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ORIGINAL ARTICLE

A Cross-Sectional Study of *Clostridium Difficile* Infection in Inpatients with Antibiotic Associated Diarrhea

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SUMMARY

Background: ***Clostridium difficile*** **(*C. difficile*) is a Gram-positive, anaerobic, spore-forming bacillus that can cause pseudomembranous colitis and other *C. difficile*-associated diseases, resulting in significant morbidity and mortality. The incidence and clinical features vary by geography.**
Methods: **In this cross-sectional study, we examined the incidence and clinical features of *C. difficile* infection (CDI) within a 2,900-bed academic medical center in a southern area of China from January 1, 2017, to December 31, 2020. All adult inpatients (aged ≥ 18 years) who submitted loose stool samples for *C. difficile* testing over this period were considered for the study.**
Results: **This cross-sectional study showed that the average incidence of CDI was 2.07 cases/100,000 hospital patient-days. The mean age of these inpatients was 71.21 ± 2.83 years (range 30 - 93 years), and 83.61% (51/61) were treated in medical units. We found that 85.25% (52/61) of inpatients with CDI were aged > 60 years. Multivariate logistic regression analysis revealed that age > 60 years, and admission to the geriatric treatment unit or neurosurgery treatment unit were indeed independent risk factors for CDI in inpatients.**
Conclusions: **The incidence of CDI in the southern area of China was low. Age > 60 years, and treatment in geriatric or neurosurgery units were independent risk factors for CDI inpatients.**
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KEYWORDS

Clostridium difficile, infection, incidence, clinical features, cross-sectional study

INTRODUCTION

Clostridium difficile (*C. difficile*) is a Gram-positive, anaerobic, spore-forming bacillus that can cause pseudomembranous colitis and other *C. difficile*-associated diseases resulting in significant morbidity and mortality [1-5]. Moreover, *C. difficile* infection (CDI) is spread by the fecal-oral route; spores, the infective form, can persist on fomites and environmental surfaces for months. Since *C. difficile* was found to be the cause of

pseudomembranous colitis in 1978 [6], CDI had become more and more important in clinical practice, and is now one of the most significant problems threatening global health. However, the incidence of CDI varies from 1.59% to 27.27% [7] in hospitalized patients, and showed great regional variation. CDI may be more prevalent in high-risk patients such as those in ICU and oncology units. Furthermore, it is well documented that the occurrence of CDI has increased worldwide [7-13]. Although historically a rare entity in Asia, this pathogen can spread quickly and will likely increase in frequency in areas where its prevalence is currently considered to be low.

This study had the following objectives: 1) to evaluate the incidence of CDI in an academic medical center in the southern area of China, and 2) to collect data on CDI risk factors in this area.

MATERIALS AND METHODS

Patients and samples

A cross-sectional study of CDI was conducted in a 2,900-bed academic medical center in the southern area of China, from January 1, 2017, to December 31, 2020. All adult inpatients (≥ 18 years) who submitted loose stool samples for *C. difficile* testing over this period were considered for the study. Loose stool samples were monitored 48 hours after admission. A total of 1,707 inpatients were included in the study. To eliminate duplicate results from the same patient, only the first results (for patients with negative results) or the most positive results (for patients with positive results) were included in data analysis. All of the 1,707 participants had received antibiotic therapy within 8 weeks of the onset of diarrhea. All data were collected from the hospital infection monitoring system data-base. This data-base drew information from numerous sources, including inpatient's electronic health record, laboratory, and microbiology records, and included admission and discharge data, age, gender, and health care exposure. Inpatients with more than one episode of CDI could be enrolled more than once. Exclusion criteria were patients with community-acquired CDI from outpatient department, inpatients < 18 years of age, those with a diverting ostomy or prior ileal pouch-anal anastomosis (IPAA) and those currently on medical therapy for CDI or CDI diagnosis within the past 4 weeks.

This study was approved by the local Ethics Committee of The First Affiliated Hospital of Xiamen University and complied with the Declaration of Helsinki (2008). Written and informed consent was obtained from all participants.

Definitions and collection of data

According to the recommended definitions in the SHEA-IDSA guidelines, a case of CDI is defined as inpatients with symptoms (usually diarrhea) from more than 48 hours after admission and having a *C. difficile* positive laboratory test result.

Stool samples were processed within 30 minutes of arriving at the laboratory and were stored at 4℃ if immediate processing was not possible. For laboratory analysis, simultaneous detection of glutamate dehydrogenase (GDH) and toxin A/B was carried out by enzyme immunoassay (EIA) with C. Diff Quik Chek Complete manufactured by TechLab (Blacksburg, VA, USA), distributed by Abbott. GDH detection in the C. Diff Quik Chek Complete test uses anti-GDH polyclonal antibodies immobilized on the membrane and enzyme labelled anti-GDH monoclonal antibodies. GDH is present in both toxigenic and nontoxigenic strains of *C. difficile*. The manufacturer's recommendations were followed. In brief, 25 mL or an equivalent volume of stool sample was added to a tube containing the diluent and conjugate (TechLab), and the mixture was transferred to the device sample well. After incubation for 15 minutes at room temperature, the wash buffer (TechLab) and then the substrate (TechLab) were added to the reaction window. The results were read 10 minutes later. GDH antigen and/or toxins were reported positive if a visible band was seen on the antigen and/or toxin side of the device display window, respectively. The cutoff values for the assay were established at concentrations of 0.63 ng/mL for toxin A, 0.16 ng/mL for toxin B, and 0.8 ng/mL for glutamate dehydrogenase. Quality control was determined with *C. difficile* ATCC700059.

Statistical analysis

The incidence of CDI was reported as the number of CDI cases per 100,000 hospital patient-days. Descriptive statistics were used to summarize the clinical and epidemiologic characteristics of CDI. For categorical variables, the percentage of inpatients or isolates in each category was calculated. The chi-squared test was used to compare categorical variables. To identify risk factors for CDI, the chi-squared test was used. Factors showing p-value < 0.05 were considered candidate predictors that were significantly related to CDI and were extracted. Then, multivariate analysis was performed for these factors using the logistic regression model. All analyses were performed using the SPSS statistical software package version 30 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA).

RESULTS

Incidence of CDI

This cross-sectional study showed that the average incidence of CDI was 2.07 cases/100,000 hospital patient-days; 2.76 cases/100,000 hospital patient-days in 2017, 2.77 cases/100,000 hospital patient-days in 2018, 1.30 cases/100,000 hospital patient-days in 2019, and 1.56 cases/100,000 hospital patient-days in 2020. The incidence of CDI thus showed a downward trend from 2017 to 2020 (Figure 1) (Table 1).

Table 1. The incidence rates of CDI patients from 2017 to 2020.

Year	CDI cases (n)	Annual hospital patient-days	Incidence of CDI (cases/100,000 hospital patient-days)
2017	19	68,7592	2.76
2018	20	72,3159	2.77
2019	10	76,7578	1.30
2020	12	77,0214	1.56

Table 2. Characteristics of *C. difficile* negative cases and CDI patients.

Characteristics	No. - *C. difficile* group (n = 1,646) n, %	CDI group (n = 61) n, %	p-value
Gender Male	1,057 (64.22)	35 (57.38)	0.275
Female	589 (35.78)	26 (42.62)	0.275
Age > 60 (year - old)	1,011 (61.42)	52 (85.25)	0.000
Underlying comorbidities			
None	224 (13.61)	7 (11.48)	0.632
Pulmonary diseases	313 (19.02)	7 (11.48)	0.138
Blood diseases	146 (8.87)	5 (8.20)	0.856
Intra-abdominal diseases	263 (15.98)	9 (14.75)	0.798
Cancer	208 (12.64)	6 (9.84)	0.517
Renal diseases	38 (2.31)	2 (3.28)	0.951
Diabetes mellitus	282 (17.13)	10 (16.39)	0.881
Hypertension	523 (31.77)	27 (44.26)	0.040
Neurological diseases	432 (26.25)	30 (49.18)	0.000
Heart diseases	593 (36.03)	19 (31.15)	0.435
BMI index			
10.0 - 18.4	369 (22.42)	11 (18.03)	0.419
18.5 - 23.9	897 (54.50)	28 (45.90)	0.186
24.0 - 27.9	297 (18.04)	16 (26.23)	0.105
28.0 - 32.0	83 (5.04)	6 (9.84)	0.174
Treatment unit			
ICU	237 (14.40)	7 (11.48)	0.522
Internal medicine			
Gastroenterology	407 (24.73)	15 (24.59)	0.981
Respiratory	164 (9.96)	1 (1.64)	0.031
Geriatrics	235 (14.28)	19 (31.15)	0.000
Hematology	151 (9.17)	5 (8.20)	0.795
Oncology	118 (7.17)	2 (3.28)	0.362
Nephrology	39 (2.37)	1 (1.64)	1.000
Neurology	25 (1.52)	1 (1.64)	1.000
Cardiology	21 (1.28)	0 (0.00)	0.767
Surgery			
Neurosurgery	37 (2.25)	8 (13.11)	0.000
Urology	21 (1.28)	1 (1.64)	1.000
Discharge disposition			
Recovery	1,509 (91.68)	58 (95.08)	0.341
Improvement	133 (8.08)	3 (4.92)	0.513
Functional status deterioration	2 (0.12)	0 (0.00)	1.000
In-hospital mortality	2 (0.12)	0 (0.00)	1.000

Table 3. Multivariable analysis to evaluate factors potentially associated with CDI.

Variables	Univariate analysis	p-value	Multi-variate analysis	p-value
	OR (95% CI)		OR (95% CI)	
Age > 60 years-old	3.638 (1.781 - 7.434)	p = 0.000	3.096 (1.463 - 6.551)	p = 0.003
Hypertension	1.705 (1.018 - 2.856)	p = 0.043		
Neurological diseases	2.720 (1.627 - 4.546)	p = 0.000		
Treatment unit distribution				
Respiratory treatment unit	0.174 (0.024 - 1.264)	p = 0.084		
Geriatrics treatment unit	2.716 (1.553 - 4.751)	p = 0.000	2.058 (1.122 - 3.777)	p = 0.020
Neurosurgery treatment unit	1.187 (1.102 - 1.277)	p = 0.000	1.195 (1.106 - 1.291)	p = 0.000

OR - odds ratio, CI - confidence interval.

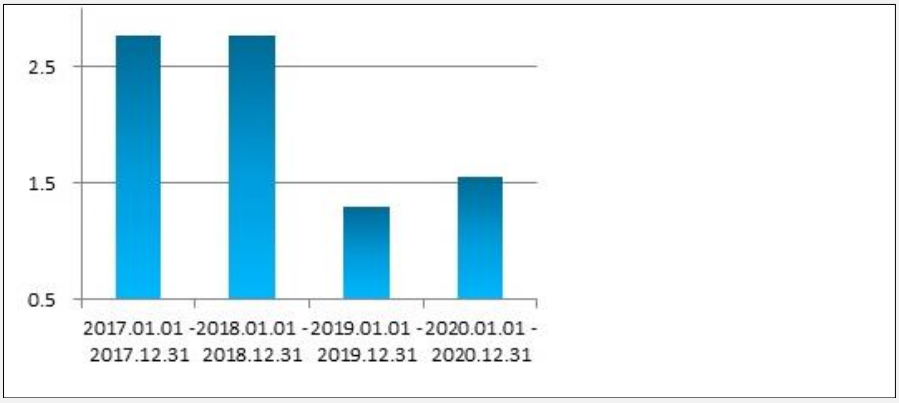

Figure 1. Comparison of incidence of CDI from 2017 to 2020.

Clinical characteristics and outcomes

A total of 61 patients were diagnosed with CDI from 2017 to 2020 (35 males, 26 females). The mean age of these inpatients was 71.21 ± 2.83 years (range 30 - 93 years). CDI occurred throughout the year without any significant seasonal variation. The majority (83.61%, 51/61) of inpatients were treated in medical units. We found that 85.25% (52/61) inpatients with CDI were > 60-years-old. In terms of clinical outcomes, all CDI cases were cured or improved. No inpatients with CDI died within 30 days of admission (Table 2).

Analysis of risk factors of inpatients with CDI

The results of univariate analysis using the chi-squared test in inpatients with CDI are shown in Table 2. Six parameters were associated with CDI inpatients, namely age > 60 years (p = 0.000), hypertension (p = 0.040), neurological diseases (p = 0.000), respiratory treatment unit (p = 0.031), geriatrics treatment unit (p = 0.000), and neurosurgery treatment unit (p = 0.00). Multivariate logistic regression analysis was applied to analyze the prognostic significance of these six factors, revealing that age > 60 years (p = 0.003, odds ratio [OR]: 3.096), geriatrics treatment unit (p = 0.020, odds ratio [OR]: 2.058), and neurosurgery treatment unit (p = 0.000, odds ratio [OR]: 1.195) were indeed independent risk factors for CDI inpatients (Table 3).

DISCUSSION

The incidence rate of CDI varies greatly between regions. Martijn et al. [14] reported that the incidence of CDI varied across hospitals (weighted mean 4.1 per 10,000 patient-days per hospital, range 0.0 - 36.3) in Europe. While Arun et al. [15] found that toxin was de-

tected in 8.8% of patients (58/660) by enzyme-linked fluorescence assay in a prospective cross-sectional study in India. However, *C. difficile* is reported as a low prevalence hospital pathogen, and its true prevalence remains unknown in Asia. A study of 17 hospitals in Korea from 2008 found an increase in incidence from 1.7 cases/1,000 adult admissions in 2004 to 2.7 cases/1,000 adult admissions [9]. This cross-sectional study showed that the average incidence of CDI was 2.07 cases/ 100,000 hospital patient-days between 2017 and 2020, which was low compared to Europe and India, and similar to Korea. Moreover, this academic medical center-based study revealed that the incidence of CDI decreased steadily from 2.76 cases/10,000 adult admissions in 2017 to 1.56 cases/10,000 adult admissions in 2020. The decline in CDI rates was the result of a combination of factors, including decreased antibiotic use, timely regulation of nosocomial infection control measures, and increased awareness, which all contributed to the decrease in CDI.

There are many reports of different risk factors of CDI patients. Kim et al. [9] found that most (91.7%) inpatients with CDI had been previously exposed to antibiotics. Sachu et al. [15] reported that diabetes, hypertension, use of proton pump inhibitors, previous hospitalization, malignancy, and chemotherapy were all risk factors. A positive history for *C. difficile*, antibiotics in the previous 4 weeks, recent hospitalization, female gender, and age were significantly associated with CDI in Italy according to a report by Cioni et al. [16]. However, in this study, age > 60 years ($p = 0.003$, odds ratio [OR]: 3.096), and a stay in the geriatric treatment unit ($p = 0.020$, odds ratio [OR]: 2.058), or neurosurgery treatment unit ($p = 0.000$, odds ratio [OR]: 1.195) were indeed independent risk factors for CDI. Our results showed that both the geriatric treatment unit and the neurosurgery treatment unit experienced high incidence rates of CDI, which were not consistent with previous reports arguing that ICU patients have a higher CDI rate than other wards [17]. This may be explained by a higher emphasis on the importance of nosocomial infection, such as aseptic surgical technique and rational use of antibiotics in the ICU over recent years.

Inpatients in geriatric and neurosurgery treatment units are generally older (> 60 years) and may suffer many underlying comorbidities, such as nervous system disease, cerebrovascular disease, or traumatic brain injury from falls, which can easily render the inpatient bedridden for long periods of time or requiring medical aids or antibiotics to maintain a normal life. For diagnosis and management of CDI, more attention should be paid to patients with diarrhea in these key departments. No CDI cases were found among these inpatients aged from 19 to 30 years old, thus old inpatients were more susceptible to CDI. Interestingly, this cross-sectional study showed that age of > 60 years was one of the risk factors for CDI acquisition, five years younger than the age of > 65 years reported by other researchers [18,19]. The reason may be that in recent years, the application of advanced medical technology, such as the use of extracorporeal membrane oxygenation (ECMO) technology, means that diseases that could not be treated before can now be treated or even cured, which makes the CDI population younger.

Although EIA for detection of toxins A and B in the stool is a relatively inexpensive, fast, and convenient method, EIA tests have variable sensitivities (63 - 73%) [20,21], suggesting that the diagnosis of CDI could be missed for many patients. Currently, there is no testing strategy that is optimally sensitive and specific for diagnosis of CDI [5]. If a proper surveillance system is introduced, more accurate data will be obtained concerning the incidence and clinical features of CDI.

There were some identified limitations to our study. Identification of toxigenic isolates was not performed in our study and therefore we could not rule out the possibility of *C. difficile* colonization. Second, most inpatients were tested with only one test (stool toxin assay) to diagnose CDI. Third, community-acquired CDIs were not included in this study.

CONCLUSION

This cross-sectional study showed that the average incidence of CDI was 2.07 cases/100,000 hospital patient-days from 2017 to 2020. Age > 60 years and a stay in the geriatric treatment unit or neurosurgery treatment unit were confirmed as independent risk factors for CDI in inpatients.

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Declaration of Interest:
The authors declare that they have no conflict of interest.

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