**Short Communication**

**Frequency of Diarrheagenic *Escherichia coli* among Children in Surabaya, Indonesia**

Kayo Osawa1,2, Dadik Raharjo2,3, Eddy Bagus Wasito2,3, Sugeng Harijono2,3, Katsumi Shigemura4, Ro Osawa4,5, Subijanto Marto Sudarmo7, Yoshio Iijima8, and Toshiro Shirakawa1,2,6*

1Division of Infectious Diseases, Department of International Health, Kobe University Graduate School of Health Sciences, Kobe 654-0142; 2Department of Urology and 6Center for Infectious Diseases, Kobe University Graduate School of Medicine, Kobe 650-0017; 3Department of Bioresource Science, Graduate School of Agricultural Science, Kobe University, Kobe 657-8501; 4Department of Microbiology, Kobe Institute of Health, Kobe 650-0046, Japan; and 5Department of Microbiology, and 7Department of Pediatrics, Faculty of Medicine, Airlangga University, Surabaya, Indonesia

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**SUMMARY:** Diarrheagenic *Escherichia coli* (DEC) is a major etiologic agent of childhood diarrhea in developing countries. We investigated the frequency of DEC in stool samples from 125 diarrheal children (age, 1–10 years) and 92 non-diarrheal children in Surabaya, Indonesia. The non-diarrheal children served as healthy controls. DEC was detected in 23 of 125 (18.4%) and 47 of 92 (51.1%) samples in the diarrheal and non-diarrheal children, respectively. Enteropathogenic *E. coli* was the most prevalent in the non-diarrheal children (25.0%), and its prevalence was significantly higher than that in the diarrheal children (0.8%) ($P < 0.0001$). Interestingly, Shiga toxin-producing *E. coli* (4.3%) was detected only in the non-diarrheal children ($P = 0.031$). This is the first study comparing between diarrheal children with non-diarrheal or healthy children to investigate the role of DEC in pediatric diarrheal diseases in Indonesia.

Diarrheagenic *Escherichia coli* (DEC) is a leading cause of diarrhea, which is one of the most common causes of morbidity and mortality among children in developing countries (1). DEC can be classified into five major categories on the basis of their specific virulence properties: Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), and enteroaggregative *E. coli* (EAEC) (2). The importance of DEC has been reported in developing countries (3–7). In Indonesia, while a high incidence of diarrhea in children has been reported (8,9), no study has compared diarrheal and non-diarrheal children to investigate the role of DEC in pediatric diarrheal diseases. In the present study, we collected a total of 178 stool specimens from 125 diarrheal children (age, 1–10 years; average ± SD, 4.0 ± 2.6 years) treated at Soetomo General Hospital, Surabaya, Indonesia. Most hospitalized patients were administered over-the-counter anti-diarrheal medications (with/without antibiotics) by a family member. In case the diarrhea persisted, then they visited primary healthcare or private clinics before visiting the hospital. Ninety-two stool specimens were collected from healthy children (age, 1–10 years; average ± SD, 5.3 ± 1.8 years) attending in kindergartens, Surabaya, who did not have diarrhea during the previous month; these children served as controls. All specimens were collected between August 2009 and September 2011.

All stool specimens were cultured for the selection of *E. coli* isolates. Three loopfuls of bacteria grown on MacConkey agar (Oxford, Basingstoke, UK) were scraped and mixed with 2 ml sterile aqua dest and then boiled at 100°C for 15 min followed by centrifugation at 6,000 rpm for 10 min. The supernatants containing DNA were then collected for DNA analysis. The DNA extracts were analyzed by real-time PCR using primer sets and TaqMan probes (Applied Biosystems, Foster City, Calif., USA) under PCR conditions described in previous studies (10–12) (Table 1). In the present study, we used the primers and probes for *eaeA* of STEC/EPEC; *stx1* and *stx2* of STEC; *aggR* of EAE; *est*, *esp*, and *elt* of ETEC; and *ipaH* of EIEC (10–12) and designed them for the *bfpA* gene of EPEC, *astA* gene of EAE, and *estH* gene of ETEC (Table 1). We defined the sample as being positive when amplification occurred in 35 cycles or less (10). In our experiment, 200 copies of targeted DNA in one reaction tube could be amplified by 35 PCR cycles, and at least 2,000 copies of DNA from each *E. coli* clone were present in the reaction tube (data not shown). We determined significant

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*Corresponding author: Mailing address: Center for Infectious Diseases, Kobe University Graduate School of Medicine, 7-5-1, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. Tel: +81-78-382-5686, Fax: +81-78-382-5715, E-mail: toshiro@med.kobe-u.ac.jp
significantly more frequently in non-diarrheal children than in diarrheal children. EPEC was detected in 23 of 125 (18.4%)
from the diarrheal and non-diarrheal children, respectively. There was no significant difference in the frequencies of EAEC, ETEC, and EIEC between diarrheal and non-diarrheal children. EPEC was detected significantly more frequently in non-diarrheal children (25.0%) than in diarrheal children (0.8%) ($P < 0.0001$). Previous studies reported that the rate of atypical EPEC isolation from children with diarrhea was not significantly different from the rate of isolation from children without diarrhea (4–7). One of the explanations may be that most of the diarrheal children were already prescribed antibiotics before hospitalization. STEC was detected only in non-diarrheal children (4.3%), and it was detected significantly more frequently in non-diarrheal than in diarrheal children ($P = 0.031$). Previous studies have suggested that EIEC and STEC may play minor roles in childhood diarrhea in developing countries (3–7). Rajendran et al. reported that STEC was detected in 2 of 99 (2.0%) non-diarrheal controls in a study conducted in southern India (13). We also detected STEC in 4 of 92 (4.3%) non-diarrheal controls in the present study. These findings suggested that a certain number of healthy children serve as chronic carriers in some developing countries. We would like to emphasize a limitation of our study. This study should have been a case-control study in which the diarrheal children should not have received antimicrobials before collecting samples. However, this study included both children who received and who did not receive antibiotics before sampling. Our prospective study will overcome this limitation.

In conclusion, this is the first study comparing diarrheal children with non-diarrheal or healthy children to investigate the role of DEC in pediatric diarrheal diseases in Indonesia. No specific category of DEC was found to be significantly associated with diarrhea in children. Therefore, further studies with a systematic surveillance for most diarrheagenic pathogens, i.e., bacteria, viruses, and parasites, including a larger number of children are required to better elucidate the role of DEC in pediatric diarrheal diseases in Indonesia.

**Conflict of interest** None to declare.

**REFERENCES**


