obtained was analyzed using SPSS statistical software. Chi-square test with Yates correction, Fisher exact test were used to compare the results, wherever applicable.

Results

A total of 32 (15.61%) children with diarrhea had positive results for *Campylobacter* antigen among which 15 samples yielded growths on culture media. The detection rate from the controls without diarrhea was 4%. The difference between the isolation rates was statistically significant ($P<0.05$). All the isolates were identified as *C. jejuni*. Among 32 positive cases, 22 (68.75%) children were infected with *C. jejuni* as a sole pathogen, whereas 10 (31.25%) cases had polymicrobial infections. Most common pathogen isolated along with *C. jejuni* was *Vibrio cholerae* O1 Ogawa (15.62%) followed by enteropathogenic *Escherichia coli* (EPEC) 9.4% and rotavirus in 3.12% cases. One case had triple infection with *C. jejuni*, EPEC and rotavirus. Mean age of children with *Campylobacter* infection was 9 months with peak incidence (18.96%) in children below 1 year (Table 1). Males were more frequently (1.5:1) infected than females.

Seasonal distribution of *Campylobacter* infection is presented in Figure 1. A total of 71.87% isolates were detected during the summer and monsoon months of which highest recovery was in the month of July (25%).

Watery diarrhea was significantly more common than inflammatory diarrhea in the *Campylobacter* infected cases (Table 2). Fever was observed in 59% cases infected with *Campylobacter* alone, but it was significantly more (90%) associated with mixed infection, $P=.0402$ (Table 3). Abdominal pain and vomiting were equally common in both the groups (75% and 90.62%). In contrast, dehydration was observed in 59% cases infected with *Campylobacter* as sole pathogen, but was much more common among mixed infection cases (90%), $P<.05$, which was

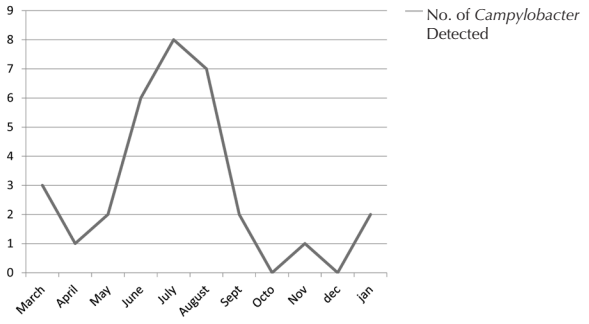

Figure 1. Seasonal Distribution of *Campylobacter* Infection.

statistically significant.

Discussion

A slightly higher isolation rate (15.61%) was found in the present study compared to previous studies from India where isolation rate varied from 7%-13.5% among acute diarrheal cases.[2,14] Variation in results may be due to the use of different techniques of detection. ELSIA as a new method was used in the present study, while other studies used only culture as method of detection. Studies from neighbouring countries like Bangladesh, Pakistan and China revealed an isolation rate of 11.8%, 18% and 25.5% and very high (62%) prevalence has been reported from Thailand.[3,4,15,16]

Another feature observed in this study was the high percentage of mixed infections of *C. jejuni* with other known enteropathogens, matching with prior studies (14). Bhadra et al reported *Vibrio cholerae* O1 Ogawa as the commonest co-pathogen of *Campylobacter* in Kolkata.[17] High number of co-infection with diarrheagenic *E. coli* and rotavirus among *Campylobacter* positive cases was found in Vellore.[18] Therefore our study reaffirms the previous finding that polymicrobial infection is common in *Campylobacter* associated diarrhea in developing countries. Similar to our study, dominance of *C. jejuni* infection among children less than 1 year (18.96%) was seen in previous studies conducted in Kolkata and Bangladesh.[17,19] In developing countries, high-level exposure to the organism early in life leading to the gradual development of protective immunity restrict symptomatic infection after the age of 2 years and duration of intestinal excretion declines with age. In order to trace the source of *Campylobacter* infection in infants, studies pointed out drinking well water, eating home prepared fruits or vegetables, exposure to pet with diarrhea, visiting or living in a farm, ridden in a shopping cart next to meat or poultry shop potential risk factors.[20,21] Indian studies documented poultry and cattle as major reservoir of this infection.[22,23] Breast feeding, drinking purified water, washing hands after contact with pets, environmental separation of household livestock, avoiding foreign travel are the protective measures to stop transmission. Data regarding in exposure to animals or poultry of the children enrolled in our study was not available; therefore, further risk assessment could not be carried out. Though *Campylobacter* infection prevailed throughout the year, higher rate of isolation (71.87%) was seen during the summer and monsoon months of which highest recovery was in the month of July (25%). Similar seasonal variation was observed in Kolkata.[17] Watery diarrhea was found to be significant-

Table 1. Age Distribution of *Campylobacter* Isolates Both From Cases and Control

	Cases With Acute Diarrhea		Controls Without Diarrhea	
Age (y)	**No. of Patients Studied**	**Positive for *Campylobacter,* No. (%)**	**No. of Controls Studied**	**Positive for *Campylobacter,* No. (%)**
<1	116	22 (18.96)	58	0 (0)
1- 2	37	6 (16.22)	19	1 (5.26)
2-5	27	3 (11.11)	13	2 (15.38)
>5	25	1 (4)	10	1 (10)
Total	205	32 (15.61)[a]	100	4 (4)[a]

[a]There was a significant difference between the isolation rates of *Campylobacter* spp. in cases and controls in total was significant ($P = .0023$) but between each age group was not significant ($P > .05$).

Table 2. Clinical Presentation of *Campylobacter* Infection

Findings	Campylobacter Positive Cases, n = 32 (%)	Campylobacter Negative Cases, n = 173 (%)
Watery diarrhea[a]	25 (78.12)	66 (38.15)
Inflamatory diarrhea	7 (21.87)	35 (20.23)
Fever (>100°F)[a]	22 (68.75)	89 (49.13)
Abdominal pain[a]	24 (75)	40 (23.12)
Vomiting[a]	29 (90.62)	53 (30.63)
Dehydration[a]	22 (68.75)	52 (30.05)
Cough and coryza	3 (9.37)	27 (15.61)
Convulsion	2 (6.25)	19 (10.98)

[a]$P < .0001$.

Table 3. Comparison of Clinical Findings Between Children With *Campylobacter* Infection Alone and *Campylobacter* Infection With Other Pathogens

Findings	Patients Infected With *Campylobacter* Alone, n=22 (%)	Patients Infected With *Campylobacter* and Other Pathogens, n=10 (%)
Watery diarrhea	17 (77.27)	8 (80)
Inflammatory diarrhea	5 (22.72)	2 (20)
Fever (>100°F)[a]	13 (59.1)	9 (90)
Abdominal pain	16 (72.72)	8 (80)
Vomiting	19 (86.36)	10 (100)
Dehydration[a]	13 (59.1)	9 (90)
Cough and coryza	3 (13.64)	0 (0)
Convulsion	1 (4.54)	1 (10)

[a] $P < .05$.

ly associated than inflammatory diarrhea in the *Campylobacter* infected cases (71.12% vs. 21.87%; $P < .0001$). Though a study from Pakistan found blood and mucus in 90% of diarrheal stools that yielded *C. jejuni*, Bhadra et al noticed watery diarrhea in 97.6% of *C. jejuni/coli* infected cases.[4,17]

In comparative analysis to investigate the difference in clinical presentation between the patients infected with *Campylobacter* alone (n = 22) and those infected with multiple pathogens (n = 10), fever and dehydration was found to be more common in mixed infections. Though Tribble et al evaluated sensitivity and specificity of various clinical presentations and stool characteristics as modality to diagnose *Campylobacter* infection, we could not associate any clinical pattern specific for *Campylobacter* enteritis.[24]

In the present study *Campylobacter* comprised a significant percentage of enteropathogens among children in India, which indicates requirement of routine identification of this pathogen. Moreover, clinical features could not be used to diagnose of campylobacteriosis per se because of the non-specific nature of the symptoms.

Conflict of Interest Disclosures

None.

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