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ORIGINAL ARTICLE

# Clinical manifestations of *Clostridium difficile* infection in a medical center in Taiwan

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*Background/Purpose:* To investigate the clinical characteristics of *Clostridium difficile* infection (CDI) at a medical center in Taiwan.
*Methods:* Patients with CDI were identified from medical records at the National Taiwan University Hospital (Taipei, Taiwan). The following information was gathered and analyzed to better understand the clinical manifestations of CDI: age; sex; underlying immunocompromised conditions; laboratory data; in-hospital mortality; and previous use of drugs such as antimicrobial agents, steroids, and antipeptic ulcer agents.
*Results:* During the years 2000–2010, 122 patients were identified as having CDI. This included 92 patients with nontoxigenic CDI (i.e., positive stool culture for *C. difficile* but negative results for toxins A and B) and 30 patients with toxigenic CDI (i.e., positive stool culture cultures for *C. difficile* and positive results for toxins A and B). Of the 122 patients, 48 (39%) patients were older than 65 years and most patients acquired the CDI while in the hospital. Active cancer was the most common reason for hospitalization, followed by diabetes mellitus, and end-stage renal disease. More than 90% of the patients had received antibiotics before acquiring

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CDI. The results of fecal leukocyte examinations were positive in 33 (27%) patients. The overall in-hospital mortality rate was 26.2%. There were no significant differences between patients with nontoxigenic CDI and patients with toxigenic CDI.
*Conclusion: Clostridium difficile* infection can develop in healthcare facilities and in community settings, especially in immunocompromised patients.


## Introduction

*Clostridium difficile* is a major causative pathogen of antibiotic-associated diarrhea. In the past 20 years, there has been a dramatic increase in the incidence and severity of healthcare-associated *C. difficile* infection (CDI).[1,2] In the United States, the incidence of nosocomial *C. difficile*-associated diarrhea increased from 31 per 100,000 people in 1996 to 61 per 100,000 people in 2003. The incidence rate, however, was significantly higher among patients with an advanced age.[3] In Canada, the mortality rate of healthcare-associated CDI increased from 1.5% in 1997 to 5.7% in 2005.[4] The results of studies from several countries have shown a rising incidence of CDI.[5–13] In Taiwan, testing for *C. difficile* toxins was not a common practice in most clinical microbiology laboratories, although cultures of *C. difficile* for stool samples were usually obtained from patients suspected of having CDI. The real situation of toxigenic CDI in Taiwan consequently is unclear.[14–17] The objective of this study is to investigate the clinical characteristics and outcomes of patients with CDI and to compare the difference between patients with nontoxigenic and toxigenic CDI.

## Materials and methods

### Setting

Patients with *C. difficile* infection were identified from medical records at the National Taiwan University Hospital, a 2500-bed medical center located in northern Taiwan. The following information was gathered and analyzed to better understand the clinical manifestations of CDI: age; sex; underlying immunocompromised conditions such as a history of immunosuppressant drug use, diabetes mellitus, liver cirrhosis, end-stage renal disease, malignancy, human immunodeficiency virus (HIV) infection, and use of drugs (e.g., immunosuppressants, steroid, antimicrobial agents, steroid, and antipeptic ulcer agents) within 30 days of the onset of symptoms; laboratory data obtained 2 days before or 1 day after diagnosis; and in-hospital mortality.

### Bacterial isolates and toxin detection

Liquid or semisolid stool samples were inoculated onto cycloserine-cefoxitin-fructose agar (BBL Microbiology Systems, Cockeysville, MD, USA).[18] After incubating the sample at 35°C for 48 hours under anaerobic conditions, *C. difficile* growth was identified by the Gram staining results (i.e., large Gram-positive rods), typical odor, and biochemical characteristics, which were identified by the Vitek Anaerobe Identification Card (bioMérieux, Inc., Baltimore, France). Before testing, the isolates of *C. difficile* were frozen at −70°C in brain-heart infusion broth (BBL Microbiology Systems) and 15% glycerol. The production of *C. difficile* toxin A or toxin B was detected by an enzyme-linked fluorescent assay (bioMérieux, France) that was performed directly on the stool.

### Definitions

Toxigenic CDI was diagnosed in patients who had clinical symptoms of CDI (e.g., fever, diarrhea, abdominal discomfort or distension, or ileus) and a stool sample that tested positive for *C. difficile* and tested positive for toxin A or toxin B.[2] Nontoxigenic CDI was diagnosed in patients who had clinical symptoms of nontoxigenic *C. difficile* and a stool culture that was positive for *C. difficile* but negative for toxin A or toxin B. The colonization of *C. difficile* was defined in asymptomatic patients who had a stool culture that was positive for *C. difficile* but negative for either toxin. Healthcare-associated infection was diagnosed in patients with the onset of CDI symptoms more than 48 hours after their admission to a healthcare facility. Community-acquired CDI was diagnosed in patients who had the onset of CDI-associated symptoms while in the community or in patients in whom symptoms appeared 48 hours or less after their admission to a healthcare facility—provided that the symptom onset was more than 30 days after the last discharge from a healthcare facility.[13] Antipeptic ulcer drugs included proton pump inhibitors and histamine 2 (H2) blockers. Active cancer was defined as a diagnosis of cancer within 6 months before enrollment, any treatment for cancer within the previous 6 months, or recurrent or metastatic cancer.[19] Patients were considered to have an immunocompromised condition if they had active cancer, liver cirrhosis, autoimmune disorders, end-stage renal disease, HIV infection, or used immunosuppressants or steroids.

### Statistical analysis

The Student *t* test was used to compare continuous variables between groups. The results are presented as the mean ± standard deviation (SD). The Chi-square test was used to compare categorical variables between groups. All statistical analyses were performed with the statistical package software SPSS for Windows, version 12 (SPSS Inc.,

Chicago, Illinois, USA). A $p$ value $< 0.05$ was considered statistically significant.

## Results

### Hospital setting and patients

During the years 2000—2010, 3024 of 26,424 (11.4%) stool specimens tested positive for *C. difficile* (Fig. 1A). Testing for *C. difficile* toxin at the National Taiwan University Hospital began in the year 2005, but this testing was not reimbursed by the National Health Insurance system. Thus, tests were not routinely performed for each patient with a suspected case of CDI. Of the 940 specimens that were examined for the presence of toxin A or toxin B, only 50 (5.3%) stool specimens were positive for the *C. difficile* toxin during the years 2005—2010; the positive rate gradually decreased over time (Fig. 1B). We identified 178 patients with stool cultures that were positive for *C. difficile* and who were administered concomitant toxin testing from 2005—2010. Among the patients, 92 patients were diagnosed as having non-toxigenic CDI, 30 patients were diagnosed as having toxigenic CDI, and colonization of *C. difficile* was diagnosed in the remaining 56 patients.

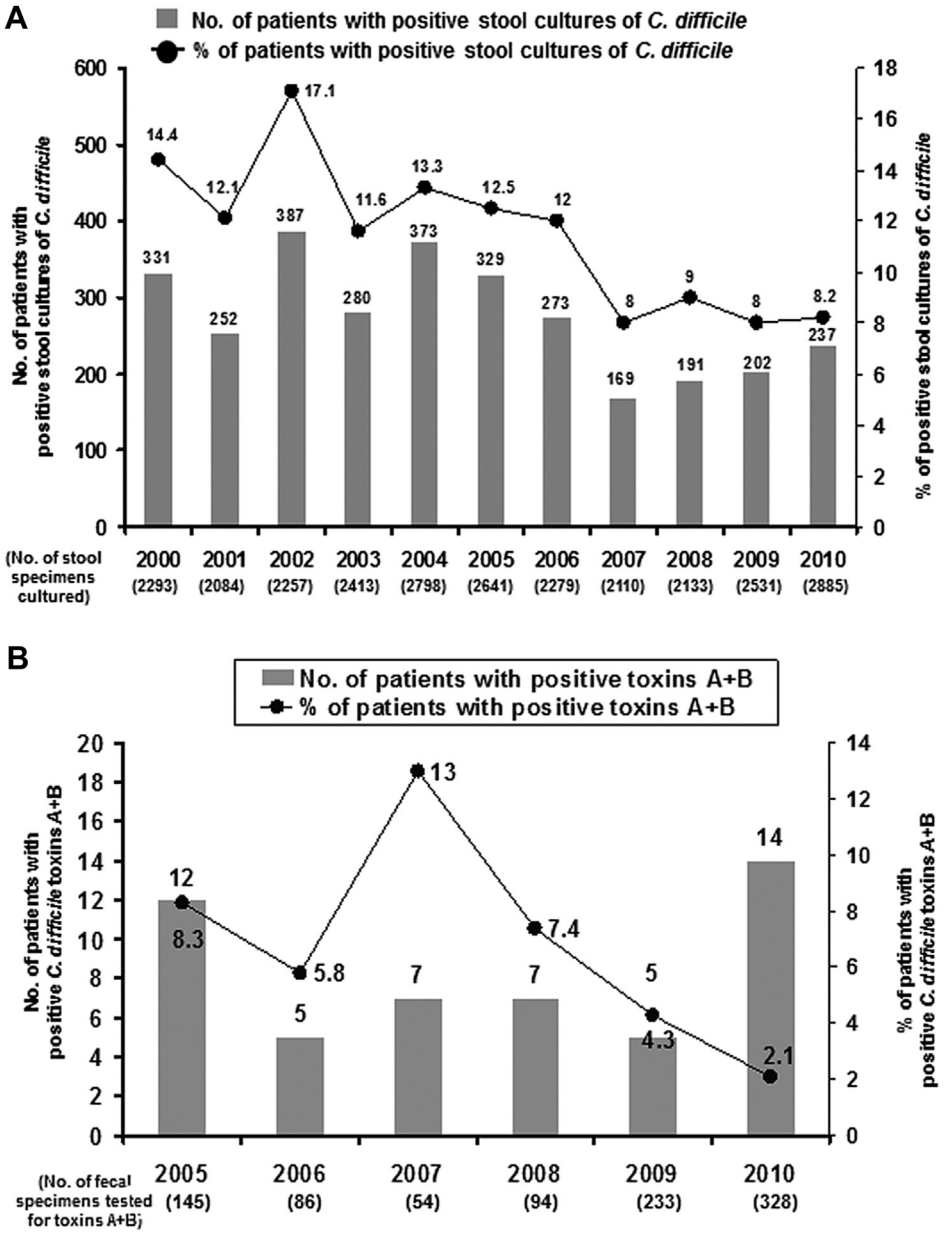


**Figure 1.** (A) The annual number of patients with stool culture testing positive for *C. difficile* in the years 2000—2010. (B) The annual number of patients with stool specimen testing positive for *C. difficile* toxins in the years 2005—2010. *C. difficile* = *Clostridium difficile*.

### Comparison between patients with colonization of *C. difficile*, nontoxigenic CDI, and toxigenic CDI

Table 1 shows the clinical characteristics of the 178 patients with a positive stool culture for *C. difficile*. We found that the patients with colonization of *C. difficile* were significantly younger than patients with nontoxigenic CDI ($p = 0.0274$). Of the 122 patients with CDI (toxigenic and nontoxigenic), 48 (39%) patients were older than 65 years and 75 (84%) patients were men. Most patients had healthcare-associated infection. Cancer was the most common underlying illness, followed by diabetes mellitus and end-stage renal disease. More than 90% of patients had received antibiotics prior to acquiring toxigenic or nontoxigenic *C. difficile* infection. Cephalosporin was the most common antibiotic used. More than one-half of the patients who acquired CDI had received antipeptic ulcer drugs. Proton pump inhibitors were the most commonly prescribed agents. The overall in-hospital mortality rate was 29.8%. To investigate the differences between patients with nontoxigenic and toxigenic CDI, we analyzed the clinical features of each group of patients (Tables 1 and 2). There were no significant differences between the two groups in age, sex, underlying diseases, laboratory findings, antimicrobial use, antipeptic ulcer drug use, or in-hospital mortality.

### Outcome analysis

Of the 30 patients with toxigenic CDI, 4 died in the hospital (13.3%) and all had an underlying malignancy and healthcare-associated CDI. Furthermore, we found a higher frequency of active cancer among patients with mortality than among surviving patients ($p = 0.003$; Table 3).

## Discussion

In this retrospective study, we found that the clinical manifestation in patients with nontoxigenic CDI was similar to the manifestation in patients with toxigenic CDI. Furthermore, the in-hospital mortality rate did not differ significantly between patients with nontoxigenic CDI and patients with toxigenic CDI. However, because of the limited number of patients in the present work, further large scale studies are still needed to clarify this finding. In the present study, healthcare-associated CDI unsurprisingly comprised most cases of CDI, although more than 20%

**Table 1** Clinical characteristics of 178 patients with positive stool cultures for *Clostridium difficile*

| Characteristic | Colonization ($n = 56$) | Nontoxigenic *C. difficile* infection ($n = 92$) | Toxigenic *C. difficile* infection ($n = 30$) |
|---|---|---|---|
| Age, mean ± SD | 38.38 ± 32.27[a] | 52.51 ± 40.21[a] | 49.47 ± 29.85 |
| Age > 65 y | 17 (30.35) | 37 (40.21) | 11 (36.67) |
| Male, *n* (%) | 28 (50.00) | 55 (59.19) | 20 (66.67) |
| Healthcare-associated infection | | 80 (86.96) | 23 (76.67) |
| Underlying disease | | | |
| Active cancer | 28 (50.00) | 44 (47.83) | 18 (60.00) |
| Diabetes mellitus | 13 (23.21) | 16 (17.39) | 6 (20.00) |
| End-stage renal disease | 1 (5.00) | 10 (10.87) | 2 (6.67) |
| Liver cirrhosis | 2 (3.57) | 6 (6.52) | 1 (3.33) |
| Autoimmune disease | 3 (5.36) | 3 (3.26) | 2 (6.67) |
| HIV infection | 0 (0.00) | 1 (1.09) | 0 (0.00) |
| Receiving immunosuppressant | 16 (28.57) | 25 (27.17) | 5 (16.67) |
| Receiving steroid | 8 (14.29) | 7 (7.61) | 3 (10.00) |
| Laboratory examinations | | | |
| White blood cell (cell/μL) | 9655.31 ± 10,139.56 | 8969.01 ± 7310.23 | 7962.59 ± 5515.18 |
| Neutrophil counts | 5771.70 ± 5661.62 | 6461.48 ± 6334.30 | 5321.66 ± 4505.09 |
| ANC < 500 (cell/μL) | 4 (7.1) | 12 (13.04) | 5 (16.67) |
| Hemoglobin (g/dL) | 10.37 ± 1.75 | 9.92 ± 2.11 | 9.98 ± 2.12 |
| AST (IU/L) | 37.42 ± 27.89 | 40.00 ± 31.74 | 36.21 ± 27.57 |
| Albumin (mg/dL) | 3.71 ± 0.84 | 3.40 ± 0.77 | 3.31 ± 0.89 |
| Creatinine (mg/dL) | 1.09 ± 1.39 | 1.45 ± 1.53 | 1.20 ± 1.08 |
| CRP (mg/dL) | 5.98 ± 8.14 | 6.32 ± 5.68 | 6.13 ± 4.78 |
| Positive stool pus cell | 15 (26.79) | 23 (25.00) | 10 (33.33) |
| Antipeptic ulcer drug | 30 (53.57) | 49 (53.26) | 16 (53.33) |
| Proton pump inhibitor | 21 (37.50) | 44 (47.82) | 15 (50.00) |
| H2 blocker | 12 (21.43) | 10 (10.86) | 1 (3.33) |
| In-hospital mortality | 19 (33.93) | 28 (30.43) | 4 (13.33) |

[a] Indicates a significant difference between patients with *C. difficile* colonization and patients with nontoxigenic *C. difficile* infection.
Data are presented as *n* (%) or mean ± SD.
ANC = absolute neutrophil count; AST = aspartate aminotransferase; *C. difficile* = *Clostridium difficile*; CRP = C-reactive protein; H2 = histamine 2; SD = standard deviation.

**Table 2** The association between antibiotic exposure and *Clostridium difficile* infection

| Characteristic | Nontoxigenic *C. difficile* infection ($n = 92$) | Toxigenic *C. difficile* infection ($n = 30$) | *p* |
|---|---|---|---|
| Any | 88 (95.65) | 29 (96.67) | 0.775 |
| Penicillin drugs | 53 (57.61) | 13 (43.33) | 0.249 |
| Any cephalosporins | 76 (82.61) | 21 (70.00) | 0.220 |
| First generation | 12 (13.04) | 7 (23.33) | 0.289 |
| Second generation | 11 (11.96) | 4 (13.33) | 0.899 |
| Third generation | 38 (41.30) | 6 (20.00) | 0.037 |
| Fourth generation | 45 (48.91) | 15 (50.00) | 0.915 |
| Fluoroquinolones | 37 (40.21) | 13 (43.33) | 0.930 |
| Carbapenems | 41 (44.57) | 11 (36.67) | 0.584 |
| Glycopeptides | 33 (35.86) | 12 (40.00) | 0.849 |
| Aminoglycosides | 13 (14.13) | 3 (10.00) | 0.787 |
| Clindamycin | 1 (1.09) | 0 (0.00) | 0.555 |
| Antifungal agent | 34 (36.96) | 13 (43.33) | 0.683 |

Data are presented as *n* (%).
*C. difficile* = *Clostridium difficile*.

of cases of CDI were classified as a community-acquired infection. In a recent population study conducted in the United States, the authors reported that community-acquired CDI accounted for 41% of 385 confirmed cases of CDI and that the incidence increased between 1991 and 2005.[20] The occurrence of community-acquired CDI was less in this study than in the United States study[20]; however, more epidemiologic studies should be performed to establish the clinical impact of community-acquired CDI.

Of the 122 patients with CDI (toxigenic and nontoxigenic), 100 (82%) patients were immunocompromised. However, we could not explore the association between CDI and the underlying immunocompromised conditions because of the small number of patients. In addition, more than one-third of the patients with CDI were of advanced age. A similar finding was reported by Viswanathan et al.[21]

In this study, we found that more than 90% of patients with CDI had a previous history of antibiotic usage. Extended-spectrum cephalosporins, including third and fourth generation cephalosporins, were the most common antecedent antibiotics. We also noted that the use of third generation cephalosporin is higher in patients with nontoxigenic CDI than in patients with toxigenic CDI. We also found that more than one-third of patients with CDI had received antifungal agents. The association between the previous use of antifungal agents and the development of CDI requires further study.

Fecal leukocyte examination is routinely performed as an initial diagnostic tool for hospitalized patients with diarrhea. We found that the test was positive in only 27% of patients with CDI. This finding is consistent with previous studies that showed that the sensitivity of fecal leukocyte testing is as low as 22.7%.[16,22] Therefore, this test is not a good predictor of CDI.

The overall in-hospital mortality rate in this study was approximately 26%. The mortality of toxigenic CDI was associated with active cancer. In a study of 86 hospitalized patients with CDI in southern Taiwan, the crude in-hospital mortality rate was 37%.[16] By contrast, the mortality rate was only 5% in a recent study of 485 cases of laboratory-confirmed

**Table 3** Risk factors associated with in-hospital mortality in patients with toxigenic *Clostridium difficile* infection

| Variable | Survival ($n = 26$) | Mortality ($n = 4$) | *p* |
|---|---|---|---|
| Age > 65 y | 10 (38.46) | 1 (25.0) | 0.9703 |
| Female sex | 8 (30.79) | 2 (50.0) | 0.8501 |
| Healthcare-associated infection | 21 (80.77) | 4 (100.0) | 0.8078 |
| Community-acquired infection | 5 (19.23) | 0 (0.0) | 0.8078 |
| Active cancer | 14 (53.85) | 4 (100.0) | 0.0031[a] |
| Diabetes mellitus | 6 (23.08) | 0 (0.0) | 0.6870 |
| Receiving immunosuppressant treatment | 4 (15.38) | 1 (25.0) | 0.8103 |
| Use of antipeptic ulcer drug | 15 (57.69) | 1 (25.0) | 0.4954 |
| Treatment | | | |
| Metronidazole | 21 (80.77) | 4 (100.0) | 0.8102 |
| Vancomycin | 4 (15.38) | 1 (25.0) | 0.8103 |

[a] Indicates a significant difference.
Data are presented as *n* (%).
*C. difficile* = *Clostridium difficile*.

CDI.[23] The variation in mortality in these studies may be because different study populations and study designs were used. In the present study, most patients with CDI had underlying immunocompromised conditions.

We also did not find that acid suppression was associated with an increased risk of death, which contrasts with the results by Morrison et al[23] (odds ratio, 4.74; 95% confidence interval, 1.57—14.37). Further large-scale epidemiological studies are needed to clarify the relationship between the use of acid suppressing agents and the outcome of CDI.

There were several limitations in this study. In this retrospective analysis, a relatively limited number of CDI cases were identified. Therefore, most findings may not reach a significant difference. In addition, only patients with a positive culture and who received toxin examinations were enrolled.

In conclusion, CDI can develop—especially in immunocompromised patients—in community settings and in healthcare facility settings. Physicians should keep this clinical entity in consideration, especially in patients with risk factors. We found that the role of fecal leukocyte testing is limited for CDI.

## Conflicts of interest

All contributing authors declare no conflicts of interest.

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