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

Raghavan P. Ramya, Subarna Roy, Ramanathan Thamizhmani, Attayar Purushothaman Sugunan

To cite this article: Raghavan P. Ramya, Subarna Roy, Ramanathan Thamizhmani, Attayar Purushothaman Sugunan (2017) Diarrheagenic *Escherichia coli* infections among the children of Andaman Islands with special reference to pathotype distribution and clinical profile, Journal of Epidemiology and Global Health 7:4, 305–308, DOI: https://doi.org/10.1016/j.jegh.2017.07.003

To link to this article: https://doi.org/10.1016/j.jegh.2017.07.003

Published online: 16 April 2019
Short communication

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

Ramya Raghavan , Subarna Roy, Ramanathan Thamizhmani, Sugunan Attayur Purushothaman

Dept. Microbiology, Regional Medical Research Centre, Port Blair, Andaman and Nicobar Islands, India

Dept. Microbiology, National Institute of Traditional Medicine, Belgaum, Karnataka, India

Dept. Epidemiology, Regional Medical Research Centre, Port Blair, Andaman and Nicobar Islands, India

Abstract
Diarrheagenic 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 diarrheal 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 diarrheal stool samples by multiplex PCR with specific primers using standard 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...
Andaman child & Nicobar Island, Deburnath 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 07471-1880.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 rpm 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 Pol1 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 deoxynucleoside triphosphates, 0.5 µL of 3 U 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 mM of each primers (Sigma-Alrich 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-Alrich 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 bfp A 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.

| 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        |

Table 2

| Primer | Target gene | Primer sequence (5’-3’) | Amplicon (bp) | Refs |
|--------|-------------|-------------------------|---------------|-----|
| LT-F   | elt         | CACACGGAGCTTCCCTCAGTC   | 508           | 7   |
| LT-R   |             | CCCCCACCTCTACGTTGTTT    | 147           | 7   |
| ST-F   | est         | TCTAACAGCAGTACCTTTCAAA  | 212           | 7   |
| ST-R   |             | GAGAACCACCTTACTATGAG    | 367           | 7   |
| BFPA-F | bfpA        | CCGGTACAGCGAGCATGTTAACA | 630           | 7   |
| BFPA-R |             | GAAATGCAATTATCTAGCTG    | 630           | 7   |
| CVD432-F| AaaA       | CTGGGCAGAAGATGCATCAT    | 630           | 7   |
| CVD432-R| eae        | CCGAGATCCGGAGCATGTTAACC | 881           | 7   |
| EAE-F  |             | CCGAGATCCGGAGCATGTTAACC | 215           | 7   |
| EAE-R  |             | ATGTGCTTCCAGGATTTAC    |               |     |
| AAIC-F | aaiC        | ACGACACCCCTGATAAACA    |               |     |
| AAIC-R |             |                        |               |     |

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

Acknowledgments

This research received no specific grant from any funding agency, commercial or not-for-profit-sector.

References

[1] Koloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric MultiCenter Study, GEMS): a prospective, case-control study. Lancet 2013;382:209–22.
[2] United Nations Children’s Fund (UNICEF)/World Health Organization (WHO). Diarrhoea: Why Children are Still Dying and What Can Be Done. New York: UNICEF/Geneva: WHO; 2009.
[3] United Nations Children’s Fund (UNICEF). Committing to child survival: A promise renewed. Progress report. New York: UNICEF; 2014.
[4] Lanata CF, Fischer-Walker CL, Olacuaga AC, Torres CX, Aryee MJ, Black RE. Global causes of diarrheal disease mortality in children, 5 years of age: a systematic review. PLoS One 2013;8:e72788.
[5] Dutta S, Guin S, Ghosh S, Pahapuri GP, Rajendran K, Bhattacharyya MK, et al. Trends in the prevalence of diarrheagenic Escherichia coli among hospitalized diarrheal patients in Kolkata, India. PLoS One 2013;8:e56988.
[6] Croxen MA, Law RJ, Scholz R, Keeney KM, Wodarska M, Finlay BB. Recent advances in understanding enteric pathogenic Escherichia coli. Clin Microbiol Rev 2013;26:822–80.
[7] Panchalingam S, Antonio M, Hossain A, Mandomando I, Ochieng B, Oundo J, et al. Diagnostic microbiologic methods in the GEMS-1 case/control study. Clin Infect Dis 2012;55:294–302.
[8] O’Ryan M, Prado V, Pickering L. A millennium update on pediatric diarrheal illness in the developing world. Semin Pediatr Infect Dis 2005;16:125–36.
[9] Ali MMM, Mohamed ZK, Klena JD, Ahmed SF, Moussa TAA, Chenglesh KS. Molecular characterization of diarrheagenic Escherichia coli from Libya. Am J Trop Med Hyg 2012;86:806–71.

Note. DEC = diarrheagenic Escherichia coli; EAEC = enteroaggregative Escherichia coli; EPEC = enteropathogenic Escherichia coli; ETEC = enterotoxigenic Escherichia coli.
Vu Nguyen T, Le Van P, Le Huy C, Gia KN, Weintraub A. Detection and characterization of diarrheagenic *Escherichia coli* from young children in Hanoi, Vietnam. J Clin Microbiol 2005;43:755–60.

Lozer DM, Souza TB, Monfardini MV, Vicentini F, Kitagawa SS, Scaletsky ICA, et al. Genotypic and phenotypic analysis of diarrheagenic *Escherichia coli* strains isolated from Brazilian children living in low socioeconomic level communities 2013;13:418.

Moyo SJ, Maselle SY, Matee MI, Langeland N, Mylvaganam H. Identification of diarrheagenic *Escherichia coli* isolated from infants and children in Dar es Salaam, Tanzania. BMC Infect Dis 2007;7:92.

Jafari F, Garcia-Gil LJ, Salmanzadeh-Ahrabi S, Shokrzadeh L, Aslani MM, Pourhoseingholi MA, et al. Diagnosis and prevalence of enteropathogenic bacteria in children less than 5 years of age with acute diarrhea in Tehran children’s hospitals. J Infect 2009;58:21–7.

Nataro JP, Mai V, Johnson J, Blackwelder WC, Hetzer R, Tirrell S, et al. Diarrheagenic *Escherichia coli* infection in Baltimore, Maryland, and New Haven, Connecticut. Clin Infect Dis 2006;43:402–7.

Ali MMM, Ahmed SF, Klena JD, Mohamed ZK, Moussa TAA, Ghenghesh KS. Enterooaggregative *Escherichia coli* in diarrheic children in Egypt: molecular characterization and antimicrobial susceptibility. J Infect Dev Ctries 2014;8:589–96.

Cio Mandomando IM, Bio Macete EV, Ruiz J, Sanz S, Abacassamo F, Vallés X, et al. Etiology of diarrhea in children younger than 5 years of age admitted in a rural hospital of southern Mozambique. Am J Trop Med Hyg 2007;76:522–7.

Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 1998;11:142–201.

Nair G, Ramamurthy T, Bhattacharya M, Krishnan T, Ganguly S, Saha D, et al. Emerging trends in the etiology of enteric pathogens as evidenced from an active surveillance of hospitalized diarrheal patients in Kolkata, India. Gut Pathog 2010;2:4.

Ghosh PK, Ali A. Isolation of atypical enteropathogenic *Escherichia coli* from children with and without diarrhoea in Delhi and the National Capital Region, India. J Med Microbiol 2010;59:1159–62.

Nguyen RN, Taylor LS, Tauschek M, Robins-Browne RM. Atypical enteropathogenic *Escherichia coli* infection and prolonged diarrhea in children. Emerg Infect Dis 2006;12:597–603.