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*Research Article*

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# Study of Clostridioides Difficile Associated Diarrhea in Acute Care Areas of a Tertiary Care Centre in Western Rajasthan- A Prospective Study

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## ABSTRACT

**Purpose:** The aim of this study is to identify Clostridioides difficile associated diarrhoea (CDAD) in patients admitted to acute care units of the hospital. It also examines different detection methods, including anaerobic culture, GDH assay, and Toxin A/B ELISA, while exploring how the infection relates to patient risk factors and clinical features.

**Materials and Method:** Cross sectional study conducted in the Department of microbiology over 2 years. Patients >1 year of age, who were admitted and passing atleast 3 unformed stool over 24 hours, with history of taking antibiotics were included in the study. Stool samples from the included patients were collected, with one part used for anaerobic culture and another part for serological testing using Glutamate Dehydrogenase (GDH) ELISA and Toxin A/B ELISA for C. difficile. Identification of isolates was performed using MALDI-TOF MS. The results were analysed using statistical methods.

**Result:** Out of 180 suspected cases of Antibiotic Associated Diarrheal (AAD), 95(52.8%) were males. Maximum cases were in age group, above 60 years (27.8%) & 51 to 60 years (15.6%). Out of the 180 samples, GDH ELISA was positive in 29 (16%) cases, however Toxin A/B ELISA was positive in 5 (2.8%) samples. Thus, the prevalence of CDAD was found to be 2.8%. Isolation of Clostridioides difficile from culture was in 3 (1.7%) cases. 80% of confirmed CDAD cases were received more than one antibiotic. The median antibiotic duration among CDAD cases was 14 days (IQR = 9). All CDAD cases had a history of consumption of BL-BLI antibiotics, while 40% were on Meropenem, and 20% were on Ceftriaxone and Levofloxacin antibiotics. A possible association between abdominal surgery and CDAD was also noted in the study. Mortality rate in confirmed CDAD cases was 60%.

**Conclusion:** This study in Western Rajasthan underscores the paramount importance of early CDAD detection and management to forestall fatal complications and mortality in critically ill patients, while fostering prudent antibiotic utilization and fortifying antimicrobial stewardship practices in hospital setting.

**Keywords:** Clostridioides Difficile, Cdad, Antibiotic, Antimicrobial Stewardship

**Abbreviations**

| | | |
|---|---|---|
| AAD | : | Antibiotic Associated Diarrhoea |
| BL-BLI | : | Beta Lactam- Beta Lactam Inhibitor |
| CDAD | : | Clostridioides difficile Associated Diarrhoea |
| CDI | : | Clostridioides difficile Infection |
| ELISA | : | Enzyme Linked Immunosorbent Assay |
| GDH | : | Glutamate Dehydrogenase |
| I.V | : | Intra venous |
| ICU | : | Intensive Care Unit |
| IDSA | : | Infectious Diseases Society of America |
| IPD | : | Inpatient Department |
| IQR | : | Interquartile Range |

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| | | |
|---|---|---|
| MALDI TOF MS | : | Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry |
| RCM | : | Robertson's Cooked Meat |
| SPSS | : | Statistical Package for the Social Sciences |
| WBC | : | White Blood Cell |

**Highlights**

No study has shown the prevalence of Clostridioides difficile Associated diarrhoea in western Rajasthan. Our study is showed a prevalence of 2.8%.

The current study revealed significant association between Abdominal surgery with incidence of Clostridioides difficile Associated Diarrhoea.

The all-cause mortality rate of Clostridioides difficile Associated diarrhoea in the current study centre is high.

**Introduction**

Antibiotic-Associated Diarrhoea (AAD) refers to diarrhoea occurring during antibiotic therapy or up to eight weeks after its discontinuation [1]. It is a significant concern in hospitalized patients, affecting 12% to 32% of individuals in this setting [2]. AAD can be categorized as infectious (10% to 30% of cases) or non-infectious. The most common infectious agents are Clostridioides difficile, Clostridium perfringens, Staphylococcus aureus, and Klebsiella oxytoca. Among these, C. difficile is the leading cause of hospital-acquired diarrhoea [1].

Clostridioides difficile Associated Diarrhoea (CDAD) is attributed to the anaerobic, gram-positive bacterium Clostridioides difficile. Diagnosis typically involves the occurrence of more than three episodes of unformed stool within 24 hours, occurring within eight weeks of antibiotic intake, alongside a positive diagnostic test [3,4]. Globally, CDAD prevalence ranges from 25% to 30%, while in India, rates vary from 3.4% to 17% [5-8]. CDAD is caused by toxigenic strains of C. difficile, primarily producing toxin A and toxin B. Improper antibiotic use and dysbiosis are major risk factors [4]. Other factors include advanced age, prolonged hospitalization, and abdominal surgery [3].

C difficile infection can either manifest as non-severe case with predominant symptom of diarrhoea (70-85%), abdominal pain and fever, or Severe case characterized by the same manifestation but with higher intensity and along with WBC > 15000 cells/mL and/or a Serum creatinine > 1.5 mg/dL [9,1]. Severe CDI can further progress into complications like hypotension, shock, intestinal perforation, acute peritonitis, pseudomembranous colitis and multiple organ failure (15-32%) [9-11]. Clostridioides difficile infection (CDI) can sometimes reappear after the initial episode. When symptoms return within eight weeks of the initial infection, it is referred to as recurrence, often due to the persistence of C. difficile spores in the gut. However, if symptoms return after eight weeks it is more likely a reinfection, usually caused by a new strain of the bacteria. Both recurrence and reinfection highlight the challenges in managing CDI and the importance of effective prevention and treatment strategies [1].

Treatment of diagnosed cases of CDI varies according to severity with the initial episode of non-severe cases of CDAD managed symptomatically and with oral Vancomycin preferably and the alternate choice being Metronidazole. In Recurrent CDAD oral Fidaxomicin and I.V Bezlotoxumab is standard Of-Care. Complicated cases require Vancomycin per oral or per rectal instillation is suggested as per IDSA guidelines [1]. Prevention of Clostridioides difficile infection (CDI) is crucial and involves reducing transmission and minimizing infection risk after exposure. Key measures include isolating patients in private rooms with dedicated bathrooms, maintaining contact precautions for at least 48 hours after diarrhoea resolves, and rigorous hand hygiene using soap and water, as alcohol-based sanitizers are ineffective against spores [4]. To prevent colonization, restoring gut flora with probiotics and avoiding unnecessary antibiotics are vital, supported by antimicrobial stewardship programs to ensure appropriate antibiotic use [1].

**Aims and Objectives**

The aim of this study is to identify Clostridioides difficile infection among patients admitted to acute care units of the hospital, characterized by experiencing more than three unformed stools within a 24-hour period and a history of antibiotic use within the previous eight weeks. Additionally, the study seeks to compare the detection of C.difficile using anaerobic culture, GDH Assay, and Toxin A/B ELISA methods and evaluate the association with risk factors and clinical characteristics of the patients.

**Materials and method**

**Study setting:** Hospital based cross sectional study conducted in the Department of microbiology from July 2021 to June 2023.

**Study Population:** Patients >1 year of age, who were admitted in the IPD/ICU and passing at least 3 unformed stool over a period of 24 hours, with antibiotics history within the last 8 weeks were included in the study.

**Sample collection and storage:** Approximately 30-40ml of stool sample was collected in a universal container and transported to the Microbiology Laboratory within 30 minutes.

**Microbiological analysis:** Grams staining by Kopeloff Beermans modification was performed on the stool sample on receiving for appreciating Gram positive bacilli with spore [5]. The received sample is divided into two parts, with one used for anaerobic culture the other for the serological tests for detection of C. difficile and its toxin.

**Anaerobic Culture:** Following the receipt of the sample, 1 gm from it was transferred directly into an RCM broth. From the remaining sample, 1gm was put in equal amount of absolute alcohol for 60 mins and then 0.1mL from this was transferred into another RCM broth labelled as alcohol treated. Both the RCMs were incubated for 24 to 48 hours at 37°C [12]. The RCM tubes were then subcultured on anaerobic basal and selective media which included Egg Yolk Agar, Cycloserine Cefoxitin Fructose Agar, and Neomycin blood agar. Suspected colonies showing grey, flat colonies with 2-3 mm diameter. Underwent Schaeffer Fulton spore staining for detection of pink bacilli with green subterminal spore (Figure:1) and was identified upto species level by either standard conventional biochemical tests or by MALDI TOF MS [12].

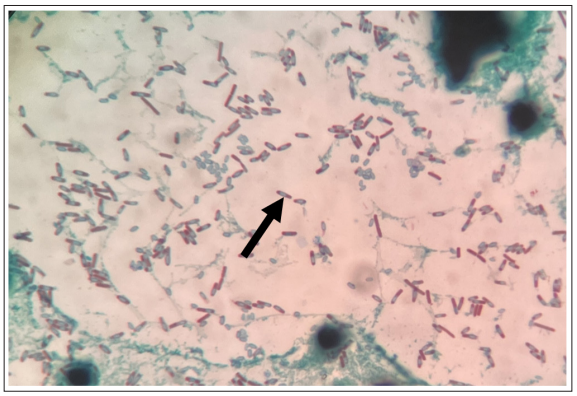

**Figure:1** Spore staining of C. difficile from culture growth by Schaeffer Fulton Showing pink bacilli with green subterminal spores under 1000x magnification. (In colour)

**ELISA for Glutamate Dehydrogenase and C. difficile Toxin A/B detection**

**Glutamate Dehydrogenase Detection:** The C. difficile GDH antigen is found in the stool sample using the CoproELISA C. difficile GDH (Savyon Diagnostics Ref: 784-01 CE). The Sensitivity is 96%, and Specificity is 100%.

**C. difficile Toxin A/B Detection:** CoproELISA C. difficile ToxA/B (Savyon Diagnostics Ref: 794-01D CE & Premier Toxin A/B by Meridian biosciences Ref: 616096) detects Toxin A (enterotoxin) and Toxin B(cytotoxin) of C. difficile in the stool specimen. The Sensitivity is 100%, and Specificity is 97.9%.

**Statistical methods:** Version 26 of SPSS was used to analyse the data. The contingency tables (2 x 2) were created. Continuous data are shown as mean value, median, and interquartile range (IQR), whereas categorical data are presented in absolute and relative (%) frequencies. Fischer's exact test was used to look at the relationship between categorical risk factors and results. A p-value of 0.05 or less was regarded as statistically significant in every instance.

**Results**

During the study period, out of the 180 suspected cases of CDAD, 95(52.8%) were males. The maximum number of suspected cases were in age group above 60 years (27.8%) followed by the age group 51 to 60 years (15.6%). Suspected cases were more from the IPD area than the ICUs. The most commonly observed risk factor among this population were co-morbidity like COPD (15.6%), Diabetes mellitus (12.85), followed by surgical intervention (11.1%). Clinically suspected cases of CDAD had various symptoms, along with diarrheal episodes. The most common among the associated symptom was fever in 94 (52.2%) cases, followed by abdominal pain in 56 (31.1%) cases, and vomiting in 18 (10%) cases.

All clinically suspected cases were subjected to microbiological diagnosis by anaerobic culture and serology by GDH ELISA and Toxin A/B ELISA. Out of the 180 samples, GDH ELISA detected C difficile in 29 (16%) samples, of which Toxin A/B ELISA was detected in 5 (2.8%) samples as depicted in Figure 2. According to the IDSA guidelines, for a case to be diagnosed as CDAD, the stool sample should be Positive for minimum two methods or test i.e. either positive for both GDH ELISA and Toxin A/B ELISA or GDH ELISA and Toxigenic Culture [1]. Hence in this study, out of 29 GDH ELISA-positive cases, a total of 5 cases were positive by Toxin A/B ELISA. Thus, giving a prevalence of 2.8%. In the study we were able to isolate C difficile from 3 (1.7%) samples by anaerobic culture. Isolation was seen on all three-culture media used, i.e. Egg Yolk Agar, Cycloserine Cefoxitin Fructose Agar (CCFA), and Neomycin blood agar. There was no variation in isolation rate between the samples directly inoculate in RCM broth and those inoculated after alcohol treatment. Figure:3 depicts growth of C. difficile on Neomycin Blood Agar showing grey, flat to slightly raised colonies with 2-3mm diameter, rugose margins.

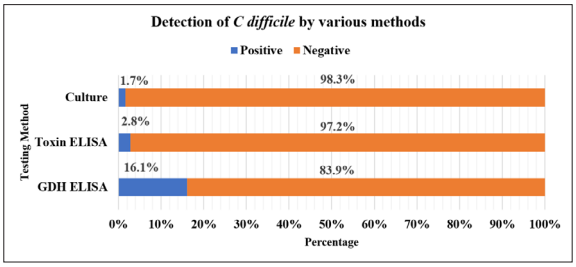


**Figure 2:** Comparison of detection of C difficile by various methods. (In colour)

**Figure 3:** Growth of C. difficile on Neomycin Blood Agar showing grey, flat to slightly raised colonies with 2-3mm diameter, rugose margins (In Colour)

The median age of the patients diagnosed as CDAD was 58.5 (IQR = 26). 80% of the CDAD cases were 51 years and above and 20% in age group 30 to 40 years. On classification according to gender, 4 among the CDAD cases were females and 1 was male. No statistical significance was found in age, gender and the area of admission. Most common symptoms in CDAD patient were abdominal pain 4 (80%) cases, (p-value = 0.033, stating significance in a study of 5 positive was a limitation of the current study) and fever (p-value = 1.00). The median days of hospital admission among the CDAD cases was 10 (IQR = 5), this did not have any statistical significance.

Clinical profile was noted of all the patients enrolled in the current study at the time of presentation to the tertiary care centre till the onset of diarrhoea. These are categorized as CDAD and non CDAD and summarized in Table 1.

**Table 1: Association of co-morbid conditions in CDAD and non-CDAD cases.**

| Risk factors (Co-morbid conditions) | CDAD (n=5) | | Non-CDAD (n=175) | | p-value |
|---|---|---|---|---|---|
| | No | % | No | % | |
| Diabetes mellitus | 0 | 0 | 23 | 13.1 | 1.00 |
| Hypertension | 0 | 0 | 17 | 9.7 | 1.00 |
| Renal failure/ Dialysis | 1 | 20 | 11 | 6.3 | 0.295 |
| Chronic Liver Disease | 0 | 0 | 6 | 3.4 | 1.00 |
| Cholecystitis | 0 | 0 | 4 | 2.3 | 1.00 |
| Surgical Intervention | 3 | 60 | 17 | 9.7 | 0.01 |
| COPD | 2 | 40 | 26 | 14.9 | 0.173 |
| Pneumonia | 0 | 0 | 9 | 5.1 | 1.00 |
| Mechanical Ventilation | 0 | 0 | 9 | 5.1 | 1.00 |
| Urinary Tract Infection | 0 | 0 | 3 | 1.7 | 1.00 |
| Fracture | 0 | 0 | 6 | 3.4 | 1.00 |
| Cerebrovascular disease | 0 | 0 | 16 | 9.1 | 1.00 |
| Intake of multiple antibiotics | 4 | 80 | 145 | 83 | - |

Majority of the patients (83%) recruited in the study were on multiple antibiotics and among CDAD cases, 4 (80%) were on multiple antibiotics. Median antibiotics duration among the CDAD cases was 14 days (IQR = 9) and among the others was 16 days (IQR = 10). Most commonly used antibiotics were in the class BL-BLI (70.1% was Piperacillin Tazobactam, 13.1% Cefoperazone Sulbactam, 7.8% Amoxicillin Clavulanic acid and the rest Ampicillin Sulbactam), followed by Carbapenems in 42.8% (Meropenem 98.7%) and Cephalosporins in 25.6% (63% of Ceftriaxone, 30.4% of Ceftazidime, 4.3% of Cefoxitin and 2.3% of Cefepime). When antibiotic usage in CDAD cases was considered, all 5 (100%) had a history of BL-BLI (Piperacillin Tazobactam and Cefoperazone Sulbactam) usage followed by Carbapenem in 2 (Meropenem - 40%), Ceftriaxone and Levofloxacin in 1(20%). Association of various groups of antibiotics with clinically diagnosed AAD cases is depicted in Figure 4.

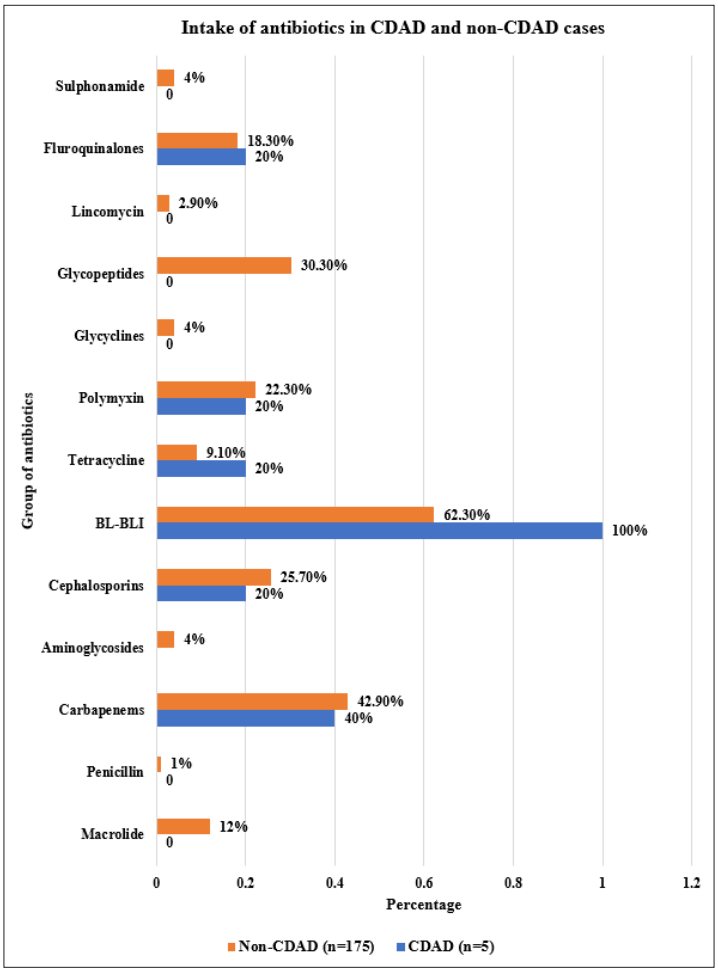


**Figure 4:** Association of various groups of antibiotics with clinically diagnosed AAD cases. (In colour)

All the diagnosed cases were treated with oral Vancomycin 125 mg QID for 10 days. During the course of treatment per rectal instillation of Vancomycin was also performed in one of the cases. The CDAD cases were followed up and complications like colitis was noted in 80% ($p < 0.001$). 3 (60%) among the 5 patients succumbed to death ($p=0.003$), this was attributed to all-cause mortality considering the various other co-morbidities.

**Discussion**

Clostridioides difficile infection, stemming from anaerobic Gram-positive bacilli, stands as the foremost etiology of antibiotic-associated diarrhoea, notably prevalent among hospitalized individuals [1]. With documented associations with elevated mortality rates and escalated healthcare expenditures, it constitutes a significant healthcare challenge. Inadequate hospital infection control practices can precipitate swift transmission, amplifying the disease burden substantially. Therefore, it is imperative to implement rigorous preventive and control measures within hospital settings to mitigate the impact of this infection effectively [4].

The present study, encompassing 180 clinically suspected cases of AAD, revealed a male predominance (52.8%), consistent with Dea et al.'s Canadian study, which reported 63% male cases [13]. However, studies by Chaudhry from India (2017) and Baines. from Italy (2008) found no significant gender difference [14,15]. The age distribution of patients in the current study aligns with findings from Pepin Canadian study which showed higher number of cases in patients >75 years of age (2005) [16]. The higher incidence in older age groups may be attributed to increased comorbidities, longer hospital stays, and greater illness severity [4]. Additionally, this study found that the majority of suspected AAD cases (55.9%) were from general wards. This contrasts with Zahar et al.'s findings in France (2012), where 68% of cases were reported in ICU settings [17].

The most frequently observed symptoms in this study were fever (52.2%) and abdominal pain (31.3%). Comparatively, De Jong et al.'s 2011 study reported that 38.46% of patients experienced fever and 28% had abdominal pain [18].

The prevalence (2.8%) found in our study is comparable to that aligns with the study by Metzger which also showed a prevalence of 2.34% and Iyer in South India (3.4%) [19,20]. However, this is low compared to a study by Vaishnavi et al in Chandigarh (17.7%) [21].

In the current study, among the three testing modalities, GDH ELISA had the highest positivity rate (16.1%), followed by toxin A/B ELISA (2.8%) and anaerobic culture (1.7%). This trend was consistent with Segar et al.'s (2017) study [22]. While Asha et al.'s (2006) was detected 12.7% and culture isolation in 12% CDAD cases [23].

In the current study the median age of CDAD cases was 58 years. The findings of present study differed from previous studies, for instance, Predrag et al. (2016) reported a mean age of 63.4, and Metzger in his study showed a broader age range [19,24].

There was higher prevalence of CDAD among females in the current study. However, some studies from India and abroad have shown equal distribution between males and females. This included a study by Metzger in 2007 and in India by Iyer in 2013 [19,20]. Hormonal fluctuations in females may contribute to changes in gut microbial composition, potentially increasing susceptibility to C. difficile infection, which could explain the higher occurrence of CDAD among females in this study [25].

Abdominal pain was the most commonly noted symptom in current study. This aligns with findings from de Jong (2011) in Europe and a study by Kannambath from South India in 2017 [18,26]. The common risk factors in laboratory-confirmed CDAD cases were found to be surgical intervention that included abdominal and genitourinary surgeries which was clinically significant. Similar findings were observed in a study by Zhao and by Vaishnavi [27,28].

The present study showed that 80% of the CDAD cases had a history of intake of multiple classes of antibiotics. These included BL-BLI (100%), followed by Meropenem (40%), and equal usage of 3rd generation Cephalosporin, Tetracycline, Polymyxin, and Fluoroquinolone. Suspected cases of AAD in the study also showed a similar pattern. The was similar to that of Metzger et al. in the USA, in which Carbapenem was the most commonly used antibiotic [19].

In this study, 80% of confirmed CDAD cases had complications like colitis, with a significant 60% mortality observed during follow-up. These findings were consistent with studies by Predrag [24]. The high mortality rate is also attributed to the existing risk factors and comorbidities among CDAD patients.

**Limitations of the study**

The study followed the two-step diagnostic algorithm recommended by IDSA, where a positive result for GDH and Toxin A/B ELISA confirms CDAD, while both being negative confirms a negative result. However, cases with GDH positive and Toxin A/B negative couldn't be categorized as non-toxigenic or toxigenic colonizers.

The study detected only 5 cases of CDAD which is insufficient to establish a solid statistical significance or draw robust conclusions.

**Conclusion**

Clostridioides difficile associated diarrhoea (CDAD) represents a formidable healthcare challenge, often commencing as mild diarrhoea but rapidly progressing to life-threatening pseudomembranous colitis. This study paramount importance of early CDAD detection and management to forestall fatal complications and mortality in critically ill patients, while fostering prudent antibiotic utilization and fortifying antimicrobial stewardship initiatives within the clinical setting.

**Consent for publication**

This work has not been published previously or under consideration for publication elsewhere. This publication is approved by all authors and the responsible authorities where the work was carried out, and, if accepted, this will not be published elsewhere in the same form, in English or in any other language.

**Conflicts of interest:** The authors declare there are no conflict of interests.

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**Ethics approval:** Institutional Ethical approval was attained dated 07/09/2021 with Certificate reference no: AIIMS/IEC/2021/3617.

**Author Contributions**

Dr Sarika P Kombade: Conceptualization, Supervision, Methodology, Validation, Formal analysis, Resources, Dr Nithya S Roy: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Data curation, Writing- original draft. Dr Kumar S Abhishek: Supervision, Resources Dr Kuldeep Singh: Resources Dr Nikhil Kothari: Resources Dr Daisy Khera: Resources Dr Deepak Kumar: Resources.

**Data Summary**

All data associated with this work is reported within the article

**References**

1. Johnson S, Lavergne V, Skinner AM, Gonzales-Luna AJ, Garey KW, et al. Clinical Practice Guideline by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA): 2021 Focused Update Guidelines on Management of Clostridioides difficile Infection in Adults. Clin Infect Dis. 2021. 73: 1029-1044.
2. McFarland LV. Epidemiology of infectious and iatrogenic nosocomial diarrhea in a cohort of general medicine patients. Am J Infect Control. 1995. 23: 295-305.
3. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, et al. Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis. 2018. 66: 987-994.
4. Dolin R, Bennett JE, Mandell GL. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. InMandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 2005. 1727-3662.
5. Procop GW, Church DL, Hall GS, Janda WM, Koneman EW, et al. Koneman's color atlas and textbook of diagnostic microbiology. Seventh edition. Philadelphia: Wolters Kluwer Health. 2017. 1.
6. Ingle M, Deshmukh A, Desai D, Abraham P, Joshi A, et al. Clostridium difficile as a cause of acute diarrhea: a prospective study in a tertiary care center. Indian J Gastroenterol Off J Indian Soc Gastroenterol. 2013. 32: 179-183.
7. Singh M, Vaishnavi C, Kochhar R, Mahmood S. Toxigenic Clostridium difficile isolates from clinically significant diarrhoea in patients from a tertiary care centre. Indian J Med Res. 2017. 145: 840-846.
8. Joshy L, Chaudhry R, Dhawan B. Detection and characterization of Clostridium difficile from patients with antibiotic-associated diarrhoea in a tertiary care hospital in North India. J Med Microbiol. 2009. 58: 1657-1659.
9. Bartlett JG, Gerding DN. Clinical recognition and diagnosis of Clostridium difficile infection. Clin Infect Dis Off Publ Infect Dis Soc Am. 2008. 46: 12-18.
10. Evans CT, Safdar N. Current Trends in the Epidemiology and Outcomes of Clostridium difficile Infection. Clin Infect Dis Off Publ Infect Dis Soc Am. 2015. 60: 66-71.
11. Ogielska M, Lanotte P, Le Brun C, Valentin AS, Garot D, et al. Emergence of community-acquired Clostridium difficile infection: the experience of a French hospital and review of the literature. Int J Infect Dis IJID Off Publ Int Soc Infect Dis. 2015. 37: 36-41.
12. Jousimies-Somer HR, Summanen P, Citron DM, Baron E, Wexler H, et al. Wadsworth-KTL Anaerobic Bacteriology Manual. 2002.
13. Shahinas D, Silverman M, Sittler T, Chiu C, Kim P, et al. Toward an understanding of changes in diversity associated with fecal microbiome transplantation based on 16S rRNA gene deep sequencing. mBio. 2012. 3: 10-128.
14. Chaudhry R, Sharma N, Gupta N, Kant K, Bahadur T, et al. Nagging Presence of Clostridium difficile Associated Diarrhoea in North India. J Clin Diagn Res JCDR. 2017. 11: 6-9.
15. Baines SD, Freeman J, Fawley WN, Harmanus C, Mastrantonio P, et al. Emergence of reduced susceptibility to metronidazole in Clostridium difficile. J Antimicrob Chemother. 2008. 62: 1046-1052.
16. Pépin J, Valiquette L, Cossette B. Mortality attributable to nosocomial Clostridium difficile–associated disease during an epidemic caused by a hypervirulent strain in Quebec. CMAJ Can Med Assoc J. 2005. 173: 1037-1042.
17. Zahar JR, Schwebel C, Adrie C, Garrouste-Orgeas M, Français A, et al. Outcome of ICU patients with Clostridium difficile infection. Crit Care. 2012. 16: 215.
18. Jong de E, Jong de AS, Bartels CJM, Melchers WJG, Sturm PDJ, et al. Clinical and laboratory evaluation of a real-time PCR for Clostridium difficile toxin A and B genes. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. 2012. 31: 2219-2225.
19. Metzger R, Swenson BR, Bonatti H, Hedrick TL, Hranjec T, et al. Identification of risk factors for the development of clostridium difficile-associated diarrhea following treatment of polymicrobial surgical infections. Ann Surg. 2010. 251: 722-727.
20. Iyer VH, Augustine J, Pulimood AB, Ajjampur SSR, Ramakrishna BS. Correlation between coinfection with parasites, cytomegalovirus, and Clostridium difficile and disease severity in patients with ulcerative colitis. Indian J Gastroenterol Off J Indian Soc Gastroenterol. 2013. 32: 115-118.
21. Vaishnavi C, Bhasin D, Kochhar R, Singh K. Clostridium difficile toxin and faecal lactoferrin assays in adult patients. Microbes Infect. 2000. 2: 1827-1830.
22. Segar L, Easow JM, Srirangaraj S, Hanifah M, Joseph NM, et al. Prevalence of Clostridium difficile infection among the patients attending a tertiary care teaching hospital. Indian J Pathol Microbiol. 2017. 60: 221-225.
23. Asha NJ, Tompkins D, Wilcox MH. Comparative Analysis of Prevalence, Risk Factors, and Molecular Epidemiology of Antibiotic-Associated Diarrhea Due to Clostridium difficile, Clostridium perfringens, and Staphylococcus aureus. J Clin Microbiol. 2006. 44: 2785-2791.

24. Stojanović Predrag. Analysis of risk factors and clinical manifestations associated with Clostridium difficile disease in Serbian hospitalized patients. Braz J Microbiol. 2016. 47: 902-910.
25. He S, Li H, Yu Z, Zhang F, Liang S, et al. The Gut Microbiome and Sex Hormone-Related Diseases. Front Microbiol. 2021. 12: 711137.
26. Kannambath R, Biswas R, Mandal J, Vinod KV, Dubashi B, et al. Clostridioides difficile Diarrhea: An Emerging Problem in a South Indian Tertiary Care Hospital. J Lab Physicians. 2021. 13: 346-352.
27. Zhao C, Guo S, Jia X, Xu X. Distribution and risk factor analysis for Clostridium difficile-associated diarrhea among hospitalized children over one year of age. Pediatr Investig. 2020. 4: 37-42.
28. Vaishnavi C, Singh M, Kapoor P, Kochhar R. Clinical and demographic profile of patients reporting for Clostridium difficile infection in a tertiary care hospital. Indian J Med Microbiol. 2015. 33: 326-327.