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Original Article

Microbiology Section

Prevalence and Outcome of *Clostridioides difficile* Infection in a Tertiary Care Hospital in Kerala, India

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ABSTRACT

Introduction: Increasing incidence of *Clostridioides difficile* Infections (CDI) has been associated with antibiotic usage. The most commonly used test for its diagnosis is Glutamate Dehydrogenase (GDH) enzyme along with toxin assay. Implementation of strict infection control practices are required to curb rising prevalence and outbreaks.

Aim: The aim of this study was to find the prevalence of CDI in the hospital and to analyse the risk factors leading to its development.

Materials and Methods: Stool samples were screened for the presence of GDH enzyme and toxins A and B. Samples positive in both tests were considered as CDI cases. For risk factor analysis, 50 cases were randomly selected (25 toxin positive cases vs 25 GDH negative cases).

Results: A total of 493 stool samples were analysed over two years of which 18.5% were toxin A and B positive. The prevalence of CDI decreased from 0.1% to 0.06% in the second year. The GDH values of all toxin positive cases were much higher than that of the cut off of 0.1 IU/mL, while among the non-toxigenic strains, high GDH value was noted in 49.7%. The significant risk factors for CDI in the present study were old age, diabetes, hypertension, renal diseases and cerebrovascular accidents.

Conclusion: The most common test used for the diagnosis of CDI is GDH enzyme along with toxin assay. CDI is significantly related to risk factors.

Keywords: Glutamate dehydrogenase, Infection control, Toxins

INTRODUCTION

Clostridioides difficile previously known as *Clostridium difficile* Infection (CDI) is the most commonly recognised cause of hospital acquired infectious diarrhoea [1]. Since 2000, increasing incidence of CDI has been noted worldwide, consequent to the emergence of hyper virulent *C. difficile* strains known as NAP1/BI/027 [1-3].

Increasing use of antibiotics particularly those with broad spectrum activity has been singularly pointed out as the most important risk factor for CDI [4]. Following prolonged antibiotic therapy, there is suppression of the normal gut flora. *C. difficile* which are resistant to these antibiotics will now colonise and proliferate in the gastro intestinal tract [2]. In a study conducted by Privitera G et al., in University of Leece, Italy, it was found that even a single dose of antibiotic given for surgical prophylaxis can increase the risk of *C. difficile* colonization [5]. These strains can be either toxin producing or non-toxigenic. The toxin producing strains are associated with a spectrum of disease ranging from self-limiting diarrhoea to more severe conditions like pseudomembranous colitis [2].

Proton pump inhibitors have been found to be an independent risk factor for CDI, particularly in those with no previous antibiotic exposure [6]. It has been suggested that this could be due to its role in acid suppression, which is an important physical barrier for *Clostridioides difficile* spores [3]. The other susceptible populations include older age groups, those with immunodeficiency states, inflammatory bowel disease, kidney disease and prolonged hospital stay [2,3].

The severity of CDI can be graded as mild, moderate and severe based on the frequency of diarrhoea per day, presence or absence of fever, abdominal symptoms and associated complications like lower gastrointestinal bleed, colon perforation, sepsis, renal dysfunction and acidosis. Leucocytosis and hypoalbuminemia also increases the severity of CDI [7]. Infectious Diseases Society of America (IDSA) recommends a multistep algorithm for the diagnosis of CDI. The first step is a screening test for the detection of GDH. This should be done in combination with toxin detection assay, as GDH is present in both toxigenic and non-toxigenic strains. Even though, Nucleic Acid Amplification Test (NAAT) is very sensitive, it should not be used as a solitary test, but done in combination with toxin detection and with or without GDH. While it is the recommended method, application of NAAT in low income countries like ours would mean increased financial burden to the patient [3].

The aim of the present study was to find the prevalence of CDI in Pushpagiri Institute of Medical Sciences and Research Centre and to analyse the risk factors leading to its development.

MATERIALS AND METHODS

This retrospective cross-sectional study was conducted in the Department of Microbiology, in a 1200 bedded tertiary care hospital in central Kerala, India. Institutional ethical clearance (PIMSRC/E1/388A/50/2016) was obtained and data regarding the patient demographics, risk factors and clinical presentation was collected for a period of two years, from July 2015 to June 2017 in a detailed proforma. A general consent was taken and stool samples of all symptomatic in-patients that were requested for CDI detection were included in the study. All formed stool samples as well as those who had diarrhoea due to other known causes like taking laxatives were excluded from this study. These samples were screened for the presence of *C. difficile* GDH enzyme and toxin A and B by ELFA, VIDAS (BioMerieux). The positive cut-off value for *C. difficile* GDH assay was > 0.10 IU/mL and for *C. difficile* toxin A and B assay was >0.37 IU/mL. Those samples positive in both tests were reported as toxigenic *C. difficile* and were immediately reported to the treating physician and infection control department. Samples that were positive for GDH enzyme but negative for toxins were reported as non-toxigenic *C. difficile* and PCR for the detection of CD toxin gene was suggested as an additional test to confirm diagnosis. The infection control department was alerted. Those that were negative for GDH enzyme and CD toxin were reported as negative for *C. difficile*.

For risk factor analysis, random 50 samples were studied (25 from toxigenic CDI group and 25 from CDI negative cases). Sample size was calculated based on hospital prevalence with a confidence

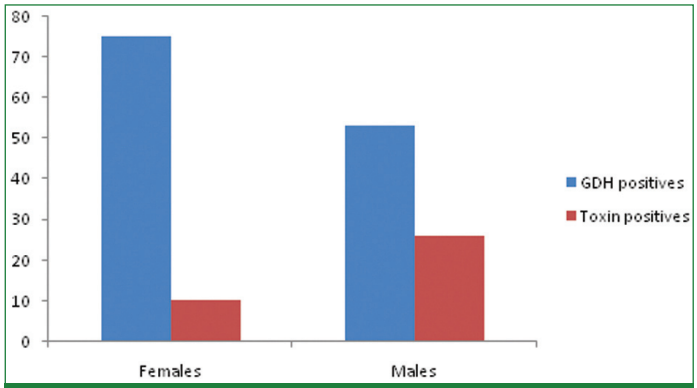
interval of 90%. The antibiotics that these CDI cases were exposed to were also analysed.

Results were collected and organised using Microsoft Excel and statistical significance (p-value) was computed by Chi-square test. A p<0.01 was considered statistically significant.

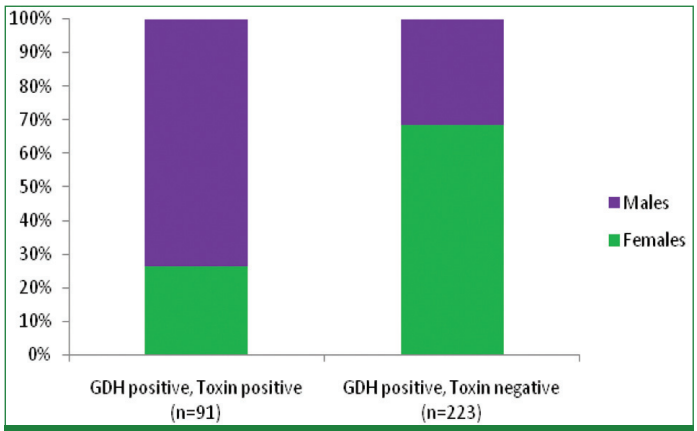
RESULTS

A total of 493 stool samples were received during a period of two years from July 2015 to June 2017. GDH enzyme was positive in 63.7% (314/493). Among these 29% (91/314) were positive for toxin too. Thus, the presence of disease due to *C. difficile* infection was attributed to 18.5% (91/493) of the total samples received.

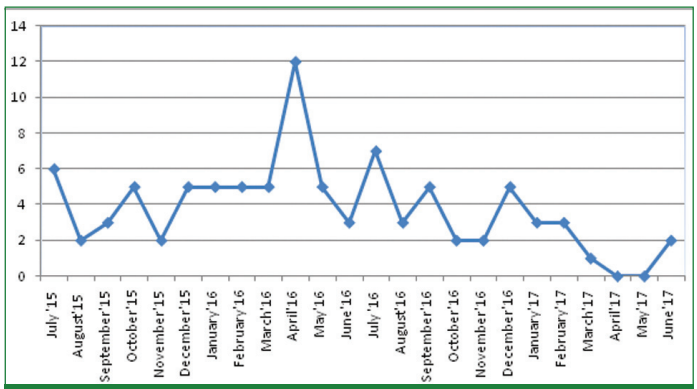
A total of 235 females and 258 males were enrolled in the study. Gender wise distribution of GDH and toxin positivity is depicted in [Table/Fig-1]. Gender wise distribution of GDH positivity among toxigenic and non-toxigenic strains is shown in [Table/Fig-2]. The monthly distribution of toxin positives across two years is depicted in [Table/Fig-3].



[Table/Fig-1]: Gender wise distribution of GDH and toxin positivity (in percentages).



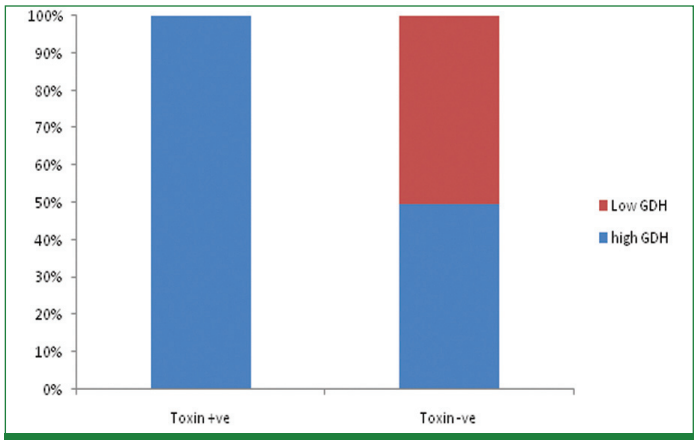
[Table/Fig-2]: Gender wise distribution of GDH positivity among toxigenic and non-toxigenic strains (in percentages).



[Table/Fig-3]: Month wise distribution of CDI cases (n=91) from July 2015 to June 2017.

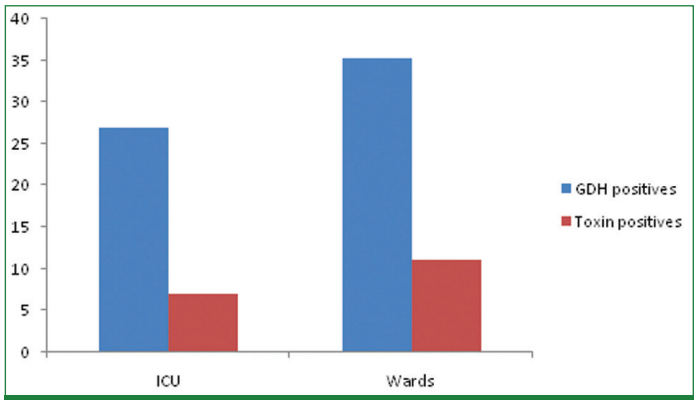
A total of 58 toxigenic *C. difficile* cases were seen in the first year and 33 cases in the second year. Hence, the prevalence per 100 admissions was calculated to be 0.1% in the first year and 0.06% in the second year.

The GDH values of all strains were noted and a comparison of GDH values among toxigenic and non-toxigenic strains were analysed [Table/Fig-4].



[Table/Fig-4]: Comparison of high GDH value (≥ 3.5 IU/ml) among toxigenic and non-toxigenic strains (in percentages).

Of the 493 stool samples received, 286 were from various ICUs and 207 from wards. Among ICU patients, 26.9% were positive for GDH and 7% positive for toxin production, whereas in the wards, GDH was positive in 35.3% and 11% for toxin production [Table/Fig-5].



[Table/Fig-5]: Comparison of GDH positives and toxin positives *C. difficile* (in percentages) among ICUs and wards.

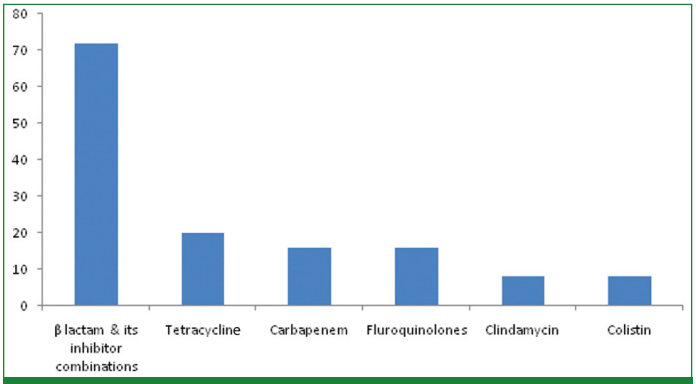
The GDH positive patients developed diarrhoea on an average of 22 days after hospital admission whereas among the GDH negative patients, the diarrhoea was seen after 10 days of hospital admission. On an average, 55.1% of GDH positives developed diarrhoea after 20 days of hospital admission, whereas it was only 15.1% (27 patients) among the GDH negatives (p-value- <0.001).

Various factors like age, gender, underlying co-morbidities, length of hospital stay, exposure to various antibiotics, surgery etc., were analysed for determining the predisposing factors to CDI [Table/Fig-6]. To calculate the significance, a total of 50 cases (25 toxin

Predisposing factors	Positive cases n=25	Negative cases n=25	p-value
Age above 60	84% (21)	52% (13)	<0.01
Males	56% (14)	52% (13)	0.67
Diabetes mellitus	80% (20)	16% (4)	<0.01
Hypertension	72% (18)	56% (14)	0.02
*ICU stay	60% (15)	48% (12)	0.08
†Antibiotic exposure	76% (19)	40% (10)	<0.01
Old CVA	32% (8)	8% (2)	<0.01
Surgery	28% (7)	32% (8)	0.53
Renal disease	36% (9)	8% (2)	<0.01

[Table/Fig-6]: Risk factor analysis for *C. difficile* toxin positivity.
*ICU: Intensive Care Unit; †CVA: Cerebrovascular Accidents

positive cases vs 25 GDH negative cases) were randomly selected. The antibiotics that these CDI cases were exposed to maximum are depicted in [Table/Fig-7]. The mortality rate among established CDI cases was 21%.



[Table/Fig-7]: Trends of antibiotic exposure (in percentages) among toxin positives (n=25).

DISCUSSION

The main virulence factor for CDI is the production of exotoxins A and B by *C. difficile*. These toxins act by causing colonic dysfunction. With the increasing incidence of NAP1/BI/027 in this 20th century, not only population at high risk is affected, but increasing incidence is also seen in those people who are otherwise healthy with little or no exposure to health-care settings or antimicrobial use [8].

In the present study 63.7% of the samples received were positive for GDH enzyme and 18.5% of the total samples were toxin positive. This is much higher when compared to various western studies were the recovery rates were as low as 8-10% [9]. Even in a study conducted in Kochi, Kerala, in 2014 only 8.8% of the total samples were toxin positive [2].

In the present study, we got more of GDH positivity from females 177/235 (75.3%), but toxin producing strains were far less 24/235 (10.2%). However, in males, even though GDH positivity was comparatively less 137/258 (53.1%), half of it were toxin producing (p-value<0.001) [Table/Fig-1]. Thus, in the present study colonization with toxin producing strains were seen more in males 67/91 (73.6%) when compared to females 24/91 (26.4%). Females were more likely to be colonized with non-toxigenic strain {153/223 (68.6%) in females vs. 70/223 (31.4%) in males [Table/Fig-2]}. This contrasts with data from CDC, where the incidence of CDI was found to be greater among females [5].

In a study conducted in a hospital in Minneapolis, the annual incidence of CDI over 10 years ranged from 0.4 to 1% [10]. The rates in the present hospital are much lower and ranges from 0.06 to 0.1%. This can be comparable with the statistics in a hospital in Paris, where the prevalence ranged from 0.07 to 0.12 per 100 admissions [8]. During outbreaks, the incidence has been shown to explosively increase to as high as 32% [11]. The prevalence in the first year of the present study was 0.1% which was higher than 0.06% in the second period of the present study. The comparatively increased prevalence in the first year could be due to an outbreak of CDI we experienced for a brief period. Once the infection is introduced by an index patient, *C. difficile* can explosively disseminate in wards where there is clustering of susceptible population [8].

Patients with CDI can heavily shed spores into the environment and these spores can survive for several months on various surfaces. In a study conducted by McFarland LV et al., in 1989 on acquisition of CDI, they found that the contamination was significantly higher in rooms of patients with diarrhoea compared to asymptomatic carriers (49 vs. 29%). Even in those rooms where *Clostridioides difficile* patients were not admitted, contamination rate was 8%, showing that spores of *C. difficile* can persist, despite routine cleaning of rooms [12].

Transmission of *C. difficile* is thought to occur via the faecal oral route. During hospital outbreaks, transmission is probably by the hands of the medical personnel. Transmission can also occur by direct contact with contaminated surfaces. The ease at which these transmissions occurs could be due to the resistance of the spores to the most commonly used disinfectants and antiseptics, the antibiotic pressure in hospitalised patients and the compromised immune status of the patients [8].

In the present study, we found out that there was an increased reporting of cases of CDI from the laboratory in April 2016 [Table/Fig-3]. A preliminary outbreak investigation was done by the hospital infection control team. The index case was identified and it was noticed that this case resulted in a propagative outbreak. This case was admitted in ICU and later shifted to ward. But following this, other patients assigned the same bed got infected and transmitted to other patients subsequently. Infection control measures implemented with immediate effect resulted in bringing down the further transmission of infection. The importance of contact-based precautions and washing hands with soap and water was emphasised by the infection control team. All the infected patients were segregated to a single ward. Wherever possible, antibiotic use was restricted. Following discharge of patients, terminal cleaning of room with soap and water followed by disinfection of the rooms with sporicidal agents helped in reducing the incidence.

The GDH values of all the toxin positives were ≥ 3.5 IU/mL, which was much higher than that of the cut off of 0.10 IU/mL, while among the non-toxigenic strains, high GDH value was noted in 49.7% (111/223) of cases (p-value - <0.01) [Table/ Fig-4]. Hence, we suggest to use GDH as a sole test initially for screening and to do toxin assay only for those with high GDH values. Highly sensitive tests like NAAT can be reserved for those cases which are negative for toxin but have a very high GDH.

In a study conducted by Brown E et al., Intensive Care Unit (ICU) admissions and increased duration of hospital stay were associated with an increased exposure to *C. difficile* [13]. But in the present study [Table/Fig-5], there was no significant difference of CDI acquisition among ICU and ward patients.

The risk of CDI among CVA patients was probably associated with their prolonged hospital stay [Table/ Fig-6]. We found that, the GDH and toxin positives were more common in those patients who had diarrhoea approximately after three weeks of hospital admission. In those patients who had diarrhoea before that GDH/toxin was seldom detected.

In the present study, the incidence of CDI was higher among elderly people as well as in chronic renal patients. This could be mainly because of their ineffectiveness in mounting a specific serum IgG immune response when first exposed to the toxins, as well as the delay taken by the normal gut flora to re-establish themselves [14,15].

In the present study, 80% of CDI cases were seen among those with diabetes mellitus. These patients are prone to increased frequency of infections which can affect any organ or system [16]. As a result they are highly likely to receive many courses of antibiotics over a prolonged period. Antibiotics are well-known risk factors for acquisition of CDI. They act by disrupting the normal intestinal flora, allowing *C. difficile*, from both endogenous and exogenous sources, to colonize and proliferate there. If the colonized strain turns out to be toxigenic, these toxins can increase the intestinal fluid secretions, resulting in inflammation and mucosal damage, leading to diarrhoea [8].

In the present study, 52% of them were on multiple antibiotics. Parenteral formulation of β-lactam and its inhibitor combinations ranked highest among the antibiotics predisposing to CDI (18/25-72%) [Table/Fig-7]. Among those on oral formulations of broad spectrum antibiotics, only 28% (7/25) developed CDI. In a study done in UK by Bignardi GE, the antibiotics most frequently implicated in CDI were orally available formulations of broad spectrum antibiotics [17]. It has been postulated that overgrowth of *C. difficile* from persistent spores occurs at a faster rate than the restoration of the normal colonic flora.

Antibiotic exposure need not necessarily be a prerequisite for developing CDI. In CDC's morbidity and mortality weekly report dated 2nd December 2005, 24% of patients reported no exposure to antimicrobial agents within three months before the onset of CDI [8].

The first step in the treatment of CDI is to stop all inciting antibiotics in use [3]. Metronidazole is the drug of choice for mild to moderate CDI, whereas vancomycin is recommended for severe cases [1]. The latest IDSA guidelines of 2017, recommends the use of either vancomycin or fidaxomicin irrespective of the severity of disease [3]. Only in those settings where the accesses to these drugs are limited, they suggest the use of metronidazole in non-severe cases. For those with multiple recurrences of CDI, faecal microbiota transplantation can be considered. In case of fulminant CDI, subtotal colectomy with preservation of rectum may improve the outcome.

Prevention of transmission of CDI mainly relies on effective implementation of infection control practices. Following an outbreak, isolation measures should be followed by strict contact barrier precautions. Devices and equipment used on these patients should be thoroughly cleaned with a sporicidal disinfectant. Terminal cleaning of room allotted to these patients should also be done [3].

Hospitalization is the single most important factor that predisposes to CDI acquisition. It brings together an environment contaminated with clostridial spores, a place where the selective pressure on antibiotic usage is very high and susceptible population. Testing for both GDH and toxin helps in detecting more cases. Initial GDH screening only, and toxin detection only for high GDH values can be cost effective. The prompt diagnosis of CDI is the first step in controlling C. difficile dissemination. Laboratory plays a prime role in first recognizing evidence-based outbreaks. Together with the infection control team, prompt implementation of contact barrier precautions will help in containing its further spread.

The most important element in transmission-based precaution is hand washing with soap and water. Audits on effective hand washing technique and disinfection of rooms using fluorescent powder and UV light should be conducted on a regular basis. Use of antibiotics should be constantly monitored with emphasis on adherence to hospital antibiotic policy. Routine rectal screening of patients for colistin, carbapenems and vancomycin resistant organisms using screen agar can give us a clue to degree of antibiotic exposure

CONCLUSION

CDI is commonly diagnosed by GDH enzyme along with toxin assay. Samples positive for both GDH enzyme and toxin are considered as CDI cases. The GDH value was much higher in toxin positive cases as compare to toxin negative cases. Risk factors such as old age, diabetes, hypertension, renal diseases and cerebrovascular accidents, gender, length of hospital stay, exposure to various antibiotics, surgery etc., play an important role in CDI.

The most common test used for the diagnosis of CDI is GDH enzyme along with toxin assay. CDI is significantly related to risk factor. Risk factors play an important role in CDI.

LIMITATION

The most important limitation of the present study was we did not have NAAT based analysis of sample. Those samples that were GDH positive and toxin negative could not be analysed further for the presence of Tox B gene by PCR.

Sensitive tests like NAAT in combination with GDH and toxin assay is currently recommended by IDSA. In a low income country like ours, we can consider subjecting only those samples that have got a high GDH value but are toxin negatives for NAAT.

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