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# Diarrheal pathogens in children under five: a cross-sectional study from Children's Hospital 1, Vietnam

Thanh Minh Ngoc Bui[1,2,†], Tri Minh Bui[3,†], Phuc Le Hoang[4], Van Hung Pham[5], Tuan Anh Nguyen[1,6,*]

[1]Department of Pediatrics, School of Medicine, University of Medicine and Pharmacy at Ho Chi Minh City, 700000, Vietnam
[2]Center for Advanced Training in Clinical Simulation, School of Medicine, University of Medicine and Pharmacy at Ho Chi Minh City, 700000, Vietnam
[3]Center for Molecular Biomedicine, University of Medicine and Pharmacy at Ho Chi Minh City, 700000, Vietnam
[4]Gastroenterology Department, Children's Hospital 1, 700000, Vietnam
[5]Vietnam Research and Development Institute of Clinical Microbiology, 700000, Vietnam
[6]Department of Internal Medicine and Gastro-Hepato Integrated Research Team (GHIRT-002.TCM2025), School of Medicine, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, 700000, Vietnam

*Corresponding author. Department of Pediatrics, School of Medicine, University of Medicine and Pharmacy at Ho Chi Minh City, 217 Hong Bang Street, Ho Chi Minh City, 700000, Vietnam. E-mail: nguyenanhtuan@ump.edu.vn
†Thanh Minh Ngoc Bui and Tri Minh Bui contributed equally as first authors.

## Abstract

**Background: Acute diarrhea remains a major cause of mortality in children under five, with over 40 known enteropathogens. In Vietnam, most studies have focused on individual pathogens or pathogen groups. Objectives: This study aimed to characterize the etiological profile of children with acute watery and acute bloody diarrhea, describe epidemiological and clinical characteristics across pathogen groups, and examine the characteristics of Rotavirus-infected children. Methodology: From 10 December 2022 to 30 April 2023, 382 children under five hospitalized with acute diarrhea at the Gastroenterology Department, Children's Hospital 1, Vietnam, were tested for enteric pathogens using multiplex real-time RT-PCR. Results: Fifteen pathogens, including viruses, bacteria, and parasites, were identified among 382 cases. Group A Rotavirus (55.2%), Adenovirus (20.9%), Norovirus II (13.9%), and diarrheagenic *Escherichia coli* (DEC) (9.2%) were the most frequently detected pathogens. Coinfections were observed in 39.0% of cases. Vomiting, watery diarrhea, and vomiting at onset were more common in viral infections, whereas bloody stools, high-grade fever, and fever at onset predominated in bacterial infections. Unvaccinated children with Rotavirus infection experienced higher diarrhea frequency and longer durations of diarrhea, vomiting, fever, and hospitalization. Conclusion: The microorganisms associated with acute diarrhea are diverse, with 15 pathogens identified, Group A Rotavirus, Adenovirus, Norovirus II, and**

**DEC being the top four most common. Clinical features showed differences among pathogen groups. Understanding epidemiological and clinical information may support early clinical decision-making in Vietnam in the absence of diagnostic testing. Further studies are needed to assess their similarity and applicability in low- and middle-income countries.**



## Introduction

Diarrhea is one of the major causes of mortality in children under five [1]. According to World Health Organization statistics, approximately 1.7 billion cases of childhood diarrhea occur each year, resulting in 443 832 deaths among children under 5 years old [1].

More than 40 diarrheal pathogens have been reported [2–4]. The causes of acute diarrhea vary over time, depending on age groups, socioeconomic conditions, and environmental sanitation [5]. In countries that have implemented Rotavirus vaccination programs, shifts in pathogen epidemiology have been observed [6, 7]. In the United States, Norovirus has become the leading cause of acute diarrhea in children [8]. Meanwhile, in Africa and Asia, Rotavirus, *Shigella*, Adenovirus, *Enterotoxigenic Escherichia coli*, *Cryptosporidium*, and *Campylobacter* have been identified as causes of moderate to severe acute diarrhea [9].

Over the past 20 years, most studies in Vietnam have only focused on a single pathogen or multiple pathogens within the same group of disease-causing agents. The most comprehensive study, which investigated 12 pathogens, was conducted in 2015 [5]. Thompson et al. carried out a prospective multicenter observational study on children hospitalized for acute diarrhea in Ho Chi Minh City, investigating: Rotavirus, Norovirus, diarrheagenic *Escherichia coli* (DEC), *Campylobacter*, *Salmonella*, *Shigella*, *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium cysts*, *Yersinia*, *Plesiomonas,* and *Aeromonas* [5]. In contrast, other studies have focused on fewer diarrheal agents. Nguyen TV investigated 6 pathogens in 2006 (Rotavirus, DEC, *Shigella*, *Salmonella*, *Vibrio Cholerae,* and *Bacteroides fragilis*), while Duong VT examined 3 pathogens in 2020 (DEC, *Shigella,* and nontyphoidal *Salmonella*) [10–12]. Additionally, it is challenging to detect pathogens from stool samples. In Ho Chi Minh City, microbiological culture is rarely performed in hospital laboratories, and only a narrow variety of bacterial pathogens is identified [13]. For parasitic detection, microscopy has low sensitivity, and real-time PCR is rarely applied due to high cost and staffing requirements [13]. Consequently, several pathogens are not routinely identified [13]. Therefore, updating the epidemiology of enteropathogens and clinical characteristics of children with acute diarrhea is essential.

In Southern Vietnam, Children's Hospital 1 is among the largest tertiary pediatric hospitals, with a capacity of 1500 beds [5, 14]. Annually, the hospital receives approximately 1.5 million outpatient visits and 95 000 admissions from the city and neighboring provinces in the Mekong Delta [14]. At present, Rotavirus vaccines in Vietnam are provided on a self-financed basis, and no national data on coverage are available [15]. A recent report from Children's Hospital 1 (2018) indicated that only 3.9% of hospitalized children with acute diarrhea had received Rotavirus vaccination [15]. Therefore, this study examines the epidemiological and clinical characteristics of causative pathogens of all acute diarrhea (acute watery diarrhea and acute bloody diarrhea) in children aged 1 month to 5 years admitted to the Gastroenterology Department, Children's Hospital 1. We aim to identify the distribution of etiological pathogens; describe the epidemiological and clinical characteristics of children according to pathogen groups (viral, bacterial, and viral–bacterial coinfections); and assess Rotavirus vaccination status and clinical characteristics of Rotavirus-infected children in Ho Chi Minh City. The findings of this study are expected to support clinicians in making treatment decisions and inform public health policy in resource-limited tropical settings where diarrhea remains a major burden.

## Materials and methods

### Sample size determination

To estimate the sample size, a single population proportion formula was used with the assumption of a 95% confidence level and a 5% margin of error. For the proportion, 41.6% was chosen based on the highest pathogen proportion observed in a prospective, hospital-based study in Ho Chi Minh City in 2015 [5]. Therefore, our calculated sample size was 374. We aimed to collect 400 samples to account for potential issues: children withdrawn from the study, children who do not meet the clinical manifestation criteria or children who do not have enough information.

## Ethical considerations

The study uses the minimally invasive method and was approved by the Ethics Committee of Children's Hospital 1, Ho Chi Minh City, Vietnam. The number of approval document was IRB-VN02.026; IORG0007285, FWA00009748 (Date: 17 November 2022, Decision No: 424/GCN-BVNĐ1). Written informed consent was obtained from parents or legal guardians.

## Patient selection

This cross-sectional study was conducted at the Gastroenterology Department, Children's Hospital 1, between 10 December 2022 and 30 April 2023.

According to the guidelines, acute diarrhea is defined as the passage of unusually loose or watery stools at least three times within 24 hours, with a duration of less than 14 days [16]. Therefore, this study enrolled children aged 1–60 months who were hospitalized in the Gastroenterology Department with acute diarrhea, defined as at least three episodes of loose or watery stools within 24 hours, possibly containing mucus and/or blood, and lasting less than 14 days.

The exclusion criteria were as follows: children diagnosed with short bowel syndrome, intestinal diseases that had undergone surgery, chronic bowel disease, continuous diarrhea since before 30 days of age, and metabolic diseases; children diagnosed with cow's milk protein allergy, lactose intolerance, and immunocompromised; children treated with long-term immunosuppressive drugs; children who lack necessary information in the study (frequency of diarrhea, frequency of vomiting, weight loss, etc.); children whose stool samples were not obtained within 24 hours after admission.

## Data collection

For pediatric patients who met the inclusion criteria, interviews concerning epidemiology, medical history, and clinical symptoms were conducted with their caregivers. Nutritional status was categorized into three groups: Malnutrition, normal, and overweight—obese. Malnutrition was defined as weight-for-height/length below –2 SD, whereas overweight—obese was defined as weight-for-height/length above +2 SD [17]. The treatment results (duration of diarrhea/vomiting/fever, length of hospital stay, initial fluid infusion duration, initial antibiotic use duration) were documented before discharge. If a patient was discharged before the diarrhea symptoms resolved, the investigator followed up by phone to collect the date of diarrhea resolution.

For sample collection, sterile containers were used to collect 2 ml of diarrheal stool from patients within 24 hours of admission. To avoid contamination with urine, stool samples was collected directly from the patient's rectum using a rectal catheter. The samples were then transferred to Nam Khoa Biotek's Laboratory for gastrointestinal pathogen identification using multiplex real-time RT-PCR.

## Detection of pathogens by real-time PCR

Total nucleic acid (DNA and RNA) was extracted from stool samples using Nam Khoa's DNARNAprep-MAGBEAD kit (Nam Khoa biotek, Ho Chi Minh city, Vietnam). After extraction, a multiplex real-time RT-PCR kit from Nam Khoa was used to identify 44 diarrheal pathogens, including 18 viruses, 15 bacteria, and 11 parasites simultaneously. The sample was considered positive when the cycle threshold value was less than 40.

## Statistical analysis

Data were analyzed using Stata software (StataCorp, version 16.0, College Station, Texas). Analysis of variance (ANOVA) was applied for parametric tests, while the Mann-Whitney U or Kruskal-Wallis test was used for the non-parametric data. A Fisher's exact test or Pearson Chi-Square test was used for categorical data. A $p$-value $< 0.05$ was considered statistically significant. When significant differences were found among the three groups of agents (viral, bacterial and viral–bacterial coinfection), pairwise comparisons were performed with Bonferroni correction.

# Results

## Baseline characteristics

In total, 382 cases that met the sampling requirements were included in the study (Fig. 1).

Children aged 12–24 months were the most common group, with a median age of 16 months (IQR: 11–26). Males were more prevalent, with a male-to-female ratio of 1.65:1 (Supplementary Table S1).

The clinical characteristics of patients were shown in Supplementary Table S2. Vomiting was the most common onset symptom (44.8%) and the major clinical manifestation (94.5%). Besides, watery stool was the predominant stool characteristic (77.8%) among children admitted to the hospital.

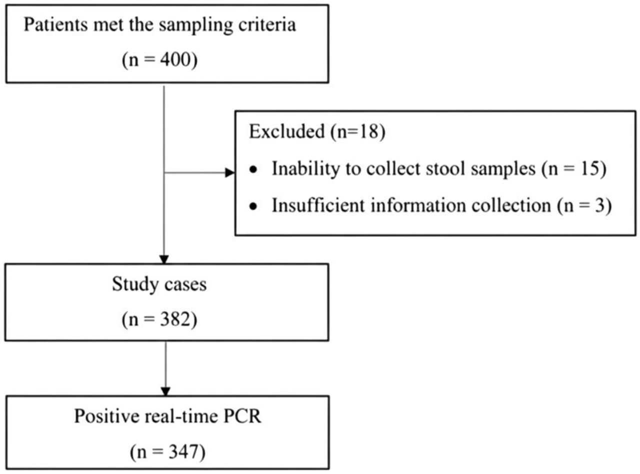


**Figure 1** Flow diagram of study design.

## Detection of diarrheal pathogens

Based on the multiplex real-time RT-PCR method, diarrheal pathogens were detected in most stool samples (347/382, 90.8%) (Table 1 and Fig. 2).

Viruses, including viral mono-infection and viral co-infection, constituted the largest proportion of detectable enteropathogens, with a positive rate of 58.1%. The main causative agent was Group A Rotavirus (55.2%), followed by Adenovirus (20.9%).

Of the 382 samples, 149 tested positive for at least two diarrhea-associated pathogens, including dual and triple infections (Table 1 and Supplementary Table S3). Group A Rotavirus was the most commonly found pathogen in the viral-viral (36/52, 69.2%) and viral–bacterial (54/90, 60%) coinfection group. In the bacterial–bacterial coinfection group, DEC (4/7, 57.1%) was the most frequently detected pathogens.

## Distribution of pathogens by clinical characteristics

Table 2 showed the association between pathogen groups (viruses, bacteria, and mixed infections) and patients' clinical characteristics. Vomiting was a common clinical manifestation at the onset of the disease. The incidence and frequency of vomiting during diarrhea were significantly higher in the viral or viral–bacterial groups compared to the bacterial group. Watery stool was mainly seen in viral infections, while bloody stool was more frequent in bacterial infections. Moderate to high fever was more common in bacterial and viral–bacterial infections, with a significant difference in high fever between the bacterial and viral groups ($p < 0.05$).

## Characteristics of rotavirus-infected children and their vaccination status

The analysis of Rotavirus mono-infection (117 cases) showed that unvaccinated children had more severe symptoms, including higher diarrhea frequency, longer durations of diarrhea, vomiting, fever, and longer hospital stays, with all differences being statistically significant ($p < 0.05$) (Table 3).

## Discussion

To the best of our knowledge, this is the first study in Vietnam using multiplex real-time RT-PCR to identify 44 diarrheal pathogens. Therefore, this surveillance study conducted at the largest children's hospital in Vietnam's biggest city may offer valuable data.

Multiplex real-time RT-PCR was used to identify the diarrheal pathogens. The results showed that 90.8% of the stool samples contained agents, which was higher than previous reports conducted in Tanzania (86.2%) [18], Peru (73.5%) [19], Colombia (71%) [20], China (69.8%) [21], and also Vietnam (67.3% and 75.2%) [5, 10]. Although the studies in Tanzania and Peru used multiplex real-time RT-PCR, the criteria for determining positive results might account for the variation in pathogen detection rate [18, 19, 22]. In other studies, a combination of methods was used: The Colombian study employed stool culture, RT-PCR, enzyme-linked immunosorbent assay (ELISA), and stool microscopy [20]; the study in China combined stool culture and RT-PCR [21]; while the study in Vietnam used stool culture, ELISA, and stool microscopy [5, 10]. These varying techniques may account for

**Table 1** Detection of diarrheal pathogens in 382 children with acute diarrhea.

| **Pathogens** | | ***n*** **(%)** |
|---|---|---|
| **Viral mono-infection** | | **170 (44.5)** |
| Rotavirus group A | | 117 (30.6) |
| Adenovirus | | 26 (6.81) |
| Norovirus II | | 18 (4.71) |
| Sapovirus | | 6 (1.6) |
| Aichivirus | | 1 (0.25) |
| Bocavirus | | 1 (0.25) |
| Enterovirus | | 1 (0.25) |
| **Viral-viral co-infection** | | **52 (13.6)** |
| Rotavirus group A | Adenovirus | 21 (5.5) |
| | Sapovirus | 5 (1.3) |
| | Enterovirus | 4 (1.1) |
| | Norovirus II | 3 (0.8) |
| | Bocavirus | 3 (0.8) |
| Adenovirus | Norovirus II | 7 (1.8) |
| | Bocavirus | 2 (0.5) |
| | Enterovirus | 1 (0.25) |
| Norovirus II | Astrovirus | 1 (0.25) |
| Sapovirus | Enterovirus | 1 (0.25) |
| Rotavirus group A + Norovirus II + Adenovirus | | 3 (0.8) |
| Rotavirus group A + Sapovirus + Adenovirus | | 1 (0.25) |
| **Bacterial monoinfection** | | **27 (7.1)** |
| *Salmonella* | | 13 (3.4) |
| *Campylobacter jejuni* | | 4 (1.1) |
| *Campylobacter coli* | | 1 (0.25) |
| *Shigella* | | 4 (1.1) |
| *Clostridioides difficile* | | 3 (0.8) |
| *Ecoli EPEC/STEC (eaeA)* | | 1 (0.25) |
| *Vibrio cholerae (CTX)* | | 1 (0.25) |
| **Bacterial–bacterial co-infection** | | **7 (1.8)** |
| *Ecoli EPEC/STEC (eaeA)* | *Ecoli O157: H7* | 2 (0.5) |
| *Ecoli EIEC (ial)* | *Campylobacter coli* | 1 (0.25) |
| | *Shigella* | 1 (0.25) |
| *Clostridioides difficile* | *Campylobacter jejuni* | 1 (0.25) |
| | *Salmonella* | 1 (0.25) |
| | *Shigella* | 1 (0.25) |
| **Parasitic mono-infection** (*Enterocytozoon bieneusi*) | | **1 (0.25)** |
| **Viral–bacterial co-infection (Table S3)** | | **90 (23.5)** |
| **Total** | | **347 (90.8)** |

Bold values indicate the total percentage for each pathogen category; the individual pathogens and their corresponding percentages are listed below each bold heading.

differences in results. Generally, the detection rates for stool microscopy, stool culture, and ELISA were lower than those for multiplex real-time RT-PCR. Additionally, these studies were often focused on identifying only a limited range of microorganisms. Seasonality and patient characteristics, such as nutritional and vaccination status, may contribute to the different results reported in studies from various countries [6, 7, 23].

Viruses were the predominant cause of acute diarrhea in children under 5 years old. Among them, Rotavirus was the leading pathogen, consistent with previous studies [5, 10, 18, 24–26]. However, surprisingly, our findings recorded a higher rate of

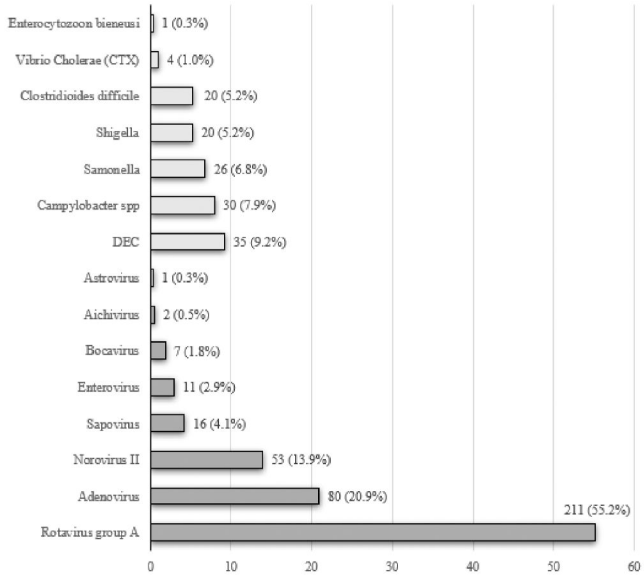


**Figure 2** Proportion of diarrheal pathogens in the study ($n$ = 382).

**Table 2** Distribution of pathogens by clinical characteristics.

| **Characteristics *n* = 346** | | **Diarrheal Pathogens** | | | ***p*** |
|---|---|---|---|---|---|
| | | **Virus *n* = 222** | **Bacteria *n* = 34** | **Virus + Bacteria *n* = 90** | |
| **Nutritional status** | Malnutrition | 23 (65.7) | 4 (11.4) | 8 (22.6) | 0.65[a] |
| | Normal | 183 (65.1) | 27 (9.6) | 71 (25.3) | |
| | Overweight—Obese | 16 (53.3) | 3 (10) | 11 (36.7) | |
| **Onset symptoms** | Vomiting[b+c] | 118 (53.2) | 3 (8.8) | 42 (46.7) | ***0.001***[a] |
| | Fever[b+c] | 26 (11.7) | 23 (67.6) | 15 (16.7) | ***0.001***[a] |
| **Stool characteristics** | Watery[b+c+d] | 201 (90.5) | 8 (23.5) | 63 (70.0) | ***0.001***[a] |
| | Mucoid[b+d] | 16 (7.2) | 11 (32.4) | 22 (24.4) | ***0.001***[a] |
| | Bloody[b+c] | 5 (2.3) | 15 (44.1) | 5 (5.6) | ***0.001***[e] |
| **Diarrhea frequency** | | 8 (6–10) | 7.5 (5–10) | 8 (6–12) | 0.18 |
| **Fever severity** | Low | 95 (42.8) | 8 (23.5) | 29 (32.2) | 0.05[a] |
| | Moderate | 61 (27.5) | 15 (44.1) | 32 (35.6) | 0.09[a] |
| | High[b] | 9 (4.1) | 9 (26.5) | 9 (10) | ***<0.001***[a] |
| **Highest temperature**[b+d] | | 38.5 (38.3–39) | 39.1 (38.6–39.8) | 39 (38.5–39) | ***0.001*** |
| **Vomiting**[b+c] | | 213 (95.9) | 28 (82.4) | 88 (97.8) | ***0.006***[a] |
| **Vomiting frequency**[b+c] | | 5 (4–8) | 3 (2–4.5) | 5 (4–10) | ***0.001*** |
| **Dehydration** | | 46 (20.7) | 4 (11.8) | 26 (28.9) | 0.092[a] |
| **Respiratory symptoms** | | 72 (32.4) | 5 (14.7) | 25 (27.8) | 0.1[a] |

All categorical data are expressed as $n$ (%). Continuous data are non-normally distributed and are presented as median (interquartile range). Bold values of $p < 0.05$ indicate statistically significant differences. a Pearson chi-square test. b Virus vs. Bacteria ($p < 0.05$). c Bacteria vs. Virus + Bacteria coinfection ($p < 0.05$). d Virus vs. Virus + Bacteria coinfection ($p < 0.05$). e Fisher's exact test. Malnutrition (weight-for-height/length < −2 SD) and overweight/obesity (weight-for-height/length > 2 SD).

[a] Pearson Chi-Square.
[b] Virus vs Bacteria ($p < 0.05$).
[c] Bacteria vs Virus + Bacteria coinfection ($p < 0.05$).
[d] Virus vs Virus + Bacteria coinfection ($p < 0.05$).
[e] Fisher's exact test.

Malnutrition (weight for height/length < −2 SD) and Overweight—Obese (weight for height/length >2 SD).

**Table 3** Characteristics of Rotavirus mono-infection in 117 children based on the vaccination status.

| **Characteristics Rotavirus mono-infection** | **Rotavirus vaccine status** | | ***p*** |
|---|---|---|---|
| | **Yes *n* = 46** | **No *n* = 71** | |
| **Diarrhea frequency** | 8.3 ± 3.3 | 9.6 ± 3.3 | ***0.02*** |
| **Vomiting** | 44 (95.7) | 70 (98.6) | 0.5[a] |
| **Vomiting frequency** | 5.9 ± 2.3 | 6.2 ± 3.2 | 0.4 |
| **Fever** | 35 (76.1) | 53 (74.7) | 0.8[b] |
| **Highest body temperature** | 38.5 (38.2–38.5) | 38.5 (38.3–39) | 0.8 |
| **Dehydration** | | | |
| None | 37 (80.4) | 51 (71.8) | 0.6[a] |
| Mild—Moderate | 8 (17.4) | 18 (25.4) | |
| Severe | 1 (2.2) | 2 (2.8) | |
| **Diarrhea duration** | 4.5 ± 1 | 5.7 ± 1.7 | ***<0.001*** |
| **Vomiting duration** | 1.9 ± 0.9 | 2.7 ± 1.5 | ***0.006*** |
| **Fever duration** | 1.9 ± 0.7 | 2.5 ± 1.2 | ***0.003*** |
| **Hospitalization duration** | 4 (3–5) | 5 (4–6) | ***<0.001*** |

All categorical data are presented as *n* (%). Normally distributed data are presented as mean ± SD; non-normally distributed data are presented as median (interquartile range). Bold values of $p < 0.05$ indicate statistically significant differences.

[a] Fisher's exact test.

[b] Pearson Chi-square.

Adenovirus compared to the study by Tuan et al. at Children's Hospital 1 in Vietnam (2003), which reported a 3.2% detection rate for this pathogen [27]. This discrepancy raised the question of whether the prevalence of Adenovirus in acute diarrhea cases in Ho Chi Minh City is increasing. In a similar context, Lilian reported an increase in Adenovirus prevalence from 21.3% to 24.8% in southern Brazil between 2018 and 2019 [24], and Chang Hailing observed an increase from 5.9% to 9.3% in Shanghai from 2016 to 2018 [28]. Therefore, future studies will be essential to address this issue and develop timely and effective intervention strategies.

Coinfections were found in more than a third of the study participants. The detected co-infections included combinations of pathogen groups (viruses, bacteria, and both). Among these groups, the mixed etiology between viruses and bacteria (23.5%) was the most common. Our infection rate was similar to reports from Tanzania [18] and India [29], while it was higher than the rate in the previous study of Thompson in Ho Chi Minh City (4%) [5]. This variance could be explained by different living conditions, lifestyle, hand hygiene practices, and even diagnostic techniques. The high rate of coinfections suggests that the detected pathogens may interact to cause disease, contributing to the overall burden. Additionally, the fact that certain pathogens are more frequently associated with coinfections implies that these pathogen combinations may share common sources of exposure [30].

Significant differences in clinical characteristics between viral and bacterial groups are documented. We hope these findings will provide valuable insights for identifying the enteropathogens of acute diarrhea during initial assessments of children. Vomiting was an initial symptom in most children infected with viruses. Angela et al. noted it occurred twice as often in viral infections compared to bacterial ones [19], and Verena reported a higher frequency of vomiting in viral infections (2.6 ± 0.2 times/day) compared to bacterial infections ($p < 0.001$) [31]. These findings are consistent with our results. Kalabamu et al. identified fever as a key predictor of bacterial diarrhea, with bacterial infections causing more severe inflammation and intense systemic response [32]. Wiegering also reported higher fever in bacterial infections (39.4 ± 0.3°C), consistent with our findings. Regarding diarrhea frequency, both Thompson [5] and our study found no significant difference between viral and bacterial infections, while Wiegering reported more daily episodes in bacterial infections [31]. This discrepancy may be due to differences in data collection methods: our data was obtained through caregiver interviews, whereas Wiegering relied on nursing staff recordings of diaper changes. Therefore, diarrhea frequency may not be a reliable symptom due to variations in collection methods.

Overall, the pathogens responsible for acute diarrhea are highly diverse and exhibit overlapping clinical features. Understanding the epidemiological and clinical characteristics of these infections can aid in guiding diagnosis and appropriate initial management, especially when laboratory testing is not available or while awaiting results. However, it should be emphasized that oral rehydration solution and zinc supplementation remain the mainstay of treatment for acute diarrhea, regardless of the causative pathogen [16]. Antimicrobials are indicated for acute bloody diarrhea and specific bacterial causes, but are not indicated for routine use in acute watery diarrhea [16, 33].

Group A Rotavirus was the most common agent and was therefore analyzed in relation to vaccination status. Despite the heavy burden of Rotavirus infection in Vietnam, at the time of this study, Rotavirus vaccination was not included in the national immunization program. Our study showed that the coverage rate had increased [15], however, the proportion of children not vaccinated remained high (44.5%) (Supplementary Table S1). Furthermore, children who had not received the Rotavirus vaccine had a significantly higher rate of Rotavirus infection ($p < 0.001$) (Supplementary Table S4). Additionally, among children infected with group A Rotavirus, vaccinated children experienced less severe symptoms and faster recovery than unvaccinated children. These findings are consistent with several studies [15, 34, 35]. However, children were infected with Rotavirus despite their vaccination status (Supplementary Table S4). This could be due to the difference in protective efficacy, since the vaccine's effectiveness was influenced by various factors, including age, geographical location, season, nutritional status, the types of Rotavirus vaccination, and the genotype of Rotavirus [15, 36]. Although genotyping was not performed in this study, which can affect both the severity of clinical symptoms and the vaccine's efficacy, it is important to note that Group A Rotavirus remains a leading cause of acute severe diarrhea. These findings underscore the ongoing need for attention from our healthcare system. Increasing Rotavirus vaccination coverage, alongside improvements in socioeconomic conditions, health, and sanitation standards, can help address this public health challenge.

## Strengths and limitations

There are several limitations in our study. First, the study population comprised children with acute diarrhea admitted to Children's Hospital 1, which may not fully represent community cases. Second, the sample was collected using a convenience sampling method, but we attempted to include all eligible hospitalized patients during the study period to minimize selection bias. Another limitation was the lack of a healthy control group, as some pathogens may also be present in asymptomatic individuals, usually in substantially lower quantities [30]. Consequently, this range of pathogens identified should be regarded as preliminary data to guide future research. Lastly, the relatively short study period prevented analysis of the monthly distribution of detected pathogens or exploration of the association between seasonality and pathogen recovery. Future studies with longer durations and monthly stratification are needed to clarify these temporal trends. Our sampling period is known as the peak circulation time for Group A Rotavirus, which was typically observed in countries with winter or the northern regions of our country. However, in the southern region and Ho Chi Minh City, the circulation rate of Rotavirus was relatively consistent throughout the year [27, 37]. Therefore, we believe this factor did not significantly impact the prevalence of microorganisms observed in our study.

Besides, our study also has several strengths. First, this is a prospective study, so we were able to proactively collect data on relevant variables. Second, this is the first study in Vietnam identifying 44 pathogenic microorganisms by multiplex real-time RT-PCR. Third, the study was conducted at Children's Hospital 1, which is a core pediatric center in the south of Vietnam. Therefore, the results can generally reflect the disease patterns not only in Ho Chi Minh City but also in the surrounding southern provinces. Finally, the diagnostic methods used in this study are relatively low-cost and feasible for replication in similar resource-limited contexts. Importantly, the study provides evidence that may inform clinical practice and public health strategies in tropical pediatrics, where cost-effectiveness and accessibility of interventions are critical considerations.

# Conclusion

The microorganisms associated with acute diarrhea are diverse, with 15 different etiologies identified among 382 cases in southern Vietnam. Group A Rotavirus, Adenovirus, Norovirus II, and DEC were commonly detected. Viral infections were more frequently associated with vomiting, watery diarrhea, and vomiting at disease onset, whereas bacterial infections more frequently presented with bloody stools, high-grade fever, and fever at disease onset. Understanding epidemiological and clinical characteristics may assist clinicians in Vietnam in guiding initial diagnosis and treatment in the absence of diagnostic testing or while awaiting results. Further studies are needed to determine whether these findings are similar and applicable to other low- and middle-income country settings.

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## Author contributions

Thanh Minh Ngoc Bui (Conceptualization [equal], Data curation [lead], Formal analysis [equal], Investigation [lead], Methodology [equal], Project administration [lead], Software [equal], Validation [equal], Visualization [equal], Writing—original draft [equal], Writing—review & editing [equal]), Tri Minh Bui (Formal analysis [equal], Methodology [equal], Software [equal], Visualization [equal], Writing—original draft [equal], Writing—review & editing [equal]), Phuc Le Hoang (Conceptualization [equal], Project administration [equal], Supervision [equal], Writing—review & editing [equal]), and Van Hung Pham (Conceptualization [equal], Funding acquisition [equal], Project administration [equal], Resources [equal], Writing—review & editing [equal]), and Tuan Anh Nguyen (Conceptualization [lead], Project administration [equal], Resources [equal], Supervision [equal], Writing—review & editing [equal])

## Supplementary material

Supplementary material is available at *Journal of Tropical Pediatrics* online.

## Conflicts of interest

The authors declare that they have no conflict of interest. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

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## Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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