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Original Article

Prevalence and clinical features of *Clostridium difficile*-associated diarrhea in a tertiary hospital in northern Taiwan

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Background and Purpose: Although the clinical manifestations of and risk factors for *Clostridium difficile*-associated diarrhea (CDAD) have been extensively investigated in western populations, data from Taiwanese patients are comparatively limited. This study investigated the incidence density of CDAD in Taiwanese patients and also the risk factors and clinical manifestations of CDAD.

Methods: From September 21, 2003 to December 21, 2003, patients hospitalized in 2 infection wards and 6 medical intensive care units at National Taiwan University Hospital who were older than 20 years, had a history of antibiotic usage within the prior 6 weeks, and developed diarrhea without another identified etiology were classified as having antibiotic-associated diarrhea (AAD), and were enrolled for further study. The diagnosis of CDAD was established when toxin A of *C. difficile* was detected in stool.

Results: The incidence density of AAD was 1/100 person-days of antibiotics usage. CDAD accounted for 12.5% of AAD. Fever and abdominal discomfort developed in only less than half of CDAD patients. Pus cell in the stool sample was found in 100 percent of patients with CDAD. Univariate analysis revealed that presence of malignancy and treatment with antifungal agents within the previous 6 weeks were risk factors for CDAD development. In multivariate analysis, use of antifungal agents was the only independent risk factor for CDAD.

Conclusion: The incidence density of CDAD in this study of Taiwanese patients with AAD was 12.5%. Prior usage of antifungal agents was the only independent factor associated with subsequent CDAD development in patients with AAD.

Key words: Antibacterial agents, *Clostridium difficile*, diarrhea, prevalence, risk factors, Taiwan

Introduction

Clostridium difficile is the cause of approximately 15-25% of all cases of antibiotic-associated diarrhea (AAD) [1-3]. In most cases of AAD, no etiologic agent is identified, and diarrhea is usually mild and not accompanied by abdominal pain [2]. *C. difficile*-associated diarrhea (CDAD) occurs in more than 300,000 patients/year in the United States and most cases occur in hospitals or long-term care facilities [1]. In addition to antibiotics use, prior usage of anti-neoplastic or immunosuppressive drugs was another previously reported risk factor for *C. difficile* infection [4-6]. Symptoms of CDAD may start on the first day of antibiotic therapy or 6 weeks or longer after antibiotic therapy is stopped [7].

Diagnosis of CDAD should be based on the detection of toxin A and/or toxin B in the stool [2]. However, test for toxin A and/or toxin B is not available in most hospitals in Taiwan. Consequently, the incidence density of CDAD in Taiwanese patients remains unclear. This study investigated the incidence density of CDAD in Taiwanese patients based on the detection of toxin A in stool. The risk factors for CDAD among patients with AAD and clinical manifestations of CDAD were also studied.

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Methods

Patients

From September 21, 2003 to December 21, 2004, patients hospitalized at 2 infection wards and 6 medical intensive care units (ICUs), who were older than 20 years and developed AAD were enrolled. A register of inclusions was established to avoid inclusion of the same patient twice. Once the patient was enrolled, 2 stool specimens were submitted for culture of *C. difficile* and to detect toxin A of *C. difficile*.

Definitions

Diarrhea was defined as more than 3 loose, watery stool passages during a 24-h period. Patients who had diarrhea which was distinctively caused by high osmotic diet or upper gastrointestinal bleeding were excluded first. All stool specimens were also sent for analysis of parasite, ameba, urobilirubin and fat droplets to exclude the possibility of steatorrhea or parasite-related diarrhea. A patient was considered to have AAD if he or she had a history of antibiotic therapy within 6 weeks prior to the onset of diarrhea and no specific etiology had been identified as the cause of diarrhea [7]. CDAD was defined as the identification of toxin A in stool, regardless of whether *C. difficile* was isolated from stool. Patients who had AAD but no toxin A was detected in stool were categorized as being enigmatic AAD. Extended-spectrum antibiotics used in this study included anti-pseudomonas pencillins, third- and fourth-generation cephalosporins and carbapenem.

Data collection

A standard case record form was designed to collect patients' demographic data, clinical manifestations, laboratory data and microbiological examination results. The demographic data included age, gender, hospital unit (medical ward or ICU), and date and cause of admission. Information on clinical manifestations included date of diarrhea onset, the severity of abdominal pain, stool consistency, the underlying infection requiring antibiotic usage, and the duration of prior antibiotic usage.

The severity of abdominal pain and discomfort was stratified into 4 grades as severe, moderate, mild and no symptoms; and the stool consistency was stratified into 5 grades as absence, very hard, hard, formed and watery. The clinical conditions included ambulatory status, gastrointestinal decontamination, hospitalization within the previous 6 months, recipient of anticancer chemotherapy and/or antibiotics within previous 6 weeks, and presence of a nasogastric feeding tube. Laboratory data, including stool occult blood, pus cells in stool, leukocyte count, and microbiological data including the isolation of *C. difficile* and toxin A in stool samples, were collected.

During the 3-month study period, whether patients received or did not receive antibiotic therapy in these wards was also recorded. Therefore, the person-days of antibiotic usage of a patient was calculated from the first day of antibiotic exposure to the latest day of antibiotic exposure, regardless of how many kinds of antibiotics were used.

Detection of *C. difficile*

Stool sample collection, storage and transport

Stool samples were collected at the indicated times by natural defecation in a sterile collection container. At least 2 g of stool was collected. The stool sample was divided into 2 different transport containers for toxin testing and culture. Approximately 1 g of the stool sample was transferred to a pre-weighed vial containing anaerobic transport medium (Port-A-Cul™ Transport Jars; Becton Dickinson, Rutherford, NJ, USA) for the culture of anaerobic organisms. Approximately 50 μL of liquid stool was transferred to a tube containing 200 μL of diluent solution, a component of the *C. difficile* Toxin A/B ELISA kit from Wampole Laboratories (Cranbury, NJ, USA). These samples were refrigerated and shipped on wet ice for delivery to the bioanalytical site within 24 h of collection. All samples were labeled with the date and time of collection as well as the date and time of processing.

Processing

Microbiology samples were processed at the bio-analytical site within 12 h of receipt. The anaerobic samples (anaerobic transport container) were opened and all manipulations performed in a glove box containing an anaerobic atmosphere of 80% nitrogen, 10% hydrogen, and 10% carbon dioxide.

Culture for C. difficile

A 0.1 mL aliquot was removed from the original tube and each of the dilution tubes and plated on a *C. difficile*-selective agar (CDSA; Becton Dickinson). Plates were placed in an anaerobic chamber and incubated at 37°C for 48 h under anaerobic conditions. Growth was examined with long-wave ultraviolet light (365 nm) for yellow fluorescence within 1 h of removal from the anaerobic atmosphere [8]. Colonies suspected to be

C. difficile on the basis of their macroscopic appearance and characteristic odor were confirmed by their biochemical characteristics (Rapid ID 32A; bioMérieux, France).

Toxin A detection

All stool samples were tested by BD Color PAC™ Toxins A Test (Becton Dickinson), which was reported by the manufacturer to have 95% sensitivity and 93% specifi-city to determine the presence of *C. difficile* toxin A. A positive result generated an unmistakable bright pink reaction against a white background in the test window in 10 min. For both positive and negative tests, a pink control line of any intensity appeared.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows (version 10.0, SPSS Inc., Chicago, USA). Continuous variables were reported as mean ± standard deviation and were compared with 2-tailed *t* test. Categorical variables were compared with chi-squared test. Logistic regression model was used for univariate and multivariate analyses to determine the independently predictive factors of CDAD in patients with AAD. The variables entered in the multivariate analysis were selected based on the results of univariate analysis, previous study results, and possible biological association. Two-sided *p* values were reported. A p value <0.05 was considered significant.

Results

During the 3-month study period, there was a total of 5930 person-days in these study wards. There were 96 stool samples collected from 48 patients who met the criteria for AAD and 4863 person-days of antibiotics usage in these wards. Nineteen patients were treated in infection wards; the other 29 patients were treated in medical ICUs. The incidence density of AAD was 1/100 person-days of antibiotics usage (48 out of 4863 person-days) and the overall incidence density of AAD among hospitalized patients was 8/1000 person-days (48 out of 5930 person-days). Eleven patients had *C. difficile* isolated from stool. Among them, only 4 patients had toxin A detected in stool. Among the other 37 patients who had no *C. difficile* isolated from stool, 2 had toxin A detected in stool. CDAD accounted for 12.5% of AAD (6 out of 48). The incidence density of CDAD was 1.2/1000 person-days of antibiotic usage (6 out of 4863 person-days of antibiotic usage), and the overall incidence density of CDAD among hospitalized patients was 1/1000 person-days (6 out of 5930 person-days). The characteristics of patients with CDAD are listed in Table 1.

Comparison of the CDAD and enigmatic AAD groups revealed no significant difference in the distribution of age, gender, source of infection and various clinical parameters including frequency and duration of diarrhea, stool consistency, fever, abdominal pain, nasogastric tube in place, ambulatory status, hospitalization and chemotherapy within the previous 6 months, as well as laboratory parameters including pus cells in stool and peripheral blood white cell count (Table 2). The underlying infections for which antibiotic treatment was indicated in these 48 patients were similar between CDAD group and enigmatic AAD group, and included bloodstream infection, pneumonia, urinary tract infection, and cardiovascular or central nervous system infection (p=1-0.07, data not shown). Malignancy and positive stool occult blood were significantly more frequent in the CDAD group (p=0.04 in malignancy and p=0.02 in stool occult blood, respectively). Among the 4 patients with malignancy in the CDAD group, 1 patient with cholangiocarcinoma died before anticancer chemotherapy was initiated, and the other 3 patients had previously received anticancer chemotherapy, including gefitinib in a patient with lung cancer, navelbine and cisplatin in another patient with lung cancer, and thalidomide in a patient with multiple myeloma.

The antibiotics used within 6 weeks prior to the diagnosis of enigmatic AAD and CDAD are compared in Table 3. Carbapenem use tended to be more frequent in patients with CDAD (66.7% versus 28.6%, p=0.09). Antifungal agents, including amphotericin B and fluconazole, were also used significantly more frequently in patients with CDAD (50% versus 7.1%, p=0.02). Duration of prior exposure to antifungal agents was also longer in patients with CDAD (1.7 days versus 1.5 days, p=0.006). In addition, patients with CDAD were exposed to more extended-spectrum antibiotics, such as anti-pseudomonas penicillins, third- and fourth-generation cephalosporins and carbapenem, than patients with AAD (2 versus 1, p=0.03).

Univariate analysis revealed that malignancy (p=0.04, odds ratio [OR] = 7.33, 95% confidence interval [CI] = 1.15-46.67), chemotherapy within the previous 6 months (p=0.05, OR = 6.00, 95% CI = 0.97-36.98), and antifungal agents usage (p=0.01, OR = 13.00, 95% CI = 1.79-94.60) were significantly associated with the

Table 1. Characteristics of patients with *Clostridium difficile*-associated diarrhea (CDAD)

Case	Age (years)/ gender	Existing conditions	Antibiotics prior to CDAD	Clinical presentations	Treatment	Outcome
Toxin (+)/culture (+)						
1	70/F	Lung cancer, C/T (+), NG (+), hospitalization (+)	Cefepime, vancomycin, metronidazole, fluconazole	Fever (+); abdominal pain (–); stool occult blood (2+)	Metronidazole	Discharged
2	86/M	Multiple myeloma, C/T (+), NG (+), hospitalization (+)	Amp/sulb, cefepime	Fever (–); abdominal pain (–); stool occult blood (1+)	Metronidazole + vancomycin (oral)	Mortality
3	75/F	Malignancy (–), C/T (–), NG (+), hospitalization (–)	Cefepime, imipenem, vancomycin, ciprofloxacin	Fever (+); abdominal pain (–); stool occult blood (4+)	No treatment	Mortality
4	34/F	Lung cancer, C/T (+), NG (+), hospitalization (–)	Amp/sulb, pip/tazo, cefpirome, imipenem, metronidazole	Fever (–); abdominal pain (–); stool occult blood (1+)	Metronidazole	Mortality
Toxin (+)/culture (–)						
5	83/F	Cholangiocarcinoma, C/T (–), NG (+), hospitalization (+)	Cefotiam, ceftriaxone, imipenem, vancomycin, ciprofloxacin, metronidazole, fluconazole	Fever (+); abdominal pain (NA); stool occult blood (1+)	Metronidazole	Mortality
6	66/M	Malignancy (–), C/T (–), NG (+), hospitalization (–)	Ticar/clav, cefuroxime, meropenem, fluconazole	Fever (–); abdominal pain (–); stool occult blood (2+)	Metronidazole	Discharged

Abbreviations: F = female; M = male; C/T = chemotherapy; (+) = positive/present; NG = nasogastric tube insertion; (–) = negative/absent; amp/sulb = ampicillin/sulbactam; pip/tazo = piperacillin/tazobactam; ticar/clav = ticarcillin/clavulanic acid; NA = not available because patient was under sedation

occurrence of CDAD among patients with AAD. However, in the multivariate analysis, only usage of antifungal agents was an independent risk factor (p=0.01, 95% CI = 1.97-181.54) and exposure to extended-spectrum antibiotics was only a borderline-significant risk factor (p=0.06, 95% CI = 0.95-12.60) [Table 3].

Discussion

Previous reviews reported that approximately 15-25% of AAD cases are due to *C. difficile* [1-3]. CDAD accounted for 12.5% of AAD in this study, and the incidence density of CDAD was 1.2/1000 person-days of antibiotics usage (6 out of 4863). Our findings about the incidence density of CDAD in Taiwanese patients demonstrate its clinical importance among patients with AAD in this population.

Fever, abdominal cramping pain, and watery diarrhea were reported to be common in CDAD [1,2,4]. In the present study, however, less than half of patients had all of these typical complaints when diarrhea developed. CDAD should be considered as the cause of diarrhea in a patient with a history of recent antibiotic treatment who presents with diarrhea, even when fever and abdominal discomfort do not develop.

Occult colonic bleeding was usually present in moderate to severe colitis [1]. In this study, occult blood was noted (1+ to 4+ by semi-quantification) in all of 6 stool specimens from patients with CDAD, although none of these patients had undergone colonscopy or panendoscopy. Although stool occult blood was not an independent predictor for CDAD in this study, this might have been due to the small number of cases; it remains an important feature of CDAD.

Several essential factors are required for patients to develop CDAD. The 2 most important factors are treatment with antibiotics and colonization or acquisition of *C. difficile*. However, most patients who had *C. difficile* isolated from stool were colonized, not infected, by *C. difficile* [2]. This suggests that other additional factors such as host susceptibility, the virulence of the particular *C. difficile* strain, and the type as well as timing of antimicrobial exposure play roles in the development of CDAD [2]. Predisposing host factors for the development of CDAD have been studied extensively,

Table 2. Characteristics of patients with antibiotic-associated diarrhea (AAD) due to *Clostridium difficile* infection and enigmatic AAD

	AAD			
	CDAD (n = 6) No. (%)	Enigmatic AAD (n = 42) No. (%)	p^a	Univariate p^b
Gender (male/female)	2/4	25/17	0.38	0.24
Age (years) [mean ± SD]	69.0 ± 18.7	63.6 ± 18.2	0.51	0.50
Residence unit (medical ward/ICU)	1/5	18/24	0.38	0.25
Laboratory parameters				
Peripheral blood white blood cell (/μL) [mean ± SD]	13,821 ± 7071	10,598 ± 7098	0.30	0.30
Pus cells in stool (/HPF)	1[c] (20)	1 (2.3)	0.24	0.16
Occult blood in stool	6 (100)	19 (45)	0.02	0.86
Clinical parameters				
Frequency (times/day) [mean ± SD]	8.5 ± 4.9	6.8 ± 3.6	0.30	0.31
Duration (days) [mean ± SD]	18.5 ± 7.2	26.2 ± 56.10	0.71	0.73
Fever	3(50)	25(59.5)	0.68	0.66
Abdominal pain	0(0)	12[d](34)	0.16	0.85
Nasogastric tube in place	6(100)	30(71)	0.32	0.86
Bedridden	4/6(66.7)	19/42(45)	0.41	0.34
Chemotherapy within 6 months	3/6(50)	6/42(14.2)	0.07	0.05
Hospitalization within 6 months	4/6(66.7)	26/42(61.9)	1.00	0.82
Comorbidity				
Diabetes mellitus	2(33.3)	10/42(23.8)	0.63	0.62
Cardiovascular disease	2(33.3)	15/42(35.7)	0.67	0.45
Liver disease	1(16.7)	1/42(2.4)	0.24	0.16
Renal disease	2(33.3)	4/42(9.5)	0.16	0.12
Malignancy	4 (66.7)	9/42 (21.4)	0.04	0.04
Overall mortality	4 (66.7)	13/42 (31.0)	0.17	0.11

Abbreviations: CDAD = *Clostridium difficile*-associated diarrhea; SD = standard deviation; ICU = intensive care unit; HPF = high-power field

[a]*p* value was calculated by chi-squared test or *t* test.

[b]*p* value was calculated by logistic regression analysis entering significant variables in the univariate analysis.

[c]Data for only 5 cases available

[d]Data for only 35 cases available.

including antibiotic exposure and other non-antibiotic-related factors such as advanced age, ICU stay, non-surgical gastrointestinal procedures, and occasionally treatment with methotrexate or paclitaxel for cancer chemotherapy [3,9-12].

Regarding antibiotic exposure, every antibiotic has the potential to predispose patients to the development of CDAD. Many reports have shown that cephalosporins are the agents most frequently implicated in CDAD; nevertheless, clindamycin is probably the most frequently used agent when relative utilization rates are taken into account [2,4,13-15]. However, in the present study, neither cephalosporin nor clindamycin were significantly associated with CDAD in the univariate analysis, while use of antifungal agent was an independent risk factor for CDAD among AAD patients (p=0.01). Furthermore, patients with CDAD were more likely to have previous exposure to broad-spectrum antibiotics (p=0.03) compared to patients with AAD only. These findings may be due to the antibiotic prescribing patterns for critically ill patients in our hospital, where antifungal agents were used either empirically when broad-spectrum antibiotics were not effective in controlling the infection, or definitely when fungal infection was documented, which usually developed after several episodes of bacterial infection. Therefore, the usage of antifungal agents may simply reflect patients' having previously received treatment with several antibiotics before CDAD developed. Although prior exposure to broad-spectrum antibiotics was not a significant risk factor for subsequent development of CDAD (p=0.06 in multivariate analysis), this might have been due to the small number of cases included in this study.

Among non-antibiotic-related factors, anticancer chemotherapy and the presence of underlying malignancy

Table 3. Antibiotic usage in patients with antibiotic-associated diarrhea (AAD) due to *Clostridium difficile* infection and enigmatic AAD

	AAD			
	CDAD (n = 6) No. (%)	Enigmatic AAD (n = 42) No. (%)	p^a	Univariate p^b
Penicillins	3 (50)	20 (47.6)	1.00	0.91
Mean use (days)	4.7	4.1	0.92	0.84
Cephalosporins	6 (100)	31 (73.8)	0.31	0.86
Mean use (days)	8.7 7.4	0.37	0.70	
Carbapenem	4 (66.7)	12 (28.6)	0.09	0.08
Mean use (days)	8	4.5	0.11	0.40
Macrolides	0 (0)	9 (21.4)	0.58	0.82
Mean use (days)	0	1.5	0.25	0.88
Clindamycin	0 (0)	4 (9.5)	1.00	0.88
Mean use (days)	0	1.3	0.44	0.90
Aminoglycosides	0 (0)	8 (19.0)	0.57	0.83
Mean use (days)	0	1.4	0.29	0.87
Sulfonamides	0 (0)	9 (21.4)	0.58	0.82
Mean use (days)	0	3.1	0.22	0.85
Quinolones	2 (33.3)	17 (40.5)	1.00	0.74
Mean use (days)	2	10.5	0.54	0.44
Glycopeptides	3 (50)	19 (45.2)	1.00	0.83
Mean use (days)	5	6.6	1.0	0.71
Metronidazole	3 (50)	13 (31)	0.33	0.36
Mean use (days)	5.5	2.9	0.36	0.36
Antifungal agents	3 (50)	3 (7.1)	0.02	0.01
Mean use (days)	1.7	1.5	0.006	0.95
Median number of classes of extended-spectrum antibiotics[c] usage	2.5	2	0.03	0.08

Abbreviation: CDAD = *Clostridium difficile*-associated diarrhea

[a] *p* value was calculated by chi-squared test or *t* test.

[b] *p* value was calculated by logistic regression mode with univariate analysis.

[c] Including anti-pseudomonas pencillins, third- and fourth-generation cephalosporins and carbapenem.

have been reported as risk factors for the development of CDAD. One possible explanation for the association between CDAD and prior exposure to anticancer agents may be the inflammatory effects of cytotoxic agents on the gut [3,4,16,17]. Although these 2 factors were significant in univariate analysis in this study, neither of them was significant in the multivariate analysis. Only 1 of the 4 patients with malignancies in the CDAD group had previously received cisplatin, which may modify the fecal flora [18]. However, all 4 of these patients had advanced stage disease with relatively poor host immunity and had received strong antibiotics for active infection. Therefore, prior exposure to anticancer chemotherapy and/or presence of malignancy might have been significant factors in univariate but not in multivariate analysis because of their confounding effects on other parameters such as strong antibiotic usage. On the other hand, the small case number in the present study might also have led to this statistical result.

The primary limitation of this study was the small number of patients, which makes the statistical implications less conclusive. Furthermore, many of our patients had received several types of antibiotics, and the effect of interaction between multiple antimicrobial agents is difficult to evaluate. Another limitation of this study was the inclusion of 2 patient populations with different disease severity. This may have overlooked some important clinical parameters, such as Acute Physiology and Chronic Health Evaluation II score, in the final analysis. In addition, only toxin A detection was considered in this study, and thus patients infected with toxin A-negative, toxin B-positive strains may have been missed. However, most previous studies in CDAD usually included toxin A-positive patients only because the toxin A-negative, toxin B-positive strain of *C. difficile* is very rare [19,20]

In summary, the incidence density of CDAD in this study of Taiwanese patients with AAD is similar to that

reported from studies in western countries. Prior use of antifungal agents was the only independent factor associated with subsequent CDAD development in patients with AAD.

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