Enterotoxigenic Clostridium perfringens and sporadic diarrhoea: a study from an Indian tertiary care hospital

Food-borne diseases present a growing health problem worldwide and over 200 different diseases are known to be transmitted by food (Bryan, 1982). Food safety has always been a very significant public health issue, but its global importance is not fully appreciated by many health authorities even now. In the United States, food-borne pathogens have been reported to cause 76 million cases and 5000 deaths each year (Mead et al., 1999). At present, the conventional means for diagnosing food-borne diarrhoea in the microbiology laboratory relies on the culture of bacteria from stool samples. Because there are many food-borne bacterial pathogens, such investigation is usually concentrated only on facultative bacteria. Analyses of food-borne anaerobic bacteria are not routinely performed due to difficulties in their isolation and identification. The role of Clostridium perfringens and its enterotoxin in food-borne diarrhoea is well known. More recently, C. perfringens enterotoxin has also been implicated as a cause of antibiotic-associated diarrhoea (Borriello et al., 1984), infectious diarrhoea (Larson & Borriello, 1988) and sporadic diarrhoea (Luzzi et al., 1998; Brett et al., 1992). So far no information exists on disease prevalence from our country, even though it is well established in the west. This prompted us to carry out a prospective study to determine the role of enterotoxigenic C. perfringens in patients with sporadic, apparently non-food-related, diarrhoea in the Indian population using a duplex PCR and to type the isolates by multiplex PCR.

A total of 200 faecal specimens comprising 100 samples from single isolated cases of acute diarrhoea (both from in-patients and out-patients) and 100 non-diarrhoeal controls received by the Anaerobic Laboratory of the All India Institute of Medical Sciences during the period January 2003–January 2005 were analysed for the presence of C. perfringens. All the specimens from which other enteropathogens (toxigenic Escherichia coli, Salmonella, Shigella, Vibrio, Staphylococcus aureus, Clostridium difficile) were isolated were excluded.

Tenfold diluted stools specimens were subcultured onto egg yolk agar (EYA) with neomycin and brain heart infusion agar (BHIA) with a 5 μg metronidazole disc (Hi media). Culture plates were incubated anaerobically at 37°C for 48 h. Identification of C. perfringens colonies was carried out by Gram stain, lecithinase production and other biochemical analyses as described previously (Joshy et al., 2006). Multiple colonies from each culture plate were stored at −20°C in RCM broth with glycerol (30%) for further analysis.

Detection of C. perfringens enterotoxin was performed on the stool specimen by reverse passive latex agglutination (RPLA) (PET RPLA kit; Denka-Sieken) and double sandwich-ELISA (R-Biopharm) using a commercial kit. The assays were performed according to the manufacturer’s instructions. The search for enterotoxin production by the isolate was performed after sporulating them in vitro in Duncan–Strong sporulation medium.

DNA was extracted from each culture-positive faecal specimen with the QIAamp rDNA stool mini kit (QIAGEN) and DNA extracts were stored at −20°C before PCR. After thawing extracted DNA was added directly to the PCR assay as template. For PCR of C. perfringens stored isolates were cultured on BHI agar. One colony was suspended in 50 μl sterile distilled water and heated to 100°C for 10 min. Supernatant (10 μl) was used as template in the PCR. For enterotoxin gene detection PCR was done on stool samples as well as on the isolates by duplex PCR (Joshy et al., 2006). Toxinotyping of C. perfringens isolates were done using specific primers corresponding to the fragments of toxin genes as described previously (Joshy et al., 2006).

During the two year study period, a total of 200 faecal specimens were examined for C. perfringens. Stool specimens from 20 out of 100 (20%) patients were positive for C. perfringens by culture. Of these 1% of samples were positive for C. perfringens enterotoxin by both RPLA and ELISA. Duplex PCR analysis for enterotoxin gene detection, also showed that 1% of samples were positive for enterotoxin gene.

Of 100 non-diarrhoeal controls 27 (27%) C. perfringens strains were isolated. None of the samples were positive for enterotoxin either by RPLA, ELISA or by PCR. Multiplex PCR indicated that all the isolates were of type A, containing only the z toxin gene, β, ε and τ genes were absent.

In this study we tried to find an answer to the question whether or not enterotoxin-producing C. perfringens strains are casually related to sporadic diarrhoea in our population. Samples known to contain other pathogens were excluded because it would be difficult to interpret the significance of the presence of C. perfringens enterotoxin in addition to other enteropathogens such as Salmonella. The RPLA kit has been used to investigate cases of sporadic diarrhoea; positivity rates for enterotoxin in faecal specimens ranged from 2.5 to 18% (Samuel et al., 1991; Brett et al., 1992; Mpamugo et al., 1995; Tompkins et al., 1999; Forward et al., 2003). However, there have been problems with the specificity of this test (Food Standards Agency, 2000) and none of those studies involved enterotoxin analysis at the molecular level.

In our study 1 of the 47 isolates gave positive results for both plc and cpe genes (the presence of 2 bands of phospholipase C gene (283 bp) and enterotoxin gene (426 bp) by duplex PCR. This suggested a prevalence of 2% of C. perfringens carrying the cpe gene. The observed prevalence of cpe gene in C. perfringens strains isolated from faecal specimens in this study was close to...
those recently reported in molecular epidemiology surveys. Those surveys suggested that only a low percentage (<5%) of all C. perfringens isolates from various origins carried the cpe gene (Kokai-Kun et al., 1994; Van Damme-Jongsten et al., 1989) whereas the percentage is higher among C. perfringens strains isolated from confirmed outbreaks of food poisoning.

None of the C. perfringens isolated from healthy controls were carrying the cpe gene, whereas positive cases were detected in patients from confirmed outbreaks of food poisoning. From this study it was clear that sporadic diarrhoea can be caused by enterotoxigenic C. perfringens. However, considering the low frequency of enterotoxin in faecal specimens in this study, C. perfringens seems to have a limited role in the analysed sporadic diarrhoeal population. The application of these molecular techniques to detect C. perfringens, as well as its enterotoxins and possible positive cases in stool, will help not only in diagnosis, but also in epidemiology of infection in various C. perfringens outbreaks. This duplex and multiplex PCR will also help us to implicate the aetiology or associating role of C. perfringens in diverse clinical syndromes. In the current situation of increasing numbers of immunocompromised patients this relatively mild organism might lead to serious/fatal diarrheas, if left unrecognized.

**Acknowledgements**

This work was supported by Indian Council of Medical Research, New Delhi, India.

**Correspondence:** Rama Chaudhry (drramach@rediffmail.com or ramach003@yahoo.com)

**References**


