www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i7.15

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

# Rapid Diagnosis of Toxigenic Clostridium difficile in elderly patients in Tertiary setup

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**Abstract**
**Background:** *Antibiotics cause diarrhoea due to infection by antibiotic resistant pathogens such as Staphylococcus aureus, Clostridium difficile, Candida albicans, Salmonella, C.perfringens type A, Klebsiella oxytoca, etc. CDAD (Clostridium difficile associated disease) is responsible for 15-25% of cases of AAD.[2] C. difficile associated disease (CDAD) is a serious condition with mortality up to 25 per cent in frail elderly people.[1] The average age of those with CDI is nearly 20 years older than the average age of those with hospitalization for other reasons.[3] Laboratory diagnosis is based on culture and toxin detection in fecal specimens. Culture is very sensitive but if carried out without toxin testing, may lead to misdiagnosis of CDAD as it detects even asymptomatic cases.[4] C.DIFF QUIK CHEK COMPLETE® test is a rapid cassette assay that simultaneously detects both glutamate dehydrogenase (GDH) antigen and toxins A & B of C.difficile in fecal specimens. This test when compared to Toxigenic Culture and PCR showed that 88% could be accurately screened as positive or negative.[5]*
**Objective:** *To approximate the frequency of CDI in elderly patients on antibiotics with diarrhoea, in whom other possible causes are ruled out.*
**Methods:** *Stool samples of patients from ICUs and surgical wards who were above 65 years of age with diarrhoea who received antibiotics for 7 days or more were tested after ruling out other possible causes of AAD using Enzyme immuno assay test.*
**Results:** *32 (64%) samples were GDH antigen positive, 8(16%) were positive both for GDH and toxin.*

## Introduction

The consequences of long-term antibiotic administration are diarrhoea, antibiotic resistance, impaired immunity, increased cost of treatment, organ failures and deaths and reduction in beneficial phytoestrogens.

Antibiotic-associated diarrhoea (AAD) is any unexplained diarrhoea associated with the use of an antibiotic. AAD may occur up to 2–3 weeks following cessation of antibiotic therapy rather than during the treatment. Antibiotics cause diarrhoea due to infection by antibiotic resistant pathogens such as *Staphylococcus aureus*,

*Clostridium difficile, Candida albicans, C.perfringens type A, Klebsiella oxytoca*, etc.

CDAD is one among the most important causes of AAD. It is responsible for 15-25% of cases of AAD.[4] *C.difficile* associated disease (CDAD) is a serious condition with mortality up to 25 per cent in frail elderly people .[1]The average age of those with CDI is nearly 20 years older than the average age of those with hospitalization for other reasons.[9]

CDAD is defined as unexplained diarrhoea occurring after 2 hours to within 2 months after antibiotic usage and is often accompanied by fever, abdominal pain and cramps. CDAD is established when toxin is identified in stool, regardless of *C.difficile* isolation from stool.

*C.difficile* is a spore bearing, gram positive bacterium, present in ample amounts in the environment. Spores of *C.difficile* are hard to eradicate as they are resistant to drying and heating, resistant to most of the antiseptic disinfectants. Ability to produce spores explains how the organism being fastidiously anaerobic, can be acquired from environment.[5] Broad spectrum antibiotics such as Clindamycin, Fluoroquinolones, Cephalosporins, etc most often cause CDAD.

*C.difficile* is acquired from the hospital environment. It causes increased burden in terms of cost and health. Risk factors involved in acquisition of CDAD are age above 65 years, treatment with broad spectrum antibiotics, exposure to an infant carrier or infected adult, Tracheostomy, Immunodeficiency, Ryle's tube feeding, antacids, intestinal surgeries, Chemotherapy, Chronic Kidney disease.[3]

*C.difficile* produces toxins that attack the lining of the intestine. Toxins A and B are the primary virulence factors contributing to the pathogenesis of CDAD and the genes responsible for these toxins are TcdA and TcdB. Toxin expression is increased by antibiotic concentration. TcdA is a potent enterotoxin. TcdB is cytotoxic and is generally more potent (~1000 fold) than TcdA. Another toxin is the binary toxin encoded by genes ctdA and ctdB. CDAD may progress to deadly pseudomembranous colitis with rapidly fatal fulminating colitis and megacolon, resulting in death.

Cultivation of the organism is difficult though it is considered as gold standard. Though stool culture has high sensitivity, as the rate of asymptomatic carriage of *C.difficile* among hospitalized patients is high, the specificity for CDAD is low. The disadvantages of the cell culture cytotoxicity neutralisation assay (CCNA) is that it is technically demanding and has a relatively long turnaround time (24–48 h). Although endoscopy is required for the specific diagnosis of PMC, it is an invasive test and it is not sufficient to diagnose all the cases of CDAD. Newer methods like toxin detection by Immunochromatography have a rapid turnaround time and are inexpensive.

The most important aspect of management in CDAD is early identification of the disease and discontinuation of the inciting agent, as it has the potential to emerge as epidemic strain causing hospital outbreaks. Treatment and isolation of infected cases, reduces the cost of treatment incurred by the patient, reduces the morbidity and halts spread of disease in the community.

**Objectives**

To approximate the frequency of CDI in elderly patients on antibiotics with diarrhoea, in whom other possible causes are ruled out.

**Materials and Methods**

The study was carried out at JSS Hospital, Mysore. Unformed stools of 50 patients with diarrhoea were tested who were elderly above the age of 65 years and who took antibiotics for more than 1 week admitted in ICU's and surgical wards, in whom other possible causes of AAD were ruled out.

All samples first tested with TECHLAB C. DIFF QUIK CHEK COMPLETE enzyme immune assay test. The TECHLAB C. DIFF QUIK CHEK COMPLETE test is a rapid membrane enzyme immunoassay for the simultaneous detection of

*Clostridium difficile* glutamate dehydrogenase antigen and toxins A and B in a single reaction well. The control line ("C") is a dotted line that contains anti-horseradish peroxidase (HRP) antibodies. The toxins A and B test line ("Tox") contains antibodies against *C.difficile* toxins A and B. The Conjugate consists of antibodies to glutamate dehydrogenase and antibodies to toxins A and B coupled to horseradish peroxidase.

The test was done as per manufacture's instructions. The sample was added to a tube containing a mixture of Diluent and Conjugate. The diluted sample-conjugate mixture is added to the Sample Well and the device was allowed to incubate at room temperature for 15 minutes. During the incubation, any glutamate dehydrogenase and toxins A and B in the sample bind to the antibody-peroxidase conjugates. The antigen-antibody-conjugate complexes migrate through a filter pad to a membrane where they are captured by the immobilized glutamate dehydrogenase-specific and toxins A and B-specific antibodies in the lines. The Reaction Window was subsequently washed with Wash Buffer, followed by the addition of Substrate. After a 10 minute incubation period, the "Ag" reaction was examined visually for the appearance of a vertical blue line on the "Ag" side of the Reaction Window. A blue line indicates a positive test.

If the "Ag" is positive, then the "Tox" reaction was examined visually for the appearance of a blue line on the "Tox" side of the Reaction Window. A blue line indicates a positive test. A positive "C" reaction, indicated by a vertical dotted blue line under the "C" portion of the Reaction Window, confirmed that the test is working properly and the results are valid.

**Results**

In the present study, a total number of 50 cases of elderly patients of both sexes above 65 years of age, on long term antibiotics and admitted in ICU or Surgical wards in JSS Hospital, Mysore were included.

**Study duration**: January 2015 – June 2016

Out of 50 suspected cases taken, 44 (88%) were male and 6 (12%) were female.

**Table 1**: Gender distribution of patients

| Gender | Number | Percentage% |
|---|---|---|
| Male | 44 | 88% |
| Female | 06 | 12% |
| Total(n) | 50 | 100 |

Gender distribution didn't show any statistical significance

Mean and median were calculated to characterise the patients based on age.

| | Age |
|---|---|
| Mean | 69.12 |
| Median | 69.0 |
| Standard deviation | 4.02 |

**Table 2**: Age distribution of the patients

| Age | Number (%) |
|---|---|
| 65-69 | 29 (58%) |
| 70-80 | 21 (42%) |
| Total | 50 (100%) |

Taking a median of 69 years, 29 (58%) are between 65-69 years and 21(42%) are between 70-80 years.

| | ICT tox | | |
|---|---|---|---|
| | Absent% | Present% | Total |
| age 65- 69 | 21(72.4%) | 8(27.5%) | 29 |
| >70 | 9(42.8%) | 12(57.3%) | 21 |
| Total | 30 | 20 | 50 |

Hence we see that in the age group of above 70 years, percentage of positivity is 12(57%) and negative 9(42%) compared to 8(27.5%) positive and 21(72.4%) in 65-69 years age group. Chi-Square value of 0.035, hence statistically significant. (p = 0.05)

Mean and median were calculated to characterise the patients based on length of hospital stay.

| | Duration of hospital stay |
|---|---|
| Mean | 10.56 |
| Median | 10.5 |
| Standard deviation | 4.28 |

**Table 3**: Duration of Hospital stay

Taking the obtained median of 10 days into consideration, 25(50%) of patients had duration of hospital stay for 4-10 days and another 25(50%) stayed for 11-21 days.

| Duration of hospital stay | Number (%) |
|---|---|
| 4-10 days | 25 (50%) |
| 11-21 days | 25 (50%) |
| Total | 50 (100%) |

| | | ICT tox | | |
|---|---|---|---|---|
| | | absent | present | Total |
| hospital duration | 1-10 days | 19(76%) | 6(24%) | 25 |
| | 11-25 | 11(44%) | 14(56%) | 25 |
| Total | | 30 | 20 | 50 |

In 14 (56%) of patients who stayed for more than 10 days ICT toxin was positive and negative in 11 (44%) compared to 6 (24%) toxin positive and 19 (76%) negative in patients stayed for 10days or lesser.

**Table 4**: ICU stay

| ICU stay | Number (%) |
|---|---|
| Present | 25 (50%) |
| Absent | 25 (50%) |
| Total | 50 (100%) |

25(50%) of patients were in the ICU while they were in hospital and another 25(50%) did not.

| | | ICT tox | | |
|---|---|---|---|---|
| | | absent | present | Total |
| ICU | no | 20(80%) | 5(20%) | 25 |
| | yes | 10(40%) | 15(60%) | 25 |
| Total | | 30 | 20 | 50 |

Percentage of positivity was 15 (60%) and negativity was 10 (40%) for those in ICU compared to 5 (20%) positive and negative 20 (80%) for those not having stayed in ICU.

Chi-Square value of 0.004, hence statistically significant. (p=0.05).

**Table 5**: On Ryle's tube

| Ryle's tube | Number (%) |
|---|---|
| Present | 25 (50%) |
| Absent | 25 (50%) |
| Total | 50 (100%) |

25(50%) of patients had ryle's tube and another 25(50%) did not.

| | | ICT tox | | |
|---|---|---|---|---|
| | | absent | present | Total |
| ryles tube | no | 19(76%) | 6(24%) | 25 |
| | yes | 11(44%) | 14(56%) | 25 |
| Total | | 30 | 20 | 50 |

Percentage of positivity was 14 (56%) and negativity was 11 (44%) for those on Ryle's tube compared to 6 (24%) positive and negative 19 (24%) for those not on ryles tube.

Chi-Square of 0.042, hence, statistically significant for ICT.

**Table 6**: Diabetes

| Diabetic | Number (%) |
|---|---|
| yes | 35 (70%) |
| no | 15 (30%) |
| Total | 50 (100%) |

35(70%) of patients were diabetic and another 15(30%) were not.

| | | ICT tox | | |
|---|---|---|---|---|
| | | absent | present | Total |
| diabetic | no | 13(86.6%) | 2(13.3%) | 15 |
| | yes | 17(48.5%) | 18(51.4%) | 35 |
| Total | | 30 | 20 | 50 |

As seen above positivity was more in diabetics 18 (51.4%), absent in 17 (48.5%) compared to 2 (13.3%) and absent in13 (86.6%)Chi-Square of 0.012, hence, statistically significant for ICT.

**Table 7**: Fever

| Fever | Number (%) |
|---|---|
| present | 35 (70%) |
| absent | 15 (30%) |
| Total | 50 (100%) |

35(70%) of patients had fever and another 15(30%) did not.

**Table 8**: On Antacids

| Antacids | Number (%) |
|---|---|
| taken | 33(66%) |
| Not taken | 17 (34%) |
| Total | 50 (100%) |

33 (66%) had taken antacids, 17 (34% ) did not.

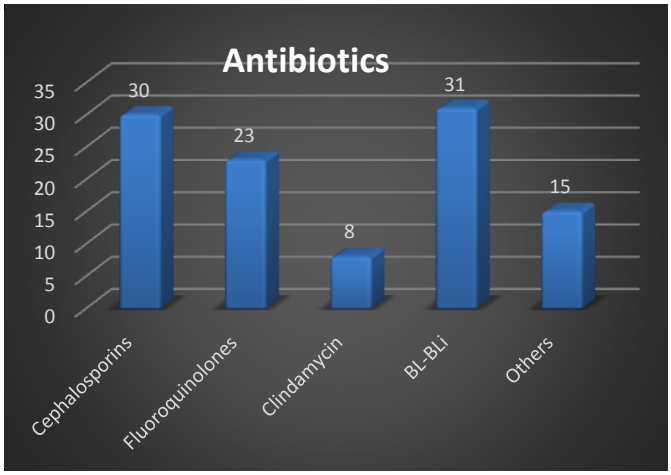
No statistical significance if had fever or taken antacids.

**Table 9 :** Leukocytosis

| Leukocytosis | Number (%) |
|---|---|
| present | 34 (68%) |
| absent | 16 (32%) |
| Total | 50 (100%) |

34 (68%) had leukocytosis, 16(32%) did not. No statistical significance was seen.

**Table 10:** Exposure to Antibiotics.




Among 50 patients 30 (60%) were on cephalosporins, 23 (46%) had taken Fluoroquinolones, 8(16%) took Clindamycin, 31(62%) took Betalactam-Betalactam Inhibitor combinations and 15 (30%) took other groups of antibiotics.

**Table 11**: GDH antigen positives.

| GDH | Number (%) |
|---|---|
| positive | 32 (64%) |
| negative | 18 (36%) |
| Total | 50 (100%) |

32 (64%) were GDH positive, 18(36%) were negative for GDH.

**Table 12**: ICT toxin positives

| ICT toxin | Number (%) |
|---|---|
| positive | 8 (16%) |
| negative | 42 (84%) |
| Total | 50 (100%) |

8 (16%) were positive for ICT toxin, 42 (84%) were negative.

**Discussion**

In India, the studies on CDAD not extensive due to difficulty in culturing the pathogen. In Indian studies prevalence of CDI was seen as follows, Dhawan B et al. (15%)[8], Gogate A et al. (18%)[9] and Joshy L et al. (12.1%).[10] Considering other geographical regions, Heimesaat MM et al and Jamal W et al.have found the prevalence rates of CDI to be 11.4%[11] and 8%[12] respectively.

Gender distribution didn't show any statistical significance in our study. Similarly no difference in sex in a retrospective study of 10,154 hospitalizations for patients and 241 cases detection of *C.difficile* toxin in stool samples.[13] But few studies say females seem to be at increased risk of CDI,[14] In our study only 6 (12%) were females which could be reason for lack of female preponderance.

Taking a median of 69 years, 29 (58%) are between 65-69 years and 21(42%) are between 70-80 years. We observed that in the age group of above 70 years, percentage of positivity is 12 (57%) and negative 9 (42%) compared to 8 (27.5%) positive and 21(72.4%) in 65-69 years age group. Chi-Square value of 0.035, hence statistically significant. (p = 0.05).

Cohort studies found that CDAD patients were about 10 years older than patients without CDAD[15]. In 101,796 patients, the average age for patients with CDAD was 65.4±16.9 years compared with 56.5±19.9 years for patients without CDAD (*P*<.001). Among 535 patients in Jerusalem, patients positive for *C difficile* toxin had a mean age of 76±20 years compared with 66±26 years in toxin-negative patients (*P*<.001).[16]

In our study, in 14 (56%) of patients who stayed for more than 10 days in hospital, ICT toxin was positive and negative in 11 (44%) compared to 6 (24%) toxin positive and 19 (76%) negative in patients stayed for 10days or lesser. 25 (100%) of patients who stayed for more than 10 days PCR toxin was positive and negative in 0 (0%) compared to 19 (76%) toxin positive and 6 (24%) negative in patients who stayed for 10 days or lesser.

A retrospective cohort study found that patients with CDAD had spent more time in the hospital, a mean of 19 days compared with 8 days for patients without diarrhoea (*P*<.001).[17] A prospective cohort study reported that the mean length of stay was 15 days (range=8.0-26.0) for

CDAD patients compared with 5 days (range=3.0-8.0) for patients without CDAD (*P*<.001).[18]

15 (60%) were positive and negativity was 10 (40%) for those in ICU compared to 5 (20%) positive and negative 20 (80%) for those not having stayed in ICU. Chi-Square value of 0.004, hence statistically significant. (p=0.05). In a study done by Patel et al, a greater proportion of patients were diagnosed with severe CDI in the ICU.[19] In the ICU, patients are generally more unwell and likely have other reasons for higher WBC and creatinine levels which are again CDI risk factors. As a result, it is difficult to determine whether the location itself or the patient's current clinical state played a role in the severity of CDI.[20]

Percentage of positivity was 14 (56%) and negativity was 11 (44%) for those on Ryle's tube compared to 6 (24%) positive and negative 19 (24%) for those not on ryles tube. Chi-Square of 0.042, hence statistically significant for ICT.

As seen in results, positivity was more in diabetics 18 (51.4%), absent in 17 (48.5%) compared to 2 (13.3%) and absent in13 (86.6%)Chi-Square of 0.012, hence, statistically significant for ICT.

In our study, no statistical significance with antacids. In a study, it was concluded that PPI was not an independant risk factor for CDI acquisition.[21] Shivashankar and colleagues, in a population-based study, found that individuals with exposure to gastric acid suppressants were 1.8 times more likely to have severe-complicated CDI.[22]

In our study among 50 patients 30 (60%) were on cephalosporins, 23 (46%) had taken Fluoroquinolones, 8(16%) took Clindamycin, 31(62%) took Betalactam-Betalactam Inhibitor combinations and 15 (30%) took other groups of antibiotics, but no statistical ssignificance was seen.

In a study by Buchler et al, Thirty patients (32.6%) had one or more complications: 20 patients (21.7%) suffered from infectious complications. Penicillins (i.e., amoxicillin, amoxicillin/clavulanate and piperacillin/ tazobactam) were the most commonly prescribed antibiotics (i.e., 38.5% took penicillins with an average duration of 4.6 days (median 4 days), followed by cephalosporins (29.5%) and quinolones (26.9%) with an average duration of 6.8 and 4.9 days, respectively (median 6 and 2 days, respectively). Further antimicrobials were carbapenems (15.4%), glycopeptides (10.3%), nitromidazoles (5.1%), lincosamides (2.6%), aminoglycosides (3.8%) and others (11.5%). Within the hospitalized patients, 25 were given one class of antibiotics while 34 patients were given more than one class before diagnosis of CDI.[18]

by combining both GDH and toxin testing into a single device (C. Diff Quik Chek Complete; TechLab improved sensitivity was seen with the newer immunochromatographic (ICT) membrane version of the test [6] it is very rare to have a GDH-negative, EIA toxin-positive result for a true-positive sample.

Screening with EIA is cost-effective as it reduce the expense of testing concordant positive (i.e., GDH positive, toxin positive)[7]

In a study done by Lyerly DM et al, they evaluated 1,152 specimens. They found that the positive and negative predictive values were 100 and 98.6%, respectively, and the correlation of the TOX A/B TEST with toxigenic culture was 98.8%.[23]

**Conclusion**

Distinguishing between asymptomatic colonization and symptomatic infection with *C.difficile* makes the diagnosis of CDAD complex. Current detection methods for *Clostridium difficile* infection (CDI) can be time-consuming and have variable sensitivities.

Combined approach of infection control and strict antibiotic policies greatly reduce the burden of CDAD. Reducing the use of injectable cephalosporins leads to significant reduction in CDAD cases as they avoid precipitation of CDAD. Use of narrow-spectrum antibiotics should be encouraged.