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Original Article

Clinical characteristics associated with the severity of *Clostridium* [*Clostridioides*] *difficile* infection in a tertiary teaching hospital from Mexico

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ABSTRACT

Background: *Clostridium difficile* infection (CDI) is a leading cause of healthcare-associated diarrhea worldwide. In this study, risk factors associated with the development of severe-complicated and recurrent outcomes in CDI patients in different age groups, including the non-elderly, were assessed in a third-level hospital.

Methods: CDI cases were detected by clinical data and polymerase-chain-reaction (PCR). Clinical, demographic, epidemiological, and microbiological risk factors for CDI were evaluated.

Results: During the study period, 248 out of 805 patients with nosocomial diarrhea were diagnosed with CDI and the majority were severe-complicated cases (87.90%). Female gender (OR 3.19, 95% CI 1.19–8.55, $p = 0.02$) and lymphoma (OR 3.95, 95% CI 1.03–15.13, $p = 0.04$) were risk factors for severe-complicated CDI. Mature adulthood (51–60 years) (OR 5.80, 95% CI 1.56–21.62, $p = 0.01$), previous rifampicin use (OR 7.44, 95% CI 2.10–26.44, $p = 0.00$), and neoplasm (solid malignant neoplasm or hematological malignancies) (OR 4.12, 95% CI 1.01–16.83, $p = 0.04$) were risk factors for recurrent infection. Autoimmune disorders (OR 6.62, CI 95% 1.26–34.73, $p = 0.02$), leukemia (OR 4.97, 95% CI 1.05–23.58,

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p = 0.04), lymphoma (OR 3.79, 95% CI 1.03–12.07, p = 0.04) and previous colistin treatment (OR 4.97, 95% CI 1.05–23.58, p = 0.04) were risk factors for 30-day mortality.

Conclusion: Newly identified risk factors for recurrent CDI were rifampicin treatment and age between 51 and 60 years; colistin treatment was identified as a risk factor for 30-day mortality. Previously identified risk factors for severe-complicated CDI were confirmed, but with a major impact on non-elderly patients.

Q3 Introduction

Clostridium difficile (recently reclassified as *Clostridioides difficile*) infection (CDI) is a leading cause of healthcare-associated diarrhea worldwide. In 2011, there were an estimated 453,000 infections and 29,000 deaths resulting from CDI in the United States [1]. CDI may be a mild, self-limiting disease or a severe, complicated, life-threatening or recurrent disease [2].

Since 2000, an increase in the overall incidence of CDI has been highlighted by outbreaks of more severe disease in the United States, Canada, England, some Asian countries, and Latin America, with rates five-fold higher in patients older than 65 years. The emergence of the strain *C. difficile* NAP1/BI/027 has been associated with an increased incidence, more severe infection, a high recurrence rate after 8 weeks of resolution, and higher 30-day mortality rates [3]. A limited number of effective antimicrobials have been approved for CDI treatment, and the highly virulent NAP1/BI/027 strain complicates current treatment protocols [2].

Known risk factors for acquiring CDI are advanced age (>65 years), the use of broad-spectrum antibiotics, extended hospital stays, stay at the intensive care unit, infection with the human immunodeficiency virus, cancer, leukemia, lymphoma, autoimmune disorders, pulmonary infections (tuberculosis or pneumonia), gastrointestinal procedures, arterial hypertension, and the use of immunosuppressive drugs, antacids and steroids [4–9].

The present study aimed to identify risk factors associated with the development of severe-complicated, and recurrent outcomes in CDI patients of different age groups, including the non-elderly, in a third-level hospital.

Material and methods

Study setting and population

This was a retrospective study at the Hospital Civil de Guadalajara Fray Antonio Alcalde, a 1000-bed tertiary-care teaching hospital. The hospital has 31 wards housed in four separate buildings. The hospital provides services to Guadalajara in the state of Jalisco, Mexico, with approximately 30,000 admissions each year.

Patient demographics and CDI diagnosis

As part of the surveillance protocol for CDI, between November 2013 and December 2015, all hospitalized patients

with a hospital stay more significant than 48 h who developed diarrhea (3 or more depositions in the last 24 h with a Bristol score of 6 or 7 or a recent hospitalization in the previous 12 weeks) were tested for *C. difficile* using the Cepheid Xpert *C. difficile*/Epi test (Cepheid, Sunnyvale CA).

Epidemiological and clinical data were reviewed in medical records from CDI patients

CDI was defined as complicated when one of the following criteria were present: admission to the intensive care unit, hypotension with or without the required use of vasopressors, fever $\geq 38.5^{\circ}\text{C}$, ileus, significant abdominal distention, mental status changes, white blood cell (WBC) count $\geq 35,000$ cells/ mm^3 or < 2000 cells/ mm^3 , or serum lactate levels > 2.2 mmol/l [10]. CDI was defined as severe because of hypoalbuminemia (serum albumin < 3 g/dl), WBC $\geq 15,000$ cells/ mm^3 , or abdominal tenderness [10].

Cases not meeting the severe or complicated infection criteria were classified as having mild to moderate CDI (without additional symptoms)

Recurrent CDI was defined by the reoccurrence of diarrhea associated with clinical and laboratory evidence of CDI within eight weeks after completing therapy or resolution of the initial CDI, [10]. The study was reviewed and approved by the Ethics Committee of the “Hospital Civil de Guadalajara Fray Antonio Alcalde.”

Statistical analyses

The patients were classified into the following age group: 11–20, 21–30, 31–40, 41–50, 51–60, and >60 years of age. For the analyses of categorical variables, Pearson's chi-squared and Fisher's exact tests were used; for continuous variables, the Mann–Whitney U test was used. The Kruskal–Wallis test was performed for comparison between severe-complicated and recurrent CDI, as well as NAP1/027-associated CDI, 30-day, and 12-month mortality frequencies among age groups.

A multivariable logistic regression was conducted over two stages: a univariable analysis to identify significant associations between independent variables and outcomes. Odds ratios with a 95% confidence interval were determined. Variables identified in the univariate analysis with odds ratios > 1.0 and $p < 0.25$ were included in a binary logistic regression model. Variables with a $p < 0.05$ were considered as independent risk factors.

We examined multi-collinearity using linear regression to study the tolerance index (Ti) and the variance inflation factor (VIF) for the absence of multicollinearity issues between independent variables by the Statistical Package for the Social Sciences (SPSS), software version 23.

Table 1 Demographic and epidemiological characteristics of patients with confirmed CDI (N = 244^a).

	N	%
<i>Gender</i>		
Male	154	63.10
Female	90	36.90
<i>Age groups (years)</i>		
11-20	29	11.88
21-30	46	18.85
31-40	25	10.25
41-50	37	15.16
51-60	45	18.44
61-70	32	13.11
71-80	21	8.60
>80	9	3.69
<i>Severity of infection</i>		
Severe-complicated	214	87.70
Moderate	30	12.29
<i>Clinical data</i>		
Abdominal pain	115	47.13
Fever (>38 °C)	103	42.21
Abdominal distention	102	41.80
Mucus in stool	102	41.80
Vomiting	37	15.16
Blood in stool	17	6.97
Pseudomembranes	9	3.69
<i>Laboratory results</i>		
Hypoalbuminemia	148	60.65
White blood cells (>15,000 cells/mm ³)	76	31.14
<i>Comorbidities</i>		
Arterial hypertension	93	38.11
Kidney disease	84	34.40
Diabetes mellitus	77	31.55
Neoplasm (solid malignant neoplasm or hematological malignancies)	63	25.82
Pneumonia	37	15.16
Lymphoma	11	4.51
Leukemia	8	3.28
Autoimmune disorder	6	2.46
Other	19	7.79
<i>C. difficile strain characteristics</i>		
NAP1/027 strain	127	52.05
non- NAP1/027	117	47.95
<i>Recurrence</i>		
At least one	22/84	26.19
Due to <i>C. difficile</i> NAP1/027 strain	10/22	45.45
Due to non- <i>C. difficile</i> NAP1/027 strain	12/22	54.55
<i>Hospitalization</i>		
General ward (before and after diagnosis)	222	90.98
In the previous 12 weeks	124	50.82
Intensive Care Unit	20	9.01
<i>Antibiotic treatment before CDI diagnosis</i>		
Overall	165	67.62
Clindamycin	128	77.58
Cephalosporins	89	53.94
Carbapenems	54	32.73
Metronidazole	35	21.21
Fluoroquinolones	23	13.94
Linezolid	21	12.73
Vancomycin	19	11.52
Rifampin	19	11.52
Amikacin	15	9.09
Piperacillin-tazobactam	15	9.09
Fluconazole	12	7.27
Others	27	16.36
<i>Another drug treatment</i>		

(continued on next page)

Table 1 – (continued)

	N	%
Histamine blockers	14	5.73
Proton pump inhibitors (PPI)	204	83.61
Immunosuppressors	36	14.75
<i>CDI cases per year^b</i>		
2014	68	27.42
2015	177	71.37
<i>30-day mortality</i>		
Overall	38	15.57
<i>12-month mortality</i>		
Overall	69	28.27

^a Clinical data were available for 244 patients only.
^b 2013 frequency was not included since only 2-month period data was available for this year.

Results

Epidemiological and clinical data

During the study period, 805 patients presented with nosocomial diarrhea, and 248 were confirmed to have CDI by PCR (62.10% were males, and 75% were <60 years old). Most patients with CDI were young adults (age, 21–30 years; n = 46, 18.85%) or mature adults (51–60 years of age; n = 45, 18.44%) [Table 1].

Most patients presented with severe-complicated disease (87.70%). Hypoalbuminemia n = 148, 60.65%), abdominal pain (n = 115, 47.13%), fever >38.5 °C (n = 103, 42.21%), abdominal distention (n = 102, 41.80%), and mucus in stool (n = 102, 41.80%) were the most common signs and symptoms associated with CDI. The most frequently observed comorbidities were arterial hypertension (n = 93, 38.11%), kidney disease, including patients that presented urinary infection or creatinine levels >1.5 mg/dl (n = 84, 34.40%), and diabetes (n = 77, 31.55%) [Table 1].

The hypervirulent *C. difficile* NAP1/027 strain was detected in 127 (52.04%) patients, and 84 of these patients were followed for the next eight weeks; among them, 22 patients (26.19%) developed at least one recurrent episode. From the recurrent episodes, 10/22 (45.40%) were caused by the *C. difficile* NAP1/027 strain and 12/22 (54.50%) by a non-NAP1/027 strain.

Most patients (n = 222) were housed in the general ward, and among them, 124 had been hospitalized during the 12 weeks previous to CDI. Twenty patients had been admitted to the intensive care unit when CDI was diagnosed. The remaining two patients were in other wards. All patients with the first episode of mild to moderate CDI were treated with metronidazole, and recurrent episodes were treated with metronidazole in combination with vancomycin according to the American College of Gastroenterology guidelines and the Guidelines for *C. difficile* Infection in Adults and Children of the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Second-line therapies, such as fidaxomicin treatment or a fecal microbiota transplant, were not used due to their unavailability at that time in our hospital.

Table 2 Risk factors for the development of severity, recurrence, and 30-day mortality.

Dependent	Variable	Univariate analysis		Binary logistic regression	
		U-OR ^d (95% CI)	p-value	A-OR ^e (95% CI)	p-value
Severe CDI ^a	Gender (females)	2.58 (1.00–6.51)	0.03*	3.19 (1.19–8.55)	0.02*
	Lymphoma	1.33 (0.89–1.99)	0.04*	3.95 (1.03–15.13)	0.04*
	Age (21–30 years)	1.51 (0.98–1.35)	0.03*	2.41 (1.00–5.78)	0.05
	Infection with 027 strain	1.67 (0.77–3.64)	0.13	0.56 (0.25–1.26)	0.16
Recurrence ^b	Age (51–60 years)	6.54 (2.07–20.68)	0.00**	5.80 (1.56–21.62)	0.01*
	Antibiotics intake	2.19 (0.57–8.44)	0.19	1.42 (0.31–6.43)	0.64
	Rifampin	4.37 (0.89–21.37)	0.07	7.44 (2.10–26.44)	0.00**
	Neoplasm	2.42 (0.79–7.47)	0.10	4.12 (1.01–16.83)	0.04*
	Fluoroquinolones	2.74 (0.74–10.12)	0.11	2.55 (0.55–11.73)	0.23
	Hypertension	1.23 (0.46–3.29)	0.67	1.62 (0.48–5.49)	0.23
	Autoimmune disorder	5.09 (0.97–26.24)	0.03*	6.62 (1.26–34.73)	0.02*
30-day-mortality ^c	Leukemia	3.90 (0.84–18.24)	0.09	4.97 (1.05–23.58)	0.04*
	Lymphoma	2.62 (0.75–9.21)	0.12	3.79 (1.03–12.07)	0.04*
	Colistin	3.79 (0.81–17.70)	0.10	4.97 (1.05–23.58)	0.04*

Goodness of fit test: Hosmer y Lemeshow, ^a $\chi^2 = 3.878$, $p = 0.567$, ^b $\chi^2 = 4.657$, $p = 0.702$, ^c $\chi^2 = 0.167$, $p = 0.683$. * $p < 0.05$, ** $p < 0.01$. ^d Unadjusted odds-ratio, ^e Adjusted odds-ratio.

Almost two-thirds of the patients had previously received antibiotic treatment ($n = 165$, 67.62%), most commonly clindamycin ($n = 128$, 77.58%) and cephalosporins ($n = 89$, 53.94%); metronidazole and vancomycin were previously administered in 21.21% and 11.52% of the cases, respectively which are usually administered for CDI treatment [Table 1]. From the CDI 248 cases, 3 (1.21%) occurred in 2013, 68 (27.42%) in 2014, and 177 (71.37%) in 2015.

Risk factors detected for CDI

Female gender (OR 3.19, 95% CI 1.19–8.55, $p = 0.02$) and lymphoma (OR 3.95, 95% CI 1.03–15.13, $p = 0.04$) were independent risk factors for severe CDI [Table 2].

Mature adulthood (51–60 years) (OR 5.80, 95% CI 1.56–21.62, $p = 0.01$), rifampin consumption (OR 7.44, 95% CI 2.10–26.44, $p = 0.00$) and neoplasm (solid malignant neoplasm or hematological malignancies) (OR 4.12, 95% CI 1.01–16.83, $p = 0.04$) were independent risk factors for the development of recurrent CDI [Table 2].

We identified the following independent risk factors for 30-day mortality rates: autoimmune disorders (OR 6.62, CI 95% 1.26–34.73, $p = 0.02$), leukemia (OR 4.97, 95% CI 1.05–23.58, $p = 0.04$), lymphoma (OR 3.79, CI 95% 1.03–12.07, $p = 0.04$) and previous colistin treatment (OR 4.97, 95% CI 1.05–23.58, $p = 0.04$) [Table 2].

Discussion

Our study addressed risk factors among patients with CDI, and we detected that 89.0% of the patients developed severe CDI. In contrast to previous reports that identified the elderly (>60 years) to be the most vulnerable age group [3,11–16], in our study, young (age 21–30 years) and mature adulthood (age 51–60 years) adults had the highest frequency of CDI, respectively 18.55% and 18.15%. Previous studies conducted in Mexico reported that patients <65 years of age had a higher risk of recurrent CDI [17–19]. This Mexican study identified

mature adulthood (51–60 years) as an independent risk factor for the development of recurrent CDI. The different conclusions with respect to the most vulnerable age group may be explained by the fact that most CDI surveillance studies have focused primarily on the elderly, excluding younger adults and children [3,20,21]. Furthermore, the healthcare facility where the study was conducted also provided medical attention mainly to traumatized younger patients and was better equipped to identify this novel, at-risk population.

The prevalence of the NAP1/027 *C. difficile* strain in about half of our CDI patients is consistent with reports from other countries where prevalence was between 45 and 61% [19].

The present study confirmed previously described risk factors for complicated or recurrent CDI [3,12,22,23], e.g., middle-aged adulthood and neoplasm illness were independent risk factors for recurrent CDI, whereas lymphoma was an independent risk factor for complicated CDI.

As far as we know, we are the first to report that rifampin and colistin are independent risk factors for recurrent CDI and 30-day mortality, respectively. It should be noted, though, that only seven patients received colistin therapy before the CDI diagnosis. This risk factor is significant because recent studies have reported infections due to multidrug-resistant strains that were only susceptible to colistin. This antibiotic was not used until recently because of its nephrotoxicity. However, during the last decade, it has been reintroduced to treat critical illnesses such as pneumonia, bacteremia, meningitis, obstructive pulmonary disease, and cystic fibrosis [24–26]. However, in the treatment of pneumonia, bacteremia, and other critical diseases related to the development of CDI, a high mortality rate (61.9%) has been attributed to colistin therapy (OR, 1.99 to 8.2; $p < 0.001$) [24,25].

It should be mentioned that our hospital had an outbreak of carbapenem-resistant enterobacteria during the study period; thus, the use of tigecycline and colistin was increased. Regarding colistin as an independent risk factor for mortality, we must consider that these patients had severe clinical conditions, and the high mortality rate may be due to the underlying infection. In this regard, a prediction model using

logistic regression for CDI in hospitalized patients identified an underlying infection as an independent predictor of CDI [27].

Still, this newly described risk factor for the development of CDI reinforced the need for control of other hospital-acquired infectious diseases as an additional means of preventing CDI. As a result, in our hospital, quinolone was reduced, and the use of levofloxacin was eliminated from almost all wards except for the hematology and urology wards.

We used the Xpert *C. difficile*/Epi assay to confirm CDI. This assay detects the genes *tcdB* and *cdt* and the 18-bp deletion in *tcdC*, but it does not identify the *tcdA* gene. Therefore, it is possible that patients infected with strains that only produced *tcdA* were not identified. On the other hand, it seems that the production of only toxin A is rare. A study that compared CDI detection by either the Xpert *C. difficile* with detection by the *tcdA*-based Illumigene *C. difficile* assay (Meridian Bioscience, Inc.) found that 157 samples were negative in both tests, 35 were positive in both assays, 7 were positive by GeneXpert but negative by the Illumigene assay, and in one case, the GeneXpert was positive. However, the result of illumigene was invalid [28]. Thus, the lack of detection of *tcdA* by the GeneXpert may have had a minimal effect in our study.

The major limitation of our study was that it was performed at a single site, and results may not apply to other hospitals. The high number of complicated CDI infections in our hospital and their associated risk factors may be advocated by increased infections caused by carbapenem-resistant enterobacteria during the study period.

Conclusion

In summary, in our study, CDI was mainly prevalent among patients <60 years, who were also more vulnerable to severe CDI. The use of colistin was identified as a new risk factor for increased 30-day mortality rates, while the use of rifampin treatment was a new risk factor for the development of recurrent episodes in patients between the ages of 51 and 60 years.

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Declaration of interest statement

The authors report no conflicts of interest.

Data statement

All the authors of this study give the rights to public the material content of this project.

No additional data are available for sharing.

Ethics in publishing

This study was reviewed and approved by the Ethics Comitee of “Hospital Civil de Guadalajara, Fray Antonio Alcalde”.

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