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Original Article

A Study of Diarrheagenic Escherichia Coli in Acute Diarrhea Among Under-Five Children with and Without Severe Acute Malnutrition (SAM)

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ABSTRACT

Background: Acute diarrhoea remains a leading cause of morbidity and mortality among under-five children, particularly in low- and middle-income countries. Severe acute malnutrition (SAM) predisposes children to enteric infections, including diarrheagenic *Escherichia coli* (DEC), which contribute significantly to diarrhoeal disease burden.

Objectives: To determine the prevalence and distribution of diarrheagenic *E. coli* among under-five children with acute diarrhoea with and without severe acute malnutrition, and to compare the associated clinical and microbiological characteristics.

Methods: This cross-sectional analytical study was conducted from May 2023 to October 2024 at a tertiary care centre in New Delhi. Children aged 2–59 months hospitalized with acute diarrhoea were enrolled and categorized into SAM and non-SAM groups. Stool samples were cultured for *E. coli* and isolates were identified using MALDI-TOF and biochemical tests. Antimicrobial susceptibility testing was performed using the VITEK-2 system. Diarrheagenic *E. coli* pathotypes were detected by multiplex real-time PCR targeting specific virulence genes. Statistical analysis was carried out using SPSS version 26.

Results: Of the 80 *E. coli* isolates studied, 50 (62.5%) were identified as DEC. A significantly higher proportion of DEC was detected in the SAM group compared to the non-SAM group ($p = 0.037$). Enterotoxigenic *E. coli* was significantly more prevalent among SAM children, while enteroaggregative *E. coli* was more common in non-SAM children. The majority of cases occurred in children aged 6–23 months. Altered sensorium was uncommon and showed no significant association with DEC.

Conclusion: Diarrheagenic *E. coli* is more frequently associated with acute diarrhoea in children with severe acute malnutrition. Early detection of DEC and targeted management, along with nutritional rehabilitation, are essential to improve outcomes in this vulnerable population.

Keywords*: Acute diarrhoea; Diarrheagenic Escherichia coli; Severe acute malnutrition; Under-five children; Enteric infections; Real-time PCR.*

INTRODUCTION

Acute diarrhoeal disease remains one of the leading causes of morbidity and mortality among children under five years of age worldwide, particularly in low- and middle-income countries [1]. Despite significant global efforts, diarrhoea continues to account for approximately 8–9% of under-five deaths, with the highest burden observed in South Asia and sub-Saharan Africa [2]. In India, diarrhoeal diseases contribute substantially to paediatric hospital admissions and remain a major public health concern [3].

Among the various etiological agents responsible for acute diarrhoea, *Escherichia coli* plays a pivotal role. While most strains of *E. coli* are commensal inhabitants of the human gut, certain pathotypes, collectively referred to as diarrheagenic *Escherichia coli* (DEC), possess specific virulence factors that enable them to cause gastrointestinal disease [4]. DEC is classified into six major pathotypes based on virulence gene profiles: enterotoxigenic *E. coli* (ETEC), enteropathogenic

E. coli (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC/STEC), and diffusely adherent *E. coli* (DAEC) [5].

DEC infections are particularly important in children under five years, where they are associated with a wide spectrum of clinical manifestations ranging from mild watery diarrhoea to severe dehydration, malnutrition, and altered sensorium [6]. The burden and distribution of individual DEC pathotypes vary geographically and are influenced by host factors, environmental conditions, and nutritional status [7].

Severe acute malnutrition (SAM) is a critical risk factor that exacerbates both the incidence and severity of diarrhoeal diseases. Malnourished children exhibit impaired gut barrier function, altered intestinal microbiota, and compromised immune responses, rendering them more susceptible to enteric infections and their complications [8]. Diarrhoea and malnutrition form a vicious cycle, wherein diarrhoeal episodes worsen nutritional status, and malnutrition, in turn, increases susceptibility to recurrent and severe diarrhoeal infections [9].

Several studies have demonstrated a higher prevalence of DEC infections among children with SAM compared to their well-nourished counterparts [10,11]. Certain DEC pathotypes, particularly ETEC and EAEC, have been frequently associated with prolonged and severe diarrhoea in malnourished children, contributing to increased morbidity and mortality [12]. However, data comparing the distribution of DEC pathotypes between SAM and non-SAM children remain limited, especially from tertiary care settings in India.

Accurate identification of DEC using molecular methods such as real-time polymerase chain reaction (PCR) has improved diagnostic sensitivity and enabled precise pathotype characterization [13]. Understanding the epidemiology, clinical associations, and pathotype distribution of DEC in children with and without SAM is essential for guiding clinical management, antibiotic stewardship, and public health interventions.

In this context, the present study was undertaken to determine the prevalence and distribution of diarrheagenic *Escherichia coli* among under-five children presenting with acute diarrhoea, and to compare DEC detection, pathotype distribution, and clinical features between children with severe acute malnutrition and those without SAM in a tertiary care hospital setting.

MATERIALS AND METHODS

Study Design and Setting

This was a cross-sectional analytical study conducted between May 2023 and October 2024 in the Department of Microbiology, in collaboration with the Department of Pediatrics, Lady Hardinge Medical College and Kalawati Saran Children's Hospital, New Delhi, a tertiary care referral center.

Study Population

Children aged 2–59 months presenting with acute diarrhoea and requiring hospitalisation were enrolled after obtaining written informed consent from parents or guardians.

Inclusion Criteria

Participants were categorised into two groups:

Group 1 (SAM group)

- Children aged 2–59 months with acute diarrhoea (duration <14 days)
- Children with severe acute malnutrition (SAM) are defined by:
    - Weight-for-height/length Z-score (WHZ) < −3 SD (WHO standards) and/or
    - Mid-upper arm circumference (MUAC) <11.5 cm and/or
    - Bilateral pitting oedema
- Escherichia coli isolated from stool culture

Group 2 (Non-SAM group)

1. Children aged 2–59 months with acute watery diarrhoea (<14 days)
2. Children without SAM (WHZ ≥ −3 SD and/or MUAC >11.5 cm)
3. Escherichia coli isolated from stool culture

Exclusion Criteria

4. Children who received antibiotics or other medications within the previous 7 days
5. Children with known immunosuppression, including HIV infection, malignancy on chemotherapy, primary immunodeficiency, or nephrotic syndrome

Sample Size

Based on a previous study by Jain et al. (2019), the expected prevalence of diarrheagenic *E. coli* was assumed to be 85% in SAM children and 60% in non-SAM children. With a 95% confidence interval and 80% power, the calculated sample size was 47 per group. For uniformity, 40 *E. coli* isolates from each group (SAM and non-SAM) were included.
The sample size was calculated using the formula:

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \times [p_1(1-p_1) + p_2(1-p_2)]}{(p_1 - p_2)^2}$$

Ethical Approval
The study was approved by the Institutional Ethics Committee, Lady Hardinge Medical College, New Delhi.

Case Definitions
Acute Diarrhoea
Passage of three or more loose or liquid stools per day for less than 14 days, as per WHO definition.

Severe Acute Malnutrition (SAM)
- Children ≥6 months: WHZ < −3 SD and/or MUAC <11.5 cm and/or bilateral pitting oedema
- Infants <6 months (>49 cm length): WHZ < −3 SD and/or bilateral pitting oedema
- Infants <49 cm: visible severe wasting

Diarrheagenic *Escherichia coli* (DEC)
Pathogenic *E. coli* strains possessing specific virulence genes causing gastroenteritis, classified into ETEC, EPEC, EAEC, EIEC, EHEC/STEC, and DAEC.

Sample Collection and Transport
Approximately 1–2 mL of fresh liquid stool was collected in sterile, wide-mouthed, screw-capped containers free of preservatives. Urine contamination was avoided. Samples were transported to the laboratory and processed within 60 minutes of collection**.**

Microbiological Processing
Culture and Isolation
Stool samples were enriched in Selenite-F broth for 4–6 hours and sub-cultured onto MacConkey agar, XLD agar, and Bismuth Sulphite agar (HiMedia, India). Lactose-fermenting colonies suggestive of *E. coli* were further purified on MacConkey agar.

Identification of *E. coli*
Presumptive isolates were identified using MALDI-TOF MS and confirmed by conventional biochemical tests (Indole, Methyl Red, Voges-Proskauer, Citrate, and Urease). Isolates positive for indole and methyl red and negative for VP, citrate, and urease were confirmed as *E. coli*.

Antimicrobial Susceptibility Testing
Antibiotic susceptibility testing was performed using the VITEK-2 Compact system with AST-N405 cards against 12 antibiotics. Results were interpreted according to CLSI guidelines 2024.

Additionally, ampicillin susceptibility was tested by the Kirby–Bauer disc diffusion method.

Preservation of Isolates
Confirmed *E. coli* isolates were preserved in Tryptic Soy Broth with 20% glycerol and nutrient agar slants and stored at −80°C for molecular analysis.

Molecular Detection of Diarrheagenic *E. coli*
DNA Extraction
DNA was extracted from overnight cultures using the Qiagen DNeasy Blood & Tissue Kit following manufacturer's instructions.

Real-Time PCR for DEC Pathotyping
Multiplex real-time PCR was performed using:
- VIASURE E. coli Real-Time PCR Kit
- Allplex™ GI-Bacteria (II) Assay (Seegene)

PCR amplification was carried out on a CFX96 real-time PCR system (Bio-Rad). A Ct value ≤38 was considered positive for DEC virulence genes.
Statistical Analysis

Data were analysed using SPSS version 26. Categorical variables were expressed as frequency and percentage and analysed using Pearson's chi-square test or Fisher's exact test, where applicable. Quantitative variables were expressed as mean ± standard deviation. A p-value ≤0.05 was considered statistically significant.

RESULT AND OBSERVATIONS

Table 1: Distribution of patients with respect to sex by groups, and results of the Pearson chi- square test

Sex		Total	Groups		p-value
			SAM	NSAM	
Male	F	35	15	20	.367[FET]
	%	43.8%	37.5%	50.0%	
Female	F	45	25	20	
	%	56.3%	62.5%	50.0%	

The study population consisted of 35 males (43.8%) and 45 females (56.3%). The Pearson chi- square test revealed a non-significant p-value of .367[FET] indicating a similar distribution of patients in SAM and NSAM with respect to sex

Table 2: Distribution of patients with respect to age group by groups, and results of the Pearson chi-square test

Age		Total	Groups		p-value
			SAM	NSAM	
6 to 23 months	F	52	34	18	.001[FET]
	%	65.0%	85.0%	45.0%	
2 to 5 years	F	28	6	22	
	%	35.0%	15.0%	55.0%	

There were 65% (52) patients who were between 6 to 23 months of age, while 35% (28) patients were between 2 and 5 years. The Pearson chi-square test revealed a significant p-value indicating that a significantly higher number of patients between 2 to 5 years was found in the NSAM group, and vice versa in the case of the SAM group, i.e. higher in the lower age group between 6 to 23 months, respectively

Table 3: Distribution of patients with respect to sex by DEC, and results of the Pearson chi-square test

Sex		Groups		p-value
		Non- DEC	DEC	
Male	F	16	19	0.245[FET]
	%	53.3%	38.0%	
Female	F	14	31	
	%	46.7%	62.0%	

Among males, 53.3% (16) were classified as non-DEC while 38% (19) were DEC. In contrast, for females, 46.7% (14) were non-DEC and a higher 62% (31) were DEC. The p-value of 0.245 suggests non-significant difference between the sexes regarding their association with DEC

Table 4: Distribution of patients with respect to age group by DEC, and results of Pearson chi-square test

Age		Groups		p-value
		Non- DEC	DEC	
6 to 23 months	F	17	35	.238[FET]
	%	56.7%	70.0%	
2 to 5 years	F	13	15	
	%	43.3%	30.0%	

In the age group of 6 to 23 months, a higher number of patients i.e. 70% (35) had DEC while 56.7% (17) were non-DEC. Conversely, in the 2 to 5 years age group, a smaller number of patients i.e. 30% (15) had DEC, while 43.3% (13) were non-DEC. The p-value of 0.238 indicates that the differences observed between these groups are not statistically significant

Table 5: Distribution of patients with respect to DEC by groups, and results of Pearson chi- square test

			Total	Groups		p-value
				SAM	NSAM	
Total number of patients with DEC	No DEC	F	30	10	20	.037 FET
		%	37.5%	25.0%	50.0%	
	DEC	F	50	30	20	
		%	62.5%	75.0%	50.0%	

Out of total 80 positive *E coli* patients, 62.5% (50) were found to have DEC while 37.5% (30) had no DEC. The Pearson chi-square test revealed a significant p-value of 0.037^{FET} indicating that patients with SAM had more possibility to have DEC than NSAM patients

Table 6: Distribution of patients with respect to DEC pathotypes by groups, and results of Pearson chi-square test

DEC pathotype		Total detected	Groups		p-value
			SAM	NSAM	
EAEC	F	15	3	12	$.020^{FET}$
	%	18.8%	7.5%	30.0%	
EPEC	F	10	5	5	1.00^{FET}
	%	12.5%	12.5%	12.5%	
ETEC	F	22	21	1	$.001^{FET}$
	%	27.5%	52.5%	2.5%	
EIEC	F	11	5	6	1.00^{FET}
	%	13.8%	12.5%	15.0%	
STEC	F	0	0	0	-
	%	0.0%	0.0%	0.0%	
EHEC	F	0	0	0	-
	%	0.0%	0.0%	0.0%	

Overall, 18.8% (15) of the patients had EAEC, 12.5% (10) of the patients had EPEC, 27.5%

(22) had ETEC and 13.8% (11) had EIEC while STEC and EHEC were not detected in any patient. The Pearson chi-square test revealed a significant p-value for EAEC ($p = .020^{FET}$) and ETEC ($p = .0010^{FET}$) indicating that EAEC was detected significantly higher in NSAM while ETEC was detected more in SAM patients. While other DEC pathotypes did not revealed a significant p-value indicating similarity of detection between SAM and NSAM

Table 7: Distribution of patients with respect to DEC pathotypes (multiple) by groups, and results of Pearson chi-square test

DEC – Multiple		Total detected	Groups		p-value
			SAM	NSAM	
EAEC + EPEC	F	2	0	2	.225
	%	25.0%	0.0%	50.0%	
EAEC + EIEC	F	1	0	1	
	%	12.5%	0.0%	25.0%	
EPEC + ETEC	F	1	1	0	
	%	12.5%	25.0%	0.0%	
EPEC + EIEC	F	3	2	1	
	%	37.5%	50.0%	25.0%	
ETEC + EIEC	F	1	1	0	
	%	12.5%	25.0%	0.0%	

Overall, multiple DEC pathotypes were detected in 8 patients. Out of which, 2 (25%) had EAEC + EPEC, 3 (37.5%) had EPEC + EIEC while 1 (12.5%) patient each had EAEC+EIEC, EPEC+ETEC and ETEC+EIEC respectively. The Pearson chi-square test revealed a non- significant p-value of 0.225 indicating similarity in the detection of these pathogens between SAM and NSAM patients

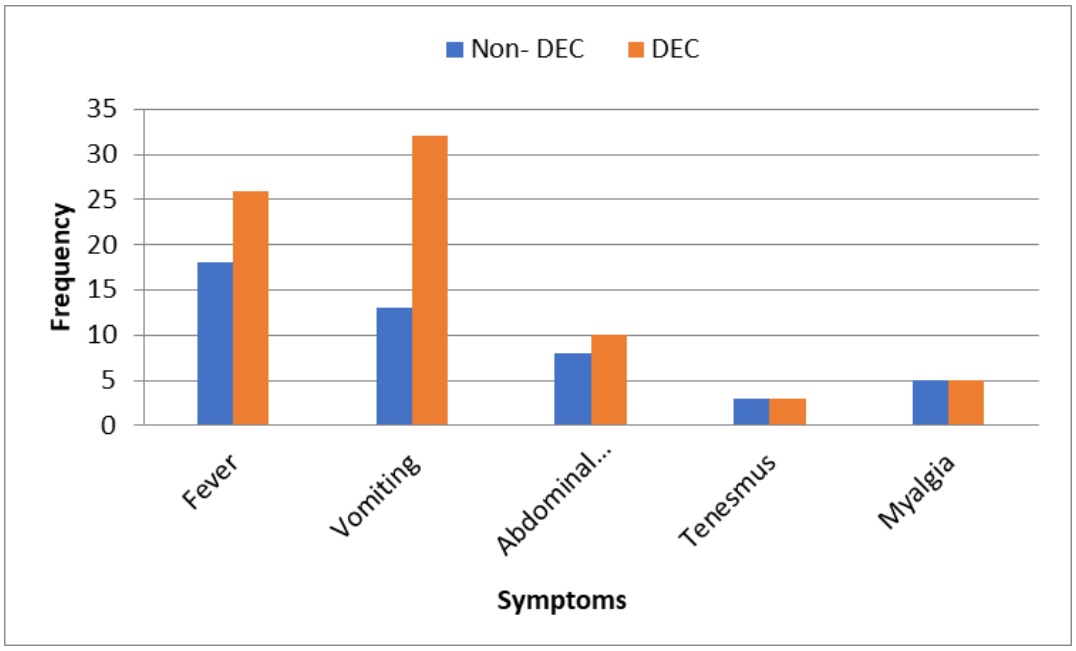

Figure 1: Distribution of patients with respect to symptoms by DEC

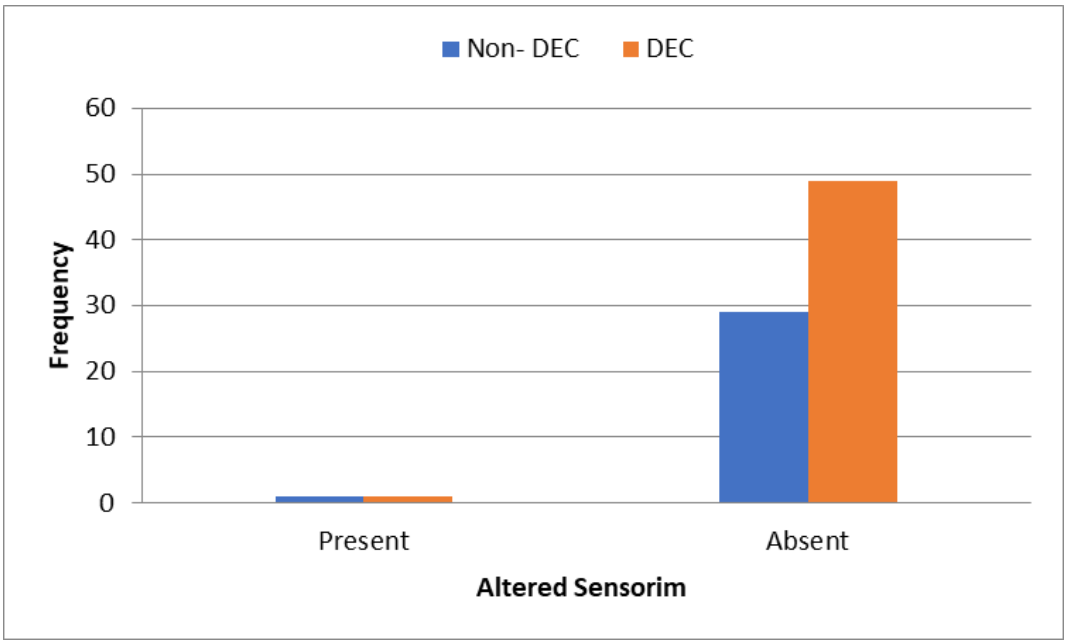

Figure 2: Distribution of patients with respect to Type of diarrhoea by DEC

Table 8: Distribution of Patients According to Altered Sensorium in DEC and Non-DEC Groups

Altered Sensorium	DEC (n)	Non-DEC (n)
Present	**1**	**1**
Absent	**49**	**29**
Total	**50**	**30**

DISCUSSION

Acute diarrhoeal illness remains a significant cause of hospitalisation among under-five children, particularly in developing countries where malnutrition continues to be prevalent. The present study evaluated the prevalence, distribution, and clinical associations of diarrheagenic *Escherichia coli* (DEC) among children with acute diarrhoea, comparing those with severe acute malnutrition (SAM) and those without SAM, using culture-based identification complemented by molecular diagnostics.

In the present study, females constituted a slightly higher proportion (56.3%) than males (43.8%); however, no statistically significant difference was observed between SAM and non-SAM groups with respect to sex distribution.

This finding is consistent with previous studies that have reported no gender predilection in paediatric diarrhoeal diseases or DEC infections [1,2]. Similarly, the distribution of DEC did not show a significant association with sex, suggesting that exposure and susceptibility to DEC are largely independent of gender in this age group.

Age-wise analysis revealed that the majority of patients (65%) were between 6 and 23 months of age, with a significantly higher proportion of SAM children falling within this younger age group. This observation is in agreement with earlier reports indicating that the weaning period represents a critical window of vulnerability due to immature immunity, exposure to contaminated complementary foods, and declining maternal antibody protection [3,4]. The predominance of DEC in younger children underscores the importance of early preventive strategies targeting this age group.

Overall, DEC was detected in 62.5% of the *E. coli* isolates, indicating a substantial burden of pathogenic *E. coli* among hospitalised children with acute diarrhoea. Notably, DEC was significantly more prevalent among children with SAM (75%) compared to non-SAM children (50%). This finding corroborates previous studies that have demonstrated a higher susceptibility of malnourished children to enteric pathogens due to impaired mucosal immunity, altered gut microbiota, and compromised intestinal barrier function [5,6]. The significant association between SAM and DEC detection highlights the synergistic relationship between malnutrition and enteric infections.

With regard to DEC pathotypes, enterotoxigenic *E. coli* (ETEC) was the most frequently detected pathotype (27.5%), followed by enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), and enteroinvasive *E. coli* (EIEC). ETEC was detected significantly more often in the SAM group, while EAEC showed a significantly higher prevalence in the non-SAM group. These findings are in accordance with earlier Indian and international studies that have identified ETEC as a predominant pathogen in malnourished children with acute watery diarrhoea [7,8]. The higher prevalence of EAEC in non-SAM children may be attributed to its association with persistent or recurrent diarrhoea rather than acute severe disease [9].

EPEC and EIEC were detected in comparable proportions in both groups, and no statistically significant differences were observed. The absence of STEC and EHEC in the present study is consistent with several Indian studies reporting a low prevalence of these pathotypes in paediatric diarrhoea, possibly due to geographical variation and differences in exposure patterns [10]. This also suggests that routine screening for STEC/EHEC in similar settings may have limited yield unless clinically indicated.

Multiple DEC pathotypes were identified in 10% of patients, with combinations such as EPEC + EIEC and EAEC + EPEC being most common. Although multiple infections were more frequently observed in SAM children, the difference was not statistically significant. Co-infection with multiple DEC pathotypes has been previously reported and is believed to contribute to increased disease severity, prolonged diarrhoea, and poorer nutritional outcomes [11]. However, larger studies are required to establish the clinical significance of such mixed infections.

Clinical analysis showed that altered sensorium was rare and observed in only one patient each in DEC and non-DEC groups, indicating that neurological manifestations were uncommon and not specifically associated with DEC infection. This finding aligns with earlier reports suggesting that altered sensorium in diarrhoeal illness is more often related to severe dehydration, electrolyte imbalance, or sepsis rather than the specific enteric pathogen involved [12].

The strengths of this study include the use of molecular methods for accurate DEC pathotyping and the comparative evaluation of SAM and non-SAM children in a tertiary care setting. However, certain limitations should be acknowledged. The study was hospital-based and may not reflect the true community burden of DEC. Additionally, viral and parasitic causes of diarrhoea were not evaluated, which may have led to an underestimation of mixed infections.

CONCLUSION

Diarrheagenic *Escherichia coli* was significantly more common among under-five children with severe acute malnutrition than in non-malnourished children presenting with acute diarrhoea. Enterotoxigenic *E. coli* predominated in the SAM group, while enteroaggregative *E. coli* was more frequent among non-SAM children. Most affected children were below two years of age. These findings highlight the need for early microbiological diagnosis of DEC and integrated nutritional and infection management to reduce diarrhoeal morbidity in malnourished children.

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