

Original article

Detection of *Clostridium difficile* infection in patients with antibiotic associated diarrhea in a tertiary care hospital

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Abstract-

Background- *Clostridium difficile* is a major cause of hospital acquired diarrhea and is responsible for pseudomembranous colitis.

Aims and objectives- 1] To isolate *C.difficile* from stool samples of patients, on long term antibiotics, admitted in the hospital 2] to detect the toxin production by ELISA.

Material and Methods- A total of 50 stool specimens were processed for culture and for detection of *C. difficile* toxin A and toxin B via *C.difficile* A+B Stool Antigen Elisa kit from patients on long term antibiotics for a period of 8 months. All *C.difficile* suspected cases were from Medicine ward and were on multiple antibiotics for more than 7 days.

Keywords- *C.difficile*, AAD, *C.difficile* toxin A+B

Introduction-

Diarrhea is one of the most common complications associated with antibiotic therapy⁽¹⁾. *C.difficile* is a primary pathogen responsible for 15-25% cases of nosocomial Antibiotic associated Diarrhea (AAD)⁽²⁾. The risk of *C.difficile* acquisition increases in proportion to the length of the hospital stay. *C.difficile* produces both an enterotoxin, toxin A and a cytotoxin B⁽¹⁾. The prevalence of *C.difficile* spores in the environment is higher among hospital and thus the patients have higher rate of *C.difficile* colonization than the healthy adults in the general population. Prolonged hospital stay, elderly age, immunosuppressive conditions have been recognized as risk factors for *C.difficile* infection [CDI]^(2,3).

Elderly patients are at higher risk of acquiring disease rate for patients as much as 20 fold higher than those for younger patients⁽²⁾. Other factors that increase the vulnerability include underlying disease severity, non surgical gastrointestinal procedures, use of antiulcer medications. Also the patients who have suppressed immune response to *C.difficile* toxins are at increased risk^(1,2). Further antibiotic therapy causes imbalance of intestinal tract bacterial flora, which predisposes *C.difficile* proliferation and colonization of intestinal tract mucosa⁽⁴⁾.

The toxigenic *C.difficile* may excrete exotoxins, enterotoxin (toxin A) and cytotoxin (toxin B) and both can cause diarrhea and colitis. Toxin B being major virulence factor according to recent studies⁽¹⁾.

C.difficile associated diarrhea (CDAD) covers a wide range of diseases from asymptomatic to mild diarrhea to moderately severe diarrhea and pseudomembranous colitis which can be fatal ⁽⁴⁾ . Frequent indiscriminate use of broad spectrum antibiotics has increased the incidence of *C.difficile* associated diarrhea in the recent years⁽¹⁾ . CDAD is the most common cause of antibiotic associated diarrhea responsible for one third of AAD cases, 50-70% for antibiotic associated colitis and 90-100% cases of pseudomembranous colitis which is the most severe manifestation of CDAD ^(1,2) . CDAD is one of the commonest causes of nosocomial diarrhea⁽¹⁾ .Clindamycins, Penicillins and Cephalosporins and Fluroquinolones are frequently used antibiotics ^(1,2) .The prevalence of CDAD is global and the incidence varies from place to place. However CDAD is still under diagnosed in India and Asia ⁽²⁾ .Present study was undertaken to detect the presence of *C.difficile* and/or the toxin produced from the stool sample of patients on long term antibiotic. The presence of organism and/or toxin can initiate precise and effective antibiotic therapy. Also it will help to undertake infection control measures in the hospital.

Aims and Objectives-

- To isolate the *C.difficile* from stool samples of patients on long term antibiotics admitted in hospital.
- To detect the toxin production by ELISA.

Materials and Methods-

A prospective study was conducted on 50 patients presenting with diarrhea who were on antibiotic therapy. Other etiologies of diarrhea were ruled out by microscopy and culture.

1) Inclusion criteria-Patients on antibiotics for more than 5 days and with diarrhea.

2) Exclusion criteria-

- Patients without antibiotics or on antibiotic less than 5 days.
- Patients with other known cause of diarrhea.
- Patients suffering from TB and AIDS.
- Paediatric patients.
- Obstetrics patients.

A total of 50 stool samples were processed for a period of 8 months in Anaerobic section in Microbiology Laboratory, Sassoon General Hospital, Pune. The study was approved by Institute's Ethical committee.All samples were collected after taking consent of the patient. Most of the patients in the present study were from medicine wards and were on multiple antibiotics for more than 7 days. Stool sample was inoculated in Robertson's cooked meat medium with Thioglycollate broth and was incubated at 37⁰C for 4 hours. Then was subcultured on cycloserine, cefoxitin fructose agar (CCFA) and incubated in anaerobic jar for 48 hrs. The colony smear was Gram stained and subjected to aero-tolerance test by plating on blood agar and incubated aerobically. Further identification of anaerobes was done as per Wardsworth anaerobic manual ⁽⁵⁾ .

Toxin detection-Stool sample was centrifuged at 1000 rpm for 10 min and supernatant was stored at -20⁰c for toxin detection ⁽⁶⁾ .Toxin was detected by using *C.difficile* ELISA Antigen detection kit (IVD Reasearch INC, Carlsbad , A92010 USA).The test was performed as per manufacturer's instructions.

Results-

Table 1: Number of *C.difficile* isolates and toxin production in patients with AAD-

	NO.(n= 50)	Percentage (%)
Culture positive	0	0
Toxin positive	3	6

Toxin was detected in 6% of patients.

Table 2: Antibiotics associated with AAD-

Antibiotics used	No. of patients	Cases of AAD
Cephalosporins	26	1
Clindamycin	6	2
Penicillin	8	0
Fluroquinolones	10	0

Cephalosporins and clindamycin were the most common cause of AAD.

Discussion-

CDAD is considered to be the commonest cause of nosocomial diarrhoea. All antibiotics have the potential to cause CDAD though certain antibiotics such as fluroquinolones, clindamycin and beta lactam are more no-torious in causing the same. Emergence of hypervirulent strains due to indiscriminate use of antimicrobials and inad-equate infection control measures in hospitals are the main factors responsible for the recent outbreaks of *C.difficile* infection⁽²⁾. *C.difficile* caused large outbreaks in US and European countries where half a million infections occur-ed in a year⁽⁷⁾. CDC hopes to prevent this deadly infection by supporting State Antibiotic Resistance Prevention Pr-ogrammes based on which *C.difficile* infection has become notifiable to CDC since 2013⁽⁷⁾. *C.difficile* associated di-arrhea is prevalent globally, but its incidence varies from place to place. CDAD is largely under recognized in India and Asia, due to lack of clinical suspicion, difficulty in culturing the organism and the cost of toxin assay⁽²⁾. Increa-sing prevalence of *C.difficile* has

been reported in Europe and USA during past 10 yrs⁽⁶⁾. Most previous studies ab-out *C.difficile* toxin in India have shown prevalence rate ranging from 7 to 30%⁽⁸⁾. Niyogi et al. and Bhattacharya et al reported prevalence of *C. difficile* toxin to be 8.4%⁽⁹⁾ and 7.3%⁽¹⁰⁾ respectively.

In the present study, 6% stool sample showed the presence of *C.difficile* toxin which co relates with the study by Bhattacharya et al.⁽¹⁰⁾. Shashidhar Vishwanath et al.⁽¹¹⁾ documented the prevalence rate of *C.difficile* as 16% , which was higher than the present study. Also, a study done by Daniel M. Musher et al.⁽¹²⁾ documented the higher prevalence rate of 24%.

Varous studies have shown that, ICU stay, underlying diseases like malignancies and inflammatory bowel diseases, exposure to chemotherapy and immunosuppressive therapy are additional risk factors⁽¹³⁾. In the present study, patients with malignancies such as cancer of rectum, ulcerative colitis were at risk. Studies have shown that older age and female gender have been identified as the risk

factors⁽⁶⁾. In the present study, older male patients (more than 55 yrs) were at major risk. Out of 50 samples processed, 3 patients were positive for CDT (Clostridium difficile toxin) and were male patients. Prolonged course of antibiotic treatment have been related to an increased risk of AAD. Other risk factors reviewed in the present study were history of previous antibiotic treatment. In the present study, Cephalosporins and Clindamycin were the antibiotics associated with AAD. Thus, the present study supports the significance of broad spectrum antibiotics as one of the risk factors for CDAD^(14,15). CDT positive result was more likely to occur in those with prolonged hospital admissions (> 14 days) than in those who had shorter hospital stay (< 7 days). In the present study the time for the occurrence of symptoms was 8 days after the start of antibiotics which was in accordance with other studies⁽⁶⁾. This suggests that disturbance of the normal colonic flora eventually results in diarrhea takes place within about a week after the antibiotic treatment⁽⁶⁾. Prolonged duration of hospital stay has also been reported to be associated with AAD and CDAD⁽⁶⁾.

Proton pump inhibitors(PPI) may be the risk factors for CDAD. They are implicated because the survival of spores is facilitated by elevated gastric PH levels and due to effect of PPIs on immune function or on the toxin production of the organism⁽⁶⁾. In the present study all the AAD patients were on PPIs. The tests conventionally recommended to diagnose CDAD(Clostridium difficile associated diarrhoea) are stool culture, toxin assay, Enzyme immuno assay for toxins and endoscopy where toxin assay is negative^(1,6). However stool culture for *C.difficile* lacks specificity due to possible faecal carriage of

non toxigenic isolate⁽¹⁾. In the present study none of the sample was grown on culture. Laboratories thus rely on toxin detection rather than culture for the diagnosis of *C.difficile*⁽¹⁾. ELISA is the method of choice for the detection of toxin A+B. This method is highly specific and sensitive.

CDAD is a growing nosocomial and public health challenge⁽⁶⁾. Prevention of *C.difficile* infection is challenging⁽¹⁾. Continued surveillance for *C.difficile* infections is important to monitor success of prevention methods. Established guidelines should be followed to minimize exposure to the pathogen which include injudicious use of antibiotics, rapid detection of *C. difficile* by Immunoassays for toxin A+B, isolation of patients who have CDAD, barrier nursing, proper disinfection of objects and education of staff members^(1,2). The most successful control measure to reduce the asymptomatic diseases is to restrict antimicrobial use⁽²⁾.

Conclusion-

Prompt and precise diagnosis is an important aspect of effective management of *C.difficile* infection. *C.difficile* toxin detection can be used for early diagnosis of CDI (Clostridium difficile infection)^(1,2). Immediate antibiotic treatment can be started to avoid long hospital stay which can worsen the infection⁽¹⁾. CDAD being an important nosocomial and public health challenge, prevention of *C.difficile* plays crucial role⁽¹⁾. Continuous surveillance for *C.difficile* infections needs to be done to monitor progress in prevention. The most successful control measure to reduce the asymptomatic diseases is to restrict antimicrobial use⁽²⁾.

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