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INCIDENCE AND MICROBIOLOGICAL PREDICTORS OF *CLOSTRIDIUM DIFFICILE* ASSOCIATED DIARRHOEA IN A TERTIARY CARE HOSPITAL OF NORTHERN INDIA

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Abstract: **Background/Aims:** Nosocomial infection with *Clostridium difficile (C.difficile)* increases morbidity and mortality among hospitalized patients. This study aimed to determine the incidence and microbiological predictors of developing *C.difficile* infection among hospitalized patients with antibiotic associated diarrhoea (AAD). **Settings and Design:** This prospective study was conducted in a tertiary care hospital over a period of two years. **Methods and Material**: Fresh stool samples of patients with history of AAD were processed. *C.difficile* toxin A & B by enzyme linked immunosorbent assay (ELISA) and culture on Cycloserine Cefoxitin Fructose Agar (CCFA) were done. In addition, fecal leukocyte test (FLT), Gram's staining for predominance of Gram positive bacilli (GPB) and fecal occult blood test (FOBT) were done. **Statistical Analysis:** Done using Chi square test. **Results**: Out of 331 stool samples received from AAD patients, 9 (2.7%) were found to be *C.difficile* toxin positive. Of these, *C.difficile* was isolated in one (11.1%). Percentage of males with AAD who developed *C.difficile* associated diarrhoea (CDAD) (3.2%; 7/219) was more than percentage of females (1.8%; 2/112) (P = 0.181). FLT, Gram's staining for predominance of GPB, and FOBT were positive in 30%, 44.4% and 0% of *C.difficile* toxin positive patients respectively compared to 18.0%, 22.4% and 11.5% of toxin negative patients (P = 0.129, 0.106, 0.152 respectively) **Conclusion**: Incidence of CDAD among patients with AAD was 2.72%. A relatively increased incidence of CDAD among male patients was observed. FLT, Gram's staining for predominance of GPB and FOBT were found to be poor predictors of development of CDAD.

Keywords: *Antibiotic associated diarrhea, Clostridium difficile, Clostridium difficile associated diarrhoea,* Cycloserine Cefoxitin Fructose Agar

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INTRODUCTION

Clostridium difficile (*C. difficile)* is now recognized as a frequent cause of antibiotic associated diarrhea (AAD) and colitis, and the incidence of *C. difficile*-associated diarrhoea (CDAD) appears to be increasing. Frequent use of broad spectrum antibiotics in hospitalized patients has increased the incidence of CDAD in recent years. AAD occurs in about 5-30% of patients either early during antibiotic therapy or up to two months after the end of the treatment.[1] *C. difficile* is the most commonly identified cause of AAD, accounting for 15%–25% of cases.[2]

Major risk factors for *C. difficile* infection include advanced age, hospitalization, and exposure to antibiotics, immunosuppressants and proton pump inhibitors.[3] Rate of colonization is 20% to 30% in hospitalised adults as compared to 3% in outpatients.[2,4-5] Clindamycin, cephalosporins, and penicillins are the antibiotics most frequently associated with *C. difficile* diarrhea, although they also cause diarrhea that is unrelated to superinfection with this organism.[2]

The cornerstone of the laboratory diagnosis includes isolation of *C. difficile* and/or a positive stool cytotoxin test from a fresh fecal specimen. Stool culture for *C. difficile* is a very sensitive and most specific test but its limitations are: longer time for testing (2-5 days), lack of standardization of methods & culture media in different laboratories and the need for anaerobic culture.[6,7]

The most sensitive and specific test available for diagnosis of *C. difficile* infection is a tissue culture assay for the cytotoxicity of toxin B, using preincubation with neutralizing antibody to show the specificity of the cytotoxicity.[8,9] This test can detect as little as 10 pg of toxin in stool and has a high sensitivity (94%-100%) and specificity (99%).[8,10] However, the most widely used method in the clinical setting to diagnose *C. difficile* infection is the enzyme-linked immunosorbent assay (ELISA) for detection of *C. difficile* toxins A and B.[6]

Recently, polymerase chain reaction (PCR) methods for the detection of *C. difficile* toxin A or B, or both, have been developed with excellent sensitivity (91.5%–97%) and specificity (100%) as compared with the tissue culture cytotoxicity assay. The PCR method is promising for rapid diagnosis of CDAD.[11,12]

Though many reports of both culture and toxin detection of *C. difficile* are available from western countries, few documented reports are available from this region of India.[13-16] Thus we undertook this study to determine the incidence and microbiological predictors of developing of *C. difficile* infection among hospitalized patients with AAD.

MATERIALS & METHODS

This prospective study was conducted over a period of two years (Jan 2007 to Dec 2008) in patients clinically suspected to have AAD admitted in various wards & Intensive care units (ICU) of Dayanand Medical College and Hospital, Ludhiana.

Patient demographic data, including name, age, sex, hospital identification number, different classes of antibiotics administered were recorded. A patient was considered to have *C. difficile* infection, if stool culture and/or toxin assay was positive for *C. difficile*.

Stool specimens of patients with suspicion of AAD were collected and transported immediately to the department of Microbiology. In case of delay, the specimens were refrigerated at 4°C for a maximum period of 72 hrs. Gram's stain was done to look for the abundance of Gram-positive bacilli (GPB) and wet mount was prepared for fecal leukocyte count (FLT). The fecal occult blood test (FOBT) was done using rapid card test method (Hemospot standard, Guaiac method, Tulip Diagnostics (P) Ltd, India).

Culture for *C. difficile* was done on Cycloserine Cefoxitin Fructose Agar (CCFA) after giving ethanol shock (using 95% ethanol) within one hour of receipt of sample. The plates were incubated anaerobically at 37^0C for 48-72 hours. After incubation, the suspected colonies were examined under Wood's lamp for fluorescence. Large (4mm) colonies, with yellowish rhizoids with "Speckled opalescence" and horse manure odour were considered to be suggestive of *C. difficile*. Identification of *C. difficile* was done by Gram's stain and biochemical reactions. If no growth was obtained in 48-72 hrs, plates were reincubated for seven days.

All stool samples were tested for enterotoxin and cytotoxin (Toxin A and B) of *C. difficile* by ELISA (RIDASCREEN® *C. difficile* Toxin A/B kit, R-Biopharm AG, Germany). The assay was performed according to the manufacturer's instructions. Sensitivity, specificity, positive predictive value and negative predictive value given by the manufactures are 96.8 %, 100.0 %, 100.0 % and 98.0 % respectively.

The study was approved by the institutional ethics committee and was carried out in accordance with the guidelines of Helsinki declaration of 1975. The data obtained in this study was analyzed statistically using Chi square test. $P< 0.05$ was taken to be statistically significant.

RESULTS

A total of 331 patients with AAD were enrolled over a period of two years (147 patients in 2007 and 184 in 2008). Maximum number of patients i.e. 71 (21.4%) and 70 (21.1%) were present in age group of 41-50 and 51-60 years respectively with the least percentage 0.3% (1 patient)

falling in the age group of 1-10 years [Figure 1]. In our study, 219 (66.2%) patients were males and 112 (33.8%) were females (M:F = 1.9:1).

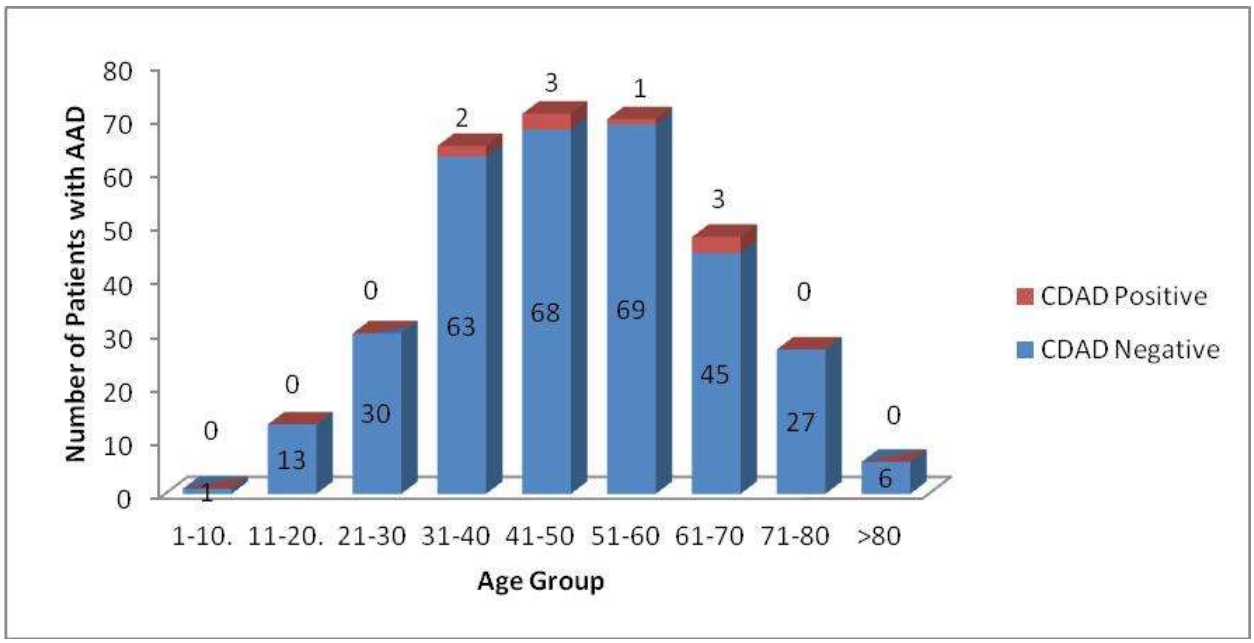

Figure 1: Age distribution of patients with antibiotic associated diarrhoea (AAD) and *Clostridium difficile* associated diarrhoea (CDAD)

Stool specimens from all patients (n=331) were analyzed for *C. difficile* infection from suspected cases of AAD. Enzyme-immuno assay for detection of *C. difficile* toxin A & B was positive in 9 (2.7%) cases. Of these 6 (4.1%) & 3 (1.7%) were positive in 2007 & 2008 respectively. Out of the 9 toxin positive stool samples, 1 (11.1%) showed growth of *C. difficile* on anaerobic culture.

The age distribution of patients with CDAD is shown in Figure 1. The male to female ratio of CDAD patients was 3.5:1. The percentage of males with AAD who developed CDAD (3.2%; 7/219) was more than the percentage of females (1.8%; 2/112) (P = 0.181)

Fecal leukocytes were present in 33.3% (3/9) of the toxin positive patients, compared to (18.01%; 58/322) of toxin negative patients (P = 0. 129). Predominance of Gram positive rods on Grams staining was seen in (44.4%; 4/9) of the toxin positive patients, compared to (22.36%; 72/322) of toxin negative patients (P = 0.106). FOBT was positive in none (0/9) of the toxin positive patients compared to (11.5%; 37/322) of toxin negative patients (P = 0.152).

Two of the CDAD patients underwent sigmoidoscopic examination before the *C. difficile* toxin reports were evaluated. Sigmoidoscopic findings in these patients were suggestive of *C. difficile* infection [Figure 2].

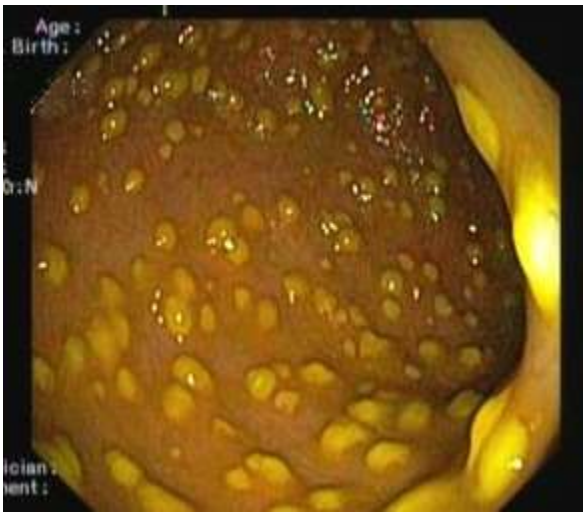

Figure 2: Sigmoidoscopic view of *Clostridium difficile* colitis showing dirty yellow exudates (some coalescing to form pseudomembrane) on colonic mucosa.

A detailed analysis of the antibiotics used in our study population was done. Almost all the patients were treated with more than one antibiotic either concomitantly or sequentially. Overall metronidazole was used in (42.0%; 139/331), ciprofloxacin in (38.7%; 128/331), cefotaxime in (29.3%; 97/331), teicoplanin in (27.2%; 90/331), ofloxacin in (25.4%; 84/331), Piperacillin/tazobactam in (21.7%; 72/331), meropenem in (10.3%; 34/331), ceftazidime in (5.4%; 16/331) patients [Figure 3]. In our study group, CDAD infection developed in (7.1%; 6/84) patients treated with ofloxacin, (5.7%; 8/139) patients on metronidazole, (5.1%; 5/97) on cefotaxime, (2.78%; 2/77) on piperacillin/ tazobactam, (2.34%; 3/128) on ciprofloxacin, and (2.22%; 2/90) on teicoplanin [Figure 4]. All the patients enrolled in our study group improved with conservative management and were discharged from the hospital.

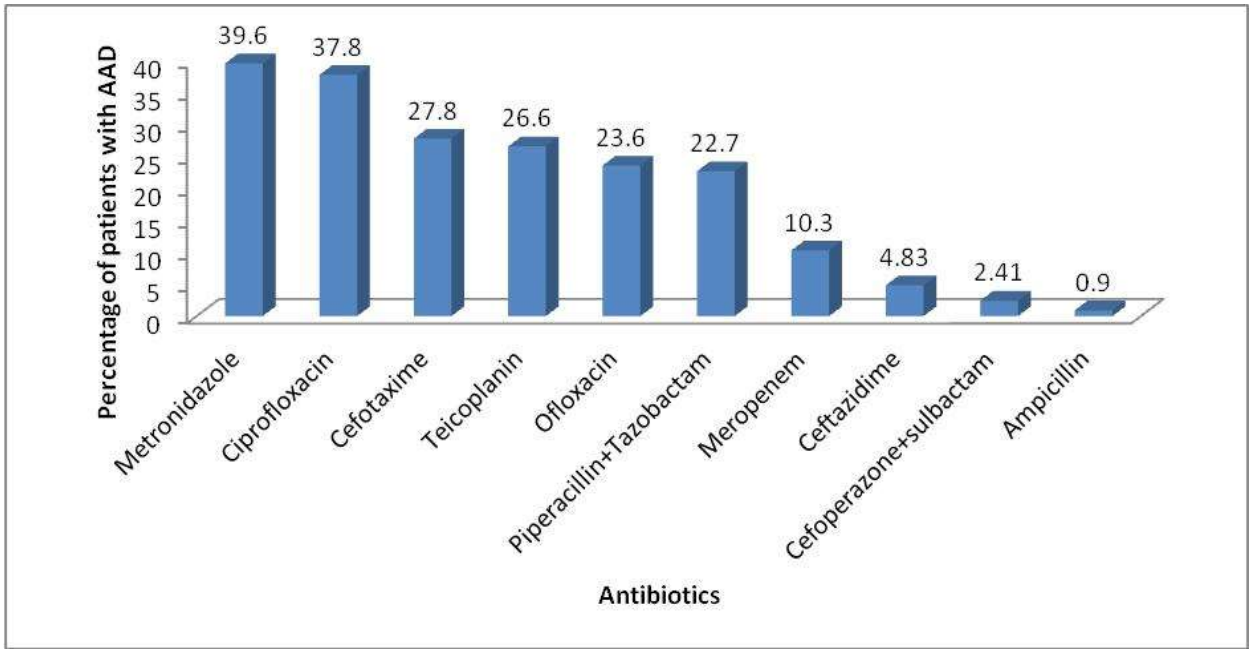

Figure 3: Distribution of Antibiotics used in patients with Antibiotic associated diarrhoea (AAD)

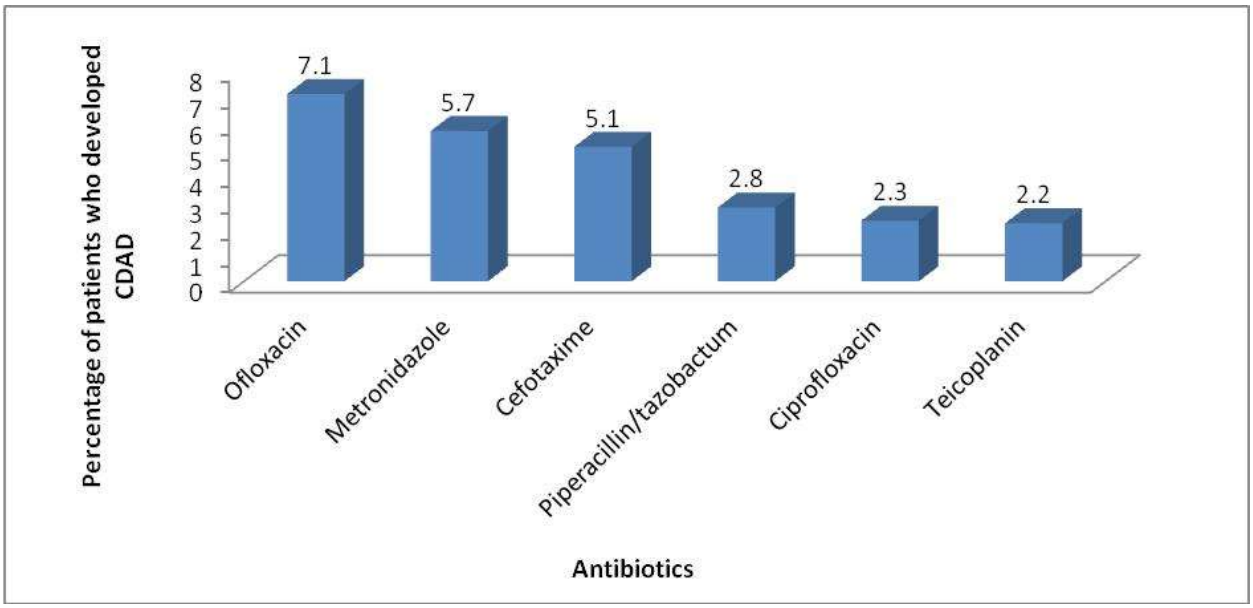

Figure 4: Frequency of development of *Clostridium difficile* associated diarrhoea (CDAD) with use of various antibiotics in patients with antibiotic associated diarrhoea (AAD)

DISCUSSION

C. difficile is considered as the most frequent aetiological agent of nosocomial diarrhoea occurring in hospitalized patients, spreading easily to the environment, the hands of health care

workers and subsequently to other patients, particularly in large hospitals. The study of *C. difficile* infection is important because of its nosocomial implications and association with antibiotic use.

The laboratory diagnosis of AAD due to *C. difficile* is based on culture and toxin detection in fecal specimens. Culture is very sensitive (>92%)[6] but, when used alone without toxin detection, leads to low specificity and under diagnosis of cases when high rates of asymptomatic carriage exist.

In our study, the overall incidence of CDAD among the patients with AAD was found to be 2.7% by toxin detection assays [6 (4.1%) in 2007 and 3 (1.7%) in 2008]. Chaudhry *et* al[13] found that 7.1% patients were positive for *C. difficile* infection by the toxin dependent assay. Out of these, 11.2% were positive in 2001, 9.4% in 2002, 8.6% in 2003, 5% in 2004 and 4% in 2005.[13] Dhawan *et al* found that *C. difficile* was responsible for 15 % of cases of nosocomial diarrhoea. Out of the 210 stool samples tested, 12 were positive by at least one assay. Of these, 11 were positive for the presence of toxin A & B, 8 by culture and 7 by both methods.[14] In our study, out of the 9 positive cases, 8 were positive by toxin and 1 by both toxin & culture, and none by culture alone. This might be due to the reason that most of the culture negative but toxin positive cases were on metronidazole at the time of collection of sample. Similar observation has also been reported by Chaudhry *et al*.[13] In a recent study done by Kaneria *et al* on 50 consecutive, hospitalized patients with AAD and sex matched controls, incidence of 10% of CDAD was observed.[16]

We found a relatively increased incidence of CDAD among male patients as compared to the female patients but it was statistically insignificant. Kaneria *et al* also found increased incidence of CDAD among male patients in their study (80% of CDAD cases were males as compared to 20% females).[16] In contrast, Al-Eidan *et al* reported female gender to be a risk factor for CDAD in hospitalized patients.[17] Chaudhry *et al* found no significant gender difference among the CDAD cases.[13] A retrospective study was done on 49 patients having *C.difficile* infection requiring colectomy for fulminant *C.difficile* colitis (FCDC) by Girotra *et al*.[18] They found that FCDC patients were significantly older (mean age 77±13 years) as compared with CDAD group (65±20 years) without any significant difference in reference to gender or ethnicity.[18] In another study done by Mc Farland *et* al, older age was found to be associated with increased risk of *C.difficile* carriage and diarrhea.[19] Similar findings were reported in our study.

The data on association of presence of fecal leukocytes in CDAD patients is controversial. The results of our study showed that the FLT is a poor predictor of CDAD; because only 33.3%(3/9) of stool specimens positive for *C.difficile* toxin were positive for fecal leukocyte as compared to (18.01%; 58/322) of toxin negative patients. These findings are in concordance with the study

done by Savola *et al* which has shown that FLT was a poor predictor of *C.difficile* infection overall, regardless of the patient status.[20] In similar studies, Marx *et al* and Shanholtzer *et al* have also concluded that testing stool specimens for fecal leukocytes was not useful for predicting the presence of *C. difficile* toxin, because 72% of stool specimens positive for *C. difficile* toxin were negative for fecal leukocytes.[21,22] Manabe *et al* also reported that *C. difficile* toxin-positive patients were no more likely to have fecal leukocytosis than control group.[23] In contrast to these studies, few other studies, have shown that clinical signs of AAD may be limited to watery stools; however, evidence of colitis e.g. fecal leukocytes etc suggests *C. difficile* infection and FLT might be a useful predictor in CDAD.[24,25]

A prospective examination of a stool Gram's stain was performed by Shanholtzer *et al* to determine its utility as a simple and rapid aid in the diagnosis of CDAD. In their study, they found that interpretation of Gram's stain for a predominance of Gram positive rods is not useful in predicting the outcome of culture or toxin assays.[22] Previous study by Silva *et al* have also stated that Gram's staining is not useful in the diagnosis of *C. difficile*-related disease.[26] Our study also showed no significance of screening stool smears for predominance of Gram positive rods in predicting the incidence of CDAD among AAD patients.

In the present study we have found that presence of fecal occult blood is not a predictor for CDAD as none of the FOBT positive patients were found to be positive for CDAD infection. Similarly, a prospective study done by Peled N *et* al with a cohort of 217 hospitalized patients who had received antibiotics and developed diarrhea, also reported that FOBT did not predict the CDAD infection (6% FOBT positive cases in CDAD toxin positive patients as compared to 9% FOBT positive patients in CDAD toxin negative patients, P = 0.333).[27]

The antibiotics most frequently implicated in diarrhea associated with *C. difficile* infection are clindamycin, expanded spectrum penicillins, and cephalosporins. However, virtually any antibiotic may cause CDAD, including brief courses of antibiotics that are given prophylactically before surgery. In our study the most common antibiotics used in patients who developed CDAD were ofloxacin, metronidazole, cefotaxime, ciprofloxacin, piperacillin/tazobactum, and teicoplanin.

CONCLUSION

To conclude, the incidence of CDAD among patients with AAD in our institute was 2.7%. A relatively increased incidence of CDAD among male patients was observed as compared to female patients. FLT, Gram's staining for predominance of GPB and FOBT were found to be poor predictors of development of CDAD infection.

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