**Clostridium perfringens** type A & antibiotic associated diarrhoea

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**Background & objectives:** *Clostridium perfringens* type A (CPA) isolates produce lethal necrotizing antigens and the heat resistant forms of the organism are associated with pathogenic outcome in humans. CPA has also been implicated in antibiotic associated diarrhoea (AAD). We therefore undertook this study to investigate the presence of CPA in stool samples of patients with AAD in a tertiary care setting in north India.

**Methods:** A total of 285 stool samples obtained from patients suspected for *Clostridium difficile* aetiology were examined for the presence of CPA antigens. Four sets of reagents (CP-I, CP-II, CP-III and CP-IV) comprising latex beads coated with polyvalent immune sera to 17 serotypes of heat resistant CPA were used in the study. Agglutination reaction was carried out using the reagents with the stool supernatants.

**Results:** Of the 285 stool samples tested, 25 (8.77%) were positive for at least one or more of the four polyvalent sets. Briefly, 48 per cent were positive for all the four sets, 12 per cent for 3 sets, 28 per cent for 2 sets and 12 per cent for only one set, indicating the prevalence of multiple serotypes of CPA. Twenty three (92%) of the 25 positive samples came from patients who were on antibiotics. *C. difficile* toxin was also present in 9 of 25 (36%) of the samples positive for CPA antigens.

**Interpretation & conclusion:** In our setting, CPA could thus be associated with AAD either by itself or in synergy with *C. difficile* infection. Assessment of true burden of CPA associated AAD would be required to take appropriate steps for its control in our country.

**Key words** Antibiotic associated diarrhoea - *Clostridium perfringens*

*Clostridium perfringens* is a common intestinal inhabitant of man. However, *C. perfringens* type A (CPA) isolates produce lethal necrotizing antigens and the heat resistant forms of the organism are associated with pathogenic outcome in humans. It has been reported that about 2 to 5 per cent of all CPA produce enterotoxin1-3. It may be responsible for 5 to 20 per cent of all cases of antibiotic associated diarrhoea (AAD) and sporadic non-food borne diarrhoea4 though *C. difficile* is implicated in the most severe cases of
AAD and specific treatment is usually successful. *C. difficile* is much less often found in diarrhoea or colitis where pseudomembranes are absent and interestingly, some *C. difficile* negative cases of AAD present with bloody diarrhoea.

Borriello *et al* were the first to demonstrate *C. perfringens* enterotoxin with high counts of toxigenic organisms in patients with diarrhoea after antibiotic treatment. It is not known whether antibiotics permit infection by enterotoxigenic *C. perfringens* or allow overgrowth of small numbers of the organism that may be normally resident in the gut of these patients. Sparks *et al* have reported that regardless of geographic origin, *C. perfringens* enterotoxin (CPE) positive isolates caused most CPE linked AAD cases. No information on the role of CPA in AAD is available from this part of the country. We therefore conducted a preliminary study to investigate the presence of the pathogenic forms of CPA in the stool samples of patients with AAD in our tertiary care centre so that optimum control and treatment measures could be defined in the future.

**Material & Methods**

**Patients and samples:** During April 2000 to October 2002, a total of 285 random faecal specimens suspected for *C. difficile* aetiology were submitted to the Microbiology Section of the Department of Gastroenterology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh. These samples were collected from patients who developed diarrhoea after hospitalization and/or antibiotic usage. These patients were undergoing treatment for various ailments like septicemia, pancreatitis, cirrhosis, pneumonia, tuberculosis, meningitis, urinary tract infection, postoperative management etc. The duration of hospitalization ranged from 4 to 15 days with a median of 7 days. The antibiotics used were mainly cephalosporins, penicillins, fluoroquinolones, aminoglycosides, tetracyclines and antituberculous drugs. There were 167 males and 118 females in the study. The age of the patients varied from 2 months to 98 yr with a median of 31 yr.

Our laboratory does not receive samples from patients with infective diarrhoea caused by other enteric pathogens. However, all stool samples received are routinely investigated for *Salmonella*, *Shigella*, *Escherichia coli*, *Staphylococcus aureus*, *Candida* etc. Other pathogens are looked for whenever requested or suspected. In addition to *C. difficile* toxin (CDT) assay done, the stool samples were checked for *C. perfringens* type A heat resistant antigens. Faecal samples taken from healthy volunteers (n=15) who were attendants of patients and who had not received any antibiotic for at least 6 wk prior to testing were also screened.

**C. perfringens type A antigen testing:** Based on our previous experience, 0.81µ latex beads (Difco Labs, USA) were coated separately with four sets of CPA polyvalent immune sera raised against a range of 17 serotypes of CPA (Denka Seiken, Japan). Set I covered serotypes 1 to 5, sets II, III and IV covered serotypes between 6 to 9, 10 to 13 and 14 to 17, respectively. Slide agglutination tests were carried out using 50 µl of 1:5 diluted faecal supernatant on a clean glass slide to which latex beads with pre-titrated CPA antisera coated with all the four sets (CP-I, CP-II, CP-III and CP-IV) were added separately. The slide was gently rocked manually and looked for macroscopic agglutination. A sample was considered CPA antigen positive when agglutination occurred within 2 min. Positive assay control consisted of supernatant from sonicated CPA and negative control consisted of uncoated latex beads plus diluted test sample.

**C. difficile toxin assay:** *C. difficile* toxin assay was done by our indigenous method described elsewhere. Briefly, 50 µl of 1:5 diluted faecal supernatant was taken on a clean glass slide to which ready to use *C. sordelli* antitoxin coated latex beads (Difco Labs, USA) were added. The slide was gently rocked manually and checked macroscopically for agglutination. A sample was considered CDT positive when agglutination occurred within 2 min. A known positive faecal sample obtained from a patient with antibiotic associated diarrhoea constituted the positive control. Negative controls comprised of either or both of (i) an unreactive faecal sample from a healthy volunteer who had no antibiotic exposure for 6 wk prior to testing; and (ii) uncoated latex beads plus diluted test sample. All positive samples were further titrated by doubling dilutions (ranging from 1:10 to 1:640) and repetition of CDT agglutination tests. The titre of the toxin was recorded as the highest dilution of the faecal supernatant which gave a positive
agglutination reaction. The advantages of using *C. sordelli* coated beads for CDT assay has been described in details earlier\(^\text{10}\).

**Statistical analysis:** Chi square method was used to compare the presence of CPA antigens with antibiotic intake and CDT positivity.

**Results & Discussion**

Twenty five (8.77%) of the 285 stool samples tested were positive for at least one or more polyvalent sets identifying 17 different serotypes of CPA antigens. Of these, 18 (72%) of the samples belonged to males and 7 (28%) to female patients. The infants with positive CPA were 7 (28%) whereas the remaining 18 (72%) were children or adults. Interestingly, 48 per cent (n=12) of the CPA positive samples were positive for all the four sets of reagents. Similarly CPA antigen of three sets were present in 12 per cent samples; of 2 sets in 28 per cent samples and 1 set in 12 per cent. Twenty three (92%) of the 25 *C. perfringens* positive samples came from patients who were hospitalized and were on antibiotics before the onset of diarrhoea and none of them had clinical symptoms typical of food poisoning. *C. difficile* toxin was also detected with a titre ranging from 1:10 to 1:320 in 9 (36%) of them. The presence of CPA antigens was significantly associated (\(P<0.01\)) with antibiotic intake. However, the presence of CPA did not influence the presence of CDT (\(P>0.05\)). None of the samples from healthy volunteers had detectable level of CPA. Other causes of AAD like *S. aureus* and *Candida* were also looked for, and only one case with gastroenteritis (14 months male infant) was positive for candida, but negative for CPA and CDT.

A total of 57 samples (20%) were positive for CDT (Table) of whom 47 were on antibiotics. Sporadic reports are available on the role of *C. perfringens* in the aetiopathogenesis of diarrhoea in hospitals of India. Gupta and Gulati\(^\text{11}\) isolated *C. perfringens* from 43 per cent of hospital patients among which 29 per cent were heat resistant isolates. Prasad \etal\(^\text{12}\) reported 3 of 11 isolates of *C. perfringens* from cases of diarrhoea to be enteropathogenic in the rabbit ileal loop. Chakrabarty \etal\(^\text{13}\) reported significantly higher CPA isolation with in diarrhoeal cases than in controls.

<table>
<thead>
<tr>
<th>Antibiotics used*</th>
<th>Diarrhoea n (%)</th>
<th>Pain abdomen n (%)</th>
<th>Fever n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples (n=285)</td>
<td>239 (83.8)</td>
<td>268 (94.0)</td>
<td>73 (25.6)</td>
</tr>
<tr>
<td>CPA positive (n=25)</td>
<td>23 (92.0)</td>
<td>24 (96.0)</td>
<td>7 (28.0)</td>
</tr>
<tr>
<td>CDT positive (n=57)</td>
<td>47 (82.4)</td>
<td>55 (96.5)</td>
<td>9 (15.8)</td>
</tr>
</tbody>
</table>

*Multiple antibiotics were used in almost all cases. They were cephalosporins, penicillins, fluoroquinolones, aminoglycosides, tetracyclines and anti-tuberculosis drugs.

Borriello \etal\(^\text{7}\) were the first to suggest that the association of *C. perfringens* with antibiotic treatment is causal rather than fortuitous. Samuel \etal\(^\text{14}\) tested 721 diarrhoeal stool specimens, of which 25 were shown to be positive for enterotoxigenic *C. perfringens* with most of them being hospital inpatients. Pituch \etal\(^\text{15}\) isolated both *C. difficile* and *C. perfringens* from the stool samples of 4 of 158 patients suspected of AAD. Joshy \etal\(^\text{16}\) reported 10 per cent positivity of *C. perfringens* in *C. difficile* negative samples in patients with AAD and suggested that it might result in extended hospitalization particularly in frail elderly patients.

The antibiotics used in our setting, were mainly penicillins, cephalosporins and fluoroquinolones with most of the patients (n=23, 92%) having received multiple antibiotics. However, two of the remaining patients also had detectable CPA antigens although there was no history of antibiotic treatment. The reason for this could be the acquisition of *C. perfringens* spores in a hospital setting similar to the acquisition of *C. difficile* spores.

*C. perfringens* AAD is a distinct entity\(^\text{17}\), though it is unclear if antibiotic exposure primarily permits the proliferation of small numbers of resident *C. perfringens* strains or allows their acquisition. In our study, CPA antigens of one to four different sets were found in a majority of the samples, implying thereby that many different serotypes of CPA could be simultaneously proliferating in the intestine after antibiotic intake. Sparks \etal\(^\text{8}\) provided important
evidence that plasmid cpe isolates caused mostly C. perfringens associated AAD and chromosomal cpe isolates caused C. perfringens type A food poisoning cases.

C. difficile may account for only approximately 20 per cent of all cases of AAD and C. perfringens up to 15 per cent. It is not surprising that we found the presence of both C. difficile and C. perfringens in some of our patients. This is consistent with the reported literature. It is likely that both the anaerobes might work either in combination or in synergy for production of AAD. The presence of CDT in 36 per cent of our CPA positive samples is similar to those reported by other workers. Though statistically the CPA positivity did not influence the CDT positive status, it is likely that CPA as well as other clostridial species such as C. difficile might work in synergy for production of AAD.

The incidence of C. perfringens diarrhoea is expected to increase with the growing population of immunocompromised individuals and the increased use of antibiotic intake. As detection of CPA is not a part of the routine laboratory investigation due to resource constraints, one tends to miss out on the diagnosis of AAD due to this organism. Further studies need to be done to assess the true burden of CPA associated AAD in India.

Acknowledgment

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