Campylobacter and Rotavirus co-infection in diarrheal children in a referral children hospital in Nepal

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Abstract

Diarrhea, although easily curable, is a global cause of death for a million children every year. Rotavirus and Campylobacter are the most common etiological agents of diarrhea in children under 5 years of age. However, in Nepal, these causative agents are not routinely examined for the diagnosis and treatment. The objective of this study was to determine Campylobacter co-infection associated with Rotaviral diarrhea in children less than 5 years of age. 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 diarrheal children were processed to detect Rotavirus using rapid Rotavirus Ag test kit, and Campylobacter by microscopy, culture and biochemical tests. Antibiotic susceptibility test of Campylobacter isolates was performed according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines 2015. Of 303 samples, 91 (30.0%) were positive for co-infection with Rotavirus and Campylobacter; Rotavirus monoinfection was detected in 61 (20.1%), and Campylobacter monoinfection was detected in 81 (26.7%). 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 monoinfections in multivariate analysis. The study shows a high rate of Rotavirus and Campylobacter coinfection in children with diarrhea. Diagnostic based management of diarrheal cases can guide the specific treatment. In addition, the associated factors identified in this study can guide clinicians for clinical
judgement, diagnosis and treatment.

Introduction

Diarrhea remains a serious health burden in under five children in developing countries. Globally, diarrhea kills around 525,000 children (<5years) each year (1). The commonest etiological agents of acute watery diarrhea in young children in developing countries are Rotavirus, enterotoxigenic Escherichia coli, Shigella spp, Campylobacter jejuni and Cryptosporidium parvum (2). Viruses are primary agents of diarrhea during the winter in developed countries whereas bacterial diarrhea remains a rainy season disease and is the predominant cause of diarrhea in developing countries (3). However, Rotavirus has been found as a single dominant enteric pathogen among children in most developed and developing countries (4). Rotavirus accounts for 1.34 million deaths, more than a third of diarrhea deaths in children younger than 5 years worldwide (5). 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). Rotavirus infections are an important cause of hospitalization, causing significant economic impact on poor countries (7). 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 (8-11). Various studies conducted from 2008–2017 reported different prevalence rates of Rotavirus infections in diarrheal children, ranging from 22-53% (12–15). Studies revealed that co-infection does exist between enteric bacteria and viruses (16–17). 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 (18), 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. This article focuses on co-infection of Campylobacter in Rotavirus infected cases and explores different associated risk factors and clinical features.

Materials and 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 consent and clinical and demographic information was obtained from guardians/caretakers of the patient. Samples were collected from children of below the age of 5 years, presenting with diarrhea. 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 same samples were taken in the laboratory for the detection of Campylobacter causing infection by culture on Campylobacter blood-free selective agar base supplemented with 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 hours 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 observing 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 minutes), oxidase test,
catalase test and sensitivity to nalidixic acid (30µg) to differentiate *Campylobacter* spp. from other Enterobacteriaceae. Hippurate hydrolysis test was performed for differentiation of *Campylobacter* spp. 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 SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA). The association among the study groups 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 <0.05 was considered statistically significant.

**Results**

Three hundred and three diarrhoeal patients less than 5 years of age were included in the study with age from 10 days to 59 months. The highest number of patients (n = 118) belonged to age group 7–12 months. In total, there were 207 (68.3%) samples from males and 96 (31.7%) from females.

*Detection rate of pathogens*

The study was focused on detection of Rotavirus and *Campylobacter* spp., and at least one of these pathogen was detected in 233 (76.9%) samples. Among 303 children with acute watery diarrhea, Rotavirus monoinfection was detected in 61 (20.1%), *Campylobacter* monoinfection was detected in 81 (26.7%), co-infection was detected in 91 (30.0%) of the cases (Figure 1).

*Figure 1: Distribution of different infections*

Age wise distribution of different infections in children
The highest number of Rotavirus monoinfection was detected in 7–12 months age group category which accounted 29 (47.5%) of total Rotavirus monoinfection. Similarly, highest number of Campylobacter monoinfection 34 (42.0%) was found in the <6 months age group. The co-infection was observed highest (35; 38.5%) on 7–12 months of age (Table 1). Distribution of different infections in age groups was statistically not significant (p>0.05).

**Risk factors for different infections in children**

In multivariate analysis, infection in February was associated with a decreased risk of Rotavirus monoinfection (adjusted odds ratio AOR = 0.26, 95% CI = 0.07–0.98, P = 0.047) than in November. Except 25–36 months age, all age were significantly associated with decreased risk of Campylobacter monoinfection 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 monoinfection 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 diarrhea, clinical presentations were as follows: 23.8%, 49.2% and 27.1% cases had 3–6, 7–9 and >9 loose motions/day patients, 64.7% cases with fever, vomiting in 85.8% cases and 75.2%, 23.8% and 1% cases with no-minimal dehydration, mild-moderate and with severe dehydration respectively. 31.4% cases had abdominal pain, 35.6% with <3 days long diarrhea, 45.9% cases with 3–5 days long, 17.5% with 6–9 days long, 1.0% with >9 days long diarrhea, 70.6% from OPD, 29.4% from IPD, 60.7% had mucus, 30.0% had pus cells in stool.
Abdominal pain and presence of pus cells in stool were less common features, which were significantly associated with Rotavirus monoinfection in multivariate analysis. Pus cells in stool was common clinical feature, while fever and vomiting were less prevalent but significantly associated with *Campylobacter* monoinfection in multivariate analysis. More than 9 loose motions per day, fever, vomiting, presence of mucus in stool were most striking clinical features, while mild-moderate dehydration was less common compared with no-minimal dehydration but significantly associated with co-infections in multivariate analysis (Table 3).

**Antibiotic resistance patterns in Campylobacter spp.**

*Campylobacter* isolates were also resistant to ampicillin (93.6%), cephalexin (88.4%), erythromycin (73.8%), nalidixic acid (72.1%) and cotri-moxazole (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%) (Figure 2).

**Discussion**

This hospital based cross sectional study explored the association between *Campylobacter* and Rotavirus, possible risk factors and specific clinical features. In this study, most cases of acute gastroenteritis were infants. Studies also revealed that diarrhea incidence peaks at age 6–11 months and then decreases with age (19–21).

Our study indicated that a higher frequency of diarrhea was seen in males consistent with past studies (9, 12, 22, 23). Males’ preponderance to develop diarrhea 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 (24).

*Campylobacter* and Rotavirus co-infection was responsible for one third of acute gastroenteritis cases among children <5 years visiting and hospitalized at the KCH in Kathmandu, Nepal during November 2017- April 2018. Findings are consistent with past studies where *Campylobacter* co-infection with Rotavirus in children <5 years of age were reported (25, 26). *Campylobacter* spp isolation in diarrheal cases was high. *Campylobacter* is not on the detection system in routine laboratory investigations on the etiology of diarrhea in Nepal. *Campylobacter* was most prevalent in February then in January and least in November. Rotavirus monoinfection was detected in one fifth of the children with highest in the age group: 7–12 months, consistent with other studies in Nepal and South-East Asian countries (15, 27), however, contrasted with other studies from Nepal (12, 14). It appears that infants less than 6 months of age were initially protected against severe diarrhea, to some extent, by maternal antibodies and they seem to have acquired adequate immunity between 12 and 16 months of age.

Infants and young children aged 6–12 months appear to be at greater risk because of declined levels of maternal antibodies to rotavirus infection (12).

Age, sex, household income, breast feeding, meat consumption, food habit, water source, drinking water type, hygiene and education level of parent were not predictors of Rotavirus monoinfection in these children. While, the month of diarrheal illness does possess a risk for Rotavirus monoinfection, a higher infection during January-February contradicts with our study (15). In our study, age group was the significant predictor of *Campylobacter* monoinfection. No soil contact was
associated with a reduced risk of Campylobacteriosis as compared to frequent soil contact. In Nepal, *Campylobacter* is not included in routine microbiological testing in patients with diarrhea for identification of cases of Campylobacteriosis. We did not find effects of age, sex, occupation, household income, breast feeding, meat consumption, food habit, water source, drinking water, contact with soil, hygiene and parent’s education level as risk factor for co-infection in children.

In multivariate analysis, clinical picture of children with co-infection was more severe as compared to monoinfection for most clinical signs taken into examination, fever, vomiting, abdominal pain, duration of illness, hospitalization, frequency of loose motion/day and presence of mucus in stool. Our observations are consistent with study among Korean children (28). 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 the findings of study in Odisha, India (27). Disease severity is the most important criteria to assess the possibility of a synergistic effect due to co-infections in childhood diarrhea. A recent study from China also supports the above fact where virus–bacteria co-infections are the aggravating factors of severe diarrhea in children (29).

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 (30).

**Conclusion**

In conclusion, this hospital-based cross-sectional study highlighted the burden of Rotavirus, *Campylobacter* and co-infection in childhood diarrhea in Nepal. Rotavirus
and *Campylobacter* associated with gastroenteritis in children was found relatively high percentage of co-infections. *Campylobacter* spp, which are normally not screened in diarrhoeal patients in Nepal, should also be suspected. Diagnosis of the right causative agents among possible multiple infectious etiologies can help in a better management of acute childhood diarrhea.

**Declarations**

**Ethical approval and consent to participate**

*Ethics approval*

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 were securely stored and identified by Study Number.

**Consent for publication**

Not applicable.

**Availability of supporting data**

All data pertaining to this study are within the manuscript.

**Competing interests**

The authors declare that they have no competing interests.

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**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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References

1. World Health Organization. Diarrhoea, fact sheet. 2017. Available from: http://www.who.int/topics/diarrhoea/en. Accessed on: 20 June, 2019.

2. Platts-Mills JA, Babji S, Bodhidatta L, et al. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). Lancet Glob Health. 2015,3: e564-e575.

3. Mackenjee MKR, Coovadia YM, Coovadia HM, Hewitt J and Robins-Browne RM. Aetiology of diarrhoea in adequately nourished young African children in Durban, South Africa. Ann Trop Paediatr. 1984,4:183-187.

4. Nyaga MM, Jere KC, Esona MD, et al. Whole genome detection of rotavirus mixed infections in human, porcine and bovine samples co-infected with various rotavirus strains collected from sub-Saharan Africa. Infect, Gene and Evol. 2015,31: 321-34.
5. Patel MM, Steele D, Gentsch JR, Wecker J, Glass RI and Parashar UD. Real-world impact of rotavirus vaccination. Pediatr Infect Dis J. 2011,30: S1-S5.

6. Bonkoungou IJ, Damanka S, Sanou I, et al. Genotype diversity of group A rotavirus strains in children with acute diarrhea in urban Burkina Faso, 2008–2010. J Med Virol. 2011,83:1485-90.

7. Bourdett-Stanziola L, Jiménez C and Ortega-Barria E. Diversity of human rotavirus G and P genotypes in Panama, Costa Rica, and the Dominican Republic. Am J Trop Med Hyg. 2008,79: 921-24.

8. Pun SB, Nakagomi T, Sherchand JB, et al. Detection of G12 human rotaviruses in Nepal. Emer Infect Dis. 2007,13: 482.

9. Shariff M, Deb M and Singh R. A study of diarrhoea among children in eastern Nepal with special reference to rotavirus. Indian J Med Microbiol. 2003, 21: 87.

10. Sherchand JB and Haruki K. Rotavirus diarrhoea in children and animals of urban and rural Nepal. J Nepal Health Res Council. 2004,2: 1-4.

11. Uchida R, Pandey BD, Sherchand JB, et al. Molecular epidemiology of rotavirus diarrhea among children and adults in Nepal: detection of G12 strains with P [6] or P [8] and a G11P [25] strain. J Clinic Microbiol. 2006,44: 3499-505.

12. Sherchand JB, Nakagomi O, Dove W, et al. Molecular epidemiology of rotavirus diarrhea among children aged < 5 years in Nepal: predominance of emergent G12 strains during 2 years. The Journal of Infectious Diseases. 2009,200: S182-S87.

13. Pandey BD and Pun SB. Trends of rotavirus in Nepal. KUMJ. 2012,9:32-35.

14. Sherchan JB, Ohara H, Sherpa K, et al. Rotavirus Nosocomial Infection in Children under 5 years of age: A Preliminary study in Nepal. J Nepal Paedtr Soc. 2011, 31:30-34.

15. Dhital S, Sherchand JB, Pokhrel BM, et al. Molecular epidemiology of Rotavirus
causing diarrhea among children less than five years of age visiting national level children hospitals, Nepal. BMC Pediatr. 2017, 17:101.

16. Calvo C, Gallardo P, Torija P, et al. Enterovirus neurological disease and bacterial coinfection in very young infants with fever. J Clin Virol. 2016, 85:37-39.

17. Grimprel E, Rodrigo C and Desselberger U. Rotavirus disease: impact of coinfections. Pediatr Infect Dis J. 2008, 27: S3-S10.

18. The United Nations Children’s Fund (UNICEF). Levels & Trends in Child Mortality. Report; Estimates Developed by the UN Inter-agency Group for Child Mortality Estimation. 2014. New York, United Nations Children’s Fund.

19. Naficy AB, Abu-Elyazeed R, Holmes JL, et al. Epidemiology of RVA diarrhea in Egyptian children and implications for disease control. Am J Epidemiol. 1999, 150(7): 770-77.

20. Amer MA, Abdel Salam SM, Ibrahim HA and Farag MA. Detection of group A Rotavirus and characterization of G type among Egyptian children with diarrhea. Egyptian J Med Microbiol. 2007, 16(1): 123-132.

21. Fischer Walker CL, Perin J, Aryee MJ, Boschi-Pinto C, Black RE. Diarrhea incidence in low- and middle-income countries in 1990 and 2010: a systematic review. BMC Public Health. 2012, 12: 220.

22. Klein EJ, Boster DR, Stapp JR, et al. Diarrhea etiology in a children’s hospital emergency department: a prospective cohort study. Clin Infect Dis. 2006, 43(7): 807-13.

23. Moyo SJ, Gro N, Matee MI, et al. Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dares Salaam, Tanzania. BMC Pediatrics. 2011, 11:19.

24. Salim H, Karyana PG, Sanjaya-putra GN, Budiarsa S, Soenarto Y. Risk factors of
rotavirus diarrhoea in hospitalized children in Sanglah Hospital, Denpasar: a prospective cohort study. BMC Gastroenterol. 2014,14:54-60.

25. Rajendran P, Babji S, George AT, Rajan DP, Kang G and Ajjampur SS. Detection and species identification of Campylobacter in stool samples of children and animals from Vellore, south India. Indian J Med Microbiol. 2012,30: 85.

26. Zhu XH, Tian L, Cheng ZJ, et al. Viral and Bacterial Etiology of Acute Diarrhea among Children under 5 Years of Age in Wuhan, China. Chin Med J (Engl) 2016;129(16):1939-44.

27. Shrivastava AK, Kumar S, Mohakud NK, Suar M, Sahu PS. Multiple etiologies of infectious diarrhea and concurrent infections in a pediatric outpatient-based screening study in Odisha, India. Gut Pathogens. 2017,9:16.

28. Koh H, Baek SY, Shin JI, Chung KS, Jee YM. Coinfection of viral agents in Korean children with acute watery diarrhea. J Korean Med Sci. 2008,23:937-40.

29. Zhang J, Duan Z, Payne DC, et al. Rotavirus-specific and overall diarrhea mortality in Chinese children younger than 5 years: 2003 to 2012. The Pediatric Infectious Disease Journal. 2015,34: e233.

30. Ghimire L, Singh DK, Basnet HB, Bhattarai RK, Dhakal S, Sharma B. Prevalence, antibiogram and risk factors of thermophilic Campylobacter spp. in dressed porcine carcass of Chitwan, Nepal. BMC Microbiol. 2014,14: 85.

Tables

Table 1: Age-wise distribution of different infections
| Age (months) | Type of infection | Rotavirus only | Campylobacter only | Co-infections | Undetected |
|--------------|-------------------|----------------|--------------------|---------------|------------|
| <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 mono-infection | Campylobacter mono-infection | Co-infection |
|--------------|--------------------------|-------------------------------|--------------|
|              | AOR (95%CI)               | P-value                       | AOR (95%CI)  | P-value         | AOR (95%CI) | P-value |
| Months       |                          |                               |              |                 |              |         |
| November     | 1                         |                               |              |                 |              |         |
| December     | 0.59 (0.15-2.30)          | 0.451                         | 1            | 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                         | 1            | 6.06 (0.68-53.96) | 0.106       | 11.34 (1.27-101.27) | 0.030 |
| February     | 0.26 (0.07-0.98)          | 0.047                         |              | 5.36 (0.58-48.94) | 0.137       | 25.32 (2.68-238.69) | 0.005 |
| Age (in months) |                         |                               |              |                 |              |         |
| <6           | 1                         |                               |              |                 |              |         |
| 7-12         | 1.70 (0.39-7.33)          | 0.471                         | 1            | 0.19 (0.04-0.90) | 0.037       | 1.26 (0.32-4.87) | 0.738 |
| 13-24        | 1.64 (0.31-8.49)          | 0.553                         | 1            | 0.07 (0.01-0.42) | 0.004       | 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       | 0.47 (0.03-5.74) | 0.556 |
| Sex          |                          |                               |              |                 |              |         |
| Male         | 1                         |                               |              |                 |              |         |
| Female       | 1.09 (0.52-2.26)          | 0.817                         | 1            | 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         |
|                         | AOR (95% CI) | 95% CI | 1     | P     |
|-------------------------|--------------|--------|-------|-------|
| **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      | 0.64 (0.21-1.94) | 0.438 | 1.47 (0.55-3.89) | 0.436 |
| Boiled                  | 0.81 (0.28-2.28) | 0.695  | 1     |       |                   |       |
| 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 | 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      |       |       |                   |       |
| **Hygiene**             |              |        |       |       |                   |       |
| Poor                    | 1            | 1      | 0.97 (0.33-2.80) | 0.964 | 1.01 (0.40-2.55) | 0.973 |
| Good                    | 0.45 (0.16-1.24) | 0.125  | 1     |       |                   |       |
| **Parent’s education level** |          |        |       |       |                   |       |
| Illiterate              | 0            | 0.999  | 0     | 0.999 | 2.12 (0.72-6.22) | 0.169 |
| 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      |       |       |                   |       |

AOR = Adjusted odds ratio, 95% CI = 95% confidence interval, 1 = Reference, P<0.05 was considered 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) |
| **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) |
| 7-9               | 1.57 (0.56-4.38) | 0.385             | 0.98 (0.38-2.52) | 0.971   | 0.33 (0.13-0.85) |
| >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) |
| 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) |
| 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) |
| Severe            | 0              | 0.999             | 0            | 0.999   | 0           |
| **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) |
| 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) |
| 6-9               | 0.67 (0.24-1.89) | 0.458             | 0.54 (0.21-1.41) | 0.211   | 1.68 (0.67-4.16) |
| >9                | 0              | 0.999             | 2.15 (0.13-33.69) | 0.584   | 0           |
| **Mucus**         |                |                   |              |         |             |
| Absent            | 1.39 (0.74-2.61) | 0.399             | 0.81 (0.45-1.49) | 0.514   | 0.46 (0.25-0.86) |
| 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) |
| Present           | 1              |                   | 1            |         | 1           |

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

Figures
Figure 1
Distribution of different infections

Figure 2
Antibiotic resistance patterns in isolated Campylobacter spp.
