ORIGINAL ARTICLE

Health care associated Clostridioides difficile infection and colonization in patients admitted at tertiary care hospital Pakistan

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Abstract

Objective: To evaluate the epidemiology of clostridioides difficile infections and colonisation in a tertiary-care setting.

Method: The cross-sectional study was conducted at the Combined Military Hospital, Rawalpindi, Pakistan, from June 1, 2017, to October 31, 2019, and comprised adult patients admitted in high-risk units of the hospital for any disease experiencing watery stools after 48 hours of hospital admission and passing more than 3 stools per day with no other recognised aetiology. Stool samples of the participants, diagnosed with antibiotic associated diarrhoea, were submitted for glutamate dehydrogenase antigen assay and clostridioides toxin A/B assay detected by enzyme-linked immunosorbent assay and clostridioides difficile toxin gene detection by polymerase chain reaction. Clostridium difficile-associated diarrhoea was diagnosed by a positive toxin assay or polymerase chain reaction. Data was analysed using SPSS25.

Results: Of the 715 subjects, 322(45%) were males and 393(55%) were females. The overall mean age was 56.64±8.57 years, and 488(68.3%) were aged <60 years, while 227(31.7%) were aged >60 years. The incidence of clostridioides difficile-associated diarrhoea was found in 10(1.4%) patients and was highest in oncology unit 3(4.3%). No positive case was detected from the high dependency unit and the surgical ward. All the10(1.4%) positive cases were on >2 antibiotics with a combination of oral vancomycin and intravenous metronidazole. Mortality rate was significantly higher in the positive cases compared to those with clostridioides difficile colonisation ($p<0.05$).

Conclusion: The incidence of clostridioides difficile-associated diarrhoea was found to be low.

Keywords: Clostridioides difficile, Clostridium difficile, Antibiotic-associated diarrhoea, Hospitalized elderly patients, Antimicrobial therapy. (JPMA 72: 610; 2022)

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Introduction

Clostridioides difficile (C. Diff) infection (CDI) is among the most common healthcare-associated infections (HCAIs). The clinical features have a variable range from asymptomatic colonisation to self-limiting diarrhoea to more life-threatening conditions, like toxic megacolon and fulminant colitis.[1] During the last couple of decades, C. Diff has emerged as the chief cause of antibiotic-associated nosocomial diarrhoea and the incidence and severity has been rising in recent years.[2] The prevalence of disease in various regions varies from 20% to 30%. The disease has now been graded as the 5th most important cause of HCAIs and significant scientific issues fronting healthcare epidemiology.[3,4]

Antibiotics represent the major but modifiable known risk factor for the disease. Almost all classes of antibiotics tend to increase the risk of infection, but certain classes are found to have strong association with the disease, like third-generation cephalosporin (79.2%) and clindamycin (28.5%), second-generation cephalosporin (48.4%), quinolones (64%) and penicillin combinations (54%).[5] Researchers have also evaluated that increased duration, number and dosage of antibiotics poses high risk for the acquisition of the disease.[6] Antibiotic-associated diarrhaea (AAD) caused by C. Diffis due to the alteration in the normal gut flora, resulting in-growth of this opportunistic organism. The prime virulence factors for this pathogen include toxins A and B,[7,8] among which the common type of toxin is toxin A-positive/toxin B-positive strain (77.5%), whereas toxin A-negative/toxin B-positive strains are less common (15.4%).[9]

CDI diagnosis involves various microbiological methods with variable specificity and sensitivity. Diagnostic modalities include molecular methods, cell culture-cytotoxicity neutralisation assays, enzyme-linked immunosorbent assay (ELISA) and lateral flow immunochromatographic (ICT). ELISA and ICT-based tests

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detect the presence of glutamate dehydrogenase (GDH) antigen and toxin A/B. GDH is a group of enzymes which plays an important role in C. Diff colonisation and pathogenesis of the disease.[10] Many laboratories prefer enzyme-based immunological methods that detect GDH, but the approach has much lower specificity as a positive test cannot differentiate between colonisation and actual disease, and the final diagnosis of CDI is based on the presence of free toxins in patient's stool samples. The most widely used laboratory test is still the identification of toxins A and B via ELISA or by ICT device.[11]

Since CDI remains poorly investigated in Pakistan, the current study was planned to evaluate the epidemiology of CDI and colonisation in a local tertiary-care setting.

Materials and Methods

The cross-sectional prospective study was conducted at the Combined Military Hospital (CMH), Rawalpindi, Pakistan, from June 1, 2017 to October 31, 2019. The CMH is a 2200-bed teaching hospital comprising health care centres and units for various specialties. After approval from the institutional ethics review board of the Armed Forces Institute of Pathology (AFIP), Rawalpindi, 7 high-risk CMH units were selected, including medical and surgical wards comprising 174 beds, 2 intensive care units (ICUs)having 62 beds,[1] oncology ward with 82 beds, 1 bone marrow transplant centre (BMTC) comprising 50 beds and 1 high dependency unit (HDU) consisting of 20 beds. The sample size was calculated using Raosoft sample size calculator[12] by taking prevalence of C. Diff-associated diarrhoea (CDAD) as 29.1[13] margin of error 3.5% and confidence interval (CI) 95%. To cover up from missing data, the sample was inflated by an additional 100 patients.

Throughout the study period, one of the authors actively surveyed all potential subjects hospitalised in the high-risk units who developed diarrhoea after 48 hours of hospitalisation and who were on antibiotic therapy based on a predefined protocol. Those included were adult patients admitted in the high-risk units for any disease experiencing watery stools after 48 hours of hospital admission and passing more than 3 stools per day with no other recognised aetiology. Patients with non-diarrhoeal stools, and those who were not on any antibiotics and in whom the cause of diarrhoea was well established were excluded.

After taking informed consent, data was collected from all the subjects regarding details of hospital admission, duration of hospital stay before diarrhoea, antibiotics usage, number and dose of antibiotics taken. Faecal samples were tested for C. Diff GDH antigen and toxins A and B.

The detection of toxins was done using ELISA (Xpect™) or real time polymerase chain reaction (PCR). The presence of GDH antigen in stool was labelled as C. Diff colonisation.

Antibiotics prescribed during hospitalisation were reviewed retrospectively in toxin-positive as well as toxin-negative stool samples. Antibiotics were grouped into following classes: penicillin, cephalosporin, quinolones, clindamycin and miscellaneous comprising tetracycline, aminoglycoside, carbapanem, etc.

All stool samples were tested by an enzyme immunoassay for the presence GD Hantigen (C. DiffCHEK™-60, United States), and all positive samples were tested for the presence of toxins A and B by enzyme immunoassay (Xpect™). Toxin A/B assay has 85.9% sensitivity and 99.5% specificity, while the GDH assay provides 97.6% sensitivity and 90.1% specificity.[14]

The real time PCR was done according to the manufacturer's instructions. All GDH-positive specimens were tested with Gene Xpert C. Diff PCR assay. Gene Xpert is a nucleic acid amplification test (NAAT) multiplex PCR with multiple gene targets, including toxin b (tcdB), binary toxin (cdtA) and the toxin c (tcdC) deletion at base 117 associated with the ribotype 027 strain, a predictor of severe CDI and mortality. This assay works on the principal of 3 easy steps; first, the stool specimen is taken into a swab, next it is placed in sample reagent after the capped sample and reagent mixture were vigorously mixed by vortex for about 10s. Later, all the liquid from the sample reagent mixture was shifted by large transfer pipette into the 'S' chamber of the cartridge. Reagents 1 and 2 were added to the respective chambers of the test cartridge followed by closure of the lid. The cartridge was placed in Gene Xpert instrument after scanning. Results were interpreted as negative, positive or invalid.

Data was analysed using SPSS 25. Mean and standard deviations were computed for quantitative variables, and frequency and percentage were calculated for qualitative variables. Chi square and Fisher exact test were applied to check association between the qualitative variables, as appropriate. Mean comparison was done by independent t-test. Odds ratios (ORs) were calculated with the help of univariate binary logistic regression. $P \leq 0.05$ was considered significant.

Results

Of the 715 subjects, 322(45%) were males and 393(55%) were females. The overall mean age was 56.64±8.57 years, and 488(68.3%) were aged <60 years, while 227(31.7%) were aged >60 years. CDAD was found in 10(1.4%) patients, while 26(3.63%) had C. Diff colonisation only (Figure).

Table-1: Baseline characteristics of patients with antibiotic-associated diarrhoea (AAD).

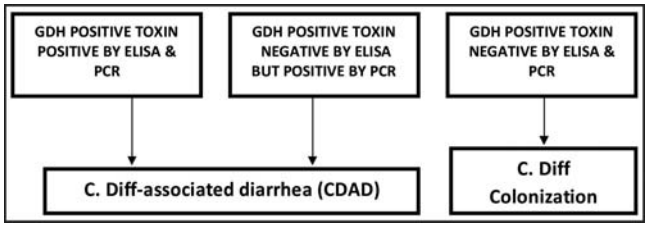
	n(%)
Age (years); Mean±SD	56.64±8.57
Age Group	
≤60 years	488(68.3)
>60 years	227(31.7)
Gender	
Male	322(45)
Female	393(55)
Sepsis	
Yes	42(5.9)
No	673(94.1)
Abdominal Surgery ForCholethiasis	
Yes	39(5.5)
No	676(94.5)
Hypertension	
Yes	107(15)
No	608(85)
Diabetes Mellitus	
Yes	107(15)
No	608(85)
Chronic Kidney Disease	
Yes	27(3.8)
No	688(96.2)
Chronic Liver Disease	
Yes	15(2.1)
No	700(97.9)
Antibiotic given after CDAD (n=26)	
Yes	11(42.3)
No	15(57.7)
Combination therapy Vs Monotherapy (n=11)	
I/V Metro + Oral Vanco	3(27.3)
Oral Vanco	8(72.7)
Survival Status	
Discharge	711(99.4)
Expired	4(0.6)
Chemotherapy Use	
Yes	10(1.4)
No	705(98.6)
H2 blockers Use	
Yes	19(2.7)
No	696(97.3)
Abdominal Surgery	
Yes	695(97.2)
No	20(2.8)
Lukemia	
Yes	3(0.4)
No	712(99.6)
Lymphoma	
Yes	1(0.1)
No	714(99.9)
Bone Marrow Transplant	
Yes	3(0.4)
No	712(99.6)
C diff colonization	
Yes	26(3.6)
No	689(96.4)
CDAD	
Yes	10(1.4)
No	705(98.6)

SD: Standard deviation, CDAD: Clostridium difficile-associated diarrhoea, C. Diff: Clostridium difficile, I/V: Intravenous.

Table-2: Association of Clostridium difficile-associated diarrhoea (CDAD) with risk factors.

	CDAD		P-value
	Yes	No	
Age (Years); mean±SD	56.72±8.60	51.00±1.94	0.000*
Age Group			
≤60 years	10(100)	478(67.8)	0.035**
>60 years	0(0)	227(32.2)	
Gender			
Male	4(40)	318(45.1)	1.000**
Female	6(60)	387(54.9)	
Abdominal Surgery ForCholethiasis			
Yes	8(80)	31(4.4)	0.000**
No	2(20)	674(95.6)	
Survival Status			
Discharge	8(80)	703(99.7)	0.001**
Expired	2(20)	2(0.3)	
Chemotherapy Use			
Yes	2(20)	8(1.1)	0.007**
No	8(80)	697(98.9)	
H2 blockers Use			
Yes	3(30)	16(2.3)	0.002**
No	7(70)	689(97.7)	
Abdominal Surgery			
Yes	9(90)	686(97.3)	0.248**
No	1(10)	19(2.7)	

*Independent t test was applied.
**Fisher exact test was applied.
P-value ≤0.05 considered as significant.
SD: Standard deviation.



GDH: Glutamate dehydrogenase, ELISA: Enzyme-linked immunosorbent assay, PCR: Polymerase chain reaction.

Figure: Flow-chart showing criteria for Clostridium difficile-associated diarrhoea (CDAD) and Clostridium difficile colonisation.

There were 69(9.65%) cases in the oncology unit, 23(3.2%) medical ward, BMTC69(9.6%), medical intensive care (MITC) 292(41%), surgical intensive care (SITC) 240(33.5%), and the remaining 22(3%) were in HDU and surgical ward.

The CDAD incidence was highest in the oncology unit 3(4.3%), followed by medical ward 1(4.3%), BMTC 1(1.4%), MITC 3(1.02%), and SITC 2(0.8%). No positive case was detected either at HDU or the surgical ward.

Overall, 117(16.4%) patients were on cephalosporin, 238(33.3%) were on penicillin, 249(34.8%) were on

Table-3: Association of Clostridium difficile (C.Diff) colonisation with risk factors.

	C Diff colonization		P-value
	Yes	No	
Age (Years)	52.46±2.90	56.78±8.68	0.000*
Age Group			
≤60 years	26(100)	462(67.1)	0.000
>60 years	0(0)	227(32.9)	
Gender			
Male	4(15.4)	318(46.2)	0.002
Female	22(84.6)	371(53.8)	
Abdominal Surgery ForCholethiasis			
Yes	24(92.3)	15(2.2)	0.000**
No	2(7.7)	674(97.8)	
Survival Status			
Discharge	24(92.3)	687(99.7)	0.000**
Expired	2(7.7)	2(0.3)	
Chemotherapy Use			
Yes	2(7.7)	8(1.2)	0.048**
No	24(92.3)	681(98.8)	
H2 blockers Use			
Yes	3(11.5)	16(2.3)	0.028**
No	23(88.5)	673(97.7)	
Abdominal Surgery			
Yes	25(96.2)	670(97.2)	0.528**
No	1(3.8)	19(2.8)	

Chi-Square test was applied.
*Independent t test was applied. **Fisher exact test was applied.
P-value ≤0.05 considered as significant.

Table-4: Odds ratio for Clostridium difficile-associated diarrhoea (CDAD).

	p-value	OR (95% CI)
Age Group		
>60 years	0.995	0.00 (NA)
≤60 Years®		1
Gender		
Male	0.748	0.811(0.227-2.900)
Female®		1
Abdominal Surgery For Cholethiasis		
Yes	0.000	86.968(17.721-426.79)
No®		1
Survival Status		
Discharge	0.000	0.11(0.001-0.091)
Expired®		1
Chemotherapy Use		
Yes	0.000	21.781(3.983-119.110)
No®		1
H2 blockers Use		
Yes	0.000	18.455(4.371-77.930)
No®		1
Abdominal Surgery		
Yes	0.198	0.249(0.030-2.068)
No®		1

®Reference group.
Univariate binary logistic regression was applied. P-value ≤0.05 considered as significant.
OR: Odds ratio, CI: Confidence interval.

Table-5: Odds ratio (OR) for Clostridium difficile (C.Diff) colonisation.

	p-value	OR (95% CI)
Age Group		
>60 years	0.995	0.000(NA)
≤60 years®		1
Gender		
Male	0.005	0.212(0.072-0.622)
Female®		1
Abdominal Surgery ForCholethiasis		
Yes	0.000	539.20(116.692-2491.492)
No®	1	
Survival Status		
Discharge	0.001	0.035(0.005-0.259)
Expired®		1
Chemotherapy Use		
Yes	0.017	7.094(1.429-35.208)
No®		1
H2 blockers Use		
Yes	0.01	5.486(1.493-20.157)
No®		1
Abdominal Surgery		
Yes	0.742	0.709(0.091-5.508)
No®		1

®Reference group.
Univariate binary logistic regression was applied.
P-value ≤0.05 considered as significant.

quinolones, and 111(15.5%) patients were on miscellaneous antibiotics. All the 10(1.4%) CDAD-positive patients were treated with antibiotics; 3(30%) were on combination therapy of intravenous (IV) metronidazole plus oral vancomycin, and 7(70%) were on oral vancomycin monotherapy. Regarding clinical outcome, mortality rate was significantly higher in CDAD-positive patients compared to those with C. Diff colonisation.

There was significant association of CDAD with age group (p=0.035), abdominal surgery for cholethiasis (p=0.000), survival status (p=0.001), chemotherapy use (p=0.007) and H2 blockers use (p=0.002) (Table-2). Significant association of C difficile colonisation was found with age group (p=0.000), gender (p=0.002), ward (p=0.000), abdominal surgery for cholethiasis (p=0.000), chemotherapy use (p=0.048) and H2 blockers use (p=0.028) (Table-3).

Male patients were less likely to have CDAD compared to female patients (OR: 0.811) (Table-4). Detailed odds for C. Diff colonisation were also noted (Table-5).

Discussion

C. Diffis a prime cause of nosocomial and iatrogenic disease. Accordingly, it is important to establish its diagnosis as promptly as possible.[15,16] During the past 20 years, the organism has emerged as a major cause of

antibiotic-associated nosocomial diarrhoea and has been responsible for large outbreaks in hospital settings.

The prevalence of CDAD is global and the incidence varies from place to place. The current 2-year, prospective, active surveillance study of patients with AAD in high-risk units at a large teaching hospital demonstrated a lower incidence of CDAD. These result were different from an increasing incidence in other parts of the world.[17] Previous studies have indicated that there is paucity of data regarding CDI prevalence in this region.[18,19] Another metanalysis suggests that C. Diff incidence in East Asia is 19.5%, with the lowest being in Japan and South Korea (1.5% to 2.5%) and is as high as 15% to 25%in China. Similar difference is seen in southeast Asia where the incidence is 10.5%; the highest in Thailand 43% and the lowest 1.5% in Indonesia.[20] A very high prevalence of CDAD (29.18%) in comparison to our study was reported from Pakistan,[13] but the methodology used for diagnosis was nonspecific culture technique. The culture lacks specificity due to the possible faecal carriage of non-toxigenic isolates, which cannot differentiate between actual disease and colonisation.[21,22]

Moreover, neither attributable mortality nor morbidity of CDAD was recognised in the current study. The attributable mortality ranged between 5.7% and 6.9% in previous studies probably because of epidemic hypervirulent NAP1/B1/027 or North American pulse-fild gel electrophoresis type1, restriction endonuclease analysis type B1, polymerase chain reaction ribotype 027, specific strain of C. Diff in those specific regions. This was associated not only with higher incidence, but also with the fatal outcome.[23,24] However, the NAP1/027 strain has not been identified in this region.

The current study coupled the use of a sensitive GDH antigen assay, detection of toxin gene by PCR and toxin detection by ELISA. Toxin detection by ELISA was found to be less sensitive and missed more than half of the cases which were later found positive by PCR. These findings are consistent with literature.[25]

CDAD patients in the current study were treated with monotherapy of oral vancomycin, with only a few having taken IV metronidazole along with oral vancomycin which is in accordance with other studies.[26]

Future studies with a large sample size are needed to confirm the findings of the current study. Future studies should also look for the specific strain type of C. Diff and to study the intestinal microbiota to explore the presence of all the risk factors, especially because despite excessive use of antibiotics the incidence of C. Diffws found to be low in the current study.

Conclusion

The incidence of CDAD was found to low compared to other parts of the world.

Disclaimer: The text is based on an academic dissertation and part of it was presented at the 39th Annual Pakistan Association of Pathologist (PAP) Conference in Peshawar, Pakistan in November 2017.

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