Original Article

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Microbiology Section

Prevalence and Clinical Features of *Clostridioides difficile* Infections among Inpatients in a Tertiary Care Teaching Hospital: A Retrospective Cross-sectional Study from Southern India

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ABSTRACT

Introduction: *Clostridioides difficile (C. difficile)*, once considered a nosocomial pathogen, is now increasingly being observed in the community. The organism is known to cause Antibiotic-associated Diarrhoea (AAD), Pseudomembranous Colitis (PMC), megacolon, and even death.

Aim: To determine the prevalence of *C. difficile* in stool samples and to associate the findings with the clinical presentation and risk factors of the patients.

Materials and Methods: This retrospective cross-sectional study analysed 208 stool samples received for *C. difficile* detection at Father Muller Medical College Hospital, Mangaluru, Karnataka, India from January 2021 to January 2023 using the CerTest *C. difficile* Glutamate dehydrogenase (GDH)+ Toxin A+B onestep combo card test. The results were then associated with the clinical profiles of the patients retrieved from the hospital software, Backbone. Statistical analysis was performed using frequency, percentage, the Chi-square test, and the z-test.

Results: Out of the 208 samples analysed from patients belonging to all ages, 20 samples (9.62%) harboured toxigenic *C. difficile*, while 22 samples (10.58%) contained non toxigenic *C. difficile*. Proton Pump Inhibitor (PPI) use and underlying diseases/conditions were identified as highly significant risk factors (χ^2=32.28, p-value<0.001, HS) among patients with toxigenic *C. difficile*. AAD was found to be statistically significant (p-value=0.030, Sig) in toxigenic patients compared to those with non toxigenic *C. difficile*.

Conclusion: The increasing presence of *C. difficile* in our community is a matter of concern. Continuous surveillance, vigilance, and appropriate preventive measures are crucial in mitigating the impact of *C. difficile* Infections (CDI) in hospitals and in the community.

Keywords: Antibiotic associated diarrhoea, Glutamate dehydrogenase, Nosocomial, Pseudomembranous colitis

INTRODUCTION

Clostridioides difficile (formerly known as *Clostridium difficile*) is a Gram-positive, anaerobic, spore-forming bacillus. The pathogenicity of *C. difficile* is primarily attributed to two toxins, toxin A and toxin B, and the organism is considered as one of the major culprits of nosocomial diarrhoea. In addition to diarrhoea, it has also been implicated in PMC, megacolon, and death [1]. The strains of *C. difficile* that do not produce toxins A and/or B are known as non toxigenic *C. difficile*, which are regarded as colonisers [2]. Recurrent *C. difficile*-associated disease has also been a significant clinical threat [1]. CDI is reported to be undoubtedly troublesome in cancer patients and solid organ transplantation recipients [3,4]. *C. difficile* has also been discovered in patients following surgery, leading to a high mortality rate, longer hospital stays, and higher costs [5]. A study has illustrated the significant role of various predisposing factors for CDI, such as the usage of antibiotics, PPIs, immunosuppressive agents, the presence of underlying diseases, old age, and duration of hospital stay [6]. Although it was perceived as a nosocomial pathogen, the role of the organism in causing community-acquired infections has been documented in the literature [7].

The diagnosis of CDI still remains a major challenge. Despite various algorithms being employed, a test that is quick, sensitive, specific, and economical is still lacking. Laboratory confirmation of *C. difficile* is based on the detection of toxigenic strains or toxins in stools [8]. Polymerase Chain Reaction (PCR) techniques are used to detect the toxin genes which requires expertise and may also lead to over diagnosis of cases [8]. Many authors have evaluated the C. Diff Quik Chek Complete assay, which has proven to be a rapid and cost-effective method for diagnosing CDI [9-13].

The present study was conducted to determine the prevalence of *C. difficile* in our hospital over the previous two-year period using the CerTest *C. difficile* GDH+ Toxin A+B one-step combo card test, which is a rapid and economical method. Timely reporting of CDI is essential for limiting patient complications and implementing effective preventive measures in the hospital, given that *C. difficile* is a spore producer. This approach also helps to reduce the length of hospital stay for patients and the unnecessary costs involved. The clinical spectrum and risk factors of those patients who tested positive in the test were also reviewed to determine the pathogenic and coloniser status of the organism.

MATERIALS AND METHODS

This retrospective cross-sectional time-bound study analysed the stool samples that arrived at the Microbiology Central Laboratory of Father Muller Medical College Hospital in Mangaluru, Karnataka, India, between January 2021 and January 2023. The study was approved by the Institutional Ethics Committee (IEC) (Ref No: FMIEC/CCM/246/2023).

Inclusion criteria: The unformed stool samples from patients of all ages that arrived for *C. difficile* testing were included in the study.

Exclusion criteria: Formed stool samples and samples from patients with incomplete records were excluded from the study.

As the study was retrospective, all the samples received between January 2021 and January 2023 were included based on the inclusion and exclusion criteria. Therefore, determining the sample size was not applicable in this case. A total of 208 stool samples were included for the analysis.

The CerTest *C. difficile* GDH+ Toxin A+B one-step combo card test (CerTest BIOTEC S.L, Zaragoza, Spain) [14] is a coloured chromatographic immunoassay for the simultaneous qualitative detection of *C. difficile* GDH, toxin A, and toxin B in stool samples. The test kit contains three stripes, each for the detection of GDH, toxin A, and toxin B, which are coated with anti-GDH monoclonal antibodies, antitoxin A monoclonal antibodies, and antitoxin B monoclonal antibodies, respectively.

The test was performed according to the manufacturer's instructions. Briefly, the test kit and stool samples were allowed to reach room temperature. Stool samples were thoroughly homogenised. A loopful of the sample was added to the stool collection tube with diluent, which was then shaken for good sample dispersion. Three drops each were added to the sample windows corresponding to GDH, toxin A, and toxin B, respectively. The results were read in 10 minutes. The test was interpreted based on the presence of red-coloured bands in the test lines. If both GDH and toxins A and B were negative, the sample was reported as negative for *C. difficile*. If both GDH and toxins A and B were positive, the sample was interpreted as positive for toxigenic *C. difficile*. If GDH and either toxin A or toxin B were positive, the samples were still reported as positive for toxigenic *C. difficile*. If GDH was negative and toxin A or toxin B was positive, the test was repeated with a fresh sample, and if toxin A or toxin B was positive again, the sample was considered positive for toxigenic *C. difficile*.

The demographic data and clinical details, such as age, sex, severity of diarrhoea, usage of antibiotics or other drugs, other co-morbidities, and length of hospital stay, of those patients who tested positive were extracted from the hospital software, Backbone. The study analysed the predisposing factors to CDI, such as usage of antibiotics, immunosuppressive agents and PPIs, old age, presence of underlying diseases, previous hospital admission, and prolonged hospital stay, based on previous literature [6,15].

STATISTICAL ANALYSIS

The collected data were all categorical in nature and were summarised by frequency and percentage. For comparison of proportions within clinical features and risk factors among patients with toxigenic *C. difficile*, the Chi-square goodness-of-fit test was used. A comparison between patients with toxigenic and non toxigenic *C. difficile* was performed using the z-test to compare proportions. The analysis was conducted using Statistical Package for the Social Sciences (SPSS) version 23.0 software.

RESULTS

A total of 208 samples met our criteria and were included in the study. Out of these, 20 samples (9.62%) tested positive for both GDH and toxin A/B [Table/Fig-1]. Thus, the toxigenic *C. difficile* rate obtained was 9.62%. Out of the 20 patients with toxigenic *C. difficile*, 13 (65%) were females and 7 (35%) were males. Among the 208 samples, 22 samples (10.58%) tested GDH positive and toxin A/B negative [Table/Fig-1]. These are non toxigenic *C. difficile*, considered as colonisers. Out of these, 13 (59.09%) were females and 9 (40.91%) were males. One sample out of the 208 samples was GDH negative and toxin A positive, prompting a repeat test with a fresh sample following kit instructions. The retest showed that the sample was GDH and toxin A negative, resulting in the sample being considered negative for both GDH and toxin.

Clinical features and risk factors were analysed among the patients with toxigenic *C. difficile* using data from the Backbone software. It was observed that PPI use and underlying diseases/conditions were highly significant risk factors (χ^2=32.28, p-value<0.001) among those patients [Table/Fig-2].

Total number of GDH positive and toxin negative samples; n (%)	Total number of GDH positive and toxin A/B positive samples; n (%)		Total number of GDH negative and toxin negative samples; n (%)	Total number of samples
22 (10.58)	20 (9.62)	Toxin A only positive: 10 (50)	166 (79.81)	208
		Toxin B only positive: 6 (30)		
		Toxin A & B positive: 4 (20)		

[Table/Fig-1]: Positivity for *C. difficile* using the CerTest Clostridium difficile GDH+ Toxin A+B one-step combo card test.

Clinical features and risk factors	Patients with toxigenic *C. difficile* (20) n (%)
Abdominal pain	4 (20)
Fever	6 (30)
Vomiting	3 (15)
Antibiotic associated diarrhoea	9 (45)
Pseudomembranous colitis (PMC)	9 (45)
Death	3 (15)
Age ≥65 years	9 (45)
Prolonged hospital stay	4 (20)
Proton Pump Inhibitor (PPI) use	14 (70)
Previous hospital admission	1 (5)
Chemotherapy	0
Patients with underlying diseases/ conditions	16 (80)

[Table/Fig-2]: Clinical features and risk factors associated with patients with toxigenic *C. difficile*.
Statistical analysis: By Chi-square goodness of fit test; χ^2=32.28, p-value<0.001, Highly significant

Underlying diseases/conditions exhibited by patients with toxigenic *C. difficile* have been presented in [Table/Fig-3].

Underlying diseases/conditions	n (%)
Kidney diseases	4 (20)
Diabetes mellitus	9 (45)
Heart diseases	5 (25)
Hypertension	6 (30)
Hypothyroidism	2 (10)
Carcinoma	2 (10)
Anaemia	2 (10)
Sepsis	7 (35)
Septic shock	1 (5)
Jaundice	1 (5)
Pleural effusion	2 (10)
Urinary tract infection	2 (10)
Hypoalbuminaemia	2 (10)
Chronic obstructive pulmonary disease	1 (5)
Parkinson's disease	1 (5)
Burns	1 (5)
Retroperitoneal abscess	1 (5)
COVID	1 (5)
Pulmonary tuberculosis	1 (5)
Bronchopneumonia	1 (5)
Oesophageal candidiasis	1 (5)
Fracture	1 (5)
Ascites	1 (5)
Human immunodeficiency virus positive	1 (5)
Fibroid uterus	1 (5)
Subarachnoid haemorrhage	1 (5)
Hepatitis B positive	2 (10)

[Table/Fig-3]: Underlying diseases/conditions exhibited by patients with toxigenic *C. difficile*.

Patients with non toxigenic *C. difficile* exhibited the following underlying diseases/conditions: kidney diseases (3), diabetes mellitus (9), heart diseases (4), hypertension (6), hypothyroidism (3), carcinoma (4), anaemia (2), sepsis (7), septic shock (3), Urinary Tract Infection (UTI) (1), pneumonia (1), encephalopathy (1), pulmonary oedema (1), stroke (3), fracture (1), hypoglycaemia (1), hypokalemia (1), rheumatoid arthritis (1), proctitis (1), external haemorrhoids (1), COVID-19 (1), hepatitis B positive (1), and bronchiectasis (1).

A comparison of the clinical features and risk factors among the patients with toxigenic and non toxigenic *C. difficile* was done, noting that AAD was statistically significant (p-value=0.030) among toxigenic patients compared to the latter [Table/Fig-4].

Clinical features and risk factors	Patients with toxigenic *C. difficile* (20), n (%)	Patients with non toxigenic *C. difficile* (22), n (%)	p-value
Abdominal pain	4 (20)	3 (13.64)	0.584, NS
Fever	6 (30)	4 (18.18)	0.375, NS
Vomiting	3 (15)	4 (18.18)	0.784, NS
Antibiotic associated diarrhoea	9 (45)	3 (13.64)	0.030, Sig
PMC	9 (45)	4 (18.18)	0.068, NS
Death	3 (15)	6 (27.27)	0.339, NS
Age ≥65 years	9 (45)	8 (36.36)	0.572, NS
Prolonged hospital stay	4 (20)	5 (22.73)	0.831, NS
PPI use	14 (70)	15 (68.18)	0.899, NS
Previous hospital admission	1 (5)	2 (9.09)	0.610, NS
Chemotherapy	0	2 (9.09)	0.175, NS
Patients with underlying diseases/conditions	16 (80)	15 (68.18)	0.389, NS

[Table/Fig-4]: Comparison of clinical features and risk factors observed in patients with toxigenic and non toxigenic *C.diffcile*.
Statistical analysis: By z-test to compare proportions

DISCUSSION

C. difficile, an anaerobic, spore-forming Gram-positive bacillus, causes a variety of clinical syndromes such as diarrhoea, colitis, PMC, megacolon, and death [1]. Previous literature features varied predisposing factors for CDI, such as antibiotic usage, prolonged hospital stay, usage of PPIs, old age, and underlying diseases [6]. According to a study, patients with a recent hospitalisation history (within three months) were more susceptible to developing CDI [15]. The organism gained significant importance due to the mutant hypervirulent strain, North American Pulse-field gel electrophoresis type 1/restriction endonuclease analysis BI/ribotype 027 (NAP1/BI/027), which caused outbreaks in the USA, Europe, Canada, and many parts of the world [1].

Different researchers have reported diverse prevalence/incidence rates globally based on a variety of factors such as patient selection criteria, the laboratory test or the algorithm employed for detection of the pathogen, and geographical disparities. A prevalence of 7.9% was reported by a Qatar-based study, and a study from Jordan observed a prevalence of 12.65% [16,17]. A cross-sectional study by Djuikoue IC et al., from five hospitals in Cameroon showed a higher prevalence of 27.33% [18]. Prevalence ranging from 4.32 to 15.7% has been reported by a few Indian studies in recent years [19-22].

In the present study, the toxigenic *C. difficile* obtained was 9.62%, which was in agreement with Rajabally N et al., who reported a prevalence of 9.2%, and Lall S et al., who published a prevalence of 8.67% [23,24]. In the present study, the prevalence was lower than the rates observed in two other Indian studies by Justin S and Antony B, and Kannambath R et al., which were 12.79% and 18.67%, respectively, and from a Malaysian study, which was 13% [25-27]. A retrospective study from South India by Sukhwani KS et al., reported a CDI prevalence of 16% [14]. However, present study demonstrated a higher prevalence than other Indian studies which revealed a prevalence of 4.9% and 4% [28,29].

PPI use and underlying diseases/conditions were the risk factors found to be highly significant statistically (χ^2=32.28, p-value<0.001) among the patients with toxigenic *C. difficile*. The present results were in complete agreement with the study by Aukes L et al., which demonstrated PPI use and presence of co-morbidities as predisposing factors for CDI [30]. Other researchers also reported similar findings in their analysis [15,31].

Advanced age and prolonged hospital stay are well-established risk factors for CDI, as observed by many researchers [25,30]. In the present study, these two factors were not statistically significant among the patients with toxigenic *C. difficile*. But present study was consistent with another study in which no association was found between age and CDI [32]. Similarly, the association between elderly age and a greater occurrence of CDI could not be established in a study by Yu H et al., [15].

The present study also compared the clinical features and risk factors among patients who harboured toxigenic and non toxigenic *C. difficile*, and it was observed that AAD was statistically significant (p-value=0.030, Sig) among the former when compared to the latter. *C. difficile* has been significantly associated with AAD according to many investigators. Gogate A et al., described *C. difficile* as an important pathogen for AAD in children aged 5-12 years [33]. A Chinese study reported the isolation of toxigenic *C. difficile* from 63 out of 206 (30.6%) patients with AAD [34]. This study did not find any statistically significant difference in terms of clinical features like abdominal pain, fever, and vomiting among the two groups. PMC was detected among 9 out of 20 (45%) patients with toxigenic *C. difficile* and in four out of 22 (18.18%) patients with non toxigenic *C. difficile*, but the difference was not statistically significant.

Among the 20 patients with toxigenic *C. difficile*, 13 (65%) were females and 7 (35%) were males in the present study. Thus, according to the findings, the prevalence of toxigenic *C. difficile* was not gender-biased (p-value= 0.263, not significant), which was in agreement with another study in which the authors did not observe any significant difference in CDI positivity based on gender [31]. No statistically significant difference was observed among gender in terms of non toxigenic *C. difficile* as well (p-value=0.523, NS). On the contrary, higher CDI incidence was noted among females by a few workers in the USA, whereas male gender was proposed as a risk factor for CDI by Lessa FC et al., and Khanafer N et al., [35,36]. A few authors noticed that the risk of in-hospital death was three times higher in patients with CDI compared with the control group, while no such association between CDI patients and death was detected in this study (p-value=0.339, NS) [15].

Abundant literature available until now presented either a single method or an algorithm for the detection of the organism which was suitable for a particular setting. According to a review, molecular methods led to overdiagnosis, and detection of toxins in stool was essential to prove the causative role of the pathogen [8]. An immunochromatography assay, the C.Diff quik chek complete, exhibited high sensitivity and specificity for toxin detection, with high positive and negative predictive values [37]. This test was rapid as well as effortless to perform compared to PCR [37].

A similar detection method was employed, the CerTest *Clostridium difficile* GDH+ Toxin A+B one-step combo card test, for the simultaneous qualitative detection of *C. difficile* GDH, Toxin A, and Toxin B in stool samples. This technique was feasible, with a shorter turnaround time and could detect toxin A and B separately in the samples. The kit claimed high sensitivity and specificity to detect GDH, toxin A, and toxin B of *C. difficile*. The sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) were 100.0% for GDH detection. The sensitivity, specificity, PPV, NPV were 96.6%, 100.0%, 100.0%, and 99.0%, respectively for toxin

A detection. For toxin B detection, the sensitivity, specificity, PPV, and NPV were 100.0%, 98.9%, 97.3%, and 100.0%, respectively. Sukhwani KS et al., had reported the usage of the same test kit for the detection of *C. difficile* from Southern India [14].

Recently, many authors have described the employment of test kits which simultaneously detected GDH and toxins of *C. difficile* either alone or as part of a two-step algorithm [11,26]. Qutub M et al., concluded that effective reporting of 89% of the samples was possible by Quik Chek Complete Enzyme Immunoassay (EIA) [12]. In another analysis, about half (45.7%) of the samples positive for *C. difficile* needed no further confirmation when *C. difficile* quik chek complete assay was used as a screening test [13]. Yazisiz H et al., confirmed that *C. difficile* quik chek complete EIA was efficient as a standalone test due to its high sensitivity and specificity [10].

Among 208 samples included in the present study, 20 samples (9.62%) were both GDH and toxin A/B positive, thus accounting for the prevalence of 9.62% for toxigenic *C. difficile*. Among these samples, 10 samples (50%) were only toxin A positive, six samples (30%) were only toxin B positive, and four samples (20%) were positive for both toxin A and toxin B. Djuikoue IC et al., reported the presence of toxin A in 37.8% of samples and toxin B in 7.3% from Cameroon using the same test kit [18]. Out of 208 samples, 22 samples (10.58%) were GDH positive and toxin A/B negative, whereas another Indian study reported a positivity rate of 16% for GDH [29].

The kit used in the study facilitated prompt reporting of the specimens, thereby helping to minimise the complications of CDI and thus implement preventive measures. Only samples with inconclusive results from this test need to be retested by PCR, which would reduce the cost and infrastructure required for testing all the specimens by molecular methods.

Limitation(s)

No retest of the samples with non toxigenic *C. difficile* was done using a NAAT method, as it was not routinely used for *C. difficile* detection in the laboratory. Consequently, *C. difficile* present in those samples was determined to be non toxigenic or colonisers without further testing, which was a major drawback of the study.

CONCLUSION(S)

C. difficile is predominantly responsible for AAD and PMC, along with a variety of clinical syndromes ranging from an asymptomatic carrier state to megacolon and death. The combo card test used in the study was simple to perform, had a shorter turnaround time, and could detect toxins A and B separately in the samples. In the present study, toxigenic *C. difficile* obtained using this kit was 9.62%. The impact of CDI on hospitals and patients could be minimised by timely reporting of specimens on a regular basis, achievable by employing an assay that detects both GDH and toxins of the organism. This assay does not require expertise as in the case of PCR or is less time-consuming compared to anaerobic culture. Indeterminate results should be retested by PCR if facilities are available. In addition to routine laboratory testing, factors such as antimicrobial stewardship, hand hygiene, and constant vigilance would significantly contribute to the reduction in CDI rates. These measures are crucial for reducing the prolonged hospital stay of patients and for avoiding unnecessary costs associated with CDI.

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