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Incidence of *Clostridium difficile* in Patients with Antibiotic Associated Diarrhea

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ABSTRACT

Background: This study was performed to determine the magnitude of *Clostridioides difficile* infection (CDI) in a tertiary care hospital in patients with antibiotic associated diarrhea (AAD) and to study the risk factors associated with this disease.

Methods: A descriptive study was conducted in the department of Microbiology in a tertiary care hospital during December 2019 to May 2021. Stool samples were collected from patients with signs and symptoms of AAD who had been consuming antibiotic or anticancer drugs durng six weeks before the sampling. The samples were subjected to *C. difficile* glutamate dehydrogenase (GDH) enzyme and CD toxin A & B detection by Enzyme Linked Fluorescent Assay (ELFA). Patient's demographic features and clinical details were noted and statistically correlated with the test results.

Results: Among the total 70 samples tested 20 (28%) were positive for GDH alone and 12 (17%) were positive for both GDH and CD toxin A and B. Fluoroquinolones was a significant risk factor in the study. Sepsis and colitis was found to have significant association with C.difficile infection in our study. The crude mortality rate was 17%.

Conclusion: Prompt and precise diagnosis and knowledge about the risk factors of CDI helps in effective management and prevention of CDI.

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Introduction

Clostridioides difficile is an enteric pathogen which is now emerging as the leading cause of antibiotic associated diarrhea in the hospital setting as well as in the community population. It accounts for about 15 to 25 % of nosocomial antibiotic associated diarrhea cases (1). It is known to cause self-limiting antibiotic associated diarrhea, antibiotic associated colitis and more serious conditions like pseudomembranous colitis and toxic megacolon. Major risk factors associated with CDI include advancing age of patient, prolonged hospital stay, immune deficiency state, use of chemotherapeutic drugs and proton pump inhibitors.

C. difficile is a gram positive strictly anaerobic spore forming bacillus., seen in normal gastrointestinal flora in 2-10% humans (2). During colonization two toxins, CD toxin A & B acts as major virulent factors, which act as glucosyltransferases that modifies Rho and Ras proteins in the intestinal epithelial cells. This disrupts the actin cytoskeleton, causing loss of intercellular junctions and leads to secretory diarrhea associated with CDI (3). Emergence of many hypervirulent strains of *C. difficile*, especially ribotype 027 has led to many outbreaks worldwide and it is difficult to treat and more infectious (4).

The diagnosis of *C. difficile* infection is based on the presence of clinical signs and symptoms, followed by the two step strategy or 3 step strategy of laboratory diagnosis. (5) Though nucleic acid amplification tests have the highest sensitivity and specificity, and provide quick results it is expensive.

C. difficile associated diarrhea (CDAD) is a key indicator for monitoring the success of antibiotic stewardship programs in hospitals. Indiscriminate use of antimicrobial coupled with emergence of hypervirulent strains and inadequate infection control measures in hospitals have led to rise in incidence of CDAD. In India CDAD, is still an under recognized cause of diarrhea due to lack of clinical suspicion, difficulty in culturing organisms and non-availability of other diagnostic assays due to their high costs. So this study was attempted to find out the magnitude of CDI in our hospital setting and to analyse the associated risk factors.

Materials and Methods

The present study is a descriptive study, conducted in the Department of Microbiology, Jubilee Mission Medical College and Research Institute, Thrissur from December 2019 to May 2021 following approval from the Institutional ethics committee. The study included all hospitalized patients with diarrhea, who had a history of exposure to antibiotic drugs and anticancer drugs in the previous six weeks. Samples from children less than 2 years and other proven cases of diarrhea were excluded from the study. Stool samples were collected from all these patients and sent to the Microbiology department for CDI diagnosis.

A two-step test protocol was followed (5). All the samples were screened for glutamate dehydrogenase (GDH) initially followed by testing for CD toxin A and B by Enzyme linked fluorescent assay (ELFA) (miniVIDAS, bioMerieux India Pvt Ltd). All the instructions of the manufacturer were strictly followed.

Samples which were positive in both tests were noted as toxigenic *Clostridium difficile*. Samples positive for GDH and negative for toxin A and B were reported as non-toxigenic *Clostridium difficile*. Socio demographic characteristics and clinical details such as age, sex, duration of hospital stay, comorbidities associated, laboratory findings, antibiotic number and duration, treatment provided and outcome of the patient were collected from medical records.

Statistical analysis

Qualitative data was analyzed using frequency proportion and association using chi square test. Quantitative data was analyzed using measures of central tendency like mean, median, standard deviation with 95% confidence interval.

Result

A total of 70 clinically suspected cases of CDI admitted in different specialities of our hospital were enrolled in our study. Twenty (28%) of the samples were GDH positive of which 12 (17%) were positive for *C. difficile* A & B toxin also. The incidence for toxigenic *C. difficile* was found to be 12 (17%) and non-toxigenic *C. difficile* was eight (11%).

Among the total toxin positive cases, seven (58%) were males and five (42%) were females. Six (50%) cases belonged to 61-80 years, four (34%) to 41-60 years of age, whereas one (8.3%) belonged to each of the age 20-40 years and above 80 years group. Mean age of toxin positive cases were 63.4+13.7 years with minimum age being 31 years and maximum 84 years.

Majority of toxin positive cases (5/12; 41.7%) were from oncology department followed by neurology (3/12; 25%), critical care unit (2/12; 16.7%), medicine and geriatric one each (8.3%). Out of the 12 toxin positive cases, six (50%) were admitted in ICU and rest in various wards. Six (50%) of them had both diabetes mellitus and hypertension and four (33%) had diabetes mellitus alone .

The association between primary illness and risk factors among the *C. difficile* toxin positive cases at the time of admission is shown in Table 1. Of the total toxin positive cases 11 (91.7%) were immunocompromised, eight (75%) were having sepsis, seven (58.3%) had colitis, six (50%) had history of prior surgery in past one year and six (50%) had malignancy. Sepsis (p value 0.041) and colitis (p value - 0.001) were found to be statistically significant risk factors for developing. The consumption of various therapeutic drugs during current hospital stay were analysed and shown in Table 2. Nine out of the 12 toxin positive cases were administered fluoroquinolones during current admission compared to 21 (36.2%) of the negative cases which was found to be statistically significant with p value 0.032.

Among the 12 toxin positive cases, two (17%) of cases had only one antibiotic during the present hospital stay and rest all 10 (93%) were on more than one antibiotic. On analysing the length of hospital stay among the total 12, nine (75%) of the toxin positive cases were admitted for 2-4 weeks, two (16%) for 1-2 weeks and one (8.3%) for 4-8 weeks. For treatment of CDI, 10 patients (92%) were given vancomycin alone, one (8%) was given metronidazole alone and one was given both these. Among the 12 toxin positive cases, 10 recovered with appropriate treatment (83.3%) and two expired (16.7%).

Discussion

Clostridiodes difficile infection (CDI) is the primary cause of antibiotic-associated diarrhea. Disruption of normal bacterial flora by antibiotic use permits overgrowth of endogenous or nosocomially acquired pathogens like C. difficile. The major CDI risk factors reported includes advanced age, prolonged hospital stay, use of multiple antibiotics and unsafe exposure to health care facilities. So, diagnosis of *C. difficile* associated diarrhea is important to initiate early treatment as it is not possible to establish the diagnosis by history and clinical examination alone. A descriptive study was conducted in the department of Microbiology to detect *C. difficile* infection by using combined *C. difficile* GDH and *C. difficile* Toxin A and B assay by miniVIDAS (bioMerieux) and the risk factors associated with the infection was also assessed.

Table 1. Association between primary illness/risk factors and *C. difficile* toxin positive cases.

Presenting illness/Risk factors	***C. difficile* Toxin positive**	**Percentage**	**P value**
Malignancy	6	50.0	0.189
Sepsis	8	66.7	**0.041***
Gastroenteritis	12	100	0.132
Immunocompromised	11	91.6	0.210
Colitis	7	58.3	**0.001***
Surgery	6	50.0	0.282

Table 2. Association between drugs administered and *C. difficile* toxin positive cases.

Drugs	Toxin Positive (N=12)	Negative (N=58)	p value
Proton-pump inhibitors			
Yes	9	44	0.604
No	3	14	
Chemotherapeutic agent			
Yes	5	15	0.222
No	7	43	
Fluoroquinolones			
Yes	9	21	0.032*
No	3	37	
Amoxicillin Clavulanic acid			
Yes	2	9	0.605
No	10	49	
Clindamycin			
Yes	0	4	0.496
No	12	54	
Piperacillin Tazobactam			
Yes	4	27	0.305
No	8	31	
Meropenem			
Yes	7	20	0.112
No	5	38	
Colistin			
Yes	2	5	0.344
No	10	53	
Cephalosporins			
Yes	6	29	0.624
No	6	29	

In the present study 70 stool samples were processed, of which *C. difficile* GDH assay was positive in 20 subjects (28%). GDH is a constitutive enzyme produced in large amounts by all strains of *C. difficile* independent of its toxigenicity. Since it can be easily detected in feces, it is used as a good screening marker for *C. difficile* infection. In order to improve the laboratory diagnostic capacity for CDI investigation, studies have recommended the use of *C. difficile* GDH as a preliminary screening test, followed by further confirmatory tests for toxin production.

C. difficile toxin A and B detection was done simultaneously in all the samples; 12 out of the total 70 (17%) samples were tested positive by the toxin assay and all these samples were positive for GDH assay too. In a study by Lukas Fenner et al, all GDH screen-positive specimens were retested by the rapid toxin A/B immunoassay, and they detected the presence of toxin A/B in 36.89% by the rapid toxin A/B test (6).

Of the 20 (28%) GDH assay positive samples eight (11%) were negative for *C. difficile* toxin A & B and were reported as non-toxigenic *Clostridium difficile* (7). As per latest IDSA/SHEA (8, 9) guidelines those samples which are only GDH positive, should be confirmed by NAAT which unfortunately was not done in our study due to non availability. In a prospective study done by Lee YC, GDH positive and CD A&B toxin negative cases were reported as *C. difficile* colonization (8). According to latest CDI management guidelines PCR positive and toxin negative patients have lower levels of *C. difficile* colonization and may not need therapy. In view of infection control measures, they can be kept in enteric isolation. Treatment is considered only in severe, non-resolving, or otherwise unexplained diarrhea strongly suggestive of CDI (9).

In our study a total of 12(17%) subjects out of 70 were positive for both GDH and CDAB toxin, the incidence of toxigenic *C. difficile* was 17%, which was concordant with the reports in other studies around the world. Studies shows that the incidence of *C. difficile*-associated diarrhea in hospitalized patients ranges from 3% to 29% (10-13). Wilcox et al. used laboratory positives (only in diarrheal patients) from medical clinics and reported annual incidence of 29.5 % and 20.2% cases per 100,000 individuals in urban and semi-rural settings, respectively (14). Recently Tanu Singhal et al reported that a total of 67 patients had CDI in the study period with a mean incidence of 0.2/1000 patient days (15). A halving of the CDI incidence was reported in their study after intensification of the CDI prevention bundle.

Among the 12 toxin positive cases, seven (58%) were males and five (42%) were females. In other studies, also a similar male predominance was reported (7).

In the current study, six (50%) of toxin positive cases belonged to 60-80 years,concordant finding was reported by Vijay Kumar et al (16). The mean age of the *C. difficile* positive cases was 64 years, where lowest was 31 and highest was 84 years in our study. The mean age of affected patients in Tanu Singhal et al study was also found to be same (15). There was one toxin positive patient above the age of 80 years in our study. Though in the present study we could not find any significant association, previous studies have shown advanced age also as a significant risk factor in developing CDAD (17).

On analyzing the location wise distribution in the present study, five (41.7%) of the toxin positive cases belonged to oncology department, followed by neurology department three (25%) multidisciplinary critical care unit two (16.7%), medicine and geriatrics one each (8.3%). This finding was concordant with the study conducted by Gulnaz et al in 2014 where maximum number of *C. difficile* positive cases were reported from the oncology department (18).

Associated risk factors and the comorbidities among the *C. difficile* toxin positive cases at the time of present admission were assessed. Out of the 12 toxin positive cases 10 (83.3%) had diabetes and six (50%) had both diabetes and hypertension. Though majority of them were diabetic and hypertensive, the present study did not show any

significant association between diabetes mellitus, hypertension, and *C. difficile* infection. But in contrast to our finding, many studies have reported diabetes as a significant risk factor in CDI. Studies conducted by N. Eliakim Raz et al reported 30.6% were diabetic (19). Another major risk factor assessed in our study was malignancy. Of the total *C. difficile* toxin positive cases, six (50%) were cancer patients who were on antibiotics during the current admission. Among these patients five (41.7%) were on chemotherapeutic agents too. Similar finding has been reported by Kamthan et al also (20). Although antibiotics are clearly linked to the development of C. difficile–associated diarrhea, there is also evidence that cytotoxic chemotherapeutic agents can promote CDAD, even in the absence of antibiotics (21).

Among the *C. difficile* toxin positive cases with sepsis, there were both *C. difficile* infection complicating to sepsis and sepsis with secondary *C. difficile* infection. Eight (66.7%) toxin positive cases were treated for sepsis during the hospital stay. This difference was found to be of statistically significance (p value=0.041). Rachel lutz et al suggested that among patients with more recurrent *C. difficile* infection, there was a parallel trend for higher rates of sepsis (21).

In the present study seven (58.3%) of *C. difficile* toxin positive cases were suffering from colitis. There was a significant association between colitis and *C. difficile* infection in our study (p value=0.001) and of these three were on chemotherapy. This result is in concordance with the study of Arun et al, who reported four cases of colitis and 1 case of pseudomembranous colitis (7).

Six (50%) cases had a history of surgical intervention during the previous one year of admission. Though surgery is also considered as one of the risk factors there was no significant association in our study. The mean duration of length of hospital stay among the *C. difficile* toxin positive patients in our study was 19.5 days. Similarly in the study done by Tanu Singhal et al reported the median duration of hospitalization as 14 days following which patients developed CDI in their study (range: 4–70 days) (15). Vijayakumar et al also reported that prolonged hospital stay as an important risk factor for developing CDI (16).

While analyzing the clinical presentation for which the patients was admitted, gastroenteritis with or without fever was the commonest symptom among the *C. difficile* toxin positive cases reported and this finding has been well supported by literature evidences. We also analyzed blood routine laboratory parameters in all the subjects and among them, total leukocyte count was increased in nine (75%) of positive cases and renal function test was deranged in seven (58.3%) cases. Leukocytosis is common in CDI and may be quite elevated, which is a finding that indicates a worse prognosis. Patients with *C. difficile* are also prone to acute kidney injury. Therefore, total leukocyte count and serum creatinine were measured in patients with C. difficile, because the presence of leukocytosis and renal impairment are indicators of severe infection.

The association of antimicrobials with AAD such as clindamycin, quinolones, third generation cephalosporins, piperacillin tazobactam, amoxicillin-clavulanic acid, meropenem and colistin were analyzed. Since many patients in the present study had concurrently received multiple antimicrobials, the risk associated with the individual drugs could have been confounded by other drugs. Among the total toxin positive cases nine (75%) of them were on fluoroquinolones, significant association was found between the use of fluoroquinolones and development of C difficile toxin positivity in our study (p value=0.016). In a study by Arun et al conducted in 2017, they reported significant association between use of piperacillin tazobactam and fluoroquinolones and development of AAD with 47% of the toxin positive cases on fluoroquinolones (7). This was concordant with our finding. It was observed that nine (75%) of the *C. difficile* toxin positive cases in our study were on proton pump inhibitors (PPI) prior to developing diarrhea. Though this was not found to

be statistically significant in our study, previous studies showing significant association with PPI are reported worldwide. Overuse of PPIs was seen in more than 50% in a retrospective study conducted by McDonald et al (23).

Majority of the toxin positive cases in our study were on multiple antibiotics prior to developing diarrhea. There was no significant difference in the onset of diarrhea among the *C. difficile* positive cases who were on multiple antibiotics when compared to those who were on single antibiotic.

According to the IDSA guidelines, vancomycin is the drug of choice for an initial episode of severe CDI. The current IDSA recommendation is to use oral vancomycin for all cases irrespective of severity (9). Notably, the 125 mg formulation of vancomycin is not available in our hospital, and hence, all the patients were treated with 250 mg oral vancomycin in divided doses daily for 10-14 days. Likewise, the drug fidaxomicin which is currently recommended as first line therapy at par with oral vancomycin for mild/severe/recurrent CDI was also unavailable. In a review by Nelson et al it was suggested that vancomycin is superior to metronidazole for treatment of CDI (24). In our study ten out of the 12 toxin positive cases were treated with vancomycin alone, one was given both vancomycin and metronidazole and one was given metronidazole only. The vancomycin treatment success rate was 90 % in our study. Of the 12 (91.6%) *C. difficile* toxin positive cases, two (17%) patients expired and the mortality rate was 17% in our study. This is lower when compared to the crude mortality in Tanu Singhal et al study which was 22% (15). Among the two patients expired, one had preexisting active malignancy and was on chemotherapy and other one succumbed to sepsis with preexisting multiple comorbidities. All the toxin positive cases were followed up for one month period to find out any CDI recurrence. No recurrence was found.

One of the important limitations of the present study was the small sample size available for correlation. Most of the cases were referred from other institutions prior to admission in this institution. This had a deficit in proper detailing of previous history and the details of previous antimicrobial therapy could not be procured in all. Another limitation encountered in our study was that we could not verify the results with any molecular studies. Incidence of CDI in patients seeking medical care on outpatient (op) basis also could not be studied.

Conclusion

The control of *Clostridium difficile* infections is an international clinical challenge. Prevention of CDI requires implementation of various strategies at different levels. Correctable risk factors need to be reduced, which finally decrease susceptibility of a patient to CDI. However, the rapid surge of CDI incidence and severity in recent years due to hypervirulent and multi antibiotic resistant *C. difficile* strains strongly suggests that current antibiotic treatment strategies cannot keep pace with the rate at which these bacteria develop resistance. Therefore, prompt, and precise diagnosis is mandatory for the effective management of CDI, along with immediate implementation of infection prevention and control strategies, and the optimization of treatment in the management of this infection.

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Ethics approval and consent to participate

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Conflict of interest

The authors notified that there are no conflicts of interest.

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