

REVIEW ARTICLE

Clostridium difficile Associated Diarrhea: Diagnostic ChallengesNeelam Taneja¹, Kamran Zaman²**Abstract**

Clostridium difficile associated diarrhea (CDAD) is a key indicator for monitoring the success of antibiotic stewardship programs in hospitals. Indiscriminate use of antimicrobials coupled with emergence of hyper virulent 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 the organism and nonavailability of other diagnostic assays due to their high costs. In Indian scenario, we need to generate data on the burden of the disease by conducting robust epidemiological studies. Diagnosis and management of CDAD is a challenge in best of the setups all over the world. There is a need for development of rapid, affordable, point of care gold standard diagnostic assays.

Introduction

In an era of prophylactic/therapeutic antibiotic misuse the incidence of antibiotic-associated diarrhea (AAD) is on the rise.¹ *Clostridium difficile* is the commonest organism implicated in AAD and is a key indicator for monitoring the success of antibiotic stewardship programs in hospitals.² *C. difficile* infection (CDI) has been recognised historically as a nosocomial infection; however, it is now being increasingly reported in community setup, thereby CDI can be either community-associated (CA-CDI) or healthcare facility associated CDI (HCFA-CDI).³ *C. difficile* associated diarrhoea (CDAD) is defined as the passage of three or more unformed stools in 24 hours with detection of *C. difficile* toxins/ positive culture or presence of pseudomembranous colitis features on colonoscopy and/or histopathology.^{3,4}

Epidemiology

A lot of literature is available from developed countries on the burden, epidemiology, risk factor and management of CDAD. According to a point-prevalence survey carried out in Europe, the estimated burden of health-care-associated CDI was 124,000 cases each year and the incidence varied from a range of 4.2–131.8 per

10,000 discharges and 0.6–18.5 per 10,000 patient days.⁵ In the USA, an active surveillance carried out across ten geographic areas showed that *C. difficile* accounted for half a million cases and 29,000 deaths in 2011.⁶ The emergence of new hypervirulent strains (*C. difficile* BI/ NAP1/027) that produce binary toxin in addition to toxins A and B have led to marked increased mortality and morbidity due their high-level fluoroquinolone resistance, efficient sporulation and markedly toxin production.^{7,8} In India, CDI is usually under-diagnosed because of low clinical suspicion, lack of laboratory facilities and other diagnostic challenges. Though there is a paucity of systematic studies, the prevalence of CDAD in India has been shown to be 2 to 4% in patients without diarrhea and 7 to 30% in patients with diarrhea in different hospital based studies.^{9–15} The emerging community onset CDAD is also a major concern and the exact prevalence of which is yet to be estimated in India.

Pathophysiology

Pathogenesis of infection caused by this spore-forming, strict anaerobic,

gram-positive bacillus includes the acquisition and overgrowth of the organism in the gut followed by the release of two enterotoxins: *C. difficile* toxin (CDT) A & B, encoded by *tcdA* and *tcdB* genes located on PaLoc pathogenicity island.¹⁶ The presence of this PaLoc region decides the toxigenicity of *C. difficile*. However, it's important to note that the mere presence of these strains is not synonymous to infection as it is a common colonizer of the neonatal gut (60–70%) and adults (2–3%). *C. difficile* can also be isolated in stools of asymptomatic patients at the time of hospitalization (10–24%) and inpatients (20–40%)^{17–20}. It has been noted that patients colonized with *C. difficile* are less likely to suffer from infection because of the development of protective antibodies.^{21,22} The major risk factors include antibiotic administration, elderly persons (>65 yr. of age), prolonged hospitalization, underlying gastrointestinal malignancy or chronic gastrointestinal disease, previous history of ADAD and immunosuppressive states. The major reason for *C. difficile* outgrowth and toxin production is the antibiotic-induced dysbiosis of the intestinal microbiota.²³ All antibiotic classes have been shown to be associated with CDI, the most frequently cited are clindamycin, cephalosporins and fluoroquinolones.²⁴ Proton pump inhibitors (PPIs) are also considered to increase the risk of CDAD by decreasing the acidic barrier for organisms.^{16,17,25}

Clinical Features

The clinical presentation of CDAD varies from mild, self-limiting diarrhea to fulminant colitis or pseudomembranous colitis. Bowel perforation, toxic megacolon, sepsis, and/or multiple organ dysfunction syndrome are life threatening

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complications associated with CDAD.
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Diagnostic Challenge

Diagnosis and management of CDAD is a challenge in best of the setups all over the world.^{3,27-30} The laboratory testing of CDAD is rapidly evolving and there is no single gold standard diagnostic test.^{31,32} The efficacy of the diagnostic tests is evaluated on the basis of its sensitivity, specificity, turnaround time (TAT), cost, and easy availability of tests which can be combined with clinical features to give best possible results. The diagnostic assays can be divided into those detecting *C. difficile* by toxigenic culture/ its byproducts like glutamate dehydrogenase (GDH), toxins *tcdA* and/or *tcdB*; neutralization of toxin and *C. difficile* nucleic acid amplification using targets like 16S rRNA or the genes encoding GDH and the toxin.^{33,34} The tests that demonstrate the presence of *C. difficile* components are less specific than the toxin detection assays as they cannot differentiate *C. difficile* colonization from active disease.³⁴ There is a paucity of a single good test and the available tests have their limitations and thereby none of them is a gold standard test. However many of diagnostic guidelines consider detecting toxigenic *C. difficile* by culture in stool to be the gold standard assay.^{4,17,28}

Toxigenic Culture (TC)

Culture of *C. difficile* followed by toxin detection is considered gold standard due to its high sensitivity.³⁰ The specificity is low and many studies with high culture positivity have been seen in asymptomatic carriers, infants and patients recently receiving antibiotics.²¹ The limitation of culture is its inability to distinguish between toxin and non-toxin producing strains, therefore a further demonstration of toxin production becomes imperative. Stool specimens are treated with heat or alcohol to increase the culture yield. Stool specimens are cultured on specialized media for 24 to 48 hours anaerobically.³⁵ Detection of toxin can be done by enzymatic immunoassays (EIA), or polymerase chain reaction assay (PCR) or by cell cytotoxicity assay (CCA) after 48 hours of incubation. Isolation in

culture enables susceptibility testing and molecular typing epidemiological surveillance. Despite being the gold standard test, TC has the limitation of being laborious, time consuming, requiring specialized equipment and trained personnel.³³ Diagnostic delays due to prolonged turnaround time have significant implications on treatment initiation decisions and infection control.^{36,37} This limitation can be overcome by rapid testing methods that detect the *C. difficile* toxin and/or GDH usually by EIA/ ELISA and the Nucleic acid amplification test (NAAT) based assays.

Toxin Detection

C. difficile toxin A and B are the major virulence determinants of the disease, as these are responsible for producing symptoms in the patients. Cell cytotoxicity assay (CCA) is considered the gold standard for detecting *C. difficile* toxins.³⁵ It can be performed directly on stool specimens or on a culture isolate. The stool suspension filtrate or supernatant of the culture are inoculated onto a cell line and looked for cytopathic effects like rounding of cells after 24 or 48 hours.³⁴ This test is highly sensitive (94-100%) and specific (99%),^{35,38} but has the limitation of long turnaround time and procedural complexity. EIAs have become the new standard diagnostic test for CDI detection in most laboratories due to its high sensitivity (98%), wide availability and very short TAT.³³ The sensitivity and specificity of EIA for toxin A and/or B has been reported to be variable. Recent studies have shown *C. difficile* toxin A and B EIA has poor sensitivity with a fairly good positive predictive value in contrast to earlier studies.³⁹ Repeat testing following the first positive sample is not recommended during the same diarrheal episode. However, repeated testing after the first negative sample can be done in cases with high clinical suspicion or during the epidemic situation.⁴⁰ However currently, EIA/ ELISA for toxin detection are no longer recommended as stand-alone tests.⁴¹

Glutamate Dehydrogenase (GDH) Assay

GDH is a cell-wall associated component of *C. difficile* existing in both toxigenic and non-toxigenic strains.

Thereby GDH positive specimens subsequently require testing with more specific assays like toxin detection and/or NAAT. Studies have shown that 46% of *C. difficile* isolates in asymptomatic colonizer are non-toxigenic.⁴² However, GDH detection assay has a high negative predictive value approaching 100% with additional features of being rapid, widely availability and affordable. Therefore it is useful as an initial screening step in a multistep approach.^{33,43}

Combined Assay (GDH + Toxin A/B Detection)

The combination of the high specificity of toxin A/B EIAs and high sensitivity of GDH can be clubbed together as a single confirmatory test. Recently, single step combined GDH and toxin (toxin A/B EIA) testing assays have become available and have shown favorable results.⁴⁴ *C. Diff* Quik Chek Complete Assay was evaluated in 174 samples, showing a sensitivity (78.3%) better than toxin A/B EIA (70%) and specificity (100%) with a turnaround time of approximately 50 minutes.⁴⁵ However, few studies have shown that GDH negative and toxin A/B positive samples by this assay, were rarely true positive when evaluated by gold standard assays. The toxin detection component of this combined assay may be redundant.⁴⁶

NAAT Assay

With the advances in developing diagnostic tools for CDI, Nucleic acid amplification testing (NAAT) was introduced. The NAAT assays target the *tcdA* / *tcdB* genes (a positive regulator of toxin A/B production) or the *tcdC* gene (a negative regulator) and the toxigenic *C. difficile* in a single step reaction. Later on, FDA approved different rapid testing PCR and loop-mediated isothermal amplification (LAMP) based assays.^{28,30,47,48} Detection of BI/NAP1/027 strain is possible in few of the NAAT based assays, which in turn may guide the therapy as fidaxomicin is associated with lower recurrence with non-hyper virulent strains only when compared with vancomycin.¹⁶ The assays have shown higher sensitivity (80 to 100%) and specificity (87 to 99%) and have rapid TATs when compared to GDH and EIA. In recent times, NAATs have become a stand-alone approach for the

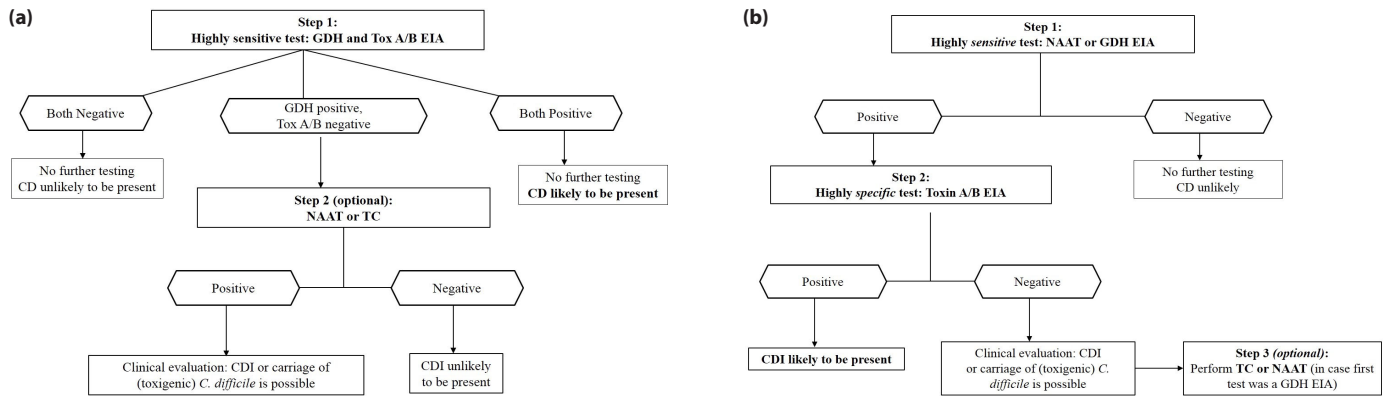


Fig. 1: (a & b) Diagnostic algorithms for diagnosis of CDI (adapted from Crobach MJ et al, ESCMID 2016 guidelines)⁴⁰

Table 1: The recommendation for CDAD diagnosis by the different diagnostic guidelines

Guideline	Screening assay	Confirmation assay
American college of Gastroenterology (ACG) ²⁹	NAAT	GDH / EIA TOX
SHEA / IDSA ³	GDH	CCTA / CC
United Kingdom National Health Service ⁵¹	NAAT / GDH	EIA TOX
European Society of Clinical Microbiology and Infectious Diseases (ESCMID) ⁴⁰	TOX/ GDH	NAAT/ TC

diagnosis of CDI.^{33,49} Early detection of the disease by NAAT has led to a reduction in CDI-related complications. However, the applicability of NAATs have been limited due to its overly sensitive nature, and thereby its use as a stand-alone test has led to the controversial hiked incidence of CDI.³⁶ False positives can occur with NAATs because it detects the genes encoding toxin rather than the active toxins. This led to over-diagnosis of *C. difficile* among colonized patients and increased antibiotic treatment for the colonized or limited infection patients which may not have required treatment.⁵⁰

Diagnostic Algorithm Approach

Due to the non-availability of standard-alone test for CDI diagnosis, the diagnostic algorithms seem to be useful. The important aspect of these algorithms is that the first step includes tests with high sensitivity (high negative predictive value) so that the non-CDI sample can be declared negative without the need of retesting. The diagnostic assays in the second step have high specificity (high positive

predictive value) so that the positive result samples are confirmed as CDI. The different guidelines have differing opinions about the choice of first and second tests, as mentioned in Table 1.

In the first algorithm (Figure 1), first step tests positive and second tests negative suggest a low threshold of CDI / a false negative toxin assay / *C. difficile* carriage. *C. difficile* carriers can be a threat for the nosocomial spread. TC in such cases will be helpful and also important for epidemiological tracing. In the second algorithm, the first test includes GDH and toxin A/B EIA, which can be carried out by a combined assay (e.g *C. diff* Quick Chek Complete, Techlab). Samples with GDH positive and toxin negative need further evaluation by NAAT/ TC. The samples with toxin positive and GDH negative need to be retested.⁴⁰

Conclusions

Indiscriminate use of antimicrobials coupled with the emergence of hyper virulent strains and inadequate infection control measures in hospitals have led to rise in the incidence of CDAD. In India, CDAD is still an under recognized cause of diarrhea, due to lack of clinical suspicion, difficulty in culturing the organism, nonavailability of other diagnostic assays due to their high costs. In Indian scenario, we need to generate data on the burden of the disease by conducting robust epidemiological studies. There is a need for the development of rapid, affordable, point of the care gold standard diagnostic assay. Of the available tests, TC and CCA are restricted to well-equipped laboratories. The NAAT based assays have shown better results, however,

their non-availability in resource poor settings due to high costs are limiting factors. Use of toxin detection and GDH assays will be helpful for establishing the diagnosis, which in turn will help taking infection control measures to prevent the spread. Overuse and repeat and frequent testing should be avoided. Tests should be ordered only in symptomatic patients with clinical risk factors having frank diarrheal stools. The colonization studies should be restricted to patients undergoing bone marrow /solid organ transplants where poor outcomes are expected due to more aggressive nature of the disease following transplantation.⁵² Using a cost-effective, reliable (e.g FDA approved), rapid diagnostic assays with clinical evidence of infection is the best approach. The “clinical cure or follow up” testing is not recommended, as the patients tend to shed spores even after cure and such testing can lead to inappropriate courses of treatment.^{3,32}

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