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Co-infection with Campylobacter and rotavirus in less than 5 year old children with acute gastroenteritis in Nepal during 2017–2018

Vishnu Bhattarai[1], Saroj Sharma[2], Komal Raj Rijal[1] and Megha Raj Banjara[1*]

Abstract

Background: Diarrhoea, although easily curable, is a global cause of death for a half million children every year. Rotavirus and *Campylobacter* are the most common etiological agents of diarrhoea in children less than 5 years of age. However, in Nepal, these causative agents are not routinely examined for the diagnosis and treatment. The main objective of this study was to determine *Campylobacter* co-infection associated with rotavirus diarrhoea in children less than 5 years of age.

Methods: A cross-sectional study was conducted at Kanti Children's Hospital (KCH), Kathmandu, Nepal from November 2017 to April 2018. A total of 303 stool specimens from children affected with diarrhoea were processed to detect rotavirus using a rapid rotavirus antigen detection test kit, and *Campylobacter* by microscopy, culture and biochemical tests. Antibiotic susceptibility tests of *Campylobacter* isolates were performed according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines 2015.

Results: Of 303 samples, 91 (30.0%) were positive for co-infection with rotavirus and *Campylobacter*. Rotavirus mono-infection was detected in 61 (20.1%), and *Campylobacter* mono-infection was detected in 81 (26.7%) samples. Patient's age, month of infection, untreated water and frequent soil contact were the major risk factors for infections. Clinical features such as > 9 loose motions per day, fever, vomiting, mild to moderate dehydration, diarrhea persisting 6–9 days and presence of mucus in stool were significant ($p < 0.05$) clinical features, and were more severe in coinfection compared to mono-infections in multivariate analysis.

Conclusion: The study shows a high rate of rotavirus and *Campylobacter* coinfection in children with diarrhoea. Diagnosis based management of diarrhoeal cases can guide the specific treatment.

Keywords: *Campylobacter*, Rotavirus, Co-infection, Diarrhoea, Children

Background

Diarrhoea remains a serious health burden in children less than 5 years of age in developing countries. Globally, diarrhoea kills around 525,000 children less than 5 years of age each year [1]. The commonest etiological agents of acute watery diarrhoea in young children in developing countries are rotavirus, enterotoxigenic *Escherichia coli, Shigella* spp., *Campylobacter jejuni* and *Cryptosporidium parvum* [2].

Viruses are primary agents of diarrhoea during the winter in developed countries whereas bacteria are the main agents of diarrhoea in rainy season in developing countries [3]. However, rotavirus is found to be a single dominant enteric pathogen among children in most of the developed and developing countries [4]. The number of deaths attributable to rotavirus infection and associated disease in children younger than 5 years in 2008 was estimated to be 453,000 (95% CI 420,000-494,000) [5]. With the introduction of rotavirus vaccine, the

* Correspondence: banjaramr@gmail.com
[1]Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
Full list of author information is available at the end of the article

number of deaths in children less than 5 years of age declined from 528, 000 in 2000 to 215, 000 in 2013; and decreased the percentage of hospitalization due to acute gastroenteritis caused by rotavirus [6, 7].

World Health Organization recommended integrating rotavirus vaccine into national immunization program [8, 9]. Although the incidence of rotavirus infection among children in developed and developing countries is similar, outcomes often vary widely with 82% of fatalities estimated to occur in developing countries [6, 10]. Rotavirus infections are an important cause of hospitalization, causing significant economic impact on poor countries [11]. Studies published on rotavirus infection in Nepal from 1999 to 2007 showed rotavirus positivity rates ranged from 17 to 39% among all hospitalized children less than 5 years [12–15]. Various published studies from 2008 to 2017 reported different prevalence rates of rotavirus infections in diarrhoeal children, ranging from 22 to 53% [16–19].

Studies revealed that co-infection does exist between enteric bacteria and viruses [20, 21]. This evidence collectively demonstrates that co-infection by bacterial and viral pathogens play a critical role in disease progression. Infectious diseases cause most of the child deaths in developing countries [22], but the etiological agents are usually unknown and can lead to overuse/misuse of antibiotics, which may exacerbate the antibiotic resistance, already a global threat [23]. This study focuses on co-infection of *Campylobacter* in rotavirus infected children and explores associated risk factors and clinical features.

Methods

A hospital based cross-sectional study was conducted from November 2017 to April 2018. A total of 303 stool samples were collected from Kanti Children's Hospital (KCH), Kathmandu, Nepal. Written informed consents and clinical and demographic information were obtained from guardians/caretakers of the patient. Samples were collected from children less than 5 years of age, presenting with diarrhoea as reported by parents in hospital. The samples were tested for rotavirus using Onsite rotavirus Ag Rapid Test kit (CTK Biotech, Inc. San Diego, USA) for rapid diagnosis of rotavirus, and the samples were taken in the laboratory for the detection of *Campylobacter* causing infection by culture on *Campylobacter* blood-free selective agar base supplemented with Campy blood free selective medium (Charcoal cefoperazone deoxycholate agar, CCDA) Selective Supplement (SR0155, containing cefoperazone and amphotericin B antibiotics) (Thermo-Fischer, Oxoid, UK). The inoculated medium was incubated at 37 °C and 42 °C in microaerophilic condition for 24 to 48 h using Campy gas pack (Oxoid, UK). After incubation, colonies appeared colorless or grey and spread like droplets. A presumptive diagnosis was made by wet mount preparation for darting motility. The isolated bacteria were identified based on the morphological character of the colonies, Gram staining of the isolate (Staining was performed with the application of carbol fuchsin as gram counter stain for 5 min), oxidase test, catalase test and sensitivity to nalidixic acid (30 μg) to differentiate *Campylobacter* spp. from other Enterobacteriaceae. Hippurate hydrolysis test was performed for differentiation of *Campylobacter* spp. A total of 4 days was required for confirmed diagnosis of infection with *Campylobacter*. Modified Kirby-Bauer Disc diffusion technique was used for testing the susceptibility pattern of different isolates towards various classes of antibiotics in Mueller Hinton Agar (MHA) with 5% defibrinated sheep blood. Antibiotics were used according to EUCAST guidelines (2015). Data analysis was done using IBM Statistical Package for Social Sciences version 20.0 (SPSS, Inc., Chicago, IL, USA). The association among co-infection and mono-infection with rotavirus and *Campylobacter* was tested using the Chi-square test for differences in proportions; and logistic regression analysis was used to assess the association between infection and the risk factors. A *p*-value less than0.05 was considered statistically significant.

Results

The study included three hundred and three diarrhoeal children less than 5 years of age during the study period. The highest number of patients ($n = 118$) were from age group 7–12 months. There were 207 (68.3%) male and 96 (31.7%) female patients.

Detection rate of pathogens

The study was focused on detection of rotavirus and *Campylobacter* spp., and at least one of these pathogens were detected in 233 (76.9%) samples. Among 303 children with acute watery diarrhea, rotavirus mono-infection was detected in 61 (20.1%), *Campylobacter* mono-infection was detected in 81 (26.7%), co-infection was detected in 91 (30.0%) (Fig. 1).

Age wise distribution of different infections in children

The highest number of rotavirus mono-infection was detected in 7–12 months age group category which accounted 29 (47.5%) of total rotavirus mono-infection. Similarly, highest number of *Campylobacter* mono-infection 34 (42.0%) was found in the < 6 months age group. The co-infection was observed highest (35, 38.5%) in 7–12 months of age (Table 1). Distribution of different infections in children less than 5 years was not statistically significant (χ^2test, $p > 0.05$).

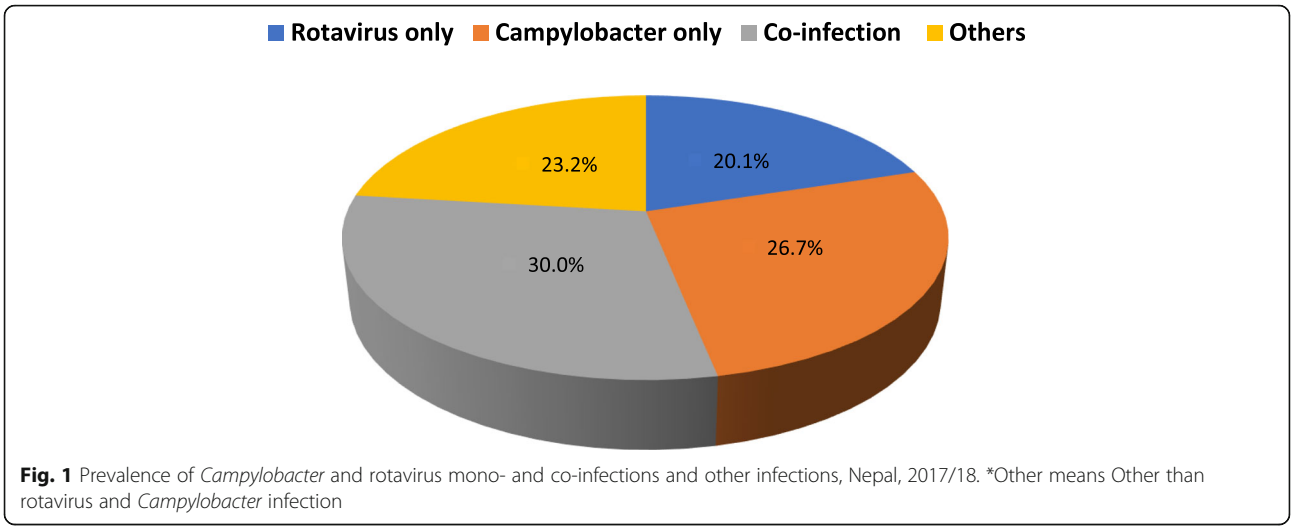

Fig. 1 Prevalence of *Campylobacter* and rotavirus mono- and co-infections and other infections, Nepal, 2017/18. *Other means Other than rotavirus and *Campylobacter* infection

Risk factors for different infections in children

In multivariate analysis, infection in February was associated with a decreased risk of rotavirus mono-infection [adjusted odds ratio (AOR) = 0.26, 95% CI = 0.07–0.98, $p = 0.047$] than in November. Except for the age group of 25–36 months, all age groups were significantly associated with decreased risk of *Campylobacter* mono-infection compared to < 6 months age. No soil contact (AOR = 0.06, 95%CI = 0.01–0.47, $p = 0.008$) was significantly associated with a decreased risk of *Campylobacter* mono-infection compared to frequent soil contact. Infection in January (AOR = 11.34, 95%CI = 1.27–101.27, $p = 0.030$) and February (AOR = 25.32, 95%CI = 2.68–238.69, $p = 0.005$) were significantly associated with a higher risk of co-infections compared to November (Table 2).

Clinical features in different infections with diarrhea

Among children with diarrhoea, clinical presentations were as follows: 23.8, 49.2 and 27.1% cases had 3–6, 7–9 and > 9 loose motions/day respectively. 64.7% cases had fever, and 85.8% had vomiting. 75.2% had no dehydration, 23.8% had mild to moderate dehydration and 1% had severe dehydration. 31.4% cases had abdominal pain. 35.6% had diarrhoea for less than 3 days, 45.9% had diarrhoea for 3–5 days, 17.5% had diarrhoea for 6–9 days, and only 1% had diarrhoea for more than 9 days. 70.6% of patients were from Out Patient Department (OPD) and only 29.4% were from In Patient Department (IPD). 60.7% had mucus in the stool and 30.0% had pus cells in the stool.

Abdominal pain and presence of pus cells in stool were less common features, which were significantly associated with rotavirus mono-infection in multivariate analysis. Pus cells in stool was common clinical feature, while fever and vomiting were less prevalent but significantly associated with *Campylobacter* mono-infection in multivariate analysis. More than nine loose motions per day, fever, vomiting, and presence of mucus in stool were most striking clinical features. Mild to moderate dehydration were less common compared with no dehydration but were significantly associated with co-infections in multivariate analysis (Table 3).

Antibiotic resistance patterns in *Campylobacter* spp.

A total of 172 *Campylobacter* isolates were tested for antibiotic susceptibility. Among them, resistance to different antibiotics were: ampicillin (93.6%), cephalexin (88.4%), erythromycin (73.8%), nalidixic acid (72.1%) and

Table 1 Age-related distribution of *Campylobacter* and rotavirus mono- and co-infections

Age (months)	Type of infection			
	Rotavirus only n (%)	*Campylobacter* only n (%)	Co-infections n (%)	Undetected n (%)
< 6	14 (23.0)	34 (42.0)	26 (28.6)	19 (27.1)
7–12	29 (47.5)	29 (35.8)	35 (38.5)	25 (35.7)
13–24	10 (16.4)	7 (8.6)	19 (20.9)	13 (18.6)
25–36	3 (4.9)	6 (7.4)	7 (7.7)	5 (7.1)
37–60	5 (8.2)	5 (6.2)	4 (4.4)	8 (11.4)
Total	61 (100.0)	81 (100.0)	91 (100.0)	70 (100.0)

Table 2 Risk factors for Rotavirus and *Campylobacter* mono-infection and co-infection in multivariate analysis

Risk factors	Rotavirus monoinfection		*Campylobacter* monoinfection		Co-infection	
	AOR (95%CI)	*P* -value	AOR (95%CI)	*P*-value	AOR (95%CI)	*P*-value
Months						
November	1		1		1	
December	0.59 (0.15–2.30)	0.451	5.57 (0.56–54.98)	0.141	8.98 (0.94–85.86)	0.057
January	0.39 (0.11–1.30)	0.127	6.06 (0.68–53.96)	0.106	11.34 (1.27–101.27)	0.030[a]
February	0.26 (0.07–0.98)	0.047[a]	5.36 (0.58–48.94)	0.137	25.32 (2.68–238.69)	0.005[a]
Age (in months)						
< 6	1		1		1	
7–12	1.70 (0.39–7.33)	0.471	0.19 (0.04–0.90)	0.037[a]	1.26 (0.32–4.87)	0.738
13–24	1.64 (0.31–8.49)	0.553	0.07 (0.01–0.42)	0.004[a]	1.42 (0.31–6.45)	0.649
25–36	3.28 (0.23–45.27)	0.374	0.10 (0.01–1.21)	0.070	0.89 (0.09–8.20)	0.925
37–60	6.04 (0.33–107.97)	0.221	0.04 (0.01–0.73)	0.029[a]	0.47 (0.03–5.74)	0.556
Sex						
Male	1	–	1		1	
Female	1.09 (0.52–2.26)	0.817	0.90 (0.42–1.90)	0.791	1.59 (0.80–3.12)	0.179
Parent's occupation						
Agriculture	0.40 (0.13–1.21)	0.107	1.09 (0.31–3.86)	0.886	2.62 (0.90–7.66)	0.077
Service	0.55 (0.15–1.96)	0.359	1.30 (0.32–5.29)	0.707	1.92 (0.53–6.90)	0.318
Business	0.82 (0.24–2.82)	0.759	1.35 (0.36–4.96)	0.651	0.70 (0.21–2.34)	0.569
Others	1		1		1	
Breast feed						
No	0.30 (0.04–2.11)	0.230	1.63 (0.34–7.72)	0.534	1.18 (0.29–4.74)	0.814
Yes	1		1		1	
Water source						
Non-pipe borne	1		1		1	
Pipe borne	0.70 (0.27–1.75)	0.448	0.95 (0.39–2.30)	0.924	1.00 (0.45–2.22)	0.997
Drinking water						
Untreated	1		1		1	
Boiled	0.81 (0.28–2.28)	0.695	0.64 (0.21–1.94)	0.438	1.47 (0.55–3.89)	0.436
Filtered	0.24 (0.03–1.56)	0.137	0	0.998	4.45 (0.92–21.33)	0.062
Chlorinated	0.70 (0.22–2.22)	0.550	0.78 (0.25–2.46)	0.681	1.45 (0.50–4.16)	0.487
Soil contact						
No soil contact	1.86 (0.35–9.84)	0.460	0.06 (0.01–0.47)	0.008[a]	3.02 (0.62–14.73)	0.171
Infrequently	1.39 (0.51–3.75)	0.510	0.49 (0.18–1.33)	0.162	1.01 (0.41–2.42)	0.990
Frequently	1		1		1	
Hygiene						
Poor	1		1		1	
Good	0.45 (0.16–1.24)	0.125	0.97 (0.33–2.80)	0.964	1.01 (0.40–2.55)	0.973
Parent's education level						
Illiterate	0	0.999	0	0.999	0	0.999
Literate	0.57 (0.19–1.72)	0.324	0.78 (0.24–2.53)	0.687	2.12 (0.72–6.22)	0.169
Higher level	1		1		1	

AOR Adjusted odds ratio, 95% *CI* 95% confidence interval, 1 = Reference, $P < 0.05$ was considered significant,[a] = significant

Table 3 Clinical features in different infections in multivariate analysis

Clinical features	Rotavirus only		*Campylobacter* only		Co-infection	
	AOR (95%CI)	*p*-value	AOR (95%CI)	*p*-value	AOR (95%CI)	*p*-value
Stool/day						
3–6	2.60 (0.79–8.55)	0.115	1.42 (0.47–4.22)	0.526	0.06 (0.01–0.23)	< 0.001
7–9	1.57 (0.56–4.38)	0.385	0.98 (0.38–2.52)	0.971	0.33 (0.13–0.85)	0.021
> 9	1		1		1	
Fever						
No	1.39 (0.69–2.79)	0.355	2.07 (1.12–3.83)	0.020	0.25 (0.12–0.50)	< 0.001
Yes	1		1		1	
Vomiting						
No	0.51 (0.16–1.57)	0.241	4.48 (1.99–10.09)	< 0.001	0.26 (0.07–0.88)	0.005
Yes	1		1		1	
Dehydration						
No-minimal	1		1		1	
Mild-moderate	2.53 (0.98–6.52)	0.053	1.22 (0.47–3.15)	0.675	0.29 (0.12–0.73)	0.009
Severe	0	0.999	0	0.999	0	0.999
Abdominal pain						
No	2.53 (1.16–5.52)	0.019	1.41 (0.75–2.62)	0.279	0.52 (0.27–1.01)	0.051
Yes	1		1		1	
Duration of diarrhea (in days)						
< 3	1		1		1	
3–5	0.87 (0.41–1.87)	0.737	1.02 (0.52–1.96)	0.952	0.66 (0.33–1.31)	0.241
6–9	0.67 (0.24–1.89)	0.458	0.54 (0.21–1.41)	0.211	1.68 (0.67–4.16)	0.262
> 9	0	0.999	2.15 (0.13–33.69)	0.584	0	0.999
Mucus						
Absent	1.39 (0.74–2.61)	0.399	0.81 (0.45–1.49)	0.514	0.46 (0.25–0.86)	0.016
Present	1		1		1	
Pus						
Absent	7.26 (2.47–21.36)	< 0.001	0.45 (0.25–0.81)	0.008	0.81 (0.43–1.50)	0.508
Present	1		1		1	

AOR Adjusted odds ratio, 95%*CI* 95% confidence interval, *NA* Not applicable, 1 = Reference

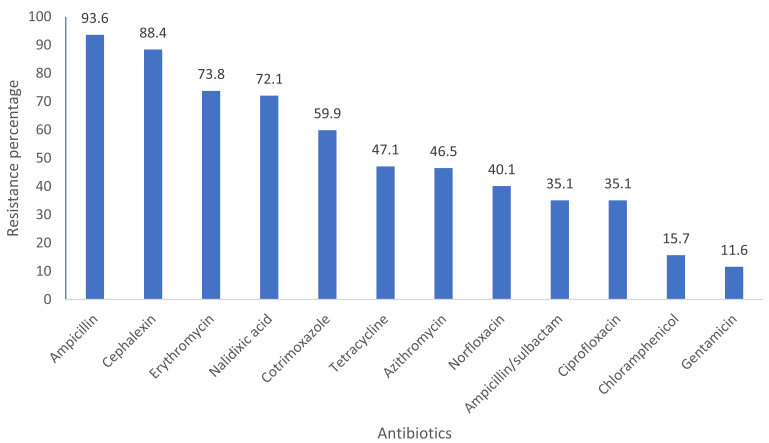

Fig. 2 Antibiotic resistance patterns in isolated *Campylobacter* spp. (*n* = 172)

cotrimoxazole (59.9%). Resistance to ampicillin/sulbactam, norfloxacin, azithromycin and tetracycline were 35.1, 40.1, 46.5 and 47.1% respectively. *Campylobacter* isolates were resistant to gentamicin (11.6%), chloramphenicol (15.7%) and ciprofloxacin (35.1%) (Fig. 2).

Discussion

This hospital based cross sectional study explored the association between *Campylobacter* and rotavirus infections, possible risk factors and specific clinical features. In this study, most cases of acute gastroenteritis were infants. Past studies have echoed with our study that shows that diarrhea incidence peaks at age 6–11 months [24–26].

Our study identified that a higher frequency of diarrhoea was seen in males consistent with other studies [13, 16, 27, 28]. Males' preponderance to develop diarrhoea can be explained by their increased susceptibility to outdoor physical activities thus exposure to unhygienic surroundings and flood-water during rainy seasons. In addition, it could be due to simply increased presentation of male patients at the hospital more than females [29].

Campylobacter and rotavirus co-infections were responsible for the one third of acute gastroenteritis cases among children less than 5 years of age visiting at the OPD and IPD clinic/vaccination unit at the KCH in Kathmandu, Nepal during November 2017–April 2018. This study confirmed previous studies where *Campylobacter* co-infection with rotavirus in children were high in less than 5 years of age [30, 31].

Campylobacter spp. isolation in diarrhoeal cases was high. *Campylobacter* is not included in the routine diagnosis protocol thus obviates the routine laboratory investigations on the important causative agents of diarrhoea in Nepal. *Campylobacter* was most prevalent in February. Rotavirus mono-infection was detected in one-fifth of the children, highest in the age group: 7–12 months and was consistent with other studies in Nepal and South-East Asian countries [19, 32], however, findings contrasted with few other studies from Nepal [16, 18]. It appears that infants less than 6 months of age were initially protected against severe diarrhoea, to some extent, by maternal antibodies and they seem to have acquired adequate immunity between 12 and 16 months of age [16].

Age, sex, breast feeding, water source, quality of drinking water, hygiene and education level of parents were not found to predict rotavirus mono-infection in these children. While the month of diarrhoeal illness does possess a risk for rotavirus mono-infection, a higher infection during January–February contradicts with one past study [19]. In our study, age group was the significant predictor of *Campylobacter* mono-infection. No soil contact was associated with a reduced risk of Campylobacteriosis compared to frequent soil contact. In Nepal, *Campylobacter* is not included in routine microbiological testing in patients with diarrhoea. Conventional culture of *Campylobacter* is time consuming and hence affects the management of diarrhoeal cases. Therefore, use of rapid sensitive molecular techniques could be useful for timely management of Campylobacteriosis [33].

In multivariate analysis, clinical picture of children with co-infection was more severe compared to mono-infection for most clinical signs taken into examination that included fever, vomiting, abdominal pain, duration of illness, hospitalization, frequency of loose motion/day and presence of mucus in stool. Our observations are consistent with a study among Korean children [34]. We observed that especially with rotavirus and *Campylobacter* co-infections, there was an increase in the episodes of loose motions per day, which is consistent with a study in Odisha, India [32]. A study reported from China revealed synergistic effect due to co-infection in severe childhood diarrhoea [35].

Increasing antimicrobial drug resistance by *Campylobacter* limits the number of therapeutic options, which makes empirical treatment more difficult. High proportions of antibiotic resistant *Campylobacter* isolates in our study reveals either there is antibiotic pressure or transmission of resistant bacteria from foods of animal origin [36].

Conclusion

In conclusion, this hospital-based cross-sectional study highlights the burden of rotavirus, *Campylobacter* and co-infections in childhood diarrhoea in Nepal. Rotavirus and *Campylobacter* associated co-infections were found to be high in this study. *Campylobacter* spp., which are generally not screened for in diarrheic patients in Nepal, should also be suspected and considered into the routine diagnosis protocol. Diagnosis of the right causative agents among possible multiple infectious etiologies can help to better manage the acute childhood diarrhoea.

Abbreviations

Ag: Antigen; AOR: Adjusted odds ratio; CCDA: Campy blood free selective medium, Charcoal cefoperazone deoxycholate agar; CI: Confidence interval; EUCAST: European committee on antimicrobial susceptibility testing; IPD: In patient department; KCH: Kanti children's hospital; MHA: Mueller hinton agar; OPD: Out patient department; OR: Odds Ratio

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Ethical approval and consent to participate
The study obtained ethical approval from Ethical Review Board of Kanti Children Hospital, Maharajgunj, Kathmandu. All aspects of the study were conducted according to Good Clinical Practice (GCP) and Good Laboratory Practice (GLP) guidelines. Written informed consent and clinical and demographic information was obtained from guardians/caretakers of the patient. Participant information was securely stored and identified by Study Number.

Authors' contributions
VB conducted the lab work and drafting of the manuscript. KRR, MRB reviewed the subsequent version of manuscript and finalized. KRR, MRB contributed in statistical analysis and revision of the manuscript. Coordination and implementation of the study: SS, KRR, MRB contributed in conceptual design and overall method of the study. All authors read and approved the final version of manuscript.

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Availability of data and materials
All data pertaining to this study are within the manuscript.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal. [2]Kanti Children's Hospital, Maharajgunj, Kathmandu, Nepal.

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