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Original article

Bacterial etiology of acute diarrhea in Vietnamese children under five: A cross-sectional study using real-time polymerase chain reaction

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Abstract. *Background and aim:* Bacterial acute diarrhea remain a significant health concern in Vietnam, necessitating further research on infection prevalence and clinical characteristics in pediatric patients. This study aimed to determine the prevalence of bacterial pathogens using real-time polymerase chain reaction and to describe clinical and paraclinical characteristics associated with bacterial infections. *Methods:* This cross-sectional study was conducted on 271 children diagnosed with acute diarrhea at Can Tho Children's Hospital between November 2022 and July 2023. *Results:* Bacterial pathogens were identified in 45.4% of cases, with *Escherichia coli* (18.5%) being the most prevalent, followed by *Salmonella spp.* (10.7%) and *Clostridium difficile* (8.5%). *E. coli* infection was significantly associated with fever (p=0.012), while *Salmonella* sp. was linked to vomiting (p=0.004) and loose watery stools (p=0.008); *Vibrio cholerae* infection showed a significant correlation with dehydration (p=0.028). Additionally, *C. difficile* infections were associated with a higher white blood cell count compared to the uninfected group (p=0.017). Multivariable analysis showed that elevated WBC and fever with *E. coli* (aOR=3.39 and 0.15), vomiting and watery stool were associated with *Salmonella spp.* (aOR=2.54 and 2.71), and dehydration with *V. cholerae* (aOR=8.92). *Conclusions:* Clinical features such as fever, vomiting, and leukocytosis showed associations with presumed bacterial etiology in pediatric diarrhea. However, these exploratory findings are hypothesis-generating and do not support diagnostic or therapeutic decisions. Microbiological confirmation remains essential for accurate etiologic diagnosis and appropriate clinical management. (www.actabiomedica.it)

Key words: bacterial etiology, acute diarrhea, children, Vietnam, real-time PCR

Introduction

Acute diarrhea in children is a significant public health concern due to its morbidity and mortality. According to a 2018 World Health Organization report, this condition accounts for approximately 440,000 deaths annually among children under five (1). The etiologies of acute diarrhea are diverse, including viruses, bacteria, parasites, and fungi, each with distinct infection prevalence and pathogenic roles (2). In developing countries like Vietnam, bacterial pathogens remain the leading cause of medically treated diarrhea, with *Shigella, Campylobacter, Escherichia coli*, and *Salmonella* sp. being the most prevalent (3, 4). The selection of specific pathogens for analysis was based on their established but variable prevalence in previous studies, with *E. coli* ranging from 4.7% to 45.2%, *Salmonella sp.* around 10.7%, and Shigella and Campylobacter contributing modestly to the overall burden of disease (5, 6). Given the evolving

epidemiological landscape, continuous surveillance is necessary to update pathogen infection prevalence (2). Acute diarrhea is defined as the passage of three or more loose or watery stools per day (7). In addition to diarrhea, affected children often present with vomiting, fever, and dehydration. A 2006 study by Nguyen Vu Trung on Vietnamese children with acute diarrhea reported vomiting in 53.8%, fever in 43.6%, and dehydration in 82.6% of cases (8). Different pathogens contribute to varied clinical manifestations, making it crucial to identify key clinical features for early suspicion of bacterial etiology, particularly in settings lacking microbiological testing. Laboratory tests such as white blood cell (WBC) count, electrolyte analysis, and C-reactive protein (CRP) levels aid in assessing inflammation, dehydration, and electrolyte imbalances (9). In clinical practice, the severity and management of diarrhea are often guided by observable clinical signs (dehydration, vomiting) and laboratory markers (WBC, hematocrit, CRP). However, the association between specific pathogens and these clinical and laboratory variables has rarely been thoroughly investigated in Vietnam (8, 10). Bacterial culture, though a traditional diagnostic method, is time-consuming and has limited sensitivity, especially in antibiotic-exposed cases (11). In contrast, real-time polymerase chain reaction (PCR) provides a rapid and accurate approach for pathogen detection and is increasingly utilized in pediatric infectious disease research (12, 13). Given that *Escherichia coli* has historically been the most common bacterial cause of acute diarrhea, we hypothesize that *E. coli* remains the predominant bacterial pathogen in our study population (14). In contrast, Salmonella and Shigella are expected to account for a smaller proportion of cases. Furthermore, infections caused by E. coli are hypothesized to be significantly associated with more severe dehydration and higher levels of inflammatory markers, including white blood cell count and C-reactive protein, compared to other bacterial pathogens (15). The primary objective of this study was to determine the prevalence of common bacterial pathogens among pediatric patients hospitalized with acute diarrhea by real-time PCR. The secondary objective was to compare the association between clinical and paraclinical features and some common bacterial agents detected.

Patients and Methods

Patients

This study included pediatric patients aged 2 months to 5 years who were diagnosed with acute diarrhea and received inpatient treatment at the Department of Gastroenterology, Can Tho Children's Hospital - the largest pediatric referral center in the Mekong Delta, Vietnam - between November 2022 and July 2023. Inclusion criteria: Children aged 2 months to 5 years diagnosed with acute diarrhea and hospitalized within 48 hours. Acute diarrhea was defined as the passage of three or more loose or watery stools within 24 hours, lasting no more than 14 days (7). Exclusion criteria: Patients with diarrhea due to digestive disorders, malabsorption syndromes, chronic diseases, or immune disorders were excluded.

Method

- Research design: Cross-sectional description.
- Sample size: $$n = \frac{Z^2_{1-\frac{\alpha}{2}}}{d^2} p(1-p)$$

With: n represents the minimum sample size; α as the probability of Type 1 error (α= 0.05); Z as the value of the standardized normal distribution for a 95% confidence level (Z= 1.96); d as the allowable error (d= 0.05); and p as the rate of detecting the agent causing acute diarrhea due to *E. coli*, as reported by Mohd Zulkifli Salleh et al in Asian in 2022 (p= 0.228) (16). Therefore, the calculated sample size was n= 270. In practice, this study included 271 patients. The sampling method was convenience sampling until the required number of samples was achieved.

Research content

Children who meet the selection criteria will be invited to participate in the study. They will undergo a structured health assessment, including a clinical examination to document age, gender, fever, vomiting, the presence of loose or watery stools, and an evaluation

of dehydration status. Classification of dehydration status into: no dehydration, some dehydration, and severe dehydration according to the Integrated Management of Child Illness (IMCI) guidelines (17). Patients with some or severe dehydration were generally considered to have "presence of dehydration". Hematocrit, white blood cell (WBC) count analysis will be performed using the SIEMENS ADVIA® 2120i hematology analyzer (Siemens Healthineers, Erlangen, Germany). Serum sodium, serum potassium, and C-reactive protein (CRP) levels will be measured using the AU480 biochemical analyzer (Beckman Coulter, Brea, California, USA). In this study, elevated WBC count and hematocrit were defined as values exceeding the upper limit of the normal reference range for the corresponding age group (18),(19). We analyzed electrolyte disturbances defined as hyponatremia (serum sodium <135mmol/L) and hypokalemia (serum potassium <3.5mmol/L), and inflammatory status defined as CRP >5 mg/L (18). Rectal swabs were collected according the instructions for sampling, packaging, preserving and transporting infectious disease specimens of Department of Preventive Medicine, Vietnam Ministry of Health (20). The procedure for taking a rectal swab sample was as follows: the patient was instructed to lie in the left lateral position with the thigh flexed toward the abdomen to facilitate the procedure. A sterile saline-moistened swab was gently inserted beyond the anal sphincter and rotated to collect the sample. The swab was then withdrawn and inspected to ensure fecal matter was present on the tip. It was subsequently placed into a transport medium tube (Nam Khoa Company, Vietnam). The excess portion of the swab shaft was snapped off to fit the tube, taking care not to touch the rim, and the cap is securely tightened to maintain sterility. Rectal swab specimens were sent to the Vietnam Institute for Clinical Microbiology Research and Development, Nam Khoa Services and Trading Company Limited, Ho Chi Minh City, Vietnam. This laboratory meets Vietnamese standards ISO 9001:2015 and 13485:2017, as well as WHO-GMP (TRS 908, ANNEX 4). Real-time PCR testing was performed to identify common bacterial and viral pathogens, including *Escherichia coli (Escherichia O157:H7, Enterotoxigenic E. coli - ETEC, Shiga toxin producing E. coli – STEC, Enteropathogennic E. coli – EPEC, Enteroinvasive E. coli – EIEC), Salmonella sp., Shigella sp., Campylobacter coli, C. jejuni, Vibrio cholerae, V. parahaemolyticus, Clostridium difficile*. The real-time PCR procedure was conducted as follows: (i) Initially, the sample was homogenized by adding 3 mL of the specimen to 10 mL of purified water containing 50 mg of N-Acetyl L-Cysteine (NALC). The mixture was then subjected to centrifugation at maximum speed in a bench centrifuge for 15 minutes. Following centrifugation, the residue (approximately 300 µL) was collected for DNA extraction. (ii) Nucleic acid extraction was performed using the ZiXpress-32® system (Zinexts Life Science Corp, Taiwan) in conjunction with the NKDNARNAprep-MAGBEAD reagent set (Nam Khoa Company, Vietnam). This reagent set has been validated through comparative analysis with the BOOM method for nucleic acid extraction. (iii) Nucleic acid extractions from patient samples were subjected to Real-time PCR using the CFX96 TouchTM system (Bio-Rad Laboratories, USA) with specific primers and a TaqMan probe to detect 42 pathogens associated with acute diarrhea in children.

Data processing

Data were entered and analyzed using SPSS v.22.0 (IBM Corp., Armonk, NY, USA). Qualitative variables are presented as percentages and were compared using the χ^2 test. Quantitative variables are expressed as means and were compared using the T-test for normal distribution or the Mann-Whitney test for non-normal distribution. A p-value of < 0.05 was considered statistically significant. Variables with a p-value < 0.1 in univariable analysis, along with age and sex, were included in multivariable logistic regression models to determine independent associations with the causative agents of acute diarrhea.

Ethical considerations

The study was approved by the Ethics Council in Biomedical Research of Can Tho University of Medicine and Pharmacy (Approval No. 22.149.SV/PCT-HĐĐĐ) onNovember 30, 2022.

Results

The rate of bacteria was detected by real-time PCR.

Real-time PCR testing of 271 acute diarrhea cases revealed that 45.4% (123/271) of the cases tested positive for bacterial pathogens. *E. coli* was identified as the predominant pathogen, with the prevalence of 18.5% (50/271), with EPEC accounting for 13.3% (36/271) of the cases. This was followed by *Salmonella* spp. (10.7%; 29/271), *C. difficile* (8.5%; 23/271), and *Shigella* spp. (6.3%; 17/271). The prevalence of *C. coli* and *V. cholerae* was relatively low (Figure 1).

Clinical and paraclinical characteristics of various bacterial agent infections in children

According to Table 1, children with acute diarrhea caused by *E. coli* exhibited a significantly higher prevalence of fever (p = 0.012). Children infected with *Salmonella* spp. demonstrated a greater prevalence of vomiting and loose, watery stools (p = 0.004 and p = 0.008, respectively). Children with acute diarrhea caused by *V. cholerae* had a statistically significant higher prevalence of dehydration or severe dehydration compared to those without dehydration (p = 0.028). No significant clinical differences were observed in children with acute diarrhea caused by *Clostridium difficile*. According to Table 2, children with acute diarrhea caused by *E. coli* exhibited a statistically significant increase in white blood cell count with age (p = 0.032). In contrast, children infected with *C. difficile* had a higher white blood cell count compared to the uninfected group (p = 0.017). No significant differences were observed in the paraclinical features of children with acute diarrhea caused by *Salmonella* spp. or *V. cholerae*. According to Table 3, vomiting (aOR = 2.54; 95% CI: 1.082–5.973; p = 0.032) and watery stool (aOR = 2.71; 95% CI: 1.205–6.112; p = 0.016) were significantly associated with *Salmonella spp.* infection, while fever (aOR = 0.15; 95% CI: 0.035–0.646; p = 0.011) and elevated white blood cell count (aOR = 3.39; 95% CI: 1.143–10.076; p = 0.028) were linked to *Escherichia coli*. Dehydration remained significantly associated with *Vibrio cholerae* (aOR = 8.92; 95% CI: 1.417–56.146; p = 0.020). Other factors such as sex and age showed no significant associations across pathogens.

Discussion

The rate of bacteria was detected by real-time PCR

Real-time PCR analysis of rectal swab samples from 271 children with acute diarrhea identified 123 cases (45.4%) positive for at least one bacterial

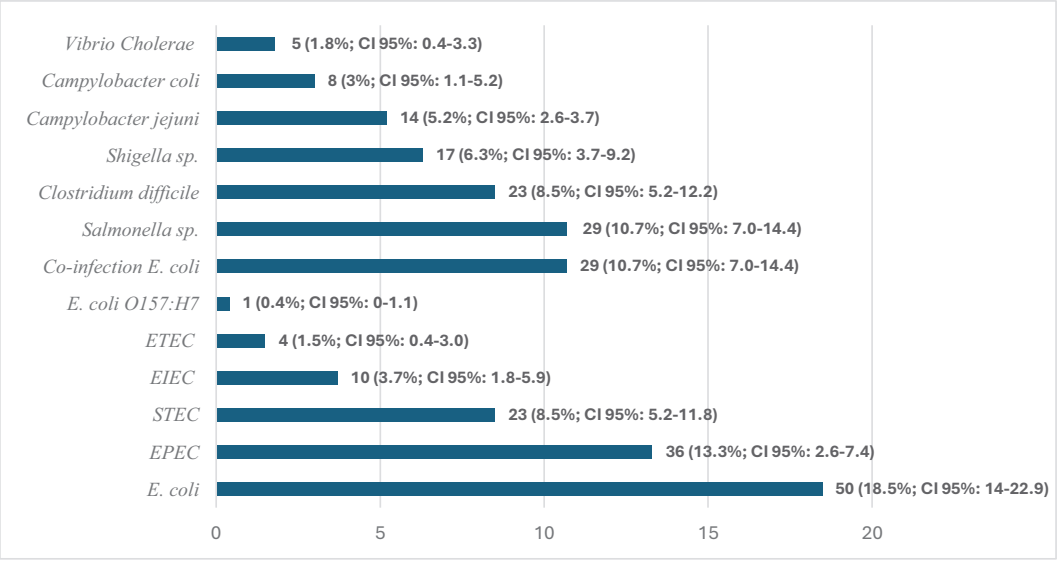

Figure 1. Summary of detected bacterial agents (n=271).

Table 1. Clinical characteristics of some common bacterial agents (n=271)

	Escherichia coli			*Salmonella* sp.			*Clostridium difficile*			*Vibrio cholerae*		
	(+)	(-)		(+)	(-)		(+)	(-)		(+)	(-)	
Agents Characteristics	**n (%) (n=50)**	**n (%) (n=221)**	**P**	**n (%) (n=29)**	**n (%) (n=242)**	**p**	**n (%) (n=23)**	**n (%) (n=248)**	**p**	**n (%) (n=5)**	**n (%) (n=266)**	**p**
Male	25 (50)	134 (60.6)	0.168	18 (62.1)	141 (58.3)	0.694	15 (65.2)	144 (58.1)	0.505	3 (60)	156 (58.6)	1.000
2-24 months	38 (76)	188 (85.1)	0.120	28 (96.6)	198 (81.8)	0.06	18 (78.3)	208 (83.9)	0.556	5 (100)	221 (83.1)	0.594
Presence of dehydration	7 (14)	35 (15.8)	0.764	7 (24.1)	35 (14.5)	0.179	4 (17.4)	38 (15.3)	0.765	3 (60)	39 (14.7)	**0.028**
Fever	47 (94)	174 (78.7)	**0.012**	25 (86.2)	196 (81)	0.494	20 (87)	201 (81)	0.587	3 (60)	218 (82)	0.230
Vomiting	36 (72)	164 (74.2)	0.748	15 (51.7)	185 (76.4)	**0.004**	20 (87)	180 (72)	0.134	2 (40)	198 (74.4)	0.115
Watery stool	32 (64)	115 (52)	0.125	9 (31)	138 (57)	**0.008**	11 (47.8)	136 (54.8)	0.518	3 (60)	144 (54.1)	1.000

p: p-value was determined by Chi-square test or Fisher ‘s Exact test.

(+): Positive; (-): Negative

Table 2. Paraclinical characteristics of some common bacterial agents (n=271)

	Escherichia coli			*Salmonella* sp.			*Clostridium difficile*			*Vibrio cholerae*		
	(+)	(-)		(+)	(-)	P	(+)	(-)	p	(+)	(-)	
Agents Characteristics	**n (%) (n=50)**	**n (%) (n=221)**	**P**	**n (%) (n=29)**	**n (%) (n=242)**		**n (%) (n=23)**	**n (%) (n=248)**		**n (%) (n=5)**	**n (%) (n=266)**	**p**
Hematocrit* (%): Median (IQR)	35.1 (32.3-37.1)	34.7 (32.6-37.7)	0.934	34.0 (31.3-36.4)	35.0 (32.6-37.7)	0.212	35.1 (31.0-37.0)	34.9 (32.6-37.7)	0.407	34.0 (33.4-48.1)	35.0 (32.3-37.6)	_
Hematocrit increase with age	20 (40.0)	70 (31.7)	0.259	9 (31.0)	81 (33.5)	0.792	9 (39.1)	81 (32.7)	0.529	2 (40.0)	88 (33.1)	1,000
White blood cell (G/L) *: Median (IQR)	9.1 (7.4-12.6)	10.0 (7.5-13.5)	0.323	9.7 (8.1-13.4)	9.6 (7.4-13.1)	0.520	13.2 (8.9-15.4)	9.5 (7.4-12.8)	**0,017**	7.9 (4.9-13.3)	9.7 (7.5-13.1)	_
White blood cells increase with age*	4 (8.3)	47 (21.9)	**0.032**	8 (28.6)	43 (18.3)	0.252	4 (18.2)	47 (19.5)	0.881	2 (40)	49 (19)	0.239
Serum sodium (mmol/l)*: Median (IQR)	132.6 (129.7-134.9)	134.4 (130.9-137.7)	0.112	128.0 (127.8-128.0)	134.3 (131.0-136.9)	0.252	135.8 (128.4-136.5)	134.3 (130.9-137.2)	0.794	140.4 (139.4-140.4)	134.3 (130.6-136.6)	0.06
Hyponatremia*	3 (23.1)	36 (46.2)	0.120	1 (33.3)	38 (43.2)	1,000	3 (60.0)	36 (41.9)	0.648	2 (100)	37 (41.6)	0.181
Serum potassium (mmol/l)*: Median (IQR)	4.0 (3.5-4.1)	3.7 (3.3-4.1)	0.332	3.8 (2.7-3.8)	3.7 (3.3-4.1)	0.903	3.7 (3.2-4.2)	3.7 (3.3-4.1)	0.910	4.1 (4.0-4.1)	3.7 (3.3-4.1)	0.267
Hypokalemia*	4 (30.8)	17 (21.8)	0.477	2 (66.7)	19 (21.6)	0.132	2 (40.0)	19 (22.1)	0.326	1 (50.0)	20 (22.5)	0.410
CRP (mg/L)*: Median (IQR)	12.2 (8.5-36.1)	10.1 (8.0-18.4)	0.071	10.5 (7.9-40.1)	10.2 (8.0-20.7)	0.866	9.4 (6.2-32.7)	10.5 (8.0-22.0)	0.599	_	10.2 (8.1-21.6)	-
CRP >5mg/L*	19 (100)	64 (91.4)	0.186	10 (100)	73 (92.4)	0.367	4 (80.0)	79 (94.0)	0.301	-	83 (93.3)	-

* The n value was less than the frequency of each reagent group because some tests were not performed due to lack of chemicals.

(a): p-value was determined by Mann-Whitney U test; (b): p-value was determined by Chi-square test or Fisher 's Exact test.

(+): Positive; (-): Negative; IQR: interquartile ranges

Table 3. Multivariable logistic regression analysis on the association between some common bacterial agents with clinical and paraclinical characteristics

Characteristics		**aOR**	**CI 95%**	**p**
Escherichia coli	Male	1.73	0.896 – 3.333	0.103
	Age 2-24 months	2.12	0.954 – 4.692	0.065
	Fever	**0.15**	**0.035 – 0.646**	**0.011**
	White blood cells increase with age	**3.39**	**1.143 – 10.076**	**0.028**
Salmonella sp.	Male	0.68	0.297 – 1.558	0.363
	Age 2-24 months	0.18	0.023 – 1.355	0.095
	Vomiting	**2.54**	**1.082 – 5.973**	**0.032**
	Watery stool	**2.71**	**1.205 – 6.112**	**0.016**
Vibrio cholerae	Male	1.07	0.167 – 6.790	0.946
	2-24 months	<0.001	0.00-0.00	0.061
	Presence of dehydration	**8.92**	**1.417 – 56.146**	**0.020**

pathogen. This prevalence was higher than that observed in a study by Lei Tian et al. conducted in China from 2014 to 2015, which reported a bacterial pathogen prevalence of 20.1% in stool samples from 508 pediatric patients under 5 years old with acute diarrhea (12). In their study, Lei Tian et al. employed culture methods to identify bacterial agents, while our study utilized real-time PCR for detecting all pathogens. Compared to traditional culture methods, real-time PCR demonstrated superior sensitivity in pathogen detection (13). Our study assessed the presence of eight bacterial pathogens, with *E. coli* classified into five distinct strains. The results revealed that the most prevalent bacterial pathogens associated with acute diarrhea in children under the age of five were *E. coli*, followed by *Salmonella* spp., *C. difficile*, and *Shigella* spp. Previous research has identified bacterial agents responsible for acute diarrhea; however, the order and relative proportions of these pathogens were different (2, 5). This difference may be attributed to differences in social, economic, and environmental factors across regions. Notably, our study identified the presence of *V. cholerae* and *Salmonella spp*., pathogens that were not detected in a prior study assessing the causes of acute diarrhea in 587 children in Vietnam (21). The most commonly detected bacterial pathogen was *E. coli*, with a prevalence of 18.5%. This prevalence was lower than that reported by Lei Tian et al. in China in 2016, which was 23.5% (5). Numerous studies have indicated that the prevalence of *E. coli* infection varies widely, ranging from 4.7% to 45.2% (5, 22). In our study, *E. coli* strains with pathogenic genes were identified in the following decreasing order: EPEC, STEC, EIEC, ETEC and E. coli O157:H7. While some studies in Vietnam have identified EAEC as the predominant factor, this pathogen was not detected in our study. EPEC was identified at a prevalence of 13.3%, which is higher than the prevalence observed in the study by Nguyen Vu Trung in Vietnam (6.6%, or 39/587 cases) and that of Lei Tian et al. in China (2.16%, or 11/508 cases) (5, 21). *Salmonella* spp. was detected with a prevalence of 10.7%, which was higher than that reported by Lei Tian et al. in China, where the prevalence was 8.5% (43/508 cases) (5). The relatively high prevalence of *Salmonella* spp. in children may be attributed to behaviors such as frequent contact with contaminated surfaces, consumption of contaminated food or water, and close interaction with asymptomatic caregivers (23). *C. Difficile* was detected in 8.5% of the cases. This pathogen has been identified in humans, animals, and the environment across Southeast Asia (6). However, reports on *C. difficile* infections in children in Vietnam are relatively limited compared to other regions. In a study by Vu Thuy Duong et al., 479 stool samples from patients hospitalized with diarrhea between 2009 and 2014 in Vietnam were collected, with 45 cases testing

positive for *C. difficile*, yielding a prevalence of 9%, which is consistent with our findings (10). *C. difficile* infections are less common in children than in adults and often remain asymptomatic (24). The use of real-time PCR testing facilitates the detection of this less common pathogen, aiding in clinical management and providing a foundation for future research. *Shigella* sp. was detected at a relatively low prevalence of 6.3%, which aligns with the findings of Lei Tian et al. in China, who reported a prevalence of 5.9% in 2016 (5). *Campylobacter jejuni* and *C. coli* were detected at prevalence of 5.2% and 3%, respectively, consistent with prior studies. The prevalence of *Campylobacter spp.* has been documented to range from 3.2% to 17.4%, with *C. jejuni* being the most commonly detected species, with a prevalence ranging from 3.2% to 11.2% (25). Overall, these three pathogens contributed modestly to the etiology of acute diarrhea. *V. Cholerae* was detected at a low prevalence of 1.8% (5/271 cases). No cases of cholera have been reported in Vietnam since 2012 (10, 26). There have been no studies focusing on cholera in children. This discovery highlights the significance of improved hygiene practices to control the spread of this dangerous pathogen, due to its high infectivity and associated mortality rate.

Clinical and paraclinical characteristics of various bacterial agent infections in children

Children with acute diarrhea caused by *E. coli* exhibited a statistically significant higher prevalence of fever (p=0.012). However, a study conducted by Tao Wang et al. in China reported no significant difference in fever prevalence among children with diarrhea caused by *E. coli* (22). This difference may be attributed to variations in sample size and study populations. No significant differences were observed in the proportions of males and females, children under 24 months of age versus those over 24 months, or in the proportions of vomiting, watery stools, or non-watery stools among children with acute diarrhea caused by *E. coli*. These characteristics align with the findings of Arpit Kumar Shrivastava in India regarding age and gender, as well as the findings of Nguyen Vu Trung in Vietnam concerning vomiting and watery diarrhea (8, 27). In terms of laboratory data, our study found that the white blood cell count did not differ significantly between children infected and not infected with *E. coli*. However, children infected with *E. coli* demonstrated a statistically significant lower increase in white blood cell count with age compared to the non-infected group. This may be attributed to the possibility that the non-infected group harbored other bacterial infections, resulting in leucocytosis. Multivariable analysis adjusted for age and sex showed that both fever and age-adjusted elevated white blood cell count were significantly associated with *E. coli* infection in children. In children infected with *Salmonella spp.*, the prevalence of vomiting was significantly higher compared to those who did not experience vomiting, a finding that is consistent with the study by Li-juan Wu et al. in China in 2021 (28). Additionally, loose and watery stools were statistically significantly associated with *Salmonella spp.* infection. While the prevalence of children with vomiting and dehydration/severe dehydration in the group infected with *Salmonella spp.* was higher than in the uninfected group, no significant differences were observed in paraclinical characteristics such as hematocrit, blood sodium, or blood potassium between the two groups. Paraclinical features indicative of infection, such as leukocytosis and CRP levels, did not exhibit significant differences, making it difficult to distinguish between children infected and uninfected with *Salmonella spp.* based on these markers. Multivariable analysis adjusted for age and sex showed that vomiting and watery diarrhea were significantly associated with *Salmonella spp.* infection. Children with acute diarrhea who tested positive for *C. difficile* were predominantly male (65.2%), with the majority aged between 2 and 24 months (78.3%). These demographic characteristics are broadly similar to those reported in children with diarrhea of various etiologies in prior studies (8, 29). No significant differences in clinical features such as fever, vomiting, or dehydration were observed between children with and without *C. difficile* infection, limiting the ability to infer specific clinical patterns. Exploratory analysis suggested that *C. difficile*-positive cases had relatively higher WBC counts than uninfected children; however, the relationship between age and WBC count appeared similar across groups. Multivariable analysis was not performed for Clostridium difficile due to the absence

of statistically significant associations in the univariable analysis of qualitative variables. The limited sample size or low event frequency may have contributed to the lack of significant associations. Children with acute diarrhea caused by *V. Cholerae* exhibited a statistically significant higher prevalence of dehydration or severe dehydration compared to those without dehydration (p=0.028). Although this pattern is consistent with the known pathophysiology of cholera, the very low prevalence of V. cholerae in the study sample limits the strength of this finding, which should be considered as a preliminary contribution and requires confirmation in larger, controlled studies (30). In the multivariable analysis, dehydration was significantly associated with *V. cholerae* infection, highlighting its clinical relevance as a key indicator of cholera in pediatric patients. Although age under 24 months showed a strong trend (aOR < 0.001; p = 0.061), the wide confidence interval and lack of statistical significance may reflect limitations in sample size or group imbalance.

Strengths and limitations

Although our study used real-time PCR, a modern diagnostic technique rarely available in Vietnamese medical settings, along with clinical data collected within 24 hours of symptom onset, several limitations remain. First, the cross-sectional design and convenience sampling are appropriate for descriptive purposes but limit causal inference and generalizability. Second, real-time PCR may detect non-viable organisms, especially C. difficile, which can be present in asymptomatic carriers. Additionally, the study did not test for EAEC, possibly underestimating the burden of E. coli infections. Lastly, antibiotic resistance was not assessed, despite its growing importance in the management of pediatric diarrhea.

Conclusion

The most commonly detected bacterial pathogens in children with acute diarrhea were *E. coli*, *Salmonella spp*., and *C. difficile*. Exploratory analyses suggested that E. coli infection tended to occur more frequently with fever, while *Salmonella spp*. were more often observed in children presenting with vomiting and loose, watery stools. Higher WBC counts were noted in cases with *C. difficile*, and *V. cholerae* appeared more frequently in children with dehydration or severe dehydration. These preliminary findings may reflect potential clinical patterns associated with specific bacterial pathogens. However, they should be interpreted with caution and require further validation in larger, controlled studies before informing diagnostic or management decisions.

Ethic Approval: This study received ethical approval from the Ethics Committee in Biomedical Research of Can Tho University of Medicine and Pharmacy under reference number 22.149.SV/PCT-HDDD.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interests, patent/licensing, arrangement etc-) that might pose a conflict of interest in connection with the submitted article.

Authors Contribution: HHTN, KQT, and UTPN were responsible for methodology, data collection, interpretation, validation, supervision, project administration, and patient management. UTPN, LTTL, and OTKN were responsible for visualization and writing the original draft. All authors were responsible for revising the manuscript. All authors have read and agreed to the final draft for publication.

Declaration on the Use of AI: None.

Consent for Publication: Consent for publication was obtained by the authors from the patient's legal guardians.

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