ORIGINAL ARTICLE

Healthcare-associated Diarrhea due to *Clostridioides difficile* in Patients Attending a Tertiary Care Teaching Hospital of North India

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Received on: 15 March 2024; *Accepted on:* 15 April 2024; *Published on:* 12 June 2024

Abstract

Background: Healthcare-associated diarrhea (HCAD) is diarrhea that develops at least after 3 days of hospitalization, with the most common infectious cause being *Clostridioides difficile*. Over the last decade, there has been a remarkable growth in the frequency and severity of *C. difficile* infection (CDI), making it one of the most prevalent healthcare-associated infections. This study aimed to analyze the prevalence and risk factors associated with CDI.

Materials and methods: A total of 107 patients with clinical suspicion of having HCAD were included in this study. Enzyme-linked fluorescent assay (ELFA) technique-based glutamate dehydrogenase (GDH) and toxin A/B assay were used as per the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) for diagnosing CDI. The details about associated comorbidities were retrieved from the hospital information system records. The presence of risk factors was noted. Risk factors associated with CDI were looked for.

Results: Out of the 107 stool samples received in the microbiology laboratory from patients with suspected HCAD eight (7.6%) samples were positive for CDI. The most frequent comorbidity observed in these patients was renal illness (acute or chronic kidney disease). In this study, a total of 7/8 cases were on multiple antibiotics most common being carbapenem.

Conclusion: The 6-year prevalence of CDI observed in this study was found to be 7.6% risk factors, associated with CDI were kidney disease, diabetes mellitus, malignancy, and exposure to broad-spectrum antibiotics.

Keywords: *Clostridioides difficile*, Diarrhea, Healthcare-associated infection, Glutamate dehydrogenase.

Euroasian Journal of Hepato-Gastroenterology (2024): 10.5005/jp-journals-10018-1429

Introduction

Diarrhea is frequently seen in hospitalized patients, and it is associated with high morbidity and low quality of life. While infectious and non-infectious etiologies of healthcare-associated diarrhea (HCAD) exist, the latter continues to prevail.[1] The World Health Organization defines diarrhea as passing three or more liquid stools each day or more frequently than is typical for an individual when healthy, whereas HCAD is diarrhea that acquired after 3 days of hospitalization, with *Clostridioides difficile* being the most common infectious cause.[2,3]

Clostridioides difficile is a spore-producing Gram-positive bacteria that grow in an anaerobic environment and thrive in the human gut as well as in the environment.[4] Over the last decade there has been a remarkable growth in the frequency and severity of *C. difficile* infection (CDI) due to the emerging hypervirulent *C. difficile* BI/NAP1/027 strain, making it one of the most prevalent healthcare-associated infections.[5] This illness spreads by fecal-oral transmission, and the most important factors for acquiring this disease include the use of broad-spectrum antibiotics, older age, chemotherapeutic and immunosuppressive medicines, and stay at a healthcare facility.[6] It has a varied presentation, ranging from a silent carrier state and mild diarrhea to severe colitis that causes mortality.[7]

Clostridioides difficile colonizes 5% of adults and 15–70% of young children with the rate of colonization being several times higher in those in hospitals.[8] Almost all antibiotics, including vancomycin and metronidazole, which are used to treat CDI, have been linked to the emergence of the disease because of disruption of gut microbiota which leads to *C. difficile* colonization.[9] Studies done by Leffler et al. and Hensgens et al. have shown that broad-spectrum penicillin, third or higher cephalosporins, lincosamides, and quinolones have a significantly greater likelihood of triggering CDI and in patients receiving antimicrobial medication, the risk of developing CDI is eight to ten times higher for the first four weeks.[10,11]

The enzyme immunoassays (EIA) detecting *C. difficile* glutamate dehydrogenase (GDH) and toxin have a short processing time of less than 3 hours, a sensitivity of 75–85%, and a specificity of 95–100%.[12] These tests are most frequently utilized in all laboratories owing to their low cost and simplicity of use.[13] European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines suggest

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How to cite this article: Raj N, Agarwal J, Singh V, *et al*. Healthcare-associated Diarrhea due to *Clostridioides difficile* in Patients Attending a Tertiary Care Teaching Hospital of North India. Euroasian J Hepato-Gastroenterol 2024;14(1):60–64.

Source of support: Nil

Conflict of interest: None

that at least two tests should be used to confirm CDI, combining two tests into a single algorithm is the best approach for confirming CDI.[14] An assay with a strong negative predictive value targeting GDH is used initially followed by a second assay having a strong positive predictive value targeting toxin A or B in the two test algorithms.[15]

In developing countries such as India, there is very limited data on CDI and its prevalence, which may be attributed to a lack of knowledge about its prevention and control, resource-limited laboratories lacking testing facilities for CDI, and insufficient surveillance methods. The present study was conducted at a tertiary care academic hospital of Northern India to determine the prevalence, contributing factors, and comorbidities linked to CDI.

Materials and Methods

This study was a retrospective study conducted at the microbiology laboratory of Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, Uttar Pradesh, India from January 2017 to December 2022, 107 individuals were admitted for at least 3 days in a healthcare facility and with a clinical suspicion of HCAD were included in this study.

According to the ESCMID guidelines, a two-test algorithm using VIDAS (bioMérieux, Marcy-l'Étoile, France) *C. difficile* GDH and toxin assay was used for the detection of *C. difficile* toxins A and B detection, respectively, for diagnosis of CDI. Furthermore, the VIDAS *C. difficile* assay consisted of two components, a monoclonal antibody-coated solid-phase receptacle and a reagent strip. Equal volumes of fresh stool sample were mixed with sample diluent and centrifuged at 3000 rpm for 5 minutes and 300 µL of supernatant was pipetted into the sample well of the reagent strip and loaded into the VIDAS system (bioMérieux, Marcy-l'Étoile, France). The testing algorithm is shown in Figure 1. The VIDAS *C. difficile* panel used in this study had a processing time of less than 2 hours. Details about associated co-morbidities were extracted from the medical records. The presence of risk factors was noted. Risk factors associated with CDI were looked for like advanced age, hospital exposure/contact, exposure to antibiotics, immunocompromised state, malignancy, and organ transplantation

The Institutional Ethics Committee of Dr. Ram Manohar Lohia Institute of Medical Sciences approved this study (Approval No. IEC 1/24; dated 27 March 2024). The complete course of this study adhered to the appropriate EQUATOR Network (http://www.equator-network.org/) criteria, in particular, the Strengthening the Report of Observational Studies in Epidemiology (STROBE) recommendations.

Results

Out of 107 stool samples from individuals with clinical suspicion HCAD, 8 (7.6%) were positive for CDI; 4 (50%) were male and 4 (50%) were females; 4 individuals (50%) with CDI were over the age of 40 years. Four patients (50%) received admission to the intensive care unit (ICU), 1 (12.5%) to the medical oncology ward, 1 out of 8 (12.5%) to the neurology ward, and 2 out of 8 (25%) to the nephrology ward. Table 1 shows the characteristics of individuals diagnosed with CDI.

In this study, we observed that the majority of CDI cases had associated comorbidities, the most common comorbidity observed in the CDI patients was renal illness (acute or chronic kidney disease) which was present in 3 (37.5%) cases, followed by diabetes mellitus seen in 2/8 (25%) cases and malignancy in 1/8 (12.5%) case of CDI. The risk factors observed in CDI patients were, broad-spectrum antimicrobial usage and antacids/proton pump inhibitors (PPI) in 7 (87.5%) cases, followed by ICU stay in 4 (50%) cases, use of immunosuppressive and chemotherapeutic drugs in 2 (25%) cases and advanced age (>60 years) in 1 (12.5%) case, gastrointestinal surgery, and transplant in 1 (12.5%) case each.

In this study, a total of 7 (87.5%) cases were on multiple antibiotics which included 3rd generation cephalosporins in 2 (25%) cases, aminoglycosides in 2 (25%) cases, carbapenem group in 4 (50%) cases, metronidazole in 3 (37.5%) cases, tigecycline, fluoroquinolones, teicoplanin, linezolid, colistin, and amphotericin B in one case each.

In this study, 1 (12.5%) case succumbed to death; the other 7 (87.5%) left the healthcare facility with advice for routine follow-up in due course after the improvement in health. Positive outcome was seen in 7 (87.5%) cases which was possible because of timely initiation of proper treatment and prompt diagnosis.

Discussion

Worldwide, hospitalized patients continue to be adversely affected by CDI. On the contrary, this highly resistant anaerobic bacterium has been largely neglected in developing countries such as India; where there is limited epidemiological evidence for evaluating the

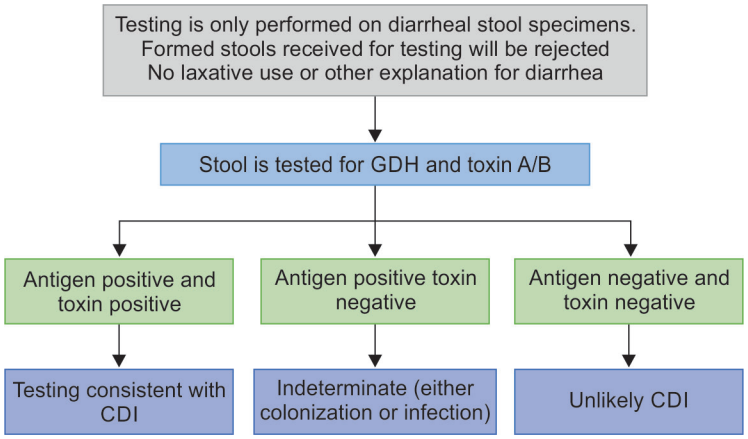

Fig. 1: Testing algorithm for *Clostridioides difficile* infection from the ESCMID
CDI, *Clostridioides difficile* infection; GDH, glutamate dehydrogenase

Table 1: Characteristics of the patients diagnosed with *Clostridioides difficile* infection

S. No.	*Age*	*Sex*	*Ward*	*Diagnosis/comorbidities*	*Medications*
1	2	Female	PICU	Intraventricular hemorrhage with gastroenteritis	IV ceftriaxone, amikacin, and metronidazole
2	13	Male	Neurology	Subacute sclerosing panencephalitis	IV sodium valproate, clonazepam, and isoprinosine
3	25	Male	Medical oncology	Malignant germ cell tumor	IV metronidazole and ciprofloxacin, and chemotherapy with carboplatin Oral pantoprazole
4	30	Female	Nephrology	Chronic kidney disease; renal failure (kidney transplant recipient)	IV Cefoperazone sulbactam, amikacin and oral linezolid Oral pantoprazole and cyclosporin
5	42	Female	ICU	Acute gastroenteritis with acute kidney injury	IV colistin and imipenem IV pantoprazole
6	55	Male	Nephrology	Chronic kidney disease (biopsy proven nodular glomerulosclerosis)	IV amphotericin B and doripenem Oral pantoprazole
7	58	Female	ICU	Acute necrotizing pancreatitis and diabetes mellitus	IV imipenem, teicoplanin, and metronidazole IV pantoprazole
8	90	Male	ICU	Thalamic bleed with septic shock	IV meropenem, teicoplanin, and tigecycline IV pantoprazole

ICU, intensive care unit; IV, intravenous; PICU, pediatric intensive care unit

Table 2: Epidemiologic studies of CDI across India*

S. No.	*Authors*	*Publication year*	*Region*	*Sample size*	*Diagnostic method used*	*Prevalence*
1	Biswas et al.[16]	2023	Maharashtra	1,683	C. diff Quik Chek	3.21%
2	Monaghan et al.[17]	2021	Maharashtra	1,223	C. diff Quik Chek	3%
3	Monaghan et al.[17]	2021	Maharashtra	179	BioFire Multiplex PCR	6.5%
4	Justin and Antony[18]	2019	Karnataka	563	Culture	12.79%
5	Vaishnavi et al.[19]	2019	Chandigarh	2,036	ELISA	22%
6	Singhal et al.[20]	2018	Maharashtra	1,361	NAAT	4.9%
7	Sachu et al.[21]	2018	Kerala	660	ELFA (VIDAS)	8.8%
8	Chaudhry et al.[22]	2017	New Delhi	791	ELISA	6%
9	Kumar et al.[23]	2014	New Delhi	237	Culture	1.2%
10	Present study		Uttar Pradesh	107	ELFA (VIDAS)	7.6%

*Studies available on PubMed/, key words used: *C. difficile* infection, epidemiology, India; ELFA, enzyme-linked fluorescent assay; ELISA, enzyme-linked immunosorbent assay; NAAT, nucleic acid amplification test

burden of CDI. High-income countries invest significant resources in diagnosing CDI and implementing preventive strategies. Given the growing elderly population, enhanced medical access, and widespread usage of antibiotics. The CDI is underreported in developing countries such as India though being highly prevalent due to the lack of testing facilities in majority of hospitals.

Various studies conducted across India have revealed that there is a wide variation in the CDI prevalence ranging from 1.2 to 22% as shown in Table 2.[16–23] In contrast, studies from developed nations such as the UK, the USA, and Germany have reported a lower prevalence ranging from 7.4 to 12.7%.[24–26] In this study, the CDI prevalence in a 6-year-long period was reported to be 7.6%, which is identical to the studies conducted across India by Monaghan et al. and Sachu et al. where they reported a prevalence of 6.5% and 8.8% respectively.[21,27] A higher prevalence was highlighted by Abuderman et al. and Vaishnavi et al., who reported a prevalence of 20.79% and 15.7%, respectively.[19,28] In comparison, a lower prevalence of 1.2% was observed by Kumar et al. in their study.[23]

This variation in prevalence can be attributed to the dissimilar study population characteristics and the variation in the testing algorithm used in different studies; those that used molecular techniques for the detection of CDI had a higher prevalence, as shown by Kannambath et al. who observed an increase from 12 to 18.68% in prevalence by using molecular assays.[29] Although these molecular assays have increased sensitivity they may lead to overdiagnosis of CDI because they only detect the toxin gene which may or may not be functional to produce toxin/disease in patients.

In this study, we observed that the male-to-female ratio of patients with CDI was 1:1, which was similar to that described by Chaudhry et al. in their study.[22] In this study, 4 (50%) CDI cases were from ICU, this finding was in agreement with those by Ingle et al., this could be due to the increased stay in hospital and enteral feeding which have been linked to the development of CDI.[30]

In this study, 7 (87.5%) cases of CDI were on multiple antibiotics and PPI. A maximum number of cases were on carbapenems, similar findings were reported by Kannambath et al., this could be

due to the broad-spectrum activity of carbapenem causing loss of normal aerobic as well as anaerobic flora of the intestine, favoring the growth of *C. difficile*.[29] Tleyjeh et al., in their study, observed a link between the use of PPI and CDI cases similar to the current study, which could be due to the increase in pH due to these drugs facilitating the growth of *C. difficile*.[31]

Various underlying comorbidities have been linked to CDI. In this study, the commonest comorbidity observed was kidney disease, similar observations were made by Kim *et al.* in their study;[32] other comorbidities observed in the CDI cases in the present study (Diabetes mellitus) were also observed by Eliakim–Raz et al.[33]

According to the Centers for Disease Control and Prevention (CDC) Emerging Infections Program surveillance data, case–fatality rates vary from 6 to 30% for CDI.[34] Our study showed favorable outcomes in 7 (87.5%) cases and mortality in only one case of CDI which was due to appropriate treatment and timely diagnosis.

One of our study's main limitations was the small sample size as it was difficult to convince treating physicians to send samples for every incidence of HCAD because many of the cases were resolved on their own.

Conclusion

The six-year prevalence of CDI was found to be 7.6% in this study. Both appropriate diagnostic algorithms and clinical correlation contribute to an accurate diagnosis of CDI. In this study, CDI cases were linked to a number of risk factors, including kidney disease, diabetes mellitus, malignancy , and exposure to broad-spectrum antibiotics. Knowledge and awareness about the contributing factors can help early identification of patients who are more likely to develop CDI.

Authors' Contributions

The full manuscript has been read and approved by all authors. Each listed author fulfills the requirements for authorship, and each author attests that the manuscript represents honest work.

Ethical Approval

This study was approved by the Institutional Ethics Committee of Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, Uttar Pradesh, India (Approval No. IEC 1/24; dated 27 March 2024). The authors followed the applicable EQUATOR Network (http://www.equator-network.org/) guidelines, specifically the STROBE guideline, during the conduct of this research project.

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