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Research Article
Nosocomial Diarrhea in Intensive Care Units: A Mortality-Based Bacteriological Study

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Abstract

Background and Objective: *Clostridium difficile* (*C. diff*) is the most frequent cause of hospital-acquired diarrhea. This study will characterize the demographics and outcome of *Clostridium difficile* infections (CDIs) in an Intensive Care Unit (ICU) population. **Materials and Methods:** Prospective, single-center study in a twelve-bed ICU in a tertiary hospital. Forty-two patients having diarrhea were investigated. Twenty-five with antibiotic-associated diarrhea (AAD) and the remaining seventeen with non-antibiotic-associated diarrhea (NAAD). As 15 healthy individuals in a control group were also studied. Three laboratory methods were used to diagnose toxigenic *Clostridium difficile* in stool samples: *Clostridium difficile* culture on cycloserine cefoxitin fructose agar, toxin A detection by a rapid immunoassay test and PCR for detection of *Clostridium difficile* toxin A and B genes. **Results:** Nine stool samples yielded positive results in at least one assay, eight (19.0%) were positive by culture, seven (16.6%) were positive according to the toxin A detection method and fifteen were positive according to PCR. One stool sample from the control subjects was positive by culture, but negative results were obtained from the other two assays (toxin A detection and PCR). The incidence of *Clostridium difficile*-Associated Disease (CDAD) using the three tested methods was estimated in the AAD group which was 34.2% and the incidence of CDAD in the NAAD group was 5.7%. **Conclusion:** In this work, a more severe form of the disease at the outset of diagnosis of infection, as indicated by a high SOFA score and age were independent predictors of morbidity within the ICU.

Key words: *Clostridium difficile*, diarrhea, intensive care unit, prediction, SOFA score

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The incidence of *Clostridium difficile* (*C. diff*) infection and associated hospitalizations are on the rise since the 2000s[1]. *Clostridium difficile*-Associated Disease (CDAD) is one of the most critical hospital acquired infection which is closely linked with prolonged antimicrobial use, advanced age, renal insufficiency, gastrointestinal surgeries or procedures and the usage of proton pump inhibitors, laxatives and antineoplastic chemotherapeutic agents[2,3]. *Clostridium difficile*-Associated Disease (CDAD) is defined as diarrhea that is not attributable to any other etiology such as infection, or medication/mechanical-related effect[4]. The use of antibiotics is associated with the vast majority of cases of CDAD. The highest risks are related to cephalosporins and clindamycin[5] and it is a widespread nosocomial infection in developed countries[6].

The patient's clinical scenario is used to distinguish *Clostridium difficile* infections (CDIs) from other causes of diarrhea[7]. The *C. diff* infections should be suspected in patients who develop diarrhea and have received antibiotics within two months or those with the onset of symptoms after hospitalization. A diagnosis of CDAD is based on testing stool specimens for the presence of leukocytes and *C. diff* toxins. However, rapid enzyme immune-absorbent and stool cytotoxin assay testing is required if symptoms persist and basic screening is negative. Invasive and non-invasive imaging modalities are required for severe and rapidly progressing cases[8].

A reduction in disease severity may be attributed to proper early treatment and intervention strategies, which are influenced by rapid, reliable and accurate diagnosis of CDAD[1]. Despite the existence of *C. diff* as a well-known significant nosocomial pathogen, the available data about its prevalence remains limited especially in developing countries, the alteration could be due to the unavailability of quick and precise diagnostic modalities. This situation prompted us to conduct this study to assess CDAD incidence in hospitalized adult patients in a single-center ICU. This work was designed to investigate the association of *C. diff* infection with mortality and to investigate the value of different diagnostic modalities.

MATERIALS AND METHODS

The patients were enrolled in the Critical Care Department of a tertiary medical center Alsaad Hospital, from January, 2016 until May, 2019 after obtaining approval from the Local Ethics Committee and Informed Consent from each patient (IRB: Reference number 2016-A89).

Subjects: Forty-two patients with diarrhea were included in this study. Their ages ranged from thirty to seventy years and the cohort included twenty-seven males and fifteen females. All patients had a history of antibiotic-associated diarrhea (AAD) and seventeen patients had different medical conditions leading to diarrhea not attributable to antibiotics (NAAD). In addition, fifteen control ICU subjects were enrolled without diarrhea in the study as a control group to assess the incidence of *Clostridium difficile*-associated diarrhea. Each patient's clinical condition was graded daily according to systemic inflammatory response syndrome (SIRS) and sequential organ failure assessment (SOFA) scores two days before the first positive stool *C. diff* toxin assay and then daily for the subsequent fourteen days[9,10]. The SOFA score was used to assess the severity of organ failure[10]. The criteria previously published by the American College of Chest Physicians/ Society of Critical Care Medicine (ACCP/SCCM) was adopted to characterize each patient's clinical condition with CDAD daily as SIRS, sepsis, or septic shock[9]. Clinical details such as the cause of diarrhea, underlying diagnosis and antimicrobial therapy was collected. In the current study patients were labelled as having AAD when they encounter significant diarrhea. The latter is defined as six or more loose stools in a period of two days associated with a recent history of antimicrobial treatment. A patient was deemed to have CDAD if AAD was present, along with a positive stool result based on a toxin-dependent *C. diff* assay or if the culture was positive for *C. diff*.

Sample collection: The specimens used in this study were loose or watery stools. The stool samples were processed immediately for the culture of CDI and toxin detection. Stool aliquots were stored at -20°C for DNA extraction.

Culture method: A culture on *C. diff* agar (oxoid) supplemented with cycloserine 125 mg L^{-1} and, cefoxitin 4 mg L^{-1} (oxoid), CCFA was conducted and incubated. All of the incubations occurred in an anaerobic chamber held at 37°C. The cultures were incubated for 48 hrs[11].

Presumptive colonies were characterized by a yellowish color, flat morphology, horsey smell, the appearance of Gram staining and ultraviolet (UV) fluorescence color. The API 20 A for anaerobes was used for further identification[12].

Toxin A detection: Toxin A was performed on the stool specimens using a commercial rapid immunoassay kit for direct qualitative detection of CDI (Oxoid Limited, Wade Road, Basingstoke, RG 248 PW, UK). The assay was performed in line with the manufacturer's instructions on fresh stool samples.

DNA extraction: A quantity of 100 mg stool was placed in 2 mL of ultra-pure water, then subjected to 10 min heating at 100°C. The mixture was subjected to short centrifugation (15,800 g, 20°C, 5 min), proteinase K (0.5 mg mL^{-1}, Sigma Chemicals, St. Louis, MO, USA) and pronase E (0.5 mg mL^{-1}, Sigma) were applied to the supernatant at 56°C for 90 min. Then, heating for five minutes was applied, followed by centrifugation and the supernatant was used as a template in the PCR reaction mixture, as reported by Kubota *et al.*[13].

The cultures on cycloserine cefoxitin fructose agar (CCFA) agar, toxin A detection immunoassays and PCR for diagnosing toxigenic CDI were used. Both toxin A and toxin B genes were detected in fifteen toxigenic *C. diff* isolates (the genes encoding the main virulence factors of CDI), which revealed a wide range of cytotoxic activity.

A simple and rapid PCR assay was used to differentiate toxigenic and non-toxigenic strains of *C. diff* reported by Kubota *et al.*[13], which permitted us to obtain reproducible results without resorting to DNA purification steps.

As reported by Jensen *et al.*[14], the advantages of the PCR method for *C. diff* enterotoxin gene detection is its prompt results (within three hours) when compared with the routine culture (at least 2 days). Cytotoxin detection necessitates 24 hrs, but a few results have been reported in as early as 4 hrs in strongly positive specimens.

PCR assay: The primers BW 69 and BW 70 amplify a 63-bp repetitive gradation of the enterotoxin gene, thereby manufacturing a characteristic staircase pattern of DNA after electrophoresis. Each single reaction mixture included either a positive control crude DNA preparation (2 µL) or crude fecal DNA preparations (2 µL) in a total reaction volume of 100 µL with 5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 5 mM KCl, gelatin 0.01% w/v, Triton×100 0.1%v/v, 200 µM of each deoxyribonucleotide, 50 pM each of primers BW69 (GAA GCA GCT ACT GGA TGG CA) and BW 70 (AGC AGT GTT AGT ATT AAA GT), which amplify toxin A and B genes and Taq polymerase (Boehringer Mannheim, Germany) 4U[14].

Amplification was conducted and the amplification end products were partitioned on 2% agarose gel containing ethidium bromide (0.5 ug mL^{-1}) and examined under UV light using a transilluminator FBTIV-88 (Fisher Scientific, Pittsburgh, PA, USA). Gels containing bands were portrayed using a built-in Polaroid camera (Photo-Documentation, Hood FB-PDH-1216, Fisher Scientific).

Statistical analysis: Skewed variables were presented as medians and interquartile ranges (IQRs), we express normally distributed continuous variables as Mean±Standard Deviation (SD). We divided the patients into two groups based on mortality. The groups were compared using parametric, nonparametric tests, or chi-squared tests, as appropriate. A significant association was defined by a probability $p \leq 0.05$. The outcome log was examined and correlated through a single-variable linear or logistic regression. The latter was submitted as a non-adjusted coefficient (NAC) and 95% confidence intervals (95% CI). Factors with a $p \leq 0.05$ according to single-variable regression analyses were included in a multivariable linear regression model presented as adjusted coefficients (AC) (95% CI). The statistical analysis using SPSS software was performed (version 22, IBM, Chicago, IL, USA).

RESULTS

The median age of our cohort was 57 years, with an IQR ranging from 36-69 years (Table 1). In addition, most of the patients (98.3%) with CDAD required metronidazole as an initial chemotherapeutic antimicrobial.

Clinical course: The septic shock occurred in 33.3% (14/42) of CDAD patients. The calculated SOFA scores were significantly higher among non-survivors (Table 1).

Laboratory results: Eight out of the forty-two studied stool samples revealed positive culture results (19%). In addition, enterotoxin A was detected in seven samples among the studied groups (16.6%) and PCR detected both toxins A and B in eight stool samples (19%) (Table 2). Most patients (59.1%) received chemotherapeutic antimicrobials before the onset of CDAD (Table 2). The clinical profile and past medical history for the eighteen clinical cases and the results of the laboratory tests were summarized in (Table 3).

Patients with AAD: Among the twenty-five patients with AAD, eight (28%) were positive by culture, six (24%) were positive by enterotoxin A detection and seven (25%) were positive by PCR. The incidence of CDAD in this group of patients was 30.

Non-AAD patients: Two positive samples were detected by both cultures and by enterotoxin A assay (5.8%), only one case was detected by PCR (5.8%) among the seventeen studied patients (i.e., an incidence rate of 5.7%) in Table 2.

Healthy adults: One individual had *C. diff* isolated via the culture method (6.6%), but a negative result was obtained for both enterotoxins A assay and PCR among the fifteen healthy adults (the control group). The correlations between medical diagnosis and positive laboratory results were listed in (Table 4).

Table 1: In-hospital mortality in CDAD patients

	Mortality (n = 11)		Survival (n = 31)		
Variables	N	%	N	%	p-value
Age, median years (IQR)[1]	61.0 (56.0-69.0)	-	53.0 (36.7-63.0)	-	0.015*
SOFA[2] score at infection onset, median (IQR)	10.5 (6.0-15.0)	-	4.0 (2.0-5.9)	-	<0.001*
Other infections concurrently	8	68.8	18	59.5	0.52
Organ failure (any)	11	100.0	20	66.7	0.01*
Septic shock	9	75.0	5	16.7	<0.001*
Respiratory failure	10	87.5	16	52.4	0.04*
Renal failure	8	68.8	4	14.3	<0.01*
Hematologic failure	2	18.8	2	7.1	0.43
Hepatic failure	4	31.3	2	7.1	0.02*

[1]IQR: Inter quartile range, [2]SOFA: Sequential organ failure assessment and *p significant if < 0.05

Table 2: Number of positive tests for detection of *Clostridium difficile* among the two studied group and the control group

Laboratory test	[1]AAD (n = 25)	[2]NAAD (n = 17)	Total (n = 42)	Control group (n = 15)
Culture positive	7 (28%)	1 (5.8%)	8 (19%)	1 (6.7%)
Toxin A detection	6 (24%)	1 (5.8%)	7 (16.6%)	0
PCR	8 (32%)	1 (5.8%)	9 (21.4%)	0

[1]AAD: Antibiotic-associated diarrhea and [2]NAAD: Non-antibiotic associated diarrhea

Table 3: Clinical presentation and past medical history of positive cases with CDI

Age/sex	Diagnosis	Past medical history	Antibiotics	Toxin A assay (7/42) 16.6%	PCR (8/42) 19%
35/M	CAP/sepsis		Cloxacillin	+	-
42/M	CAP/sepsis		Amikacin and Cefotaxime	+	+
53/M	Head trauma/CLABSI		Cloxacillin	-	+
45/F	CAP		Augmentin and Cefuroxime	+	+
41/M	VAP/sepsis		Ceftazidime and piperacillin	+	-
34/M	Head trauma, VAP		Cefotaxime and piperacillin	-	+
65/M	DKA/unknown source	Diabetes	Cloxacillin and Cefotaxime	+	-
34/M	COPD exacerbation, CAP		Cefotaxime	-	+
41/F	UTI/sepsis		Amikacin and Cefotaxime	-	+
26/F	Head trauma, VRI		Ceftazidime and piperacillin	-	+
43/F	AML	Diabetes	Chemotherapy	+	-
45/M	CAP/sepsis		Imipenem	-	+
45/F	Endocarditis		Augmentin and Cefuroxime	+	-
65/M	DKA/unknown source	Diabetes	Cloxacillin and Cefotaxime	+	-
34/M	CAP		NAAD	+	+
47/F	UTI		NAAD	-	+
39/M	VAP		NAAD	+	-

CDI: *Clostridium difficile* infection, PCR: Polymerase chain reaction, CAP: Community acquired pneumonia, UTI: Urinary tract infection, CLABSI: Central line associated bloodstream infections, VAP: Ventilator associated pneumonia, DKA: Diabetic ketoacidosis and NAAD: Non antibiotic associated diarrhea

Table 4: Relation between final diagnosis and positive results

Laboratory test results	[1] AAD (n = 25)	[2] NAAD (n = 17)	Control group (n = 15)
Culture +,Toxin -ve and PCR –ve	1 (2%)	0	1(6.6%)
Culture +,Toxin -ve and PCR +ve	1 (2%)	0	0
Culture +,Toxin +ve and PCR +ve	6 (24%)	1 (2.8%)	0
Culture +,Toxin +ve and PCR -ve	0	1 (2.8%)	0

Figures in parentheses are percentages, [1]AAD: Antibiotic-associated diarrhea and [2]NAAD: Non-antibiotic associated diarrhea

DISCUSSION

The frequency of toxigenic CDI infection among cases of AAD and NAAD was prospectively determined in a population of forty-two patients recruited from a tertiary university hospital ICU. In this study, the genes encoding the major virulence factors of *C. diff* were detected in fifteen toxigenic *C. diff* isolates that revealed a broad range of cytotoxic activity. A simple, rapid PCR assay was used to differentiate between toxigenic and non-toxigenic strains of CDI[13]. This technique allowed us to obtain reproducible results without relying on DNA purification steps. In addition, the expedited results obtained by PCR method for detecting a segment of the CDI enterotoxin gene directly from stool samples made it more preferred than the culture method[14].

In this study, toxigenic CDI was responsible for 16.6% % of cases of nosocomial diarrhea (AAD and NAAD) by PCR. Others have reported a prevalence of 20-45% out of all identified patients[2]. Zahar *et al*.[15] reported that CDI was responsible for 14.5% of cases of nosocomial diarrhea by ELISA testing and culture. *C. diff* strains were isolated in 11% of adult patients, as reported by Karanika *et al*.[16], but Dai *et al*.[17] reported that the incidence of CDI was almost 31% of the total patients who developed AAD. These variations likely arise from differences in population selection and the sensitivity of laboratory tests.

In this study, two patients had a positive stool culture, a negative toxin A assay and a positive PCR. In these cases, it is possible that the toxins output generated by the isolate was too weak to be detected by the enterotoxin assay but appropriate enough to induce diarrhea. The rapid immunoassay for CDI toxin A detection has the potential to be a quick, well-grounded laboratory examination for patients suspected of having AAD or colitis due to CDI in both inpatient and outpatient settings[18]. Toxin A is crucial for triggering human disease, therefore, it's detection is more critical than toxin B[7]. However, toxin A tests should not be used as the sole diagnostic tool due to the low specificity for toxigenic CDI and frequent false-positive results[13]. The optimal diagnostic performance will be achieved when the testing targets toxins A and B[1]. Xiao *et al*.[19] reported three methods for testing and screening CDI. The VIDAS GDH assay is useful for the initial screening of *C. difficile*. Current findings were consistent with those of Borali and Giacomo[20], Pechal *et al*.[21] and Dai *et al*.[17] that age, treatment with cephalosporins, clindamycin, or broad-spectrum penicillin are associated with an increased risk of AAD. However, gender was not associated with a difference in AAD frequency.

Since more than half of our CDI patients fell ill with associated infections, our findings predictably showed an increased rate of ICU CDAD patients expressing a maximal SIRS over the ICU course. However, no statistical difference was obtained in patients with higher SIRS with CDAD ($p = 0.17$). This observation can be attributed to the practice of delayed antimicrobial therapy initiation for patients with CDAD. In this study, ICU patients experienced diarrhea for an average duration of 7 days before microbiologic affirmation of the CDAD diagnosis and the commencement of medical treatment. Poor outcomes have been associated with metronidazole for the treatment of CDAD in some reports[22,23]. In this study, we did not set out to comment on the efficacy of metronidazole due to the existence of other contributing factors such as a co-infection and death before accomplishing the full course of metronidazole treatment. Nearly 20% of our patients took antibiotics without evidence of concurrent infection. This observation is particularly important since previous studies have reported that needless antibiotic use renders CDAD treatment more complicated and less effectual[2,3].

A high mortality rate linked with CDI diarrhea in our ICU patients were observed. Indistinguishably, in a prospective study conducted in Canadian hospitals recruiting 1,073 patients with CDAD reported a 30-day crude mortality rate of 24.8% compared to our study which reported 27.6% irrespective of critically ill patients[23]. Loo *et al*.[24] reported marked increase in the incidence of CDAD and associated mortality above the ages of fifty and sixty, respectively. Recurrent CDAD after appropriate treatment with metronidazole was associated with patients above the age of sixty-five in a study conducted by Pepin *et al*.[23]. Using univariate analysis, CDAD, increased age was observed and SOFA score were independent predictors of mortality for ICU patients. Prior reports have also corroborated the finding that higher age is a risk factor for CDAD[25].

This study provides insights on the value of diagnosing CDI in ICU settings, where we could identify possible beneficial effects and exclude another possible early outcome. The study was a single-center and the sample volume was relatively low for certain comparisons.

CONCLUSION

The CDAD is a significant burden in the ICU and strict measures to control this pathogen must be implemented. We can also conclude that the PCR assay used in this study could be a quick and specific test for detecting toxigenic CDI. Higher severity of infection at the inauguration of infection, as obtained by the SOFA score and age were independent predictors of death. Further randomized controlled studies are required to validate these results.

SIGNIFICANCE STATEMENT

This study will characterize the demographics and outcome of *C. diff* infections (CDIs) in an Intensive Care Unit (ICU) population. The study is important to prognosticate how the outcome of intensive care patients with *Clostridium difficile* infection could be influenced and the relation to the morbidity score, in the same time the study clarify the importance of diverse diagnostic modalities in these regards.

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