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ORIGINAL ARTICLE

Risk factors for *Clostridium difficile*-associated diarrhea among hospitalized adults with fecal toxigenic *C. difficile* colonization

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Clostridium difficile colonization;
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Background: Patients with toxigenic *Clostridium difficile* colonization (tCDC) are at risk of developing *C. difficile*-associated diarrhea (CDAD). However, the risk factors of hospitalized patients with tCDC developing CDAD are not clear.
Methods: We conducted an 18-month prospective study at a medical ward in a district hospital in southern Taiwan. Within 48 hours of admission, weekly stool samples from asymptomatic

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Risk factors;
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Proton-pump inhibitors

hospitalized patients were obtained to detect fecal CDC. A polymerase chain reaction for *tcdB* was performed to determine toxigenic isolates. CDAD was diagnosed if the patient had diarrhea and toxigenic *C. difficile* present in a stool sample.
Results: A total 483 patients with stool samples were eligible for the study. Eighty-six (17.8%) patients had tCDC after screening, of whom 14 (16.3%) developed CDAD during follow-up. Among those with tCDC, patients with subsequent CDAD were more likely to have diabetes mellitus ($p = 0.01$) and to have received piperacillin–tazobactam ($p = 0.04$), or proton-pump inhibitors (PPIs; $p = 0.04$) than those without developing CDAD. The variables were statistically significant as determined by multivariate analysis. However, the 60-day crude mortality rates among tCDC patients with and without subsequent development of CDAD were similar.
Conclusion: Diabetes mellitus and recent receipt of piperacillin–tazobactam or PPIs are independent risk factors for the development of CDAD among hospitalized patients with tCDC.

Introduction

Clostridium difficile is a major cause of antibiotic-associated diarrhea in hospitalized patients and causes a variety of clinical manifestations, ranging from asymptomatic carriage, infectious diarrhea, pseudomembranous colitis, toxic megacolon, to death. The incidence of *C. difficile*-associated diarrhea (CDAD) is increasingly recognized worldwide.[1–5] In Taiwan, the incidence of CDAD is also increasing.[6] The incidence of CDAD was 45 cases per 100,000 patient-days at the National Taiwan University Hospital in 2010, a tertiary hospital in northern Taiwan,[7] and 42.6 cases per 100,000 patient-days at the National Cheng Kung University Hospital in 2007–2008, a tertiary hospital in southern Taiwan.[6]

The risk factors for CDAD include antimicrobial exposure, advanced age, prior hospitalization, use of feeding tubes, gastrointestinal surgery,[5] and the use of proton-pump inhibitors (PPIs). Most classes of antibiotic exposure have been linked to CDAD, particularly third-generation cephalosporins, clindamycin, or fluoroquinolones. Besides antibiotic exposure, we had identified fecal *C. difficile* colonization (CDC) as a risk factor for CDAD.[8] We found that the prevalence of toxigenic *C. difficile* colonization (tCDC) among hospitalized patients was 16.7%, of whom 17.9% developed CDAD.[8] Michelle et al also showed 20% of initial asymptomatic carriers of toxigenic *C. difficile* isolates developed CDAD during follow-up.[9] Asymptomatic *C. difficile* carriers also had higher rates of skin (61% vs. 19%) and environmental (59% vs. 24%) contamination than did noncarriers.[9] *C. difficile* could be identified from a variety of surfaces in the hospital environment,[10] which might play a role in the transmission of *C. difficile*. Thus, CDC may be a significant issue in controlling the spread of *C. difficile* in hospital settings.

The reported prevalence of CDC varied in different populations, such as 7.6% in healthy adults,[11] 0.6-10% in hospitalized patients, and 51% in long-term care residents.[9] In the limited literature, the risk factors for CDC were similar to those of CDAD, including previous antibiotic exposure[10,12] and a history of CDAD.[9] The most commonly identified offending antibiotics were cephalosporins and fluoroquinolones.[10,12] Our previous study reported the exposure of more than one class of antibiotics was associated with CDC.[8] Besides antibiotics exposure, Loo et al found previous hospitalization, use of chemotherapy, PPIs, or H_2 blockers, and the presence of antibodies against toxin B at the time of admission, were risk factors for healthcare-associated CDC.[3]

However, the variables predisposing the patients with CDC to develop CDAD are not clear. Settle et al showed that 66% of patients with CDC would have CDAD as they had received cefotaxime or piperacillin–tazobactam. However, it is not clear if their colonized *C. difficile* isolates were toxigenic or nontoxigenic.[13] The aim of our study was to identify the risk factors of development of CDAD in hospitalized patients with tCDC.

Materials and methods

We performed a prospective study at a medical ward of Tainan Hospital, Department of Health, Executive Yuan, a district hospital in southern Taiwan. From January 2011 to June 2012, those older than 18 years old with an expected hospital stay for more than 5 days were enrolled in the present study, which included the participants in our earlier study.[8] We excluded patients who had a history of CDC or CDAD within 3 months, received metronidazole or vancomycin therapy within 3 months, had a colectomy or no stool obtained within 48 hours of admission, or had CDAD or fecal colonization or infection due to *Clostridium* species other than *C. difficile* at the time of admission.[14–16] The study was approved by the Institutional Review Board of Tainan Hospital and signed informed consent forms were obtained from all patients.

The stool samples were obtained from patients less than 48 hours after admission, weekly during hospitalization, and at the onset of diarrhea. If the patient was readmitted, the stool samples were collected again and repeated every week during hospitalization. Stool samples were plated on cycloserine–cefoxitin–fructose agar, transferred into the anaerobic chamber within less than 1 hour after collection, and incubated for 48 hours. Isolates were confirmed to be *C. difficile* on the basis of typical odor, appearance of colonies, and specific biochemical reactions. A polymerase chain reaction confirmed the presence of the toxin B gene, *tcdB* in *C. difficile* isolates, which is defines the toxigenic

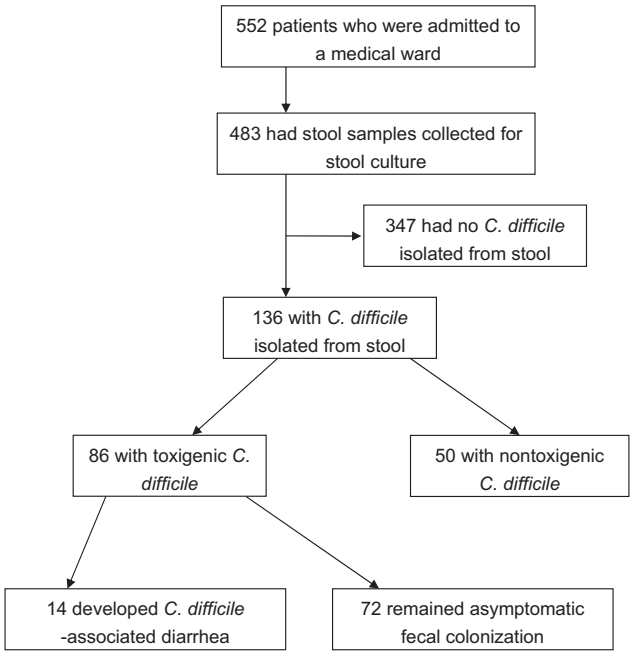

Figure 1. Results of screening for diarrhea patients with toxigenic or nontoxigenic *Clostridium difficile* isolated from stool samples.

C. difficile strain. All enrolled patients were followed until discharge or death. The primary outcome was the occurrence of CDAD, and the secondary outcome was the crude mortality rate at 60 days.

Information regarding demographic characteristics, including tube feeding, co-morbidities, and antibiotic exposure were obtained. The Charlson Comorbidity Index was used to estimate the severity of underlying diseases.[17] Chronic kidney disease (CKD) was defined as estimated glomerular filtration rates (GFR) <60 mL/min/1.73 m^2 for at least 3 months.[18] All antibiotics, steroids, H_2 blockers, and PPIs prescribed within 1 month before CDAD or at the end of follow-up were recorded. Antibiotics were grouped into the following classes: cephalosporins, fluoroquinolones, penicillins and β-lactamase inhibitor combinations, carbapenems, glycopeptides, fosfomycin, and metronidazole. Cephalosporins included cefazolin, cefuroxime, third-generation cephalosporins (ceftriaxone, cefotaxime, or ceftazidime), and cefepime. Penicillins and β-lactamase inhibitor combinations included piperacillin–tazobactam and ampicillin–clavulanic acid. Carbapenems included imipenem–cilastatin, meropenem, and ertapenem. Glycopeptides were composed of vancomycin and teicoplanin.

Diarrhea was defined as three loose stools within at least a 2-day period. Toxigenic *C. difficile* colonization (tCDC) was defined as patients with fecal *tcdB*-carrying *C. difficile* in the absence of diarrhea. CDAD was defined as the presence of diarrhea without an alternative explanation and *tcdB*-carrying *C. difficile* isolated in the feces. The tCDC duration was defined as the period between the first recognition of tCDC and the last recognition of tCDC or the development of CDAD.

Statistical analysis was performed using statistical software (SPSS, version 13.0). Continuous data were expressed as means ± standard deviations. The χ^2 test was used for categorical variables, and the Student *t* test for continuous variables. The factors with a *p* value <0.25 in the univariate analyses, and other factors that were known to be associated with CDAD,[19–21] were evaluated by the multivariate regression model. A two-tailed *p* value of <0.05 was considered to be statistically significant.

Table 1 Clinical characters of 86 cases of fecal toxigenic *Clostridium difficile* colonization who subsequently developed *C. difficile*-associated diarrhea (CDAD) or not

Characters	Total $n = 86$	No CDAD $n = 72$	CDAD $n = 14$	*p*
Male sex	47 (54.7)	39 (54.2)	8 (57.1)	> 0.99
Age	73.6 ± 13.7	73.6 ± 14.1	73.4 ± 11.7	0.96
Body weight, kg	49.9 ± 11.4	50.7 ± 11.4	46.5 ± 10.7	0.21
Body mass index, kg/m^2	19.4 ± 3.8	19.7 ± 3.9	18.1 ± 3.1	0.16
Nasogastric tube feeding	47 (54.7)	36 (50.0)	11 (78.6)	0.05
Underlying diseases				
Charlson Comorbidity Index	2.5 ± 1.8	2.4 ± 1.9	3.0 ± 1.1	0.11
Hypertension	39 (45.3)	34 (47.2)	5 (35.7)	0.62
Diabetes mellitus	37 (43.0)	27 (37.5)	10 (71.4)	0.04
Stroke history	29 (33.7)	22 (30.6)	7 (50.0)	0.22
Chronic kidney disease	14 (16.3)	11 (15.3)	3 (21.4)	0.69
Congestive heart failure	10 (11.6)	8 (11.1)	2 (14.3)	0.66
Chronic obstructive pulmonary disease	9 (10.5)	9 (12.5)	0	0.34
Malignancy	9 (10.5)	8 (11.1)	1 (7.1)	> 0.99
Liver cirrhosis	1 (1.2)	1 (1.4)	0	> 0.99
Colonization duration, d	33.0 ± 59.7	28.6 ± 43.8[a]	55.6 ± 110.5[b]	0.38

[a] Duration between the first and the last recognition of *C. difficile* colonization.
[b] Duration between the first recognition of *C. difficile* colonization and CDAD.
Data are *n* (%) of patients unless otherwise indicated.

Results

During the 18-month study period, a total of 552 patients were eligible for the study, and stool samples were obtained from 483 patients within 48 hours of admission (Fig. 1). Among the 483 patients, 136 (28.2%) had CDC. Among patients who had CDC, 50 (10.4%) were excluded because of nontoxigenic CDC. Thus, 86 (17.8%) patients with tCDC were evaluated. Among those with tCDC, 47 (54.7%) were males with a mean age of 73.6 years. No statistical difference in sex, age, body weight, or body mass index, was found between the patients subsequently developing CDAD and those remaining asymptomatic (Table 1). Use of nasogastric tube feeding was noted in 47 (54.7%) patients with tCDC. The most common co-morbidities found in patients with tCDC were hypertension (45.3%), diabetes mellitus (DM) (43.0%), and stroke (33.7%).

Of the 86 patients, 14 (16%) developed CDAD, and 72 were still asymptomatic with colonization. Patients with tCDC subsequently developing CDAD had a higher rate of having DM and tube feeding compared to those that did not develop CDAD. However, there was no difference in the prevalence of other co-morbidities, such as CKD and malignancy, or in the Charlson Comorbidity Index between the two groups. The duration from the first time of confirmed colonization to CDAD was 55.6 days, which was longer than the tCDC duration in patients who did not develop CDAD (28.6 days), although the difference was not statistically significant ($p = 0.38$).

Regarding antibiotic exposure, patients with tCDC developing CDAD were more likely to have prior exposure to piperacillin–tazobactam ($p = 0.03$). There was no difference in prior use of third-generation cephalosporin, fluoroquinolones, carbapenems, glycopeptides, or fosfomycin between the two groups. Furthermore, patients who once received more than one class of antibiotics, such as a carbapenem plus a penicillin ($p = 0.01$), a penicillin plus a carbapenem plus a glycopeptide ($p = 0.03$), or a cephalosporin plus a penicillin plus a carbapenem, had a higher risk of CDAD (Table 2). In the multivariate analysis the presence of underlying DM ($p = 0.01$), the administration of piperacillin–tazobactam ($p = 0.04$) or a carbapenem ($p = 0.05$), and the use of PPIs ($p = 0.04$), were associated with the development of CDAD (Table 3). Among the 14 patients with tCDC developing CDAD, 2 (14.3%) died within

Table 2 Medications during hospitalization in 86 cases of fecal toxigenic *Clostridium difficile* colonization with and without subsequent CDAD

Medications	No CDAD $n = 72$	CDAD $n = 14$	p
Cephalosporins	58 (80.6)	14 (100)	0.11
Cefazolin, i.v.	2 (1.7)	0	> 0.99
Cefuroxime, i.v./o	9 (12.5)	1 (7.1)	> 0.99
Ceftazidime or ceftriaxone, i.v.	43 (59.7)	10 (71.4)	0.55
Cefepime, i.v.	22 (30.6)	7 (50.0)	0.22
Fluoroquinolones, i.v./o	3 (4.2)	1 (7.1)	0.52
Penicillins	22 (30.5)	5 (35.7)	0.76
Amoxicillin-clavulanic acid, i.v.	14 (19.4)	0	0.11
Piperacillin–tazobactam, i.v.	8 (11.1)	5 (35.7)	0.03
Carbapenems, i.v.	22 (30.6)	7 (50.0)	0.22
Glycopeptides, i.v.	18 (25.0)	4 (28.6)	0.75
Fosfomycin, i.v.	3 (4.2)	1 (7.1)	0.52
Metronidazole, i.v./o	1 (1.4)	0	> 0.99
Two classes of antibiotics	32 (44.4)	6 (42.9)	> 0.99
Cephalosporin + glycopeptide	17 (23.6)	4 (28.6)	0.74
Penicillin + glycopeptide	4 (5.6)	3 (21.4)	0.08
Carbapenem + glycopeptide	10 (13.9)	3 (21.4)	0.44
Cephalosporin + penicillin	13 (18.1)	5 (35.7)	0.16
Cephalosporin + carbapenem	18 (25.0)	7 (50.0)	0.10
Penicillin + carbapenem	3 (4.2)	4 (28.6)	0.01
Three classes of antibiotics	11 (15.3)	4 (28.6)	0.26
Cephalosporin + penicillin + glycopeptide	4 (5.6)	3 (21.4)	0.08
Cephalosporin + carbapenem + glycopeptide	8 (11.1)	3 (21.4)	0.38
Penicillin + carbapenem + glycopeptide	2 (2.8)	3 (21.4)	0.03
Cephalosporin + penicillin + carbapenem	3 (4.2)	4 (28.6)	0.01
Proton pump inhibitors, i.v./o	11 (15.3)	5 (35.7)	0.13
H_2-blockers, i.v./o	8 (11.1)	2 (14.3)	0.66
Steroids, i.v./o	13 (18.1)	3 (21.4)	0.72

Data are *n* (%) of patients unless otherwise indicated.
CDAD = *C. difficile*-associated diarrhea; i.v. = intravenous; o = oral.

Table 3 Multivariate analysis of risk factors for *Clostridium difficile*-associated diarrhea in hospitalized adults with toxigenic *C. difficile* colonization

	Odds ratio	95% confidence interval	*p*
Diabetes mellitus	21.5	1.9–242.4	0.01
Stroke history	2.1	0.4–10.6	0.36
Nasogastric tube use	1.0	0.2–6.5	0.98
Body mass index	0.8	0.6–1.1	0.13
Piperacillin–tazobactam use	17.4	1.2–249.5	0.04
Proton pump inhibitor use	10.1	1.2–87.4	0.04
Carbapenem use	5.5	1.0–28.7	0.05
Cefepime use	1.1	0.2–5.5	0.95

60 days, and 10 (13.9%) of 72 patients with CDC but who did not develop CDAD died ($p > 0.99$).

Discussion

In our earlier report, adult patients with tCDC had a higher risk of developing CDAD in subsequent hospitalizations. In the present study, we further identified three independent risk factors of CDAD among hospitalized patients with tCDC: The presence of underlying DM, recent use of piperacillin–tazobactam, or PPIs. Such information is useful in designing appropriate infection or antibiotic control measures to prevent CDAD when confronted with asymptomatic adults with tCDC.

In our study, the exposure to piperacillin–tazobactam was associated with CDAD in patients with tCDC. Current literature showed that exposure to several classes of antimicrobial agents, including third-generation cephalosporins, clindamycin, and fluoroquinolones, had been linked to CDAD. To date, the influence of piperacillin–tazobactam exposure on the occurrence of CDAD is controversial. Mark et al found that piperacillin–tazobactam exposure was less likely to induce CDAD,[22] and Alston et al described an increase in the rate of CDAD during the shortage of piperacillin–tazobactam.[23] In contrast, Marisa et al observed a significant reduction in the rate of CDAD with reduced availability of piperacillin–tazobactam[24] and Stevens et al suggested that the receipt of penicillins and β-lactamase inhibitor combinations, mainly piperacillin–tazobactam, was associated with an increased risk of CDAD.[25] Likewise, the debate of the role of piperacillin–tazobactam exposure among those with tCDC who develop subsequent CDAD remains undefined. Settle et al showed a higher incidence of CDAD in patients with CDC who were once treated with cefotaxime (18/26, 69%) as compared to those treated with piperacillin–tazobactam (1/3, 33%).[13] In our study, piperacillin–tazobactam exposure was a predisposing factor of CDAD in patients with tCDC. It is easy to critique that among the above clinical studies, there is heterogeneity in terms of the study population and concurrent exposure to medications other than the targeted antibiotics, and the methodology of detection of toxigenic *C. difficile* in stools, which lead to contradictory interpretations of the interaction between prior exposure of piperacillin–tazobactam and the development of CDAD. However, the animal experiments conducted by Pultz et al, showing piperacillin–tazobactam facilitated overgrowth and toxin production by *C. difficile* in mice,[26] provide supporting evidence linking piperacillin–tazobactam therapy and CDAD.

Previous literature showed that individuals who had received PPIs were at risk of CDAD. A meta-analysis reported there was 65% increase in the incidence of CDAD among patients taking PPIs.[21] Another study found an odds ratio of 1.74 for the occurrence of CDAD in those with prior PPI use as compared with those without PPI exposure.[27] It is believed that PPI reduces gastric acidity, which in turn, makes the pH levels greater than or equal to 5, and vegetative *C. difficile* can survive exposure to gastric contents.[28] However, this could not explain such a finding that the patients with *C. difficile* presumably colonized in the colon are susceptible to CDAD after PPI therapy. Since PPIs affect gastric acidity, they may not alter the environment of colon. However, there is evidence indicating that PPIs decrease reactive oxygen production and bactericidal activity of neutrophils,[29] which mediate the defense activity against *C. difficile*. Thus, we should be cautious regarding the detrimental aspect of PPI therapy in predisposing susceptible individuals to the development of CDAD.

DM was another independent risk factor in patients with tCDC to develop CDAD found in our study. Currently, the issue that DM is a risk factor for CDAD remains controversial. Some studies did not find the link between diabetes and CDAD,[30–33] but in a *C. difficile* outbreak in Costa Rica, DM was recognized as an adjusted attributable risk for CDAD.[34] In addition, DM has been identified to be associated with severe CDAD[35] or recurrent CDAD.[36] Therefore, clinicians should pay more attention to hospitalized diabetic patients, especially with tCDC, after their receipt of antimicrobial therapy or PPIs because they are susceptible to CDAD and subsequent complications.

Our study had several limitations. First, our study was conducted in a hospital and included mainly the elderly with underlying illnesses and those who recently received antimicrobial therapy, and the representativeness was limited due to the small sample size. Multicenter studies involving more cases are needed to justify our findings. However, this study was the first study to prospectively observe the factors associated with CDAD among inpatients with tCDC. Secondly, no genetic relatedness between the colonized isolates and the isolates causing diarrhea was investigated. Thus, the possibility of acquisition of another *C. difficile* clone causing CDAD or the presence of a cluster cannot be completely excluded. The clinical significance of these risk factors will be changed given there is an unrecognized cluster of CDAD. More studies will work on the issue of genetic relationship.

In conclusion, our study showed that underlying DM and prior use of piperacillin–tazobactam or PPIs are the risk factors of CDAD among hospitalized patients with tCDC.

Conflicts of interest

The authors declare that they have no financial or nonfinancial conflicts of interest related to the subject matter or materials discussed in the manuscript.

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