**Clostridium difficile** Infection at a Tertiary Care Hospital in South India

Shashidhar Vishwanath¹, Arpita Singhal¹, Annet D’Souza¹, Chiranjay Mukhopadhyay¹, Muralidhar Varma², Indira Bairy¹

**Abstract**

**Objective:** The objective of this study was to detect *C. difficile* in patients presenting with Antibiotic Associated Diarrhoea.

**Methods:** Stool samples from twenty-five patients collected over a period of four months were processed for *C. difficile* by culture and the isolates were identified following standard methods. *C. difficile* toxins A and B and *C. perfringens* enterotoxin were detected by ELISA performed directly on stool specimens.

**Results:** Four patients (16%) were found positive for *C. difficile* infection. All patients with *C. difficile* infection received prior treatment with third-generation cephalosporins or β-lactam / β-lactamase inhibitor antibiotics. *C. perfringens* enterotoxin was found in two (8%) patients. Severe colitis was seen in one (25%) of the four patients who had co-infection with *C. difficile* and *C. perfringens*.

**Conclusion:** This study demonstrated a significant occurrence of *C. difficile* infection in this hospital population. There is a need to further evaluate the role of *C. perfringens* in causing antibiotic associated diarrhoea. Good clinical and laboratory studies to generate local epidemiological data are essential to increase awareness among the treating clinicians about *C. difficile* infection. Also limited and rational use of broad spectrum antibiotics is recommended.

**Introduction**

*Clostridium difficile* is recognised as the primary pathogen responsible for antibiotic-associated colitis and accounts for 15–25% of cases of nosocomial antibiotic-associated diarrhoea.¹ Clinical manifestations of *C. difficile* infection (CDI) range from mild or moderate diarrhoea to fulminant, pseudomembranous colitis, sepsis, multi-organ failure and death.¹,² Widespread regional outbreaks of *C. difficile* strains involving more severe and refractory disease have been reported, with greater numbers of complications, colectomies, and deaths than previously described.³ Early diagnosis is the key to prevent complications from severe CDI and to prevent transmission. Rapid diagnosis depends on maintaining a high degree of clinical suspicion for CDI in patients with diarrhoea and recent antimicrobial exposure and hospitalisation.³ Along with cytotoxin detection, culture has been a mainstay in the laboratory diagnosis of CDI which is also essential for the epidemiologic study of isolates.¹ We report our initial experience with CDI in an Indian tertiary care hospital setting.

**Material and Methods**

A prospective, pilot study was conducted from May to August 2010 with consecutive 25 patients clinically suspected with antibiotic associated diarrhoea (AAD) were included. This study was approved by our Institutional Ethical Committee. Informed consent was obtained at enrolment. Patients were suspected as having AAD if significant diarrhoea was noted along with a history...
of treatment with antimicrobial or antineoplastic agents within the previous 8 weeks. Patients were considered to have CDI, if AAD was present along with a positive toxigenic culture and/or a positive result for *C. difficile* toxin by ELISA.1 Children below two years of age and those with other proven aetiological agents of diarrhoea or diarrhoea inducing factors were excluded from the study. Stool samples collected in sterile, wide mouth containers were sent to microbiology laboratory for further processing without delay.

Stool specimens were processed for culture of *C. difficile* following standard methods4 and tested for *C. difficile* toxins A and B (Premier toxins A and B, Meridian Bioscience, Ohio, USA). Clostridium perfringens is one of the most frequently cited alternative causes of AAD.5 Hence, the stool samples were also tested for *C. perfringens* enterotoxin (Ridascreen, R-Biopharm AG, Germany) by ELISA. Culture for *C. perfringens* was not performed as it is not recommended as a diagnostic method, given the relative ubiquity of the bacterium in human faeces as normal flora, and because not all isolates will be enterotoxigenic. Also, *C. perfringens* enterotoxin production from cultured isolates can be difficult to demonstrate because it occurs during sporulation and it is difficult to make this bacterium sporulate in vitro.5

### Results

The mean age of study group was 40.7 years (range: 4 - 76 years, M:F = 1.5:1). Age group of 51 - 60 years had the highest cases of AAD (36%). Most of the patients (18, 72%) with AAD were from general medical wards, followed by oncology (3, 12%); surgery (2, 8%), and paediatric wards (2, 8%). CDI was diagnosed in four (16%) patients with suspected AAD, all from general medical wards. These four patients had received prior treatment with beta-lactam antibiotics. The clinical profile and microbiological factors of patients with CDI are summarised in Table 1. None of them received prior treatment with clindamycin or fluoroquinolones. Mean age of patients with CDI was 64 years. Mean duration of antibiotics use prior to onset of illness was 18 and 11 days respectively in patients with CDI and non CDI illness.

Among the risk factors which were analysed, prior antibiotic usage, advanced age, history of contact with child < 2 yrs of age and gastric acid suppression therapy were seen among all the four CDI cases. Three patients (75%) were hospitalised in the prior 6 months. None of them had a previous gastrointestinal surgery or a prior *C. difficile* infection or nasogastric intubation.

### Discussion

ELISA (4, 16%) had a higher positivity rate compared to culture (2, 8%). Both the cultured isolates were toxigenic. The lesser sensitivity of culture may be explained by sampling errors inherent to uneven distribution of *C. difficile* in the faecal samples.6 Also inclusion of glutamate dehydrogenase detection as screening test will be helpful to rule out false positivity of ELISA in such instances. In comparison with the positivity rates in Indian setups, this is in agreement with the earlier studies of Dhawan B et al. (15%),7 Gogate A et al. (18%)8 and Joshy L et al. (12.1%).9 Considering other geographical regions, Heimesaat MM et al.10 and Jamal W et al.11 have found the prevalence rates of CDI to be 11.4% and 8% respectively. None of the four patients diagnosed with CDI in the present analysis were on prior metronidazole or vancomycin. It is essential to know the risk factors associated with CDI, as some of them

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**Table 1**: Shows the clinical profile and microbiological factors in patients with Clostridium difficile infection

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Prior antibiotic therapy</th>
<th>Reason for choosing the Antibiotic</th>
<th>Duration of prior Antibiotic use (days)</th>
<th>Culture for Clostridium difficile</th>
<th>ELISA for Clostridium difficile Toxins A and B</th>
<th>ELISA for Clostridium perfringens Enterotoxin</th>
<th>Severe Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65 / Female</td>
<td></td>
<td>Urinary tract infection <em>(Escherichia coli)</em></td>
<td>Cefoperazone-Sulbactum</td>
<td>Based on Susceptibility results</td>
<td>12</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>55 / Male</td>
<td></td>
<td>Bilateral lower lobe pneumonia, Sepsis</td>
<td>Piperacillin-Tazobactam</td>
<td>Empirical</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Severe colitis</td>
</tr>
<tr>
<td>3</td>
<td>60 / Female</td>
<td></td>
<td>Septic arthritis</td>
<td>Ceftriaxone</td>
<td>Empirical</td>
<td>7</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>76 / Female</td>
<td></td>
<td>Urosepsis <em>(Escherichia coli)</em></td>
<td>Cefoperazone-Sulbactum; Cefixime-Clavulanic acid; Cefazidime</td>
<td>Based on Susceptibility results</td>
<td>3 months (on and off)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
may be modified to reduce the occurrence; or the presence of risk factors in a patient with AAD can raise the clinical suspicion of CDI, so that appropriate treatment and control measures can be adopted. Prevention of symptomatic \textit{C. difficile} infections requires a change in antibiotic policy with prudent use of antibiotics and implementation of standard infection control measures.\textsuperscript{12}

\textit{Clostridium perfringens} enterotoxin (CPEnt) was detected in 2 (8\%) specimens. \textit{C. perfringens} is reported to account for 2-15\% of all cases of AAD.\textsuperscript{5} Asha NJ et al.\textsuperscript{3} and Vaishnavi C et al.\textsuperscript{13} have reported similar positive rates of 8\% and 8.77\% for CPEnt. However, Joshy L et al.\textsuperscript{14} have reported lower incidence of \textit{C. perfringens} AAD (2.6\%). Both \textit{C. difficile} toxin and CPEnt were detected in one patient, who had severe colitis\textsuperscript{15} as evidenced by leucocytosis (20.3 x 10\(^9\)/L) and radiographic findings (CT abdomen - Long segment bowel thickening involving colon and rectum with pericolonic fat stranding). It is likely that both \textit{C. perfringens} and \textit{C. difficile} might work in synergy for production of AAD\textsuperscript{13} and thus may also be responsible for causing severe disease. The inciting antibiotics were withdrawn following the diagnosis of CDI and all four patients improved significantly with introduction of metronidazole therapy. There was no mortality reported among CDI cases in this study. The reported mortality rates in CDI vary from 0.6\% to 83\%.\textsuperscript{16}

\textbf{Conclusion}

Avoiding empirical therapy with β-lactam and β-lactamase inhibitors (BL-BLIs) and choosing antibiotics from the susceptibility panel with less risk for developing AAD may reduce the occurrence of CDI. In conclusion, this study demonstrated significant presence of CDI in this hospital population. BL-BLIs and third generation cephalosporins were the most common antibiotics associated with development of CDI. Being a pilot study, this study had limitation of small sample size, however good clinical and laboratory studies to generate local epidemiological data are essential to increase awareness among the treating clinicians about CDI. Limited and rational use of broad spectrum antibiotics is recommended. There is a need to further evaluate the role of \textit{C. perfringens} in causing antibiotic associated diarrhea.

\textbf{References}


