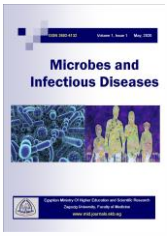
Microbes and Infectious Diseases 2024; Article-In-Press, **DOI:** 10.21608/mid.2024.276404.1854



Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Risk stratification for *Clostridioides difficile* infection among hospitalized patients with diarrhea in the Eastern Region of Saudi Arabia; with a glimpse at COVID-19 coinfection

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ARTICLE INFO

Article history:
Received 19 March 2024
Received in revised form 12 April 2024
Accepted 14 April 2024

Keywords:
C. difficile
Risk factors
Outcomes
Cancer
Antibiotic-associated diarrhea
Age
COVID-19

ABSTRACT

Background: *Clostridioides difficile* (*C. difficile*) is an anaerobic bacterium associated with considerable wide-spectrum colonic infections. Various risk factors are recognized to increase the incidence of *C. difficile* infection (CDI) in certain age groups. COVID-19 pandemic and its association with CDI remains an area of research. **Objectives:** Our aim is to investigate the risk factors and outcomes of CDI among hospitalized patients with diarrhea with special consideration for COVID-19 patients. **Results:** The study included 1515 hospitalized patients with infectious diarrhea from 2017 to 2021, 195 (13%) of them had positive CDI tests and 1320 (87%) were CDI-negative. The risk for CDI was higher in the young adults aged between 18 and 35years (OR: 2.47, 95%CI: 1.37- 4.47, p=0.0028) and older patients aged ≥ 56 years (OR: 1.95, 95% CI: 1.87-3.21, p=0.0084). Older patients' risk factors included stroke, administration of antibiotics, history of previous hospital admission within one month, and cancer. While young adults receiving two antibiotics were at greater risk of having CDI. 132 COVID-19 patients with diarrhea were identified and 7 (5%) of them were CDI positive. **Conclusions:** Investigating the risk factors of CDI in different age groups, including COVID-19 patients, is a crucial step to developing a risk-based prophylactic strategy to reduce the cost and burden on the healthcare system.

Introduction

Clostridioides difficile is a spore-forming Gram- positive bacterium that can cause symptoms ranging from nosocomial antibiotic-associated diarrhea to highly fatal inflammation of the colon in the form of pseudomembranous colitis (PMC) [1]. *Clostridioides difficile* produces two exotoxins (toxin A and toxin B). These toxins have robust proinflammatory activity, and they are able to trigger cytokines and chemokines production by the intestinal epithelial cells and immune cells resulting in severe inflammation and tissue damage [2]. The majority of CDIs are healthcare-related, however, the prevalence of community-acquired CDI (CA-CDI) is increasing. Many risk factors are associated with CDI and CDI recurrence such as old age and immunocompromised status. Commonly, the administration of antibiotics results in modifications to the normal gut flora, which are linked to CDI [3].

DOI: 10.21608/MID.2024.276404.1854
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Clostridioides difficile infection is known to contribute to extensive morbidity, mortality, and increased medical costs among hospitalized patients [4]. Nucleic Acid Tests (NATs) are the most sensitive and the least specific tests to detect *C. difficile* in patients' stool irrespective of being toxin producer or not. In the United States, nearly half the laboratories consider NATs the methods of choice in detecting *C. difficile* [5].

The incidence of hospital-acquired CDI (HA-CDI) declined during the COVID-19 pandemic according to the data published from January 2019 to September 2021 [6]. The implementation of strict hand hygiene measures, environmental cleaning programs, isolation of infected patients and using personal protective equipment (PPE), have definitely a major role in reducing *C. difficile* transmission during the pandemic [7]. Only 0.4% of COVID-19 patients had CDI, according to Italian researchers who had also highlighted the risk factors for co-infection and how its presence prolongs the hospital stays [8].

There is few information from the literature to report CDI from Asian countries, especially from South East Asia. A Middle East study in Riyadh, Saudi Arabia showed that 20.5% of the examined samples were positive for *C. difficile* using a less specific test , Enzyme Immunoassay (EIA) and 14.8% using the GeneXpert® *C. difficile* polymerase chain reaction (PCR) assay [9]. The small number of registered subjects in many studies limits the significance of the results and makes the detection of CDI in various age groups unclear. Therefore, it is recommended to have research studies with a greater significant number of subjects [10]. The emergence of COVID-19 pandemic and its impact on the health care system should be also explored. Reports of the two deadly coinfections, CDI and COVID-19 are poorly elaborated. We are trying to fill the gap by conducting this study to identify the risk factors and outcomes of CDI developed in hospitalized patients with diarrhea in the Eastern Region of Saudi Arabia during the five years of the study period with a special consideration for the diagnosed COVID-19 patients with diarrhea.

Materials and Methods

Study design and population

The Institutional Review Board (IRB) has approved this research with Reference # RA19/013/A. We used a retrospective case-control study design to identify the outcomes and risk factors of CDI-related diarrhea in patients admitted to two hospitals in the Eastern Region of Saudi Arabia between January 2017 and September 2021. The patient group includes all cases with diarrhea and positive CDI diagnosed using PCR to identify the toxigenic *C. difficile.* The control is CDI-negative patients with other infectious causes of diarrhea. The patient group included adults above 18 years old. They were further classified into three age groups: group1(young adults; age 18-35 years), group 2 (middle age, age 36-55 years), and group3 (older patients, age ≥ 56). The same age stratification was applied to the control group.

Data collection and definitions

In the present study COVID-19 cases are patients presented clinically with diarrhea and confirmed by PCR test as positive for COVID-19 infection.

Patients with severe CDI should have at least one of the following: WBC>15 x 109/L, Fever (core body temperature >38.5 ℃), Colectomy, Ileus, megacolon, peritonitis, PMC, septic shock requiring ICU admission, Serum creatinine concentration >50% above the baseline, or death [11].

In our study, HA-CDI is identified if CDI was not present on admission and was diagnosed by positive CDI assay requested at least three days after admission but before hospital discharge [12], or if patients had previous hospital stay within one month of being CDI positive. While CA-CDI is detected if positive CDI was diagnosed in the outpatient clinic or within 3 days after hospital admission in a person with no documented overnight hospital stay during the 12 weeks before sample collection [13].

The data was extracted from the electronic medical record in both hospitals by the Data Management department of the research center.

Using real-time PCR test for identification of *C. difficile* toxigenic strain

Patients' stool samples were transported immediately to the laboratory. *Clostridioides difficile* gene Xpert (Cepheid, Sunnyvale, CA) real time PCR was performed to identify the *C. difficile* toxin B gene (tcdB) according to the manufacturer's instructions [14].

Statistical analysis

After cleaning the data with a computer program written by one of the researchers in Java language, descriptive statistics were performed.

Continuous data were presented as median and interquartile range (IQR) or mean, and SD. Categorical data were recorded as frequency and percentage. For the analytical part, variables with *p* values < .05 were considered to be statistically significant. To study CDI risk factors of CDI in general or based on age group, we performed unadjusted logistic regression analysis (univariate logistic regression). The adjusted logistic regression analysis (multiple logistic regression) was conducted afterward to account for confounding between the variables. Backward elimination was employed with variables that were significant or marginally significant *(.05 <p <.1)* and not collinear excluding death and laboratory data as many laboratory results were missing. Only variables that remain significant were kept in the final adjusted models. If some variables were collinear, we chose the most significant or the general one to try first, if it was statistically insignificant, we remove it and try the next collinear variable. We kept the variables in the model that only showed statistically significant effect.

For the analysis of risk of COVID-19 coinfection and CA-CDI with HA-CDI, we used Chi-square for categorical variable and unpaired t-test for continues variables.

The data was cleaned and analyzed using Java, MS. Excel and JMP Pro 15.2.0.

Results

A total of 195 adult patients suffering from CDI-related diarrhea diagnosed between January 2017 and September 2021 were included. Their age range was between 18 and 104 years. The median age was 68 years, IQR (52-77), and 106 (54.4%) were females. Around 1320 patients with CDI-negative diarrhea were included as a control group, their age ranged between 18 and 108 years, the median age is 61 years, and IQR (46-74). Each participant was diagnosed with a minimum of one associated comorbidity. The majority of the study population; 1066 (70.4%) of 1515 total participants, 959 (72.7%) of the CDI - negative, and 107 (54.9%) CDI- positive patients were not receiving antibiotics within three months prior to the CDI test results. Of note, around 35% of the CDI- positive patients were receiving a single type of antibiotic versus 23.6% of the control group, while 10% of the CDI- positive were receiving two antibiotics versus 4% of the control group. The most frequently used antibiotics were vancomycin, metronidazole and cefuroxime. Other frequently associated comorbidities, the past history of medications and the laboratory results were listed in **table (1)**.

Missing laboratory data were excluded from the data analysis. The WBCs count and the serum creatinine level were only available for 45% of CDI- positive, and 49% of the CDI- negative patients. While the serum albumin was only available for 40% of the CDI- positive and 42% of the CDI- negative patients. The laboratory data were collected as the highest values for the WBCs count and serum creatinine level within 7 days before or after the detection of CDI. With regard to the serum albumin, it was recorded as the lowest value during the same period.

CDI-positive patients were classified into three age groups versus age matching control groups as shown in **table (1)**. A significantly higher risk of catching CDI was identified in patients related to age group 1(18-35) years (OR: 2.47, 95%CI: 1.37-4.47, *P*=.0028) and age group 3 (≥56) years (OR: 1.95, 95% CI: 1.87-3.21, *p*=.0084) compared to age group 2 (36-55) years. Further analysis of the risk factors associated with CDI in each age are shown in **tables (2, 3, 4)**.

According to the duration and past history of hospitalization, our data analysis revealed that 133 (68.21%), 49 (25.13%), and 13 (6.67%) of the CDI- positive patients were classified as HA-CDI, CA-CDI and unknown CDI (ambiguous data) respectively. The majority (68%) of CDI cases were HA-CDI, with a yearly distribution of 76 %, 69%, 72%, 52% and 79% during the period from 2017, 2018, 2019, 2020, and 2021 respectively as shown in **figure (1)**. Further data analysis revealed 132 COVID-19 patients with diarrhea; 7 cases of them were CDI positive. The risk factors associated with COVID -19, and *C. difficile* co-infection are listed in **table (5)**. A considerable group of CA-CDI patients and their associated risk factors were listed in **table (6)** compared to HA-CDI patients.

The most prominent clinical outcome of CDI-positive patients showed that 35 patients died within one month from CDI detection and the mortality rate at day 30 was 18%. With regard to COVID-19 and *C. difficile* coinfection, only one patient (14%) died within 30 days of CDI.

According to the available electronic data, patients with at least one of the following were diagnosed with severe CDI (death within one month, patients with septic shock and admitted to ICU, patients with WBCs>15 x 10^9/L, patients with megacolon or patients with PMC). WBC count was

available for around 88 CDI-positive cases and 39 (44%) of them had WBCs>15 x 10^9/L and can be categorized as severe cases. Overall severe cases could be around 67 (34%) out of 195 according to the criteria mentioned above which are available on the system. Further analysis of the risk factors associated with CDI severity is recommended.

Table 1. The unadjusted/adjusted logistic regression analysis to identify the risk factors associated with CDI among all the study population

				Unadjusted model		Adjusted model	
Variable	**All (n=1515)**	**Control (n=1320)**	**CDI (n=195)**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**p-value**
Age median (IQR)	62 (46-75)	61 (46-74)	68 (52-77)	1.01 (1.00-1.02)	**0.0108***		
Age groups							
18-35 years	249 (16.4%)	217 (16.4%)	32 (16%)	2.04 (1.15-3.64)	**0.0153***	2.47 (1.37-4.47)	**0.0028***
36-55 years	312 (20.6%)	291 (22.1%)	21 (11%)	Reference		Reference	
≥ 56 years	954 (63.0%)	812 (61.5%)	142 (72.8%)	2.42 (1.50-3.91)	**0.0003***	1.95 (1.87-3.21)	**0.0084***
Gender (Female)	824 (54.4%)	718 (54.4%)	106 (54.4%)	0.998 (0.74-1.35)	0.9927		
Hospital (Hospital 1)	1053 (69.50%)	917 (69.5%)	136 (69.7%)	1.01 (0.73-1.41)	0.9382		
Admitted to ICU	204 (13.5%)	178 (13.5%)	26 (13%)	0.99 (0.63-1.54)	0.9539		
HA- diarrhea	850 (56.1%)	717 (54.3%)	133 (68.2%)	1.80 (1.31-2.49)	**0.0003***		
Previous hospital admission	257 (17.0%)	194 (14.7%)	63 (32%)	2.77 (1.98-3.88)	**<.0001***	2.22 (1.56-3.16)	**<.0001***
Comorbidities							
Diabetes	768 (50.7%)	668 (50.6%)	100 (51.3%)	1.03 (0.76-1.39)	0.8601		
Hypertension	760 (50.2%)	651 (49.3%)	109 (55.9%)	1.30 (0.96-1.76)	0.0869		
Myocardial Infarction	75 (5%)	61 (5%)	14 (7%)	1.60 (0.87-2.91)	0.1274		
Coronary Artery Disease	28 (2%)	22 (2%)	6 (3%)	1.87 (0.75-4.68)	0.179		
CVD	858 (56.6%)	742 (56.2%)	116 (59.5%)	1.14 (0.84-1.55)	0.3893		
Pulmonary diseases	169 (11.2%)	154 (11.7%)	15 (8%)	0.63 (0.36-1.10)	0.1026		
Gastrointestinal disorders	139 (9.2%)	132 (10.0%)	7 (4%)	0.34 (0.15-0.73)	**0.0057***		
Cancer	122 (8.1%)	99 (7.5%)	23 (12%)	1.65 (1.02-2.67)	**0.0415***	1.69 (1.01-2.82)	**0.0446***
Kidney Disease	525 (34.7%)	437 (33.1%)	88 (45%)	1.66 (1.23-2.25)	**0.0011***	1.39 (1.00-.1.93)	**0.0481***
Stroke	211 (13.9%)	167 (12.7%)	44 (23%)	2.01 (1.39-2.92)	**0.0002***	1.73 (1.15-2.60)	**0.0083***
Sickle cell anemia	33(2%)	26 (2%)	7 (4%)	1.85 (0-79-4.33)	0.1542		
COVID 19	132 (8.7%)	125 (9.5%)	7 (4%)	0.36 (0.16-0.77)	**0.0091***		
Medication history							
Antibiotics	449 (29.6%)	361 (27.4%)	88 (45%)	2.18 (1.61-2.97)	**<.0001***	1.86 (1.35-2.56)	**0.0001***
Vancomycin	293 (19.3%)	222 (16.8%)	71 (36%)	2.83 (2.05-3.92)	**<.0001***		
Metronidazole	203 (13.4%)	170 (12.9%)	33 (17%)	1.38 (0.92-2.07)	0.1230		
Cefuroxime	26 (2%)	21 (2%)	5 (3%)	1.63 (0.61-4.37)	0.3333		
Antibiotics #							
0	1066 (70.36%)	959 (72.7%)	107 (54.9%)	0.51 (0.37-0.71)	**<.0001***		
1	379 (25.0%)	311 (23.6%)	68 (35%)	Reference			
2	70 (5%)	50 (4%)	20 (10%)	1.83 (1.02-3.27)	**.0417***		
PPI	524 (34.6%)	456 (34.6%)	68 (35%)	1.01 (0.74-1.39)	0.9287		
Immune suppressive	75 (5%)	66 (5%)	9 (5%)	0.92 (0.45-1.88)	0.8173		
Corticosteroids	176 (11.6%)	154 (11.7%)	22 (11%)	0.96 (0.60-1.55)	0.8757		
Lab result							
WBCs >15 x 10^9/L	230 (31.0%)	191 (29.3%)	39 (44%)	1.93 (1.22-3.03)	**0.0046***		
Creatinine >133 µmol/L	245 (33.3%)	204 (31.5%)	41 (46%)	1.85 (1.18- 2.90)	**0.0069***		
Albumin <30 gm/L	383 (60.6%)	321 (57.9%)	62 (79%)	2.81 (1.58-5.0)	**0.0004***		

Data are presented as no. (%) or median (IQR)

*Statistically significant p values <0.05, highlighted in bold

Pulmonary disease (Asthma and CPOD)

Gastroenteritis disorder (Gastroenteritis and IBD)

CVD (Hypertension, Myocardial infraction, coronary artery disease and dyslipidemia)

Table 2. The risk factors associated with CDI in old patients

Variable	All (n=954)	Control (n=812)	CDI (n=142)	Unadjusted model		Adjusted model	
				OR (95% CI)	P value	OR (95% CI)	P-value
HA- diarrhea	603 (63.2%)	497 (61.2%)	106 (74.7%)	1.87 (1.25-2.79)	**0.0024***		
Previous hospital admission	183 (19.2%)	130 (16.0%)	53 (37%)	3.12 (2.12-4.61)	**<.0001***	2.62 (1.76-3.92)	**<.0001***
Comorbidities							
Cancer	93 (10%)	73 (9%)	20 (14%)	1.66 (0.98-2.82)	0.0613	1.80 (1.02-3.17)	**0.0419***
Kidney Disease	412 (43.2%)	339 (41.8%)	73 (51%)	1.48 (1.03-2.11)	**0.0327***		
Stroke	200 (21.0%)	157 (19.3%)	43 (30%)	1.81 (1.22-2.70)	**0.0034***	1.80 (1.18-2.74)	**0.0062***
Medication history							
Antibiotics	303 (31.8%)	229 (28.2%).	74 (52%)	2.77 (1.93-3.98)	**<.0001***	2.38 (1.64-3.46)	**<.0001***
Vancomycin	222 (23.3%)	161 (19.8%)	61 (43%)	3.05 (2.09-4.43)	**<.0001***		
Metronidazole	110 (11.5%)	87 (11%)	23 (16%)	1.61 (0.98-2.65)	0.0611		
Antibiotics #							
0	651 (68.2%)	583 (71.8%)	68 (48%)	0.39 (0.27-0.57)	**<.0001***		
1	261 (27.4%)	201 (24.8%)	60 (42%)	Reference			
2	42 (4%)	28 (3%)	14 (10%)	1.68 (0.83-3.38)	0.1506		
Lab result							
WBC >15 x 10^9/L	154 (33.5%)	126 (31.8%)	28 (44%)	1.72 (1.00-2.95)	**0.0489***		
Creatinine >133 µmol/L	196 (42.8%)	162 (41.0%)	34 (54%)	1.69 (0.99-2.88)	0.0553		
Albumin <30 gm/L	293 (70.6%)	242 (67.8%)	51 (88%)	3.46 (1.52-7.87)	**0.0030***		

Data are presented as no. (%) or median (IQR)
*Statistically significant p values <0.05, highlighted in bold

Table 3. The risk factors associated with CDI in young adults.

Variable	All (n=249)	Control (n=217)	CDI (n=32)	Unadjusted model	
				OR (95% CI)	*p* value
Medication history					
Vancomycin	20 (8%)	14 (6%)	6 (19%)	3.35 (1.18-9.47)	**0.0228***
Antibiotics #					
0	187 (75.1)	163 (75.1%)	24 (75%)	1.50 (0.55-4.14)	0.4316
1	56 (22%)	51 (24%)	5 (16%)	Reference	
2	6 (2%)	3 (1%)	3 (9%)	10.2 (1.61-64.56)	**0.0136***
Lab result					
WBC >15 x 10^9/L	26 (27%)	19 (23%)	7 (50%)	3.37 (1.05-10.81)	**0.0412***

Data are presented as no. (%) or median (IQR)
*Statistically significant p values <0.05, highlighted in bold

Table 4. The risk factors associated with CDI in middle age group

				Unadjusted model	
Variable	**All (n=312)**	**Control (n=291)**	**CDI (n=21)**	**OR (95% CI)**	**P value**
Sickle cell disease	8 (3%)	6 (2%)	2 (10%)	4.99 (0.94-26.46)	0.0584
Creatinine >133 µmol/L	37 (20)	32 (19)	5 (42)	3.10 (0.92-10.41)	0.0667

Data are presented as no. (%) or median (IQR)
*Statistically significant p values <0.05, highlighted in bold

Table 5. The risk factors associated with CDI positive COVID-19 cases versus CDI negative COVID-19 patients

Variable	**All (n=132) 100%**	**CDI positive COVID-19 cases (n=7) 5.3%**	**CDI negative COVID-19 cases (n=125) 94.7%**	**P value**
Age >=50	91 (69 %)	5 (71%)	86 (69%)	0.8837
Male	66 (50%)	4 (57%)	62 (50%)	0.6977
CVD	73 (55%)	4 (57%)	69 (55%)	0.9199
Diabetes	49 (37%)	1 (14%)	48 (38%)	0.1922
Pulmonary disease	14 (11%)	1 (14%)	13 (10%)	0.7453
Stroke	7 (5%)	1 (14%)	6 (5%)	0.2758
Cancer	6 (5%)	1 (14%)	5 (4%)	0.2036
Antibiotics	15 (11%)	2 (29%)	13 (10%)	0.1404
PPI	55 (42%)	3 (43%)	52 (42%)	0.9477
Length of hospital stay (mean, SD)	(11, 15)	(7, 4)	(12, 15)	**0.0488***
Length of hospital stay after CDI (mean, SD)	(6, 9)	(3, 3)	(6, 9)	**0.0246***
CA-diarrhea	77 (58%)	4 (57%)	73 (58%)	0.9710

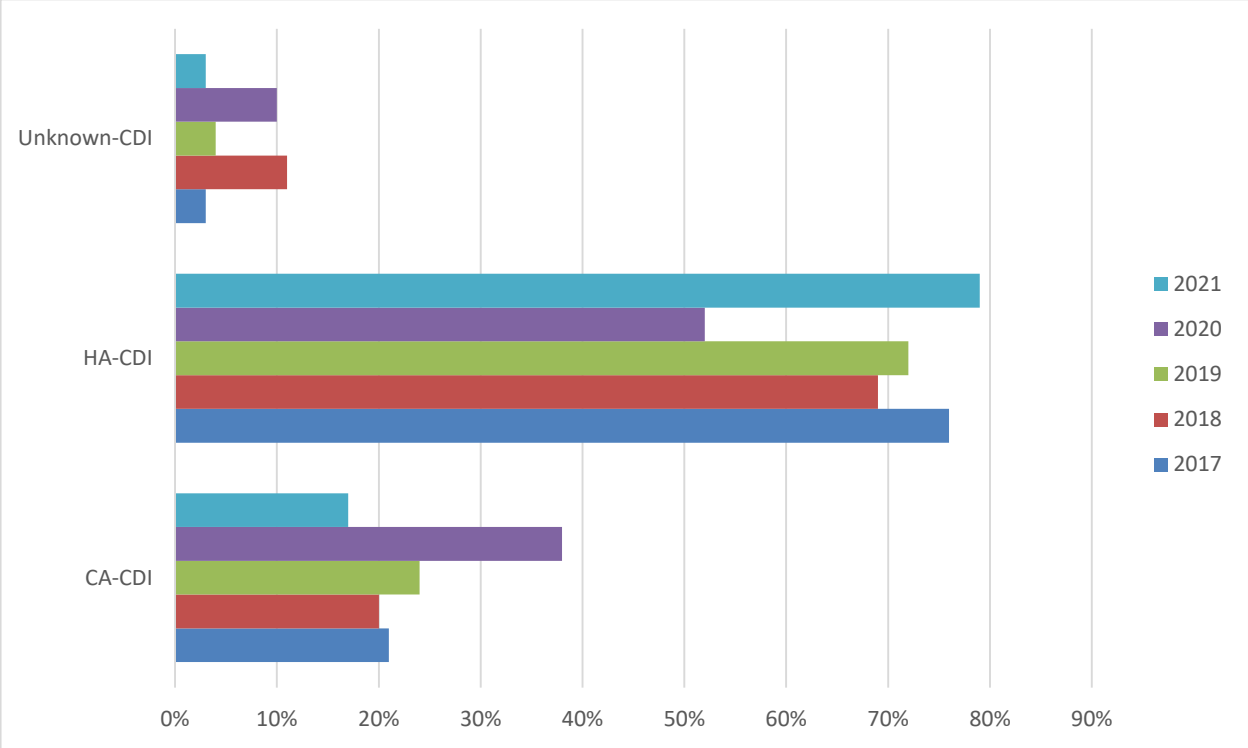
Data are presented as no. (%) or mean± standard deviation (SD)
*Statistically significant p values <0.05, highlighted in bold

Table 6. Risk factors associated with HA-CDI versus CA-CDI

Variable	**All (n=182)**	**CA (n=49)**	**HA (n=133)**	**p value**
Age Median (IQR)	69 (53-77)	61 (35-74)	70 (57-78)	**0.0091***
Age >=50	142 (78%)	29 (59%)	113 (84.9%)	**0.0002***
Female	100 (55%)	31 (63%)	69 (52%)	0.1709
CVD	109 (60%)	24 (49%)	85 (64%)	0.0683
Diabetes	93 (51%)	18 (37%)	75 (56%)	**0.0142***
Hypertension	102 (56%)	22 (45%)	80 (60%)	0.0659
Pulmonary disease	13 (7%)	1 (2%)	12 (9%)	0.1048
COVID- 19	7 (4%)	4 (8%)	3 (5%)	0.0660
Sickle cell	7 (4%)	3 (6 %)	4 (3%)	0.3324
Stroke	40 (22%)	6 (12%)	34 (26%)	0.0543
Cancer	23 (13%)	4 (8%)	19 (14%)	0.2702
Kidney disease	82 (45%)	10 (20%)	72 (54%)	**<0.0001***
Antibiotics	80 (44%)	9 (18%)	71 (53%)	**<0.0001***
PPI	61 (34%)	10 (20%)	51 (38%)	**0.0230***
Immune suppressant	8 (4%)	0 (0%)	8 (6%)	0.0791

Data are presented as no. (%) or range
*Statistically significant p values <0.05, highlighted in bold

Figure 1. Registered cases with different types of CDI across the study period



Discussion

In this study, the risk factors for CDI were assessed generally in all the patients, and the results showed a significant biphasic increase in CDI among the young adults (18-35 years) (OR: 2.47 CI: 1.37-4.47, *P*=.0028) and older patients ≥ 56 years (OR:1.95, CI: 1.87-3.21, *P*=.0084) compared to middle-aged adults. Our study took the initiative to assess the risk factors associated with CDI in patients with diarrhea, living in the Eastern Region of Saudi Arabia, according to their age group. Thus, further data analysis was done by classifying the patients into three age groups. Likewise, the identified COVID-19 patients with diarrhea were also investigated to explore the possibility of acquiring *C. difficile* coinfection and its associated risk factors. A point of strength of the current study was the large group of retrospective controls that allowed for a strong predilection matching (around 7:1).

The adjusted model indicated that previous hospital admission, kidney disease, antibiotics administration, cancer, and stroke were the most frequently associated risk factors in all the patients' group. Furthermore, a marginally significant association of sickle cell disease (SCD) with CDI was identified in middle-aged patients. The adjusted logistic regression analysis has also highlighted previous hospital admission, cancer, stroke, and antibiotics intake as the most common risk factors in older patients. The use of antimicrobial medication and increasing age in addition to other comorbidities such as liver cirrhosis, pulmonary disease, heart disease, renal dialysis, and immunocompromised status are the most common risk factors for CDI in general [15]. Cancer was found to be an eminent and significant CDI risk factor among all the current study population, and also in group 3 patients (≥ 56 years) which is consistent with the results of other studies that had mentioned malignancy as a potential extra-colonic CDI risk factor [16]. The patchy prevalence of SCD varies significantly in different regions of the Kingdom of Saudi Arabia. The eastern region shows the highest prevalence, followed by the southwestern region. The reported prevalence rate reaches up to 27% [17]. It was observed that *C. difficile* incidence among patients with SCD is increasing, moreover, the authors were able to delineate numerous significantly associated factors that increase the rate of exposure to CDI [18]. This was agreed with our study where a marginally significant (10%, *p*= .0584) number of CDI patients related to the middle age group were diagnosed with SCD (OR: 4.99, 95% CI: 0.94-26.46).

In our study, the adjusted regression analysis showed significant association between the administration of one or two antibiotics and CDI in the whole study population and in older patients ≥ 56 years. The results were insignificant in young adults and middle aged; only young adults administering two antibiotics were at higher risk of having CDI. In the unadjusted model, the most frequently used antibiotic in all the CDI-positive patients was vancomycin. Further data analysis of the three studied age groups confirmed the same significant relationship between vancomycin and CDI in young adults and older patients ≥ 56 years. Antimicrobial treatment is crucial in the pathogenesis of CDI. The development of CDI has been linked to nearly all antimicrobial classes. Several factors may influence the association of CDI with certain antimicrobials. For example, the prevalence of highly resistant strains to commonly used antimicrobials in certain locality and the increased frequency of using such antimicrobials [19].

In the 1970s and 1980s, Clindamycin was the antibiotic of choice for treating anaerobic infections. Nevertheless, in 1977, emerging toxigenic strains of *C. difficile* with clindamycin-resistance were identified as the source of hamster clindamycin-associated colitis [20]. According to the previous practice guidelines of the American College of Gastroenterology (ACG), oral metronidazole and vancomycin were recommended for treating mild-to-moderate cases with CDI while vancomycin was also recommended for severe ones [21]. In 2018 the IDSA/SHEA published a recommendation indicating using either vancomycin or fidaxomicin for non -severe CDI. However, metronidazole may be prescribed in areas where the availability of vancomycin or fidaxomicin is limited [22].

In our study, the significant increase of CDI in patients with past history of vancomycin treatment could be an alarm to prevaricate the possibility of developing vancomycin-resistant mutants through judicious use of vancomycin and metronidazole in concert with environmental and infection control–related efforts. However, underreporting of the antibiotics that patients used in addition to the lack of laboratory data for the antibiotic-sensitivity tests, or the incomplete dosage of metronidazole and/or vancomycin could be other contributing factors that limit our study. In the current study, it was also noticed that simultaneous use of multiple antimicrobials is associated with an increased risk of CDI. Nearly 10 % of CDI-positive patients were using two antibiotics versus 4% CDI-negative group, and also when compared to patients using one antibiotic (OR: 1.83; 95%CI: 1.02-3.27, *P*=.0417). Our results were also consistent with other research group [23] who reported that the incidence of CDI is increased with increasing the number of administered antibiotics (RR: 2.01; 95% CI: 1.67–2.40).

Most of CDI- positive cases included in our study were categorized as HA-CDI cases (68%). The adjusted logistic regression revealed that around 32% of CDI patients had a history of previous hospital admission within one month prior to a positive test (OR: 2.22, 95%CI: 1.56-3.16). One study conducted in Saudi Arabia elucidated an increasing trend of community onset-healthcare-associated CDIs from 17% in 2001 to 20% in 2018, while the healthcare facility–onset-associated CDI was stable and the CA-CDI was decreasing [24]. In our study, as shown in **Figure 1**, the trend of HA-CDI was almost stable 76 %, 69%, 72%, from 2017 till 2019. Then, a marked reduction was noticed in 2020 (52%). This decline is statistically significant whenever (*p*=.0057) compared to other years and it may reflect the strict infection control procedures that were implemented in 2020 during the first wave of the COVID-19 pandemic. Perhaps the excessive or inappropriate CDI testing prior to COVID-19 or more careful testing during the pandemic, in addition to the sharp decline in the hospital admissions, likely as a result of lockdown measures and the fear of infection, could be a valid explanation to the decreased or stable trend of HA-CDI during the COVID-19 pandemic [25]. During the first wave of the pandemic, several hospitals were facing unusual circumstances such as; staffing challenges, increased patient case- load, limitations of physical space, and inadequate personnel availability that may have an impact on the effectiveness of the standard precautions of the infection prevention and control program [26]. This could also explain the trend of increasing CA-CDI during 2019 and 2020 which was noticed also in our study. CA-CDI group were younger in age (< 50 years old). Thus, the young patients represent a significantly higher percentage of CA-CDI compared to HA-CDI group. On the opposite side, diabetes mellitus, kidney diseases, PPI, antibiotics intake and old age ≥50 years were significantly associated with the increased risk of HA-CDI. All

age groups are susceptible to COVID-19 infection, but it has been noted that elderly and frail people with underlying illnesses and chronic disorders are at greater risk for contracting the virus [27].

In our study, the majority of identified COVID-19 patients with diarrhea were above the age of 50 years and the commonly associated medical conditions reported were; CVD (55%), administration of PPI (42%) and diabetes mellitus (37%). With further data analysis, few COVID-19 cases 7(5%) were CDI positive, 4 (57%) were diagnosed with CA-CDI, 4 (57%) of them are male, 71% are above the age of 50 years with a high percentage of associated CVD (57%), PPI (43%) and antibiotic intake (29%). This was consistent with other study where CVD was a commonly reported comorbidity (58%) in patients with both COVID -19 and *C. difficile* coinfection [28]. It was noticed that the first publication regarding the coinfection only described nine cases of CDI in COVID-19 patients [29] which is consistent with our study. Another research revealed that 10% of COVID-19 patients got *C. difficile* coinfection. The authors of the study reported patient age, length of hospital stay, using antibiotics other than azithromycin, developing diarrhea during hospitalization, and coexistence of chronic kidney disease or nervous system disease as risk factors for developing CDI [30]. In our study, the total length of hospital stays and the length of stay after CDI-positive test in COVID-19 patients with *C. difficile* coinfection were significantly lower than that for CDI negative COVID-19 patients which is not consistent with previous reports.

Although non-significant, death (n=1, 14%) among COVID-19 patients with *C. difficile* coinfection was higher than the other group (n=6, 6%); which is consistent with the previously published data [28]. However, the small number of cases identified in our study represents a major limitation. On the other hand, American researchers reported a higher mortality rate in COVID-19 patients with *C. difficile* coinfection compared with CDI negative COVID-19 patients, but only five patients were analyzed in their study [31]. Thus, further investigation is required to explain the impact of COVID-19 pandemic on the healthcare system including hospital admission, infection control procedures, antibiotic use, and associated infections. Putting into consideration that the data available was till September 2021, which may be another contributing limitation to the present study.

The unavailability of complete laboratory data was an obstacle to determine the actual number of patients with severe CDI. **Miller et al** [32]. considered measuring leucocytic count, albumin level together with other 3 clinical variables; age, systemic antibiotics treatment, and body temperature, as prognostic predictors for the patients' response to CDI treatment with fidaxomicin and vancomycin. According to the criteria of CDI severity mentioned before, severe cases could be around 67 (34.4%) out of 195. Additionally, 17 patients (9%) had recurrent CDI within a period ranging from 2 - 8 weeks. The majority of recurrent CDI cases were related to old age group. The associated comorbidities and complications need further investigation to elucidate their actual relatedness to CDI severity and/or recurrence.

In our retrospective study, the most prominent outcome associated with CDI was death, other less common clinical outcomes include septic shock, dehydration, and toxic megacolon. 18% of the *C. difficile* infected adult patients died within one month of having a positive test. Hence, CDI may be a contributing cause of death in those cases. This rate is higher than the rate reported in other countries such as France (mortality at Day30: 4%) and the Netherlands (7.5% at Day30) [33,34]. Several studies have reported an increase in mortality since 2000 [35-37]. The 30-day mortality rate in other European countries, varied between 6.8% in Ireland to 42% in the UK [38]. It is difficult to compare the mortality rates in different countries due to the heterogeneous data published in terms of definitions, patient groups, study quality, duration of follow-up, and information collected. In our study, eight CDI-positive patients were diagnosed with unspecified sepsis during the same hospital visit. Nevertheless, fifty-five CDI-negative patients had sepsis. Therefore, it is still uncertain whether CDI is a leading cause of sepsis or not as being inferred from two epidemiologic studies conducted in 2015 and 2018 to examine the effect of CDI and broad-spectrum antibiotics on the development of sepsis. Those studies mentioned that the gut microbiome disruption may be a risk factor for sepsis but none of them had characterized the gut microbiome of the included patients [39,40].

Conclusions

The majority of CDI- related diarrhea were HA-CDI in our study area. The trend of HA- CDI during the five years of the study period was almost

stable except in 2020 with the first wave of COVID-19 pandemic where the rate of reported HA-CDI was lower compared to other years. The risk factors associated with CDI differ according to the age group. A high percentage of COVID-19 patients with CDI had CVD, history of administration of PPI and antibiotics. There is an urgent need to study the implication of the COVID-19 pandemic on the healthcare system to benefit from this experience in reducing the occurrence of hospital- acquired infections.

Declarations

Funding

This research received no external funding.

Institutional Review Board Statement

Data was collected electronically from the medical records and access is permitted only after our Research Center IRB approval (Reference # RA19/013/A).

Informed consent statement

As a retrospective case control study this was waived by IRB.

Data Availability Statement

Data will be provided upon request.

Conflicts of interest

The authors declare no conflict of interest.

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