Original Article

Isolation of Campylobacter Species in the Stool of Under Five Children With Acute Diarrhoea in a Tertiary Care Hospital of Bangladesh

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Abstract

Campylobacter species is the main etiology of gastroenteritis due to bacteria. To determine prevalence of Campylobacter species in stool of children less than five years of age with acute diarrhoea, this observational study was conducted in the Department of Microbiology, Sylhet M A G Osmani Medical College, Sylhet from January to December, 2017. Stool samples were collected from 162 under-five children with acute diarrhoea admitted in the Department of Paediatrics. Isolation of Campylobacter species were done by stool culture. About two third of the children were male (65.4%) and more than one fourth of the affected children (26.65%) were in age group 6-12 months. Campylobacter species was isolated in 24 (15%) sample and among them, Campylobacter jejuni were 22 (91.7%) and Campylobacter coli were 2 (8.3%). Isolation rate of Campylobacter species did not differ significantly between age group of 6-12 months and above 12 months (p=0.211) of age; male and female children (p=0.288); among socioeconomic status (p=0.673) and between residential status (p=0.108). Isolation rate of Campylobacter species are frequent among under five children with acute diarrhoea and most of the children came from low socioeconomic background and were male. However, a large multicenter study needs to be conducted to generate more evidence regarding the issue.

Keywords: *Acute diarrhoea, campylobacter species, prevalence, under five children*

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INTRODUCTION

Diarrhoea imparts some significant morbidity and mortality in the younger children worldwide, especially in developing countries. Among children under 5 years of age it is the second main cause of death and is estimated to have caused 0.5 million pediatric deaths per year.[1] This disease also has direct consequences in children include malnutrition, diminished growth and cognitive dysfunction in resource limited countries.[2]

In developing countries, like Bangladesh, among all the bacterial causes of diarrhoea, the five most common bacterial pathogens in children up to five years of age are *Campylobacter jejuni* followed by *Escherichia coli*, Aeromonas spp., Shigella and *Vibrio cholera*.[3] On the other hand, in developed country, Campylobacters are ranked fourth among top five pathogens in causing food borne infections and is estimated to cause more than 9.4 million cases of campylobacteriosis each year.[4]

The Campylobacter genus comprised of 26 species and is curved, Gram-negative, motile, and microaerophilic, nonspore producing bacillus[5]. *Campylobacter jejuni* and *Campylobacter coli* are the two most importatnt species.[6] In developed countries, about 5% - 20% of all cases of acute gastroenteritis in children younger than 5 years of age is caused by Campylobacter; whereas, in developed count ries incidence ranges from 5% - 35%.[7,8] In Pakistan and India, acute diarrhoea is caused by Campylobacter spp

about 18%.[9] and 4.5% to 13%,respectively.[10] In Bangladesh, isolation rate of *Campylobacter jejuni* causing acute childhood diarrhoea is 17.4%,[3] and 12%.[11]

In general, the transmission of Campylobacter occurs to humans by eating undercooked or contaminated poultry, unpasteurised dairy products and contaminated water or coming with the contact faeces of a dog or cat. But person-to-person spread of Campylobacter is not frequent.[12] Commonly the disease occurs sporadically, but outbreaks can occur if it is transmitted by contaminated water or unpasteurised milk .[13]

The *Campylobacter jejuni* can be isolated by conventional culture method but that time consuming.[14] It is a fastidious organism, uses menaquinones as their respiratory quinones, and grows in microaerophilic environment (5%O_2, 10% CO_2 and 85% N_2).[13,15] The *Campylobacter jejuni* does not ferment, or oxidize carbohydrates.[13,15] Over the past few decades, culture-independent-based diagnostics, i.e., nucleic acid test, especially qualitative polymerase chain reaction (qPCR) performed directly from diarrhoeal stools, have provided a rapid and a highly sensitive method of diagnosis in laboratories with molecular diagnostic facilities.[16] In Bangladesh few studies have been done for detection of *Campylobacter jejuni* by PCR.[17]

Although Campylobacter enteritis is a major public health problem in both developed and developing countries, there is still paucity of data related to the prevalence and burden of Campylobacter diarrhoea across vast regions of Bangladesh. Moreover, there are no available data on Campylobacter diarrhoea from north-eastern part of Bangladesh. The present study was designed to find out the isolation rate of *Campylobacter jejuni* among the hospitalized children of less than five years of age with acute diarrhoea.

MATERIALS AND METHODS

This cross sectional observational study was conducted in the Department of Microbiology, Sylhet MAG Osmani Medical College, Sylhet during the period from 1st January, 2017 to 31st December, 2017. A total 162 under five children with acute diarrhoea (the passage of three or more loose or liquid stools per day lasts for several hours or days but not more than 14 days) admitted in the Department of Paediatrics, Sylhet MAG Osmani Medical College Hospital, Sylhet fulfilling the eligibility criteria were enrolled. Children who had received antibiotic within last two weeks were excluded from the study.

After admission of an under five children with acute diarrhoea with or without blood and mucous associated with either fever or abdominal pain or both were evaluated from history and clinical examination. Informed written consents were obtained from each parents or legal guardian. Those who fulfilled the inclusion criteria were taken as sample. In this way 162 under five years of age with acute diarrhoea were enrolled. The demographic data were taken as per questionnaire.

Collection of stool samples and culturing technique: Stool sample was collected from each patient in sterile plastic, disposable bottles with proper labeling without any preservatives before starting any antibiotic. The collected specimens were immediately transported to the Department of Microbiology, Sylhet MAG Osmani Medical College, where the samples were inoculated in the primary culturing medium for Campylobacter used was Campylobacter agar base (Lot no. 0000249244, HIMEDIA, India) with defibrinated 5% sheep blood containing Vancomycin 5 (mg/l), Trimethoprim (2.50 mg/l), Cefsulodin (2.50 mg/l) and Amphotericin B (2.50 mg/l). It was incubated at 42^{0}C (microaerophilic condition) for at least 48 hours. The culture plates were re-incubated for the next 24 hours if no growth was found after initial 48 hours. The morphology of the colony, motility test, Gram staining pattern and biochemical tests like, oxidase test, catalase test and hippurate hydrolysis test were used to find out the organism.

Statistical Analysis: After collecting all the data were registered, processed and analyzed by using SPSS (Statistical Package for Social Science) for windows version 23.0. Quantitative data were expressed as mean and standard deviation and qualitative data were expressed as frequency and percentage.

Ethical Consideration: Informed written consent was taken from each parent or legal guardian after explaining the the purpose of the study. Prior to the beginning of this study. The approval of the research protocol was obtained from the Ethical Review Committee of Sylhet MAG Osmani Medical College, Sylhet.

RESULTS

Table-1 shows the distribution of socio-demographic characteristics of the children. Among the 162 children, age of the patients ranged from 6 to 60 months with the mean age of 16.20 ± 10.12 months. Highest number 89

(54.9%) patients were found in 6-12 months age group and lowest number 6 (3.7%) were seen in 49 to 60 months age group. Male preponderance with a ratio of male to female was 1.89:1.

Table-I Socio-demographic characteristics of the children (n=162)

Parameters		Frequency	Percentage
Age			
	6-12 Months	89	54.9
	13-24 Months	46	28.4
	25-36 months	14	8.6
	37-48 months	7	4.3
	49-60 months	6	3.7
Sex			
	Male	106	65.4
	Female	56	34.6

Table-II shows that the duration of diarrhoea ranging from 1 to 12 days with the mean of 5.29 ± 2.57 days and duration 1-5 days was in 107 (66%) cases and 6-12 days in 55 (34%) cases. Among the patients 116 (71.6%) were from lower class, 29 (17.9%) were from middle class and 17 (10.5%) were from upper class of socioeconomic status. In this study 128 (79%) participants were from rural areas and 34 (21%) participants were from urban areas.

Table-II: Distribution of the patient according to duration of diarrhoea and socio-economic class (n=162)

Parameters		Frequency	Percentage
Duration of diarrhoea			
	1-5 days	107	66.0
	6-12 days	55	34.0
Socioeconomic status		Frequency	Percentage
	Lower class	116	71.6
	Middle class	29	17.9
	Upper class	17	10.5
Residential status			
	Rural	128	79.0
	Urban	34	21.0

Table - III shows the association of isolation of Campylobacter and various parameters of socio-demographic and economic conditions of the children. Amongst 162 children, Campylobacter species were isolated from stool of diarrhoeal children of under 5 years in 24 (15%) cases and remaining 138 (85%) cases revealed no growth of Campylobacter species. Campylobacter jejuni was the major isolate, 22 (91.7%) cases and a tiny portion by Campylobacter coli 2 (8.3%) cases. Isolation rate of Campylobacter species did not differ significantly between age group of 6-12 months and the age group of above 12 months (χ^2=1.565; p=0.211); male and female children (χ^2=1.140; p=0.288); among socioeconomic status (p=0.673) and between residential status (χ^2=2.590; p=0.108).

Table- III: Association of isolation of Campylobacter and various parameters of socio-demographic and economic conditions of the children(n=162).

Socio-demographic parameters		Campylobacter species		p-value
		Growth	No Growth	
Age				
	6-12 months	16	73	*p=0.211
	>12 months	8	65	
Sex				
	Male	18	88	*p=0.286
	Female	6	50	
Socioeconomic status				
	Lower class	19	97	†p=0.673
	Middle class	4	25	
	Upper class	1	16	
Residential status				
	Rural	16	112	*p=0.108
	Urban	8	26	

*Chi-squared test was done

DISCUSSION

This study revealed that Campylobacter species was isolated in 24 (15%) cases from stool of acute diarrhoea of children under 5 years. Several studies in Bangladesh revealed 13.5-17.4% Campylobacter species in diarrhoeal children.[3,11,18,19] *Campylobacter jejuni* were isolated from diarrhoeic stool samples was 15.4% in Northwest Ethiopia,[20] 12.7% in South Ethiopia,[21] 18% in Pakistan,[9] which were in accordance with the present study. But higher prevalence of Campylobacter species were detected in diarrhoeic children 21%.[21] and 47.4%,[23] in Malawi and South Africa respectively; whereas lower prevalence of 2% in Sudan,[24] 4.7% in Tanzania,[25] and 7.0% in Kolkata, India.[26] The difference in frequency of

Campylobacter jejuni in different parts of the world is probably due to varying standards of living conditions, water supply and feeding habits as the infection occurs through water and food.

In this study the age of the patients ranged from 6 to 60 months with the mean age of 16.20 ± 10.12 months. The highest number 54.9% of patients were found in 6-12 months age group and lowest 3.7% of patients were in 49-60 months age group.Huda et al.[19] found that 52.5% of diarrhoea of children were aged up to one years. Which was nearly similar to the present study. But Tafa et al.[27] reported that the mean age of the children was 37.88 ± 15.96 months (ranges, one to 59 months) and 51.1% of patients were in the age group of 48–59 months followed by the age group of 36-47 months (15.4%). Campylobacter spp. were isolated in 17.98% of patients aged between 6 and 12 months; and 10.95% of cases aged above 12 months; difference was not significant (p=0.211). This result correlated with Lengerh et al.[20] that of isolation of Campylobacter species did not differ significantly between age group of <1 years and the age group of 1-5 years (p=0.16). Several other studies supported this result.[27,28] But Huda et al.[19] reported the isolation rate of Campylobacter was 19.3% in below 1 year of age group and 5.8% was above 1 year of age group. The difference was highly significant (p<0.003).

In the present study 65.4% of patients were male and 34.6% of patients were female children with a ratio of male to female of 1.89:1. Male preponderance was reported in some, [19] while female preponderance was reported in other.[27] The isolation rate of *Campylobacter species* were 16.98% of male and 10.71% of female diarrhoeal children. The isolation of *Campylobacter species* did not differ significantly between male and female (p=0.286). This result was in agreement with several studies.[19, 20, 27, 29]

The duration of diarrhoea ranged from 1 to 12 days with the mean of 5.29 ± 2.57 days. Duration of diarrhoea was 1-5 days in 66% of cases and 6-12 days in 34% of cases. This result was consistent with the study of Lengerh et al.[20] that duration of diarrhoea was 1-5 days in 91.9% cases and 6 days or more in 8.1% cases.

In this study 71.6% of patients were from lower class, 17.9% of patients were from middle class and 10.5% of patients were from upper class of socioeconomic status. *Campylobacter species* were isolated in 5.88% of case from upper class, 13.79% of cases from middle class and 16.38% of cases from lower class of socioeconomic status. Frequency of isolation of Campylobacter species in diarrhoeal children among socioeconomic status did not differ significantly (p=0.673). This may be due to the fact that people of poor socioeconomic condition constitute the major bulk of the population in Bangladesh. The government hospital facilities were mostly availed by lower class.

In this study 79% of patients were from rural areas and 21% of patients were from urban areas. This may be due to that rural people mostly poor or middle class people and availed government hospital facilities mostly. *Campylobacter species* were isolated in 12.5% of cases of rural area and 23.53% of cases of urban area.

Frequency of isolation of Campylobacter species in diarrhoeal children between residential status did not differ significantly (p=0.108). This result was supported by the study of Lengerh et al.[20] that of isolation of Campylobacter species did not differ significantly between residential status of urban and rural areas (p=0.53).

In this study, among 24 isolated Campylobacter species 91.7% of cases were *Campylobacter jejuni* and 8.3% of cases were *Campylobacter coli*. Roy et al.[11] reported 88.89% of cases were *Campylobacter jejuni* and 11.11% of cases were *Campylobacter coli* among isolated Campylobacter spp. in Bangladesh. Mshana et al.[9] reported 80.9% of cases were *Campylobacter jejuni,* 4.5% were *Campylobacter coli* and 14.6% were other species of Campylobacter in Uganda. Feizabadi et al.[30] also showed that 85.8% were *Campylobacter jejuni* and 14.2% were *Campylobacter coli* from Iran.

Limitations of the study: (1) This study was conducted in a single centre and (2) sample size was small.

CONCLUSIONS

It may conclude that infection caused by C*ampylobacter species* is very frequent among under five children with acute diarrhoea and mostly below one year of age. Majority of the patients were male and belonged to the low socio-economic status. However further large scale study involving multicentre should be carried out to evaluate prevalence of campylobacteriosis and their antimicrobial susceptibility pattern in Bangladesh.

Acknowledgments: Authors would like to thanks to all doctors and staff of Department of Microbiology, Sylhet MAG Osmani Medical College, Sylhet and Department of

Paediatrics, Sylhet MAG Osmani Medical College Hospital, Sylhet for their support in this study.

Funding: No funding sources

Conflict of interest: None declared

Author Contributions: Dr. Suborna Dey: conceptualization, methodology, data collection and original draft preparation; Dr. Rajib Das: conceptualization, methodology and revised the manuscript; Professor Dr. Md. Moynul Haque and Dr. Premananda Das supervised the study; Dr. Shantanu Das, Dr. Farjana-Binte-Habib**,** Dr. Sultana Mehnaz Rahman Chowdhury, Dr.Mohammed Mirazur Rahman and Dr. Md. Nazmul Hasan critically review the manuscript. All the authors finally contributed to the final approval of the version to be published.

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