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Factors associated with *Clostridium difficile* diarrhea in a hospital in Beijing, China

Z. Lv, G.L. Peng and J.R. Su

Clinical Laboratory Center, Beijing Friendship Hospital, Capital Medical University, Beijing, China

Abstract

Clostridium difficile is the most common cause of hospital-acquired diarrhea in patients treated with antibiotics, chemotherapeutic agents, and other drugs that alter the normal equilibrium of the intestinal flora. A better understanding of the risk factors for *C. difficile*-associated disease (CDAD) could be used to reduce the incidence of CDAD and the costs associated with its treatment. The aim of this study was to identify the risk factors for CDAD in a cohort of Chinese patients in a Beijing hospital. Medical charts of a total of 130 inpatients (62 males and 68 females) with hospital-acquired diarrhea (45 with CDAD; 85 without CDAD) were retrospectively reviewed. *C. difficile* toxins A and B were detected in fecal samples using enzyme-linked fluorescence assays. The drugs used by patients with and without CDAD before the onset of diarrhea were compared. Factors that differed significantly between the two groups by univariate analysis were analyzed by multivariate analysis using a logistic regression model. Multivariate analysis showed that cephalosporin treatment was associated with a significantly higher risk of CDAD in hospitalized patients, while treatment with glycopeptides was significantly associated with a reduction in CDAD ($P<0.001$ for cephalosporin; $P=0.013$ for glycopeptides). Our data confirmed previous findings that empirical treatment with cephalosporins is positively associated with CDAD compared to individuals using other CDAD-related drugs. Additionally, we showed that treatment with glycopeptides was negatively associated with CDAD, compared to individuals using other CDAD-related drugs.

Key words: Antibiotics; *Clostridium difficile*; Glycopeptides

Introduction

Clostridium difficile is the most common cause of hospital-acquired diarrhea in patients who are treated with antibiotics, chemotherapeutic agents, or other drugs that negatively affect the intestinal flora (1,2). *C. difficile*-associated disease (CDAD) represents a constellation of illnesses caused by the *C. difficile* toxins A and B produced in the intestine (3). CDAD is cited in up to 25% of inpatients with diarrhea in North America and Europe, and includes antibiotic-associated colitis and pseudomembranous colitis (4). The incidence of CDAD is rising, due in part to the increased use of newer-generation fluoroquinolones and changes in the demographics of hospitalized patients (2).

The increasing incidence of CDAD has been attributed in part to the emergence of a new, hypervirulent strain of *C. difficile*. This strain, designated as restriction enzyme analysis type B1, North American Pulsed Field Type 1, or polymerase chain reaction (PCR) ribotype 027 toxinotype III, involves a frameshift mutation in the negative regulator of toxins A and B, leading to very high levels of toxin production (5).

The normal human colonic flora protects against *C. difficile* colonization. Drugs that disrupt the colonic flora, including antibiotics, proton pump inhibitors, histamine receptor antagonists, steroids, and cytotoxic drugs, are among the major risk factors for CDAD (2,6,7). Exposure to antibiotics, including clindamycin, penicillins, cephalosporins, and fluoroquinolones, is one of the most well-documented risk factors for CDAD (8,9). Drugs that decrease gastric acid production are also risk factors for *C. difficile* infection (10,11). In a case-controlled study of 1672 patients with CDAD, Dial et al. (10) observed that the use of proton pump inhibitors was associated with a three-fold increase and H2-receptor antagonists with a two-fold increase in the risk of *C. difficile* infection. A lower but significant increased risk was reported in the same study for the use of nonsteroidal anti-inflammatory drugs (NSAIDs). Cancer chemotherapy is another risk factor for CDAD that is, at least in part, mediated by the antimicrobial activity of several chemotherapeutic agents (12). The risk of hospitalized patients acquiring CDAD is

Correspondence: J.R. Su, Clinical Laboratory Center, Beijing Friendship Hospital, Capital Medical University, No. 95 Yongan Road, Xicheng District, Beijing 100050, China. Fax: +86-10-6316.9181. E-mail: youyilab@163.com

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also related to the health of the patient's immune system and the duration of their hospital stay (5).

Recent studies have reported that non-CDAD antimicrobial therapy after an episode of *C. difficile* infection was associated with adverse clinical outcomes and a significant increase in the risk for recurrent disease. In addition, the previous use of second-generation cephalosporins, macrolides, fluoroquinolones, extended spectrum penicillins, and penicillins with beta-lactamase inhibitors was independently associated with an increased incidence of CDAD (13-15). In this retrospective study, our aim was to confirm these studies in CDAD patients in our hospital in Beijing, with the ultimate goal of improving clinical outcomes by identifying Chinese patients at high risk for CDAD. We investigated antibiotic prescribing patterns at our hospital and evaluated the association between the most commonly prescribed antibiotics and CDAD in these Chinese patients.

Patients and Methods

We retrospectively reviewed the records of a total of 130 inpatients (62 males and 68 females) who were consecutively admitted to a specific ward in the Beijing Friendship Hospital and who developed diarrhea during hospitalization between March 2008 and July 2010. The study was approved by the Institutional Review Board of Beijing Friendship Hospital. We analyzed the records of patients to select those who were taking antibiotics, antitumor drugs, cytotoxic drugs, acid-inhibiting agents, stool-softening agents, laxatives, NSAIDs, and steroids, all of which are regarded as risk factors for CDAD. We did not select patients taking any drugs that were regarded as unrelated to CDAD, and such drugs were not included in the analysis. Patients taking traditional Chinese herbal drugs were also excluded due to the complex nature of these preparations and the risk of side effects.

Fecal samples were collected from all patients. Patients were divided into two groups, CDAD and non-CDAD. CDAD diagnosis was based on a positive enzyme-linked fluorescence assay (ELFA) together with symptoms of diarrhea (6 or more loose or liquid yellow-green or black-green stools within 36 h, sometimes occurring with spasmodic abdominal pain or low fever). Inclusion in the non-CDAD group required negative results on both ELFA and colonoscopy.

The drugs and therapeutic strategies used to treat patients during the 28 days before the detection of *C. difficile* toxins A and B (CDAD patients) or the day of diarrhea diagnosis (non-CDAD patients) were recorded. Risk factor analysis of antibiotic use only included patients for whom antibiotics were prescribed before the onset of diarrhea. Maximum white blood cell (WBC) counts and minimum serum albumin levels over the 2 days before the detection of *C. difficile* toxins A and B (CDAD patients) or the day of diarrhea diagnosis (non-CDAD patients) were also recorded. Drugs used during hospitalization were retrospectively reviewed.

Statistical analysis

The demographics and clinical characteristics of the CDAD and non-CDAD patients are reported as means ± SD with range for continuous variables and n (%) for categorical variables. Differences in continuous data between the CDAD and non-CDAD patients were compared using the two-sample *t*-test or the Mann-Whitney U-test if data were not normally distributed; the Pearson chi-square test or Fisher's exact test was used to analyze gender variation. Drug dosages are reported as the mean defined daily dose (DDD) and mean drug utilization index (DUI; DDD/total days of drug administration). Differences between groups were compared using the Mann-Whiney U-test. We also used crude and multivariate logistic regression model analyses to identify the association of CDAD with drug use. Variables with $P<0.2$ in the crude logistic regression model were used in multivariate logistic regression model analysis considering a stepwise method for variables selection. An odds ratio with 95%CI was used for logistic regression model analysis. All statistical assessments were two-tailed, and $P<0.05$ was considered significant. Statistical analyses were performed using the SPSS 15.0 statistics software (SPSS Inc., USA).

Results

We analyzed the records of a total of 130 patients (62 males and 68 females) who developed diarrhea during hospitalization. Forty-five of these patients were diagnosed with CDAD. The remaining 85 patients, who served as controls, were negative for *C. difficile* toxins A and B and negative for CDAD by colonoscopy. The demographics and clinical characteristics of the patients are reported in Table 1. The mean age of the patients was 68.9 ± 13.1 years. There was no significant difference in demographics, age and gender, duration of hospitalization, clinical outcomes, albumin, or WBC counts between the two groups. Interestingly, WBC counts were within normal limits. Differences in the median time of hospital stay, according to the reason for hospitalization, were not significant, as determined using the Kruskal-Wallis test ($P = 0.721$, Supplementary Table S1).

A total of 44 patients (97.8%) in the CDAD group and 83 patients (97.6%) in the non-CDAD group were exposed to systemic antibacterials. ß-Lactam/ß-lactamase inhibitor compounds and cephalosporins were the most frequently used drugs in both groups (Table 1). We observed a significant association between the occurrence of CDAD and cephalosporin use ($P<0.001$). We also found a significant negative association between CDAD and exposure to glycopeptides ($P = 0.013$; Table 2).

A summary of drug schedules for the CDAD and

Table 1. Demographic and clinical characteristics of patients with and without CDAD.

	All patients (n = 130)	CDAD (n = 45)	Non-CDAD (n = 85)	P
Age (years)	68.93 ± 13.05 (26-96)	70.62 ± 12.44 (26-91)	68.04 ± 13.35 (37-96)	0.336
Gender, n (%)				0.094
Male	62 (47.7%)	26 (57.8%)	36 (42.4%)	
Female	68 (52.3%)	19 (42.2%)	49 (57.6%)	
Hospital days	32.86 ± 20.65 (8-147)	34.56 ± 22.88 (11-147)	31.96 ± 19.45 (8-89)	0.366
Albumin (g/L)	30.37 ± 5.14 (18.8-41.6)	31.39 ± 4.61 (21.02-40.00)	29.83 ± 5.34 (18.80-41.60)	0.099
WBC ($\times 10^{12}$/L)	9.57 ± 5.64 (0.05-39.42)	9.78 ± 5.06 (1.22-24.26)	9.45 ± 5.95 (0.05-39.42)	0.602
Systemic antibacterials				
ß-lactam/ß-lactamase	57 (43.85%)	12 (26.67%)	45 (52.94%)	0.004*
Inhibitor compounds				
Cephalosporins	54 (41.54%)	29 (64.44%)	25 (29.41%)	0.001*
Nitroimidazoles	23 (17.69%)	3 (6.67%)	20 (23.53%)	0.017*
Carbapenems	24 (18.46%)	5 (11.11%)	19 (22.35%)	0.116
Glycopeptides	18 (13.85%)	1 (2.22%)	17 (20.00%)	0.006*
Quinolones	21 (16.15%)	10 (22.22%)	11 (12.94%)	0.171
Aminoglycosides	13 (10.00%)	5 (11.11%)	8 (9.41%)	0.759
Lincosamides	12 (9.23%)	6 (13.33%)	6 (7.06%)	0.240
Macrolides	5 (3.85%)	2 (4.44%)	3 (3.53%)	1.000
Monocyclic β-lactam	2 (1.54%)	0 (0%)	2 (2.35%)	0.544
Systemic antimycotics	14 (10.77%)	4 (8.89%)	10 (11.76%)	0.770
Anti-viral drugs	1 (0.77%)	1 (2.22%)	0 (0%)	0.346
Drugs for peptic ulcer and GERD	66 (50.77%)	20 (44.44%)	46 (54.12%)	0.293
Probiotics	34 (26.15%)	11 (24.44%)	23 (27.06%)	0.747
Analgesic-antipyretic drugs	26 (20.00%)	10 (22.22%)	16 (18.82%)	0.645
Steroids	25 (19.23%)	8 (17.78%)	17 (20.00%)	0.760
Drugs for constipation	21 (16.15%)	8 (17.78%)	13 (15.29%)	0.714
Antidiarrheal agents	4 (3.08%)	0 (0%)	4 (4.71%)	0.298
Antineoplastic agents	3 (2.31%)	1 (2.22%)	2 (2.35%)	1.000
Coloclysis	21 (16.15%)	9 (20.00%)	12 (14.12%)	0.386

Data are reported as means ± SD (range) for continuous variables or n (%) for categorical variables. Differences between the CDAD and non-CDAD groups were compared using two-sample *t*-test or Mann-Whitney U-test if data were not normally distributed. Pearson chi-square test or Fisher's exact test was performed to analyze differences in categorical variables. CDAD: *Clostridium difficile*-associated disease; WBC: white blood cell count; GERD: gastro-esophageal reflux disease. * $P<0.05$ indicates a significant difference between CDAD and non-CDAD groups.

non-CDAD patients is presented in Supplementary Table S2. No significant difference in DDD or DUI between the two groups was observed.

Discussion

In this study, we investigated antibiotic regimens and therapeutic strategies to identify risk factors for CDAD in hospitalized Chinese patients. *C. difficile* toxins A and B, which have been implicated in the pathogenesis of *C. difficile* (6,16), are considered reliable diagnostic markers of CDAD. We used ELFA to detect *C. difficile* toxins A and B in the stools of CDAD and non-CDAD patients with diarrhea. Our data confirmed previous findings that empirical treatment with cephalosporins is positively associated with CDAD, compared to individuals using other CDAD-related drugs. Additionally, we showed that treatment with glycopeptides was negatively associated with CDAD, compared to individuals using other CDAD-related drugs.

The association between specific antibiotics and CDAD has changed over recent decades, reflecting the changing frequency of use of particular antibiotic agents (17). Through the late 1980s and 1990s, second- and third-generation cephalosporins replaced clindamycin as the highest risk factor agent for CDAD (6). A retrospective study of 1364 patients infected with *C. difficile* showed that over two-thirds had been taking a cephalosporin during the 2 months before diagnosis (16). Patients exposed to cephalosporins for 1 week have been shown

Table 2. Odds ratios and 95% confidence intervals for associations between drug treatment and CDAD (n = 130).

Drug type	Crude analysis		Multivariate analysis	
	Crude OR (95%CI)[a]	P[a]	OR (95%CI)[b]	P[b]
Systemic antibacterials				
ß-lactam/ß-lactamase	0.323 (0.147, 0.709)	0.004*	–	
Inhibitor compounds				
Cephalosporins	4.350 (2.018, 9.379)	<0.001*	5.044 (2.247, 11.321)	<0.001*
Nitroimidazoles	0.232 (0.065, 0.830)	0.025*	–	
Carbapenems	0.434 (0.150, 1.254)	0.123	–	
Glycopeptides	0.091 (0.012, 0.708)	0.022*	0.069 (0.008, 0.563)	0.013*
Quinolones	1.922 (0.746, 4.951)	0.176	–	
Aminoglycosides	1.203 (0.369, 3.919)	0.759	–	
Lincosamides	2.026 (0.613, 6.691)	0.240	–	
Macrolides	1.271 (0.205, 7.900)	0.796	–	
Monocyclic β-lactam	NA	NA	–	
Systemic antimycotics	0.732 (0.216, 2.480)	0.615	–	
Anti-viral drugs	NA	NA		
Drugs for peptic ulcer and GERD	0.678 (0.328, 1.402)	0.294		
Probiotics	0.872 (0.380, 2.003)	0.747	–	
Analgesic-antipyretic drugs	1.232 (0.507, 2.996)	0.645	–	
Steroids	0.865 (0.341, 2.194)	0.760	–	
Drugs for constipation	1.198 (0.456, 3.146)	0.714	–	
Antidiarrheal agents	NA	NA		
Antineoplastic agents	0.944 (0.083, 10.697)	1.000		
Coloclysis	1.521 (0.587, 3.940)	0.388	–	

[a]Crude logistic regression model analysis. [b]Multivariate logistic regression model analysis. Variables with significance level of P<0.2 in crude logistic regression model analysis were put into multivariate logistic regression model analysis considering stepwise selection. CDAD: *Clostridium difficile*-associated disease; NA: not assessed due to not having sufficient sample size. GERD: gastro-esophageal reflux disease. *P<0.05 indicates a significant association with CDAD.

to have a significantly higher risk of developing CDAD (3). Our data, showing that previous treatment with cephalosporins is an independent risk factor for CDAD, are consistent with these results and with those of other studies (7,15,18).

Studies investigating the correlation between glycopeptide therapy and CDAD report conflicting results. While some studies show that previous glycopeptide treatment is not related to CDAD (8,10), others indicate a significant positive association between CDAD and previous treatment with glycopeptides in combination with ß-lactams (19). In contrast to all of these reports, our univariate analysis showed that the occurrence of CDAD was significantly reduced in patients taking ß-lactam/ß-lactamase inhibitor compounds, nitroimidazoles, and glycopeptides; however, multivariate analysis showed that only the negative association between glycopeptide treatment and CDAD was significant. This apparent protective effect of glycopeptide treatment against CDAD might be explained by recent reports that the glycopeptides vancomycin, fidaxomicin, and oritavancin can inhibit the outgrowth of vegetative cells from germinated *C. difficile* spores (20,21). It has been suggested that this effect prevents some recurrences of symptomatic *C. difficile* infection that are due to germination of residual spores following antibiotic therapy (21).

The major limitations of this study are its small sample size and single-center, retrospective design. An important future goal will be to confirm our findings in a larger patient population. A larger cohort would also enable us to evaluate the correlation of CDAD with macrolides, lincosamides, and anti-tumor drugs, which we were unable to do in this study because of the small sample size. This study did not confirm the diagnosis of CDAD using PCR, which is a more sensitive test than those we employed. An additional limitation is the lack of data for our patient cohort regarding potential non-drug-related risk factors for CDAD, including gastrointestinal invasive

procedures, severe preexisting diseases, hypoalbuminemia, and diabetes.

Increasing empirical antibiotic use for prolonged periods of time is a challenge in most hospital settings. We assessed the prescribing practices at our hospital and found that the three most frequently used drugs in our patient cohort (CDAD and non-CDAD) were acid-inhibiting drugs or proton pump inhibitors, ß-lactam/ß-lactamase inhibitor compounds, and cephalosporins, closely followed by probiotic products. Although our data showed that probiotics were neither a risk factor nor a protective factor for CDAD, the observation period was before CDAD diagnosis. Further analysis is necessary to determine whether probiotics are helpful for CDAD treatment. It will be interesting to compare these data with the incidence of CDAD in different hospitals.

A recently developed clinical prediction tool identified the following as significant risk factors for CDAD: age>65 years, the presence of severe underlying disease, and continued use of antibiotics for non-*C. difficile* infections (22). The use of prediction tools to identify patients at risk would facilitate the management of CDAD in a hospital setting and reduce medical costs.

Supplementary material

Click here to view [pdf].

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