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Short communication

Diarrheagenic *Escherichia coli* infections among the children of Andaman Islands with special reference to pathotype distribution and clinical profile

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ABSTRACT

Diarrhoeagenic *E. coli* (DEC) is one of the most common causes of diarrhoeal death in children less than five years globally. It is responsible for 30%–40% of all diarrhoeal episodes in developing countries. It is estimated that 0.12 million children died of diarrhoea caused by DEC in 2011 globally. There is no baseline data on the occurrence of DEC diarrhoea in Andaman Islands, the remote islands of India. The study is particularly important as these strains are the emerging enteric pathogen in both developed and developing countries. DEC was screened from *E. coli* isolates obtained from diarrhoeal stool samples by multiplex PCR with specific primers using stasndard protocols. During the study period, among the 1394 stool samples collected, 95 (6.82%) patients were found infected with DEC. Of the 97 isolates from 95 patients, 68 (70.1%) were EAEC, 19 (19.6%) were EPEC and 10 (10.3%) were ETEC. Of the 19 EPEC isolates, 63.2% were atypical EPEC which is the emerging enteric pathogen among the children in developing as well as developed countries. More than 80% of the patients had watery diarrhoea and 6% of them had invasive diarrhoea. Persistent diarrhoea was also found in three infected children. This study documents the occurrence and type of DEC diarrhoea in Andaman Islands first time and highlights the significant proportions of *E. coli* diarrhoea being caused by EAEC and atypical EPEC strains.

1. Introduction

Diarrhea is one of the most important causes of mortality and morbidity among children, particularly from developing countries [1]. It is the second leading cause of death among children younger than 5 years worldwide [2]. Of the 5.9 million child deaths (<5 years old) in the year 2015, 0.53 million were due to diarrhea [3]. It is estimated that about 9% of the annual 1.2 million child deaths (<5 years old) in India can be attributed to various forms of diarrhea [3]. According to the Child Health Epidemiology Reference Group of WHO and UNICEF, more than half of the diarrheal deaths are caused by rotavirus, calcivirus, and diarrheagenic *Escherichia coli* (DEC) [4]. Members of DEC, such as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAEC) are responsible for 30–40% of all diarrheal episodes in developing countries [5]. ETEC is the main cause of travelers' diarrhea and is endemic in underdeveloped countries [6].

The Andaman and Nicobar Islands is an archipelago of >500 islands, situated in Bay of Bengal, inhabited by more than 350,000 people including six aboriginal tribes and settlers from mainland India. Although microbiological, clinical, and epidemiological aspects of pediatric diarrhea have been monitored in the islands by Regional Medical Research Centre (ICMR) since 1994, no attempt was made to understand the proportion of diarrhea caused by DEC. In the wake of increasing importance being attached to DEC infections, a modest study was undertaken to generate a baseline data on the status of these infections among the children of Andaman Islands.

2. Materials and methods

Pediatric patients (<5 years old) with acute diarrhea attended/admitted to G.B. Pant Hospital, Port Blair Andaman child & Nicobar Island (the only referral hospital in the Andaman & Nicobar Islands) and private clinics (Chirayu Child Care Hospital, Port Blair

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Andaman child & Nicobar Island, Debunath Clinic, Port Blair Andaman child & Nicobar Island, Swasthya Clinic, Port Blair Andaman child & Nicobar Island, and INHS Dhanvantri Hospital, Port Blair Andaman child & Nicobar Island) in Port Blair between August 2013 and January 2016 were included in the study. A total of 1394 stool samples (753 samples from G.B. Pant Hospital, 638 samples from Chirayu Child Care Hospital, and 1 sample from the rest of the each private clinic) were collected in sterile containers and processed within 2 h in the laboratory of ICMR, Port Blair using the standard protocol [7].

For the isolation of DEC, stool specimens were plated on MacConkey Agar (HiMedia Laboratories HIMEDIA LABORATORIES Corporate Office: A-516, Swastik Disha Business Park, via Vadhani Industrial Estate, L.B.S. Marg, Mumbai - 400 086, India) followed by 16–18 h of incubation at 37 °C. From three to five typical lactose fermenting colonies with different colony morphology per sample were selected and subcultured in Mueller Hinton Agar (Becton, Dickinson and Company, Becton Drive Franklin Lakes, NJ 07417-1880.United States). Cultures from this nonselective medium were tested for indole test, mannitol motility test, and triple sugar iron test. DNA templates were prepared from the colonies with typical *E. coli* biochemical reactions by rapid boiling method and subjected to multiplex PCR for the detection of different pathotypes of DEC. In this study, we investigated the prevalence of EAEC, EPEC, and ETEC pathotypes. The role of other pathotypes, such as non-lactose fermenting *E. coli*, in diarrheal diseases among the children of Andaman Islands was not included in the scope of the present study and might be a limitation.

A small portion of the bacterial growth with typical *E. coli* reactions from the colonies was emulsified in 500 μL of Tris-EDTA buffer in 1.5 mL microcentrifuge tube and boiled for 10 min, followed by snap chilling in ice for 5 min. The heat-treated bacterial suspensions were centrifuged at 10,000 rpmg for 10 min, and the supernatants were used as DNA templates for PCR.

The DNA templates were subjected to multiplex PCR using specific primers, as described previously (Table 1), for the detection of virulence genes such as *eae*, *bfp A* (structural genes of EPEC), *elt* and *est* (enterotoxins of ETEC), CVD432 (the nucleotide sequence of *Eco*-R1 *Pst*1 DNA fragment pCVD432 of EAEC), and *aaiC* (encodes a secreted protein of the EAEC pathogenicity island AAI, which is coordinately regulated by the Agg R activator) [7]. The *eae*-harbored isolates were not screened for the presence of *stx* gene [5]. PCR was performed in a 25-μL reaction mixture containing 2.5 μL of 10× PCR buffer, 0.5 μL of 2.5 mM deoxyribonucleoside triphosphates, 0.5 μL of 3U Taq polymerase (Genei,India Banglore Genei PVT ltd 6, Bda Industrial Suburb, Vi Main, Peenya, Near S R Road, Bengaluru, Karnataka 560058), 0.5 μL of 10 pM of each primers (Sigma-Aldrich Mumbai, India), 1 μL of DNA template (lysate), and 19.5 μL sterile nuclease free deionized water. The cycling condition was 96 °C for 4 min, 35 cycles of 95 °C for 20 s, 57.5 °C for 20 s, 72 °C for 1 min, with a final extension at 72 °C for 7 min following Panchalingam et al. [7] with slight modifications. Positive and negative controls were used with each PCR set up. Strains known to possess the target genes were used as the positive control, and sterile distilled water was used as the negative control. Control strains were kindly provided by National Institute of Cholera and Enteric Diseases, Calcutta, India. PCR products (10 μL) were confirmed by electrophoresis using 1% (wt/vol) agarose gel containing ethidium bromide (Sigma-Aldrich Mumbai, India). DNA bands were visualized and photographed under UV light in a gel documentation system. Ethical approval was obtained from ICMR, Port Blair Ethical Committee.

3. Results

A total of 1394 patients who attended the hospitals in Andaman Islands for the treatment of diarrhea were enrolled in this study. Among these, 95 (6.82%) patients were found to be infected with DEC. In total, 97 DEC isolates were obtained from the diarrheal specimens. Of the 95 patients, two showed infection with two different pathotypes of DEC. DEC was found to be more common in children aged 1–3 years age than in those aged <1 year or 3–5 years (Table 2). The infection with EAEC was found to be relatively less in children aged 3–5 years. No child in the age group 0–6 months was infected with ETEC. EPEC infections were also comparatively less in the children aged <6 months. Among the 95 patients, 43 (45.3%) required hospitalization.

Of the 97 isolates from 95 patients, 68 (70.1%) were EAEC, 19 (19.6%) were EPEC, and 10 (10.3%) were ETEC. Among the EAEC isolates, 32 (47.1%) strains harbored *aaiC* alone, while pCVD432 was found in 28 (41.2%) isolates. Both the virulence genes were harbored by eight (11.8%) strains. Of the 19 EPEC isolates, 12 (63.2%) were atypical EPEC, which were devoid of *bfpA* gene, while the remaining seven (36.8%) were typical EPEC with *bfp A* either along with *eae* (5 cases) or without *eae* (2 cases). ETEC strains harboring *elt* gene (8 cases) were more than the strains harboring *est* or both *est* and *elt*.

Table 2
Age-wise Distribution of DEC pathotypes among the diarrhea patients.

Age group	EAEC	EPEC	ETEC	Total (%)
0–6 mo	14	1	0	15(15.8)
7–11 mo	16	4	1	21(22.1)
1–3 y	33	9	6	48(50.5)
3–5 y	5	4	2	11(11.6)
Total	68	18	9	95

Note. DEC = diarrheagenic *Escherichia coli*; EAEC = enteroaggregative *Escherichia coli*; EPEC = enteropathogenic *Escherichia coli*; ETEC = enterotoxigenic *Escherichia coli*; mo = month; y = year.

Table 1

Primer	Target gene	Primer sequence (5′–3′)	Amplicon (bp)	Refs
LT-F	*Elt*	CACACGGAGCTCCTCAGTC	508	7
LT-R		CCCCCAGCCTAGCTTAGTTT		
ST-F	*Est*	GCTAAACCAGTAGGGTCTTCAAAA	147	7
ST-R		CCCGGTACAGGCAGGATTACAACA		
BFPA-F	*bfpA*	GGAAGTCAAATTCATGGGGG	367	7
BFPA-R		GGAATCAGACGCAGACTGGT		
CVD432-F	*AatA*	CTGGCGAAAGACTGTATCAT	630	7
CVD432-R		CAATGTATAGAAATCCGCTGTT		
EAE-F	*eae*	CCCGAATTCGGCACAAGCATAAGC	881	7
EAE-R		CCCGGATCCGTCTCGCCAGTATTCG		
AAIC-F	*aaic*	ATTGGTCCTCAGGCATTTCAC	215	7
AAIC-R		ACGACACCCCTGATAAACAA		

Table 3
Clinical presentation of diarrhea caused by DEC.

Clinical symptoms	EAEC	EPEC	ETEC-LT	ETEC-ST	ETEC LT + ST	Total (%)
No. of patients	68	18	7	1	1	95
Fever	39	9	5	1	1	55 (57.9)
Nausea	1	1	0	0	0	2 (2.1)
Vomiting	38	10	4	1	1	54 (56.8)
Abdominal pain	20	8	2	0	1	31 (32.63)
Fever + vomiting	26	5	3	1	1	36 (37.9)
Fever + vomiting + abdominal pain	6	3	1	0	1	11 (11.6)
Degree of dehydration						
None	65	16	7	1	1	90 (94.7)
Some	3	2	0	0	0	5 (5.3)
Fever + vomiting + dehydration	3	1	0	0	0	4 (4.2)
Types of diarrhea						
Watery	58	16	5	1	0	80 (84.21)
Blood, blood + mucous	1	0	2	0	0	3 (3.16)
Watery + mucous	1	0	0	0	1	2 (2.11)
Semisolid + mucous	1	0	0	0	0	1 (1.05)
Semisolid	7	2	0	0	0	9 (9.5)

Note. DEC = diarrheagenic *Escherichia coli*; EAEC = enteroaggregative *Escherichia coli*; EPEC = enteropathogenic *Escherichia coli*; ETEC = enterotoxigenic *Escherichia coli.*

More than 80% of the patients had watery diarrhea, and about 6% of the patients were suffering with invasive diarrhea characterized by the presence of blood and/or mucous. Of the 95 patients, 36 (37.9%) had both fever and vomiting, while more than half of the patients had any one of these symptoms. About 32.6% of patients complained of abdominal pain during the infection. Vomiting was a common symptom found in 56.8% of the DEC-infected patients. Some dehydration was noticed in 5.3% of the children infected with DEC; of these, four patients (4.2%) had fever and vomiting also. Three patients had persistent diarrhea that lasted for more than 14 days; of these, two were infected with EAEC and one with EPEC (Table 3).

4. Discussion

DEC has been reported to be high next only to Rotavirus among the hospitalized children in developing countries [8]. Proportion of diarrhea among children caused by DEC in this study was 6.82% which is comparable to the reports from other countries [9]. Watery diarrhea, fever, and vomiting were the most common symptoms associated with DEC diarrhea. Children aged 1–3 years were found to be more prone to infection with DEC than children aged <1 year or 3–5 years.

EAEC was the most common pathotype isolated in the islands, followed by EPEC and ETEC. Similar pattern of isolation has been reported from Vietnam and Brazil [10,11]. In our study, more than 70% of DEC cases were due to EAEC, which is higher than those reported from other countries [5,10–13]. Similar findings were observed in New Haven, Baltimore, and Egypt [14,15]. EAEC and EPEC were more common in children younger than 3 years. Similar trend had been reported from Mozambique and even from Calcutta [5,16]. Detection of ETEC was comparatively low in this study as it is found in studies from Brazil [11].

The occurrence of atypical EPEC was found to be high in this study as was reported from other developing countries [11,17]. In our study, more than 60% of the EPEC isolates were found to be atypical being devoid of *bfpA* gene. High atypical EPEC was also observed among enteric pathogens reported from Delhi and Calcutta [5,18,19]. Atypical EPEC has been considered as emerging enteric pathogen in both developed and developing countries; in view of significant proportion of infections caused by this pathogen, there is a need for further studies on these emerging pathogens [20]. Being a hospital-based surveillance study with fixed recruitment criteria, non-diarrheagenic children were not included as controls.

5. Conclusions

Because of the remote location and sparse population, the Andaman Islands provide researchers with a uniquely secluded ecosystem to study the epidemiology of strains over time that can help in understanding evolutionary trends. The present report not only documents the occurrence and types of diarrhea among children caused by DEC in the islands for the first time but also highlights the emerging importance of EAEC diarrhea and those caused by atypical EPEC strains, thereby calling for increased vigilance for such etiological agents in mainland India and other developing countries and further studies on their virulence potential, epidemiology, and antimicrobial resistance.

Conflicts of interest

None.

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