Journal of Microbiology, Immunology and Infection (2012) 45, 287–295

Available online at www.sciencedirect.com

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ORIGINAL ARTICLE

Changing incidence and clinical manifestations of *Clostridium difficile*-associated diarrhea detected by combination of glutamate dehydrogenase and toxin assay in Northern Taiwan

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Received 30 April 2011; received in revised form 13 July 2011; accepted 19 August 2011

KEYWORDS
Attributable mortality;
C difficile-associated diarrhea;
Incidence;
Taiwan

Background/Purpose: The incidence of *Clostridium difficile*-associated diarrhea (CDAD) is increasing worldwide. Spread of an epidemic hypervirulent strain in southern Taiwan was associated with poor outcome. This prospective study evaluates the incidence and clinical manifestation of CDAD following a hospital-wide hand hygiene promotion program in a 2,200-bed teaching hospital in northern Taiwan.
Patients and Methods: From June 1, 2010 to October 31, 2010, a predefined protocol was used to actively survey CDAD at 11 high-risk units. Stool samples of patients with antibiotic-associated diarrhea (AAD) were submitted for stool culture and toxin A/B assay using a combined enzyme immunoassay. CDAD was diagnosed by a positive toxin assay.
Results: The incidence of CDAD was 0.45/1000 patient-days and was highest in medical intensive care units (7.9/1000 patient-days), followed by hemato-oncology wards, and infectious disease wards. Occurrence of CDAD was associated with $\geq$3 stool pus cells per high power field ($p = 0.018$), prior use of metronidazole ($p = 0.029$), high usage of beta-lactamase stable penicillins ($p = 0.046$), and anaerobe-active antibiotics ($p = 0.029$). No attributable mortality was found. The incidence of CDAD was lower than that previously observed (1.0/1000 patient-days in 2003, $p < 0.001$).

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doi:10.1016/j.jmii.2011.12.001

Conclusion: This study showed a lower incidence of CDAD and absence of attributable mortality. The impact of hand hygiene promotion and other infection control measures on decreasing incidence of CDAD warrants further elucidation.

Introduction

Clostridium difficile-associated diarrhea (CDAD) contribute to 20% to 30% of patients with antibiotic-associated diarrhea (AAD), and more than 90% of those with antibiotic-associated pseudomembranous colitis.[1] CDAD has progressively increased in United States in the last decade.[2] Of even greater concern are the increases in severe or fatal infection.[3,4] The epidemic virulent strains of *C difficile*, North American Pulse Field type 1 and PCR ribotype 027 (NAP1/027), are associated with a higher incidence of disease and more severe and fatal disease.[3]

The epidemiological data of CDAD are limited in Taiwan. In addition to our previous prospective study conducted in 2003,[5] there was a retrospective study in southern Taiwan.[6] The former reported that the incidence of CDAD was 1/1000 patient-days. The latter found a lower incidence (0.426/1000 patient-days), but demonstrated an increase in the quarterly incidence during the study period. The incidence based on clinical practice might be underestimated because the toxin assay was not routinely performed in Taiwan and the sensitivity of the assay varied widely.[7]

Due to the low sensitivity of *C difficile* toxin assay, current guidelines suggest a two-step method with enzyme immunoassay (EIA) detection of glutamate dehydrogenase (GDH) as initial screening followed by a cytotoxic assay or toxigenic culture.[8] However, a cytotoxic assay or toxigenic culture is either expensive or turnaround takes longer. In contrast, a combined assay of both toxin and GDH provided a rapid diagnosis of CDAD with good performance.[9]

Therefore, we conducted a prospective study as a part of hospital-wide infection control program and adapted this combined assay to determine the incidence and clinical manifestations of CDAD and compared to those of our previous study.[5]

Patients and methods

Hospital setting

National Taiwan University Hospital (NTUH) is a 2200-bed teaching hospital located in Northern Taiwan. The hospital-wide hand hygiene program began in April 2004 and is promoted annually.[10] According to hospital-wide infection control surveillance data, high-risk units are defined either by the number of stool cultures that grew *C difficile* or the amount of antimicrobial usage in 2009. A total of 11 high-risk units were selected for this prospective study and included 2 infectious disease wards (73 beds), 6 medical intensive care units (ICUs; 62 beds), and 3 hemato-oncology wards (82 beds). The study was approved by the Institutional Review Board of NTUH (NTUH-201001041R). Inform consent was obtained from patients enrolled.

Patients

From June 1, 2009 to October 31, 2010, one of the authors actively surveyed all patients with diarrhea who were older than 18 years and hospitalized in high-risk units based on a predefined protocol (Fig. 1). Patients with diarrhea caused by high osmotic diet, overt gastrointestinal bleeding, steatorrhea, laxative use, or documented parasite-related diarrhea were excluded. Stool samples were sent to routine laboratory for culture

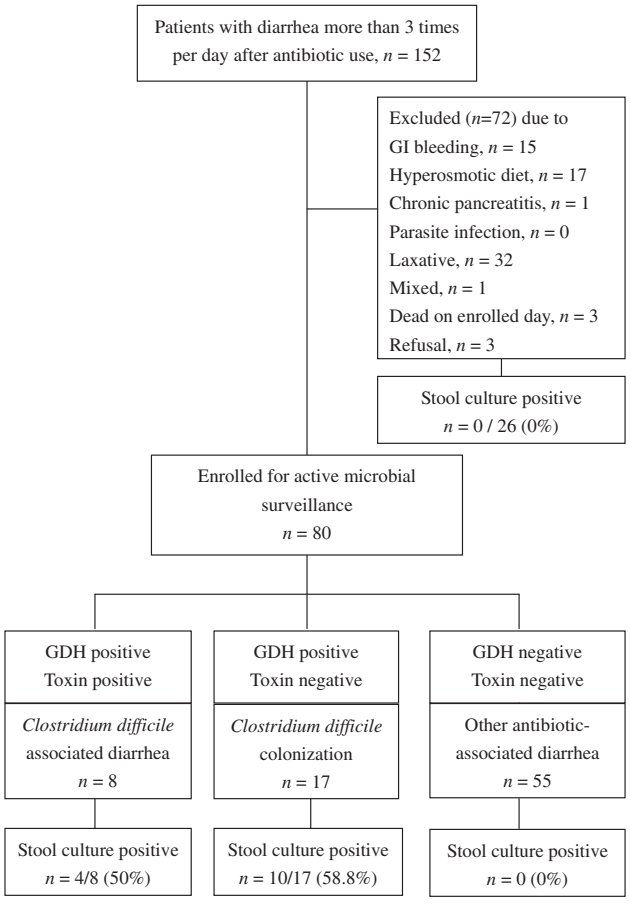

Figure 1. Enrollment of patients with antibiotic-associated diarrhea from June 1, 2010 to October 31, 2010 at National Taiwan University Hospital.

(*C difficile* and others as indicated) and occult blood or pus cell detection. All stool samples were analyzed for parasite, ameba, and neutral fat to exclude the possibility of steatorrhea or parasite-related diarrhea. In addition, active microbial surveillance for stool samples collected from patients with AAD was performed in the laboratory of the Center for Infection Control to detect toxin A/B and GDH using a combined EIA, and the presence of *C difficile* by culture.

At these high-risk units, physicians tended to prescribe antimicrobial therapy empirically and de-escalated later based on clinical course and microbiological data. If the response of oral metronidazole was unsatisfied for patients with *C difficile* infection or colonization, physicians might prescribe intravenous metronidazole or oral vancomycin according to the guidelines.[8] Contact isolation and other infection control measures were conducted immediately when the status of *C difficile* infection or colonization was confirmed.

Data collection

We used a standardized case report form to collect patient's demographic data, clinical presentations, laboratory data, microbiological examination results and treatment outcome. The demographic data included age, gender, hospital unit (medical ICU, infectious diseases wards, or hemato-oncology wards), and underlying disease. Charlson comorbidity index was calculated based on underlying comorbidities.[11] Clinical presentations included onset date of diarrhea, diarrhea frequency (times/day), febrile reaction (more than 38.0 °C), presence of abdominal pain, performance status determined by Eastern Cooperative Oncology Group (ECOG),[12] antibiotic usage within the previous 6 weeks, receipt of anticancer chemotherapy and/or antibiotic within the previous 6 weeks, and use of proton-pump inhibitors. Laboratory data included blood leukocyte count, C-reactive protein, biochemistry, stool pus cell count, and occult blood. Colonization or infection due to multi-drug resistance *Acinetobacter baumannii* (MDRAB), vancomycin-resistance *enterococci* (VRE), or *Stenotrophomonas maltophilia* were collected 30 days before and after enrollment as surrogate markers of antimicrobial selection pressure. Antimicrobial therapy and response (duration of diarrhea), crude mortality, and attributable mortality were recorded.

Definitions

Diarrhea was defined as more than three loose or watery stool passages during a 24-hour period. Patients were considered to have AAD if they had antibiotic usage within 6 weeks prior to the onset of diarrhea and no specific etiology had been identified as the cause of diarrhea.[13] CDAD was defined as the identification of toxin A or toxin B regardless of whether *C difficile* was isolated from stool. Colonization of *C difficile* was considered if there was a positive GDH test and/or positive culture result. Attributable mortality was defined as persistent diarrhea more than 48 hours before death and judged by primary care physician and infectious disease consultant.

Microbiological methods

Culture for C difficile

Diarrheal stool sample was inoculated on cycloserine cefoxitin fructose agar (CCFA).[14] CCFA plate was incubated in an anaerobic chamber at 37°C for 48 hours. Growth was examined with long-wave ultraviolet light (365 nm) for yellow fluorescence within 1 hour of removal from the anaerobic atmosphere.[15] Colonies suspected to be *C difficile* based on microscopic characteristics and specific odor were confirmed by automatic identification system (anaerobe identification, VITEK, bioMérieux, Durham, NC, USA).

Detection of toxin A/B and GDH antigen

All stool samples were tested by a combined enzyme immunoassay for toxin A/B and GDH (Techlab C Diff Quik Chek Complete, Princeton, NJ, USA). Compared to the toxigenic culture, toxin A/B assay gives 85.9% sensitivity and 99.5% specificity. The GDH assay provides 97.6% sensitivity and 90.1% specificity.[16] The toxin assay used in our previous study[5] (Becton Dickinson ColorPAC Toxin A, Cockeysville, MD, USA) had 89% sensitivity and 89% specificity.[17]

Data analysis and statistical analysis

The number of antimicrobial usage days was determined to express aggregate antimicrobial usage. One antimicrobial usage day represents the administration of a single agent on a given day regardless of the number of doses administered or dosage strength.

Statistical analysis was performed by the SPSS for Windows (version 18.0, SPSS InC, Chicago, IL, USA). Continuous variables were reported as mean $\pm$ standard deviation and compared with one-way ANOVA. Categorical variables were compared with Chi-square test or Fisher-exact test. *Posthoc* analysis was performed by Scheffe procedure and modified Bonferroni-adjust α for pair-wise comparisons if the result of ANOVA, Chi-square or Fisher-exact test was statistically significant. Incidence of CDAD between the two study periods (2003 vs. 2010) was compared by Poisson distribution method. A p value <0.05 was considered statistically significant.

Results

During the 5-month study period, a total of 17,685 patient-days in these 11 high-risk units were surveyed; 152 patients who received antibiotics and had diarrhea more than three times per day were screened. Of 80 patients with AAD who received active microbiological surveillance for CDAD (Fig. 1), eight had CDAD. Another 17 patients had *C difficile* colonization. Among 72 patients who did not receive active microbial surveillance, stool samples of 26 patients were sent for *C difficile* isolation and all were negative. In 80 patients with AAD who received active microbial surveillance, compared with stool culture only, a combined toxin and GDH assay identified 11 more patients with *C difficile* colonization or infection (total 25 vs. 14 patients).

Thus, the prevalence of CDAD was 10.0% (8 out of 80) of overall AAD. The overall incidence of AAD was 4.5/1000 patient-days (80 out of 17,685), and that of CDAD was 0.45/1000 patient-days (8 out of 17,685). The incidence was highest in medical ICUs (7.9/1000 patient-days, 4 out of 5051), followed by hemato-oncology wards (0.42/1000 patient-days, 3 out of 7213) and infectious diseases wards (0.18/1000 patient-days, 1 out of 5421).

The demographic and clinical characteristics of patients with CDAD, *C difficile* colonization and other AAD are shown in Tables 1 and 2, respectively. There was no difference in age, gender, study unit, Charlson comorbidity, underlying diseases, duration and frequency of diarrhea, febrile illness, abdominal pain, ECOG score, hospitalization, and receipt of chemotherapy or antibiotic 6 months prior to admission, and proton pump inhibitor use. Length of hospital stay either before or after enrollment was longer in patients with CDAD than in other two groups, although statistically insignificant. A stool pus cell count of 3 per high power field or more was more frequent in patients with CDAD than the other two groups (37.5%, 17.6%, and 5.5%, $p = 0.018$). Only half of the patients with CDAD or *C difficile* colonization had a positive stool culture for *C difficile*. There was no difference in prevalence of colonization or infection due to MDRAB, VRE, or *S maltophilia* among the three groups.

Table 3 shows the proportion of patients receiving indicated intravenous and oral antimicrobial agents within 6 weeks prior to enrollment and the antimicrobial usage days per patient in each group. Occurrence of CDAD was associated with more antimicrobial usage days of beta-lactamase stable penicillins ($p = 0.046$) and anaerobe-active agents ($p = 0.029$). In addition, CDAD patients were more likely to have prior use of either intravenous or oral metronidazole ($p = 0.042$).

Table 4 compares the treatment regimen, response and clinical outcome among the three groups. Patients with CDAD and *C difficile* colonization received treatment more than those with AAD ($p < 0.001$). All 8 CDAD patients and 16 of 17 patient with *C difficile* colonization received either metronidazole or vancomycin therapy. However, only 36.3% of the other AAD patientswere treated. Among 43 patients treated, 27 patients were treated with oral metronidazole, 12 with intravenous metronidazole, two with oral vancomycin and one with combination therapy (intravenous metronidazole with oral vancomycin). Oral vancomycin was more frequently prescribed in patients with CDAD ($p = 0.009$). Treatment duration was longest in patients with patients with CDAD ($p < 0.001$). Regarding clinical outcome, there was no mortality attributable to *C difficile* infection identified. Hospital mortality due to any cause was lower in patients with other antibiotic associated diarrhea ($p = 0.042$).

Table 1 Baseline characteristics of patients with *Clostridum difficile*-associated diarrhea, *C difficile* colonization, and other antibiotic-associated diarrhea

Baseline characteristics	*C difficile*-associated diarrhea ($n = 8$)	*C difficile* colonization ($n = 17$)	Other antibiotic-associated diarrhea ($n = 55$)	*p* value
Age (years), mean ± SD	63.88 ± 25.18	65.59 ± 17.34	62.85 ± 18.99	0.88
Gender				
Male, *n* (%)	5 (62.5)	10 (58.8)	30 (54.5)	0.89
Female, *n* (%)	3 (37.5)	7 (41.2)	25 (45.5)	
Study site				
Infectious disease ward, *n* (%)	1 (12.5)	3 (17.6)	13 (23.6)	0.71
Hemato-oncology ward, *n* (%)	3 (37.5)	6 (35.3)	15 (27.3)	0.73
Medical intensive care unit, *n* (%)	4 (50.0)	8 (47.1)	27 (49.1)	0.99
Charlson comorbidity index, mean ± SD	3.63 ± 2.60	4.47 ± 4.43	3.44 ± 3.25	0.56
Congestive heart failure, *n* (%)	1 (12.5)	4 (23.5)	3 (5.5)	0.09
Peripheral arterial occlusive Disease, *n* (%)	0 (0.0)	1 (5.9)	1 (1.8)	0.58
Chronic obstructive pulmonary Disease, *n* (%)	1 (12.5)	2 (11.8)	3 (5.5)	0.59
Peptic ulcer disease, n (%)	1 (12.5)	1 (5.9)	8 (14.5)	0.64
Cirrhosis of liver, *n* (%)	0 (0.0)	1 (5.9)	0 (0.0)	0.15
Chronic kidney disease, *n* (%)	2 (25.0)	4 (23.5)	5 (9.1)	0.20
Connective tissue disease, *n* (%)	0 (0.0)	3 (17.6)	5 (9.1)	0.36
Diabetes mellitus, *n* (%)	3 (37.5)	5 (29.4)	13 (23.6)	0.67
Cerebral vascular accident, *n* (%)	0 (0.0)	4 (23.5)	12 (21.8)	0.32
Solid organ malignancy, *n* (%)	2 (25.0)	3 (17.6)	10 (18.2)	0.89
Leukemia, *n* (%)	3 (37.5)	5 (29.4)	9 (16.4)	0.26
Lymphoma, *n* (%)	0 (0.0)	3 (17.6)	8 (14.5)	0.47
Acquired immunodeficiency syndrome, *n* (%)	0 (0.0)	0 (0.0)	2 (3.6)	0.63
Solid organ transplant, *n* (%)	0 (0.0)	1 (5.9)	1 (1.8)	0.58
Hematopoietic stem cell transplant, *n* (%)	1 (12.5)	3 (17.6)	8 (10.9)	0.76
Immunosuppressants use, *n* (%)	4 (50.0)	12 (70.6)	22 (40.0)	0.08

SD = standard deviation.

Table 2 Clinical manifestations of patients with *Clostridium difficile*-associated diarrhea, *C difficile* colonizaiton and other antibiotic-associated diarrhea

Clinical manifestations	*C difficile*-associated diarrhea ($n = 8$)	*C difficile* colonization ($n = 17$)	Other antibiotic-associated diarrhea ($n = 55$)	*p* value
Diarrhea				
Duration, days (mean ± SD)	3.50 ± 2.33	2.06 ± 1.30	3.45 ± 3.30	0.22
Frequency, times per day (mean ± SD)	6.25 ± 3.58	5.35 ± 2.26	5.53 ± 2.89	0.75
Fever (body temperature ≥38.0°C), *n* (%)	2 (25.0)	5 (29.4)	26 (47.3)	0.26
Abdominal pain, *n* (%)	3 (37.5)	1 (5.9)	12 (21.8)	0.15
ECOG score ≥ 3, *n* (%)	6 (75.0)	10 (58.9)	41 (74.5)	0.93
Hospitalization within 6 months prior to admission, *n* (%)	5 (62.5)	11 (64.7)	26 (47.3)	0.38
Receipt of chemotherapy within 6 months prior to diarrhea, *n* (%)	4 (50.0)	9 (52.9)	16 (29.1)	0.14
Receipt of antibiotic within 6 months prior to diarrhea, *n* (%)	7 (87.5)	12 (70.6)	38 (69.1)	0.56
Proton pump inhibitor use, *n* (%)	5 (62.5)	10 (58.8)	32 (58.2)	0.97
Length of hospital stay before enrollment (days), mean ± SD	40.00 ± 32.30	20.18 ± 17.88	21.58 ± 22.57	0.09
Length of hospital stay after enrollment (days), mean ± SD	46.13 ± 29.47	25.29 ± 20.72	34.65 ± 30.16	0.22
Laboratory data				
White blood count (x 10^9/L),mean ± SD Neutropenia	8.145 ± 7.620	10.914 ± 10.527	9.954 ± 10.937	0.83
(ANC[c] < 500 × 10^6/L), *n* (%)	2 (25.0)	3 (17.6)	7 (12.7)	0.62
Hemoglobin (g/L), mean ± SD	96.0 ± 9.8	99.4 ± 16.1	97.3 ± 15.5	0.85
Platelet (×10^9/L), mean ± SD	126.38 ± 126.24	149.88 ± 99.58	155.41 ± 137.86	0.84
C-reactive protein (mg/L), mean ± SD	61.6 ± 50.1	73.2 ± 76.4	86.5 ± 96.3	0.70
Albumin (g/L), mean ± SD	27.8 ± 6.6	33.1 ± 7.5	29.3 ± 6.1	0.07
Creatinine (mg/L), mean ± SD	13.6 ± 13.3	18.3 ± 20.1	16.2 ± 16.8	0.81
Stool occult blood ≥ 3, *n* (%)	2 (25.0)	4 (23.5)	17 (30.9)	0.24
Stool pus cell ≥ 3—5/HPF, *n* (%)	3 (37.5)	3 (17.6)	3 (5.5)	**0.018**
Microbiology data, n (%)				
Stool culture positive for *Clostridium difficile*, *n* (%)	4 (50.0)	10 (58.8)	0 (0.0)	**<0.001**
30 days before enrollment				
MDRAB [e] colonization/infection, *n* (%)	1 (12.5)	2 (11.8)	7 (12.7)	0.99
VRE [f] colonization/infection, *n* (%)	2 (25.0)	1 (5.9)	10 (18.2)	0.38
Stenotrophomonas maltophilia colonization/infection, *n* (%)	3 (37.5)	4 (23.5)	8 (14.6)	0.25
30 days after enrollment				
MDRAB colonization/infection, *n* (%)	1 (12.5)	1 (5.9)	7 (12.7)	0.73
VRE colonization/infection, *n* (%)	1 (12.5)	2 (11.8)	4 (7.3)	0.78
Stenotrophomonas maltophilia colonization/infection, *n* (%)	2 (25.0)	5 (29.4)	12 (21.8)	0.81

ANC = Absolute neutrophil count; ECOG score = Eastern Cooperation of Oncology Group score; HPF = high power field; MDRAB = multidrug resistance *Acinetobacter baumannii* defined as resistance to three or more classes of antimicrobials, including ampicillin-sulbactam, antipseudomonal cephalosporins, anti-pseudomonal carbapenems, aminoglycosides, and fluoroquinolones; SD = standard deviation; VRE = vancomycin-resistant enterococci.

Comparison of results between this study and our previous study conducted in 2003[5] showed that the incidence of AAD (4.5/1000 vs. 8.0/1000 patient-days, $p < 0.001$) and that of CDAD (0.45/1000 vs. 1.0/1000 patient-days, $p < 0.001$) was lower in this study. As the previous study did not include hemato-oncology wards, we repeated data analysis for those data collected from medical ICUs and infectious diseases wards. The conclusion remained the same (AAD incidence 5.3/1000 vs. 8.0/1000 patient-days, CDAD incidence, 0.48/1000 vs. 1.0/1000 patient-days, $p < 0.001$ for both). There was no difference of the prevalence of CDAD in AAD between 2 study periods (10.0% vs. 12.5%, $p = 0.87$).

Table 3 Antimicrobial usage within 6 weeks prior to diarrhea in patients with *Clostridium difficile*-associated diarrhea, *C difficile* colonization and other antibiotic-associated diarrhea

Antimicrobial usage[a]	*C difficile*-associated diarrhea ($n = 8$)	*C difficile* colonization ($n = 17$)	Other antibiotic-associated diarrhea ($n = 55$)	*p* value
Betalactamase-stable penicillin[b]	2 (25.0)	9 (52.94)	27 (49.09)	0.39
	8.5 ± 2.12	4.9 ± 4.49	3.3 ± 2.42	**0.046**
Cephalosporins	7 (87.50)	14 (82.35)	45 (81.82)	0.92
	6.4 ± 7.04	7.7 ± 6.53	6.9 ± 5.33	0.86
1st and 2nd generation	—	3 (17.65)	6 (10.91)	0.42
		2.0 ± 1.00	3.0 ± 2.10	0.47
3rd and 4th generation	7 (87.5)	13 (76.47)	44 (80.0)	0.81
	6.4 ± 7.04	7.8 ± 6.53	6.7 ± 5.28	0.80
Fluoroquinolones[c]	3 (37.5)	5 (29.41)	21 (38.18)	0.80
	4.7 ± 5.51	3.8 ± 3.03	8.1 ± 7.51	0.38
Carbapenems[d]	5 (62.5)	9 (52.94)	22 (40.0)	0.37
	12.8 ± 5.85	6.11 ± 4.08	9.6 ± 6.28	0.12
Glycopeptides[e]	4 (50.0)	5 (29.41)	20 (36.36)	0.61
	4.3 ± 4.57	5.6 ± 8.11	9.0 ± 8.18	0.44
Metronidazole	4 (50.0)	1 (5.88)	12 (21.82)	**0.042**
	8.5 ± 3.87	1.0	4.8 ± 4.18	0.23
Narrow spectrum antibiotic[f]	4 (50.0)	10 (58.82)	34 (61.82)	0.81
	5.5 ± 4.04	7.9 ± 10.09	8.0 ± 7.27	0.83
Broad spectrum antibiotic[g]	8 (100.0)	16 (94.12)	52 (94.55)	0.79
	16.6 ± 10.93	12.2±9.138	13.5 ± 11.05	0.63
Anaerobic-active antibiotic[h]	7 (87.5)	15 (88.24)	45 (81.82)	0.78
	15.9 ± 9.72	6.9 ± 3.83	9.5 ± 7.61	**0.029**
Anti-fungal agents[i]	5 (62.5)	9 (52.94)	29 (52.73)	0.87
	12.2 ± 12.11	18.3 ± 12.40	16.0 ± 11.303	0.64

[a] Data are shown in number (%) and antimicrobial usage days (mean ± standard deviation).
[b] Betalactamase-stable penicillins including amoxicillin-clavulanate, ampicillin-sulbactam, ticarcillin-clavulanate, piperacillin-tazobactam.
[c] Fluoroquinolones including levofloxacin, moxifloxacin, ciprofloxacin.
[d] Carbapenems including meropenem, imipenem, ertapenem.
[e] Glycopeptides including vancomycin, teicoplanin.
[f] Narrow spectrum antibiotic including penicillin, ampicillin, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, clindamycin, cephalexin, cefazolin, cefmetazole, cefuroxime, vancomycin, teicoplanin, daptomycin, linezolid.
[g] Broad spectrum antibiotic including piperacillin-tazobactam, ticarcillin-clavulanate, ceftriaxone, cefotaxime, ceftazidime, flomoxef, cefepime, cefpirome, carbapenems, fluoroquinolones, tigecycline, aminoglycosides, aztreonam.
[h] Anaerobe-active antibiotic including ampicillin-sulbactam, amoxicillin-clavulanate, clindamycin, piperacillin-tazobactam, ticarcillin-clavulanate, cefmetazole,flomoxef, metronidazole, moxifloxacin, carbapenems, tigecycline.
[i] Anti-fungal agents: fluconazole, itraconazole, voriconazole, nystatin, amphotericin B, micafungin, caspofungin, anidulafungin.

The potential risk factors[18] of CDAD in patients enrolled were compared between two study periods (Table 5). The current study population was associated with a lower rate of hospitalization within 6 months ($p = 0.001$) and higher portion of chemotherapy receipt within 6 months ($p = 0.035$). There was no difference in older age (≥65 years old), Charlson comorbidity index, or proportion of antibiotic use 6 weeks prior to diarrhea.

Discussion

This 5-month, prospective, active surveillance study of patients with AAD in high-risk units at a large teaching hospital demonstrated a lower incidence of AAD and CDAD than that of our previous study in 2003.[5] This result was different from an increasing incidence in Southern Taiwan and in Oregon, USA.[6,19] The result was concordant with the decreasing trends of MDRAB and methicillin-resistant *Staphylococcus aureus* following a hospital-wide hand hygiene promotion program in this hospital.[10] Regarding antimicrobial selection pressure, three studies have described the decreasing incidence of CDAD as related to antibiotic restriction policies.[20–22] Two studies showed that the incidence of CDAD was associated with increasing antibiotic consumption or some kind of antibiotic use, but both failed to support the association of alcohol-based hand rub usage and CDAD.[23,24] However, there was no difference on proportion of antibiotic use between the two study periods and no specific antibiotic stewardship program promoted during 2003—2010 at this hospital. Therefore, the impact of hand hygiene promotion and other infection

Table 4 Treatment response and outcome of patients with *Clostridium difficile*-associated diarrhea, *C difficile* colonization and other antibiotic-associated diarrhea

Parameter	*C difficile*-associated diarrhea ($n = 8$)	*C difficile* colonization ($n = 17$)	Other antibiotic-associated diarrhea ($n = 55$)	*p* value
Antimicrobial therapy, *n* (%)	8/8 (100)	16/17 (94.1)	20/55 (36.3)	**<0.001**
Oral metronidazole, *n* (%)	5/8 (62.5)	14/16 (87.5)	10/20 (50.0)	0.060
Intravenous metronidazole, *n* (%)	2/8 (25.0)	2/16 (12.5)	10/20 (50.0)	0.051
Oral vancomycin, *n* (%)	2/8 (25.0)	0/16 (0.0)	0/20 (0.0)	**0.009**
Duration (days), mean ± SD	16.25 ± 13.33	8.06 ± 4.14	4.80 ± 1.82	**<0.001**
Therapeutic response				
Diarrhea duration after treatment	7.13 ± 11.59	6.00 ± 6.55	3.50 ± 5.53	0.41
Outcome, *n* (%)				
7-day mortality	0 (0.0)	0 (0.0)	3 (5.5)	0.49
14-day mortality	0 (0.0)	2 (11.8)	4 (7.3)	0.58
30-day mortality	2 (25.0)	6 (40.0)	8 (16.0)	0.14
In-hospital mortality	4 (50.0)	9 (52.9)	13 (23.6)	**0.042**

SD = standard deviation.

control measures on decreasing incidence of CDAD in current study warrants further elucidation.

Furthermore, neither attributable mortality nor morbidity of CDAD was identified in this study. The attributable mortality ranged from 5.7% to 6.9% in previous studies, which were associated with epidemic hypervirulent NAP1/027 strain of *C difficile*.[3,25] The epidemic hypervirulent strain was associated with a higher incidence of disease and fatal disease.[3] However, the NAP1/027 strain has not been identified in northern and southern Taiwan,[26] which might contribute to the lower attributable mortality in the current study.

We established and confirmed a useful diagnostic protocol coupling the use of a sensitive combined antigen assay. After exclusion of other known etiologies, this flowchart identified one patient with CDAD per 10 patients with AAD and did not miss any patient with *C difficile* colonization or infection. A stool pus cell count of 3 per high power field or more was noted in only 37.5% of CDAD cases. This finding was concordant with a previous report that, compared with toxin assay, stool pus cell count gives a 30% sensitivity and 74.9% specificity.[27] Thus, stool pus cell count is not a good screening test for CDAD. This study utilizes combination of toxin and GDH assay to detect patients with CDAD or *C difficile* colonization. Only half of them had a positive stool culture for *C difficile*, which may result from difficulty in its isolation.[28] Thus, current guidelines suggest the use of GDH assay as an initial

Table 5 Comparisons of risk factors of *Clostridium difficile*-associated diarrhea in patients enrolled in this and previous studies[5] at National Taiwan University Hospital

Characteristic	Hsu et al.[5] 2003, ($n = 48$)	Current study 2010, ($n = 80$)	*p* value
Age (years), mean ± SD	64.3 ± 17.7	63.5 ± 19.1	0.82
Age (years) ≥65 years (%)	28 (58.3)	42 (52.5)	0.521
Charlson comorbidity index, mean ± SD	4.00 ± 3.69	3.68 ± 3.46	0.62
Hospitalization within 6 months prior to admission, *n* (%)	39 (81.2)	42 (52.5)	**0.001**
Chemotherapy within 6 months prior to diarrhea, *n* (%)	10 (20.8)	31 (38.8)	**0.035**
Prior antibiotics use within 6 weeks prior to diarrhea, *n* (%)			
Penicillins	23 (47.9)	39 (48.8)	0.93
Cephalosporins	37 (77.1)	66 (82.5)	0.45
Carbapenems	16 (33.3)	36 (45.0)	0.19
Fluoroquinolones	19 (39.6)	29 (36.2)	0.71
Glycopeptides	21 (43.8)	29 (36.2)	0.40
Metronidazoles	16 (33.3)	17 (21.2)	0.13

SD = standard deviation.

screening test followed by toxin assay or toxigenic culture.[8]

There was an unexpected finding that prior use of metronidazole was more common in CDAD patients than other two groups ($p = 0.042$). Due to the limitation of routine diagnostic methods, physicians tended to use metronidazole empirically for high-risk patients with AAD. Treatment without the documentation of CDAD may be incomplete or inadequate. Therefore, CDAD patients in the current study tended to have more oral vancomycin as second line therapy.

In conclusion, this study identified a lower incidence of CDAD than that in 2003 in a teaching hospital in northern Taiwan and the absence of attributable mortality following a hospital-wide hand hygiene program. This study confirmed our concern that culture methods identified only half of patients with *C difficile* colonization or infection. Cross transmission and environmental contamination from these undetected patients might at least in part contribute to the rapid spread of *C difficile* worldwide.

Financial support

This project was supported in part by a grant from the Department of Health, Taiwan (DOH99-TD-B-111-001).

Conflicts of interest statement

All authors have declared no conflict of interest.

Acknowledgment

We are grateful to members of the Center for Infection Control for the contribution of the hospital-wide healthcare-associated infection surveillance program and all staff of the hospital for their commitment for improving patient safety and reducing healthcare-associated infection. We are also grateful to Ms. Hui-Ju Pan for microbiological technical support and Ms. Le-Yin Hsu for the contribution of statistical analysis.

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