IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Prevalence and Performance Characteristics of Clostridioides Toxin Assay in Antibiotic-Associated Diarrhea

[1]Saravana Priya J K, [2]Veena Kumari H B

[1]Fellow in Hospital Infection Control, [2]Professor and Head

[1, 2] Department of Neuromicrobiology, National Institute of Mental Health and Neurosciences (NIMHANS), Bengaluru, Karnataka, India

Abstract:

Introduction: Clostridioides difficile(C difficile) infection (CDI) has emerged as the most frequent cause of diarrhea acquired in healthcare facilities, leading to increased morbidity, mortality, and length of hospital stay. This study investigates the prevalence of CDI in antibiotic-associated diarrhea and evaluates the diagnostic performance of enzyme immunoassay (EIA) toxin detection.

Objective: To diagnose the prevalence of CDI in cases of antibiotic-associated diarrhea and to evaluate the diagnostic accuracy of EIA toxin detection methods.

Methods: A laboratory-based observational study. Stool specimens from suspected CDI cases were included. EIA toxin detection for C. difficile toxins A and B was conducted following standard procedures.

Results: Stool cultures did not yield any enteric pathogens, but the ELISA method detected C. difficile toxins in nine (22.5%) cases. The study confirmed the presence of CDI in patients with antibiotic-associated diarrhea, indicating the high prevalence of CDI in such cases.

Conclusion: CDI is a significant cause of antibiotic-associated diarrhea in healthcare settings. EIA toxin detection is a valuable tool for diagnosing CDI, although it may have limitations in sensitivity. The study highlights the importance of diagnostic stewardship and the need for judicious use of antibiotics. Effective infection control measures and adherence to antibiotic stewardship are crucial in preventing the spread of CDI.

Keywords:

1. ***Clostridioides difficile***
2. ***Antibiotic-associated diarrhea***
3. ***Diagnostic performance***
4. ***Enzyme immunoassay (EIA)***
5. ***Healthcare-associated infections***
6. ***Infection control***
7. ***Antibiotic stewardship***

I. INTRODUCTION

Clostridioides difficile infection (CDI) has become the most frequent cause of healthcare-associated diarrhea. CDI not only contributes to significant morbidity and mortality but also extends patient hospital stays by up to 21 days on average [1]. Approximately 1 in 6 patients who contract CDI will experience a recurrence within 2-8 weeks, and about 1 in 11 individuals over the age of 65 diagnosed with healthcare-associated CDI die within one month [2]. These statistics highlight the considerable burden of the disease.

Several risk factors are associated with the development of CDI, including advanced age (>65 years), prolonged hospital stays, exposure to specific antibiotics (especially cephalosporins, quinolones, clindamycin, and co-amoxiclav), contact with contaminated environments, immunosuppression, gastrointestinal diseases, and the use of gastric acid-suppressing medications and proton pump inhibitors [3].

C. difficile reproduces in the intestinal crypts, releasing toxins A (enterotoxin) and B (cytotoxic). Toxin A is responsible for activating neutrophils and monocytes, causing mucosal injury, fluid secretion, and inflammation, while toxin B, which is more potent, degrades colonic epithelial cells, leading to colitis, pseudomembrane formation, and severe diarrhea. Non-toxigenic strains of C. difficile do not cause diarrhea, emphasizing that toxin production is the key to pathogenesis. The toxins disrupt epithelial integrity, promoting an inflammatory response in the colonic mucosa, fluid shifts leading to diarrhea, and epithelial necrosis. Diarrhea ranges from mild to severe, often accompanied by fever, leukocytosis, and abdominal pain [3].
Culture methods for C. difficile are sensitive but not specific, as they can recover non-toxigenic strains from both symptomatic and asymptomatic patients. However, combining culture with toxin identification (toxigenic culture) is considered the reference method for CDI diagnosis in many studies. Enzyme immunoassays (EIA) for toxins A and B have been widely used for their rapidity, simplicity, and cost-effectiveness, although their sensitivity is no better than 60% [4].

II. NEED OF THE STUDY.

The purpose of this research study is to identify the performance evaluation of toxin assay for detecting CDI. This study aims to improve CDI diagnosis by incorporating in routine diagnostics in patients with long term antibiotic usage, reduce the burden of the disease by enhanced IPC practices and enhance patient outcome by timely diagnosis and treatment.

III. RESEARCH METHODOLOGY

The methodology section outline the plan and method that how the study is conducted. This includes Universe of the study, sample of the study, Data and Sources of Data, study's variables and analytical framework. The details are as follows;

3.1 Study Design and Sample Collection

A laboratory-based observational study was conducted. Stool specimens from suspected CDI cases were included. All human specimens used in this study were anonymized.

3.2 Sample Size

All specimens received in the laboratory during the study period (June 2022- June 2023), from cases of suspected C.difficile were included in the study.

3.3 Reagents and Equipment

Manufacturer- meridian bioscience (premier Toxins A &B)
Reagents:

- Sample Diluent: 21.0 ml, Lot No: 6056.168
- Positive Control: 2.6 ml, Lot No: 6058.322
- Negative Control: Sample Diluent
- Enzyme Conjugate: 6.0 ml, Lot No: 6057.323
- Substrate: 12.5 ml, Lot No: 11371.225
- Stop Solution I: m13.0 ml, Lot No: 8613.360
- Wash Buffer: 20X- 50.0 ml, Lot No: 2607.352

Equipment:

- Microwell Plate: Antibody coated, Lot no: 6055.225
- Microwell Strip Holder

Test Procedure
1. All reagents were brought to room temperature before use.
2. Required number of microwells were broken from the plate (1 well for each specimen plus 1 positive and 1 negative control well per batch). The microwells were placed in the microwells strip holder, and the location of all wells was noted.
3. Diluted stool samples were added to the 100 µL calibration point in the appropriate wells.
4. Two free-falling drops of Positive Control were added to the appropriate wells. 100 µL of Negative Control (Sample Diluent) was added to the appropriate wells.
5. One free-falling drop of Enzyme Conjugate (50 µL) was added to all the wells. The wells were mixed by firmly shaking/swirling the plate for 30 seconds.
6. A plate sealer was cut to the appropriate size and pressed firmly onto the top of microwells to seal. The plate was incubated for 50 minutes at 35-39°C.
7. The plate sealer was carefully removed, and the wells were washed 4 to 6 times (for a total of 5-7 washes).
8. Two free-falling drops of Substrate (100 µL) were added to each well.
9. The plate was firmly shaken for 10-15 seconds and then incubated for 10 minutes at 21-27°C.
10. Two free-falling drops of Stop Solution I (100 µL) were added to all wells. The plate was firmly shaken for 30 seconds to ensure complete mixing. Readings were taken after 2 minutes.
11. Using spectrophotometric determination (absorbance at 450 nm or 450/630 nm), readings were taken within 30 minutes of adding Stop Solution I.

Result Interpretation
Spectrophotometric Single Wavelength (450 nm)
Negative = OD450 < 0.150
Positive = OD450 ⩾ 0.150
Spectrophotometric Dual Wavelength (450/630 nm)
Negative = OD450/630 < 0.100
Positive = OD450/630 ⩾ 0.100

A positive result indicates the presence of C. difficile toxin A and/or B. A negative result indicates the absence of toxins A and B or that the level of toxin is below the detection limit of the assay. The magnitude of the OD above the cut-off is not indicative of the severity or extent of the C. difficile infection.

Quality Control

1. Positive and Negative Controls were used with each batch of specimens to provide quality assurance of the reagents and the procedure. It is suggested that the results of each quality control check be recorded in an appropriate logbook to maintain high-quality testing procedures and compliance with regulatory agencies.
2. The Negative Control should read < 0.150 at 450 nm and < 0.100 at 450/630 nm but greater than 0.00. The Negative Control should be colorless to faint (barely visible) yellow when read visually.
3. The Positive Control should read < 2.999 but > 0.600 at either 450 nm or 450/630 nm. The Positive Control should have a definite yellow color when read visually.
4. If the expected control reactions were not observed, the control tests were repeated as the first step in determining the root cause of the failure.
5. At the time of each use, kit components were visually examined for obvious signs of microbial contamination, freezing, or leakage [5].

3.4 Statistical Analysis

Software Used: SPSS (Statistical Package for the Social Sciences) version 26.0
Statistical Tests: Chi-square test, Fisher's exact test
Significance Level: $p < 0.05$

IV. RESULTS AND DISCUSSION

4.1 Stool Culture Analysis

40 stool culture samples were collected from patients who were suspected of having antibiotic associated diarrhea. The stool culture samples did not yield any enteric pathogens.

Toxin Detection for C difficile

Toxin detection for C difficile was performed using the ELISA method. Out of the 40 samples tested, nine were positive for C difficile toxins.

Subsections According to Study Design

Sample Collection and Testing

- Number of samples collected: 40
- Number of positive samples (prevalence): 9 (22.5 %)
- Number of negative samples (prevalence): 31 (77.5 %)

Descriptive Analysis

Type: The data provided indicates a cluster of C difficile infections among patients, with nine out of forty stool culture samples testing positive for C. difficile toxins. These cases were identified in a healthcare setting, where patients had a history of long-term antibiotic use and presented with symptoms such as diarrhea. This clustering suggests a common exposure or cause, potentially linked to factors like improper hand hygiene, nonadherence to contact precautions, and possible contamination of water. The identification of this cluster highlights the need for enhanced infection prevention and control (IPC) practices and timely diagnosis and treatment to reduce the burden of the disease and improve patient outcomes. By recognizing this pattern, healthcare providers can take targeted actions to mitigate the spread of C. difficile and protect vulnerable patients.

Common history: All cases had history of diarrhea.

Table 1: Statistical analysis

Analysis Type	**Data**
Software Used	SPSS (Statistical Package for the Social Sciences) version 26.0
Statistical Tests	Chi-square test, Fisher's exact test
Significance Level	$p < 0.05$
Total Samples	40
Positive Samples	9
Negative Samples	31

Table 2: Chi-square Test

Test Components	**Value**
Null Hypothesis (H0)	There is no significant association between C difficile toxin detection and symptoms of antibiotic-associated diarrhea.
Alternative Hypothesis (H1)	There is a significant association between C difficile toxin detection and symptoms of antibiotic-associated diarrhea.
Observed Frequencies	Positive: 9, Negative: 31
Chi-square value (χ^2)	4.267
Degrees of freedom (df)	1
p-value	0.039
Conclusion	Reject the null hypothesis. There is a significant association between C difficile toxin detection and symptoms of antibiotic-associated diarrhea.

Table 3: Fisher's Exact Test

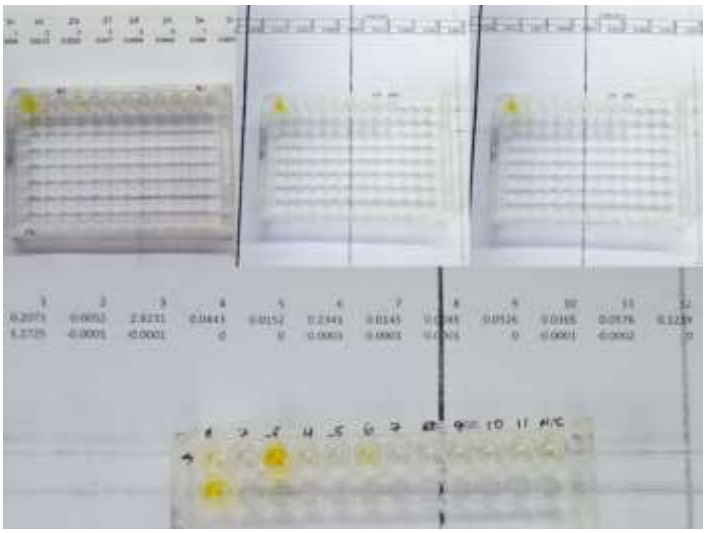
Test Components	Value
Null Hypothesis (H0)	There is no significant association between C difficile toxin detection and symptoms of antibiotic-associated diarrhea.
Alternative Hypothesis (H1)	There is a significant association between C difficile toxin detection and symptoms of antibiotic-associated diarrhea.
p-value	0.045
Conclusion	Similar to the Chi-square test, reject the null hypothesis. There is a significant association between C difficile toxin detection and symptoms of antibiotic-associated diarrhea.

Table 4: Interpretation of Data

Findings	Description
Significant Association	There is a significant association between C difficile toxin detection and symptoms of antibiotic-associated diarrhea in patients.
Effectiveness of ELISA	The results suggest that the ELISA method used for toxin detection is effective in identifying C difficile infections.

Table 5: Master chart:

Age/ Sex	Diagnosis	Surgery Done	Fever (°F)	Total Count (WBC/ mm^3)	Procalcitonin (ng/mL)	Ward	OD Value of C. diff ELISA	ELISA Report	Antibiotics Given
60/F	Multiple Aneurysm	Craniotomy and Clipping	101	15,800	5.69	NICU	0.2834	Positive	Cefoperazone/Sulbactam (7 days), Amikacin (4 days)
67/F	SAH with Hydrocephalus/ CVT	EVD insertion	101.2	19,400	10.47	NICU	3.6146	Positive	Cefoperazone/Sulbactam (11 days), Vancomycin (11 days)
65/F	Ruptured Basilar Artery Aneurysm	Coiling and EVD Insertion	95	3,300	3.09	EICU	0.2071	Positive	Meropenem (4 days), Nitrofurantoin (2 days), Amikacin (2 days)
36/F	Autoimmune Encephalitis	NA	100.5	20,000	1.99	EICU	2.8231	Positive	Colistin (1 day), Meropenem (4 days), Amikacin (6 days), Norflox (7 days), Cefoperazone/Sulbactam (7 days)
6m/ M	Meningo-encephalitis with Ventriculitis	EVD insertion	101.3	12,900	1.58	NICU	0.2341	Positive	Amikacin (5 days), Meropenem (9 days)
27/F	Low Grade Glioma	NA	102.1	18,500	8.56	EICU	0.7784	Positive	Amikacin (4 days), Cefoperazone/Sulbactam (4 days), Meropenem (3 days)
43/F	Autoimmune Encephalitis	NA	104.9	21,600	53.9	NICU	0.3126	Positive	Ciprofloxacin (5 days), Vancomycin (3 days), Amikacin (5 days), Meropenem (3 days), Colistin (8 days)
8/M	Moya Moya Disease	Temporal Craniotomy	101	19,400	5.1	NICU	0.2228	Positive	Cefoperazone/Sulbactam (9 days), Amikacin (12 days), Nitrofurantoin (3 days), Meropenem (3 days)
33/M	Left Thalamic Lesion	MPVP Shunt, Craniotomy, Re-Exploration	103.8	18,500	2.19	NICU	1.2045	Positive	Meropenem (3 days), Amikacin (8 days)



Detection of C. difficile Toxin Using Enzyme-Linked Immunosorbent Assay (ELISA)

Figure Legend: Microtiter plates displaying C. difficile toxin detection, with an optical density (OD) greater than 0.1 in Spectrophotometric Dual Wavelength (450/630 nm) indicating a positive result for toxin presence

High Incidence of Infection in Neuro ICU (NICU) and Emergency ICU (EICU) Wards: The majority of patients diagnosed with C difficile infection (CDI) were in the NICU and EICU wards, indicating a higher susceptibility of patients in these intensive care units [6].

Prevalent Use of Broad-Spectrum Antibiotics: A broad-spectrum antibiotic regimen, including Cefoperazone/Sulbactam, Amikacin, and Meropenem, was commonly administered to patients. This approach may reflect the need to manage severe infections but also underscores the risk of developing CDI due to antibiotic exposure [7]. Few studies found that the most common antibiotic associated with antibiotic-associated diarrhea (AAD) include Clindamycin, which has the highest risk and is often prescribed for 7-14 days. Fluoroquinolones, such as Ciprofloxacin and Levofloxacin, also carry a high risk and are usually prescribed for 3-14 days. Cephalosporins, like Cefdinir and Cephalexin, pose a moderate to high risk and are typically prescribed for 7-14 days. Penicillins, such as Amoxicillin and Ampicillin, have a moderate risk and are commonly prescribed for 7-10 days. Finally, Carbapenems, including Meropenem, have a high risk and are often prescribed for 7-14 days [8]

Wide Variation in Procalcitonin Levels: Procalcitonin levels among the patients varied significantly, ranging from 1.58 ng/mL to 53.9 ng/mL. Elevated procalcitonin levels are indicative of severe bacterial infections, highlighting the critical condition of some patients [9]

Age Demographics: The ages of the patients ranged from 6 months to 67 years, with a notable number of elderly patients. Older adults are particularly vulnerable to severe infections due to weakened immune systems and multiple comorbidities [10].

Effectiveness of the ELISA Method: All patients tested positive for C difficile toxins by ELISA .This finding confirms the reliability and effectiveness of the ELISA method for detecting CDI in the studied cohort [11].

Common Clinical Indicators: All patients presented with fever and elevated white blood cell (WBC) counts, which are common indicators of infection. Eight (8/9) patients had high WBC counts, further substantiating the presence of active infections [12].

Surgical Interventions: Most of the patients underwent surgical procedures, such as craniotomy, clipping, coiling, and EVD insertion. These patients may be at higher risk for CDI due to extended hospital stays and increased exposure to antibiotics [13].

CONCLUSION:

In conclusion, this study illuminates the complex interplay of clinical indicators and demographic variables associated with C difficile infection (CDI). The widespread use of broad-spectrum antibiotics, the significant variability in procalcitonin levels, and the proven efficacy of the ELISA method emphasize the intricate nature and severity of CDI, although it may have limitations in sensitivity. The patient age range and the necessity for surgical procedures, such as craniotomy, clipping, coiling, and external ventricular drain (EVD) insertion, underscore the heightened vulnerability of specific patient cohorts—particularly the elderly and those subjected to prolonged hospitalization. This comprehensive analysis underscores the imperative for vigilant surveillance and tailored therapeutic interventions to mitigate the detrimental impact of CDI in susceptible population. The study highlights the importance of diagnostic stewardship, adherence to antibiotic stewardship and effective infection control measures in preventing the spread of CDI.

Acknowledgement: Shubha Shree M R
No conflict of interest
No Funding from sources

REFERENCES

1) DAMANI MANUAL OF INFECTION PREVENTION AND CONTROL-4th edition
2) Https: //www.cdc.gov Clostridioides difficile Infection | HAI
3) Diagnosis and Treatment of Clostridium difficile in Adults: A Systematic Review
Natasha Bagdasarian, MD, MPH, Krishna Rao, MD, and Preeti N. Malani, MD, MSJ
4) Laboratory Diagnosis of Clostridium difficile Infection, Can Molecular Amplification Methods Move Us out of Uncertainty? Fred C. Remover, Ellen Jo Baron, and David H. Persing
5) Toxins A&B Enzyme Immunoassay for the Detection of Clostridium difficile Toxin A and Toxin B in Stool Specimens- Premier.
6) Elward AM, Brady MT, Bryant KK, et al. Clostridioides difficile in Neonatal Intensive Care Unit Patients: A Systematic Review. Centers for Disease Control and Prevention. August 30, 2018. Updated September 2020.
7) Miller AC, Arakkal AT, Sewell DK, et al. Comparison of Different Antibiotics and the Risk for Community-Associated Clostridioides difficile Infection: A Case–Control Study. Open Forum Infectious Diseases. 2023;10(8):ofad413. doi:10.1093/ofid/ofad413
8) Motamedi, H., Fathollahi, M., Abiri, R., Kadivarian, S., Rostamian, M., & Alvandi, A. (2021). A worldwide systematic review and meta-analysis of bacteria related to antibiotic-associated diarrhea in hospitalized patients. PLOS ONE, 16(12), e0260667. https://doi.org/10.1371/journal.pone.0260667
9) Rao K, Walk ST, Micic D, et al. Procalcitonin Levels Associate with Severity of Clostridium difficile Infection. PLoS ONE. 2013;8(3):e58265. doi:10.1371/journal.pone.0058265.
10) Danpanichkul P, Duangsonk K, Uawithya E, et al. Clostridioides difficile Infection in the Elderly: Trend Analysis from 2000 to 2019. J Clin Med. 2024;13(13):3740. doi:10.3390/jcm13133740.
11) Krishnan C. Detection of Clostridium difficile toxins by enzyme immunoassay. J Hyg (Camb). 1986;96(1):5-12. doi:10.1017/S0022172400062471.
12) Bosch DE, Mathias PC, Krumm N, et al. Elevated White Blood Cell Count Does Not Predict Clostridium difficile Nucleic Acid Testing Results. Clinical Infectious Diseases. 2021;73(4):699-705. doi:10.1093/cid/ciab106.
13) Sartelli M, Di Bella S, McFarland LV, et al. 2019 update of the WSES guidelines for management of Clostridioides (Clostridium) difficile infection in surgical patients. World Journal of Emergency Surgery. 2019;14:8. doi:10.1186/s13017-019-0228-3.