HHS Public Access

Author manuscript

Eur J Clin Microbiol Infect Dis. Author manuscript; available in PMC 2015 December 10.

Published in final edited form as:

Eur J Clin Microbiol Infect Dis. 2014 October ; 33(10): 1773–1779. doi:10.1007/s10096-014-2132-9.

Clinical characteristics of *Clostridium difficile* infection in hospitalized patients with antibiotic-associated diarrhea in a university hospital in China

F. F. Zhou,
Institute of Antibiotics, Huashan Hospital, Fudan University, Shanghai 200040, China

S. Wu,
Institute of Antibiotics, Huashan Hospital, Fudan University, Shanghai 200040, China

J. D. Klena, and
Global Disease Detection Branch, Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

H. H. Huang
Institute of Antibiotics, Huashan Hospital, Fudan University, Shanghai 200040, China

H. H. Huang: drhaihuihuang@163.com

Abstract

The purpose of this study was to identify clinical characteristics of *Clostridium difficile* infection (CDI) in patients with antibiotic-associated diarrhea (AAD). A prospective study was conducted among patients hospitalized in Fudan University Hospital Huashan from August 1, 2012 to July 31, 2013. Toxigenic *C. difficile* isolates were characterized by PCR ribotyping and multilocus sequence typing. AAD developed in 1.0 % (206/20437) of the antibiotic-treated hospitalized patients and toxigenic *C. difficile* was isolated from 30.6 % (63/206) of patients with AAD. The frequency of AAD was highest in the intensive care unit (10.7 %); however the proportion of CDI in AAD was highest in the Geriatric Unit (38 %). AAD ranged in severity from mild to moderate. One case with pseudomembranous colitis was identified. Use of carbapenems was found to significantly increase the risk of CDI (OR, 2.31; 95 % CI, 1.22–4.38; p= 0.011). Patient demographics, presumed risk factors, clinical manifestations and laboratory findings revealed no significant difference between patients with CDI and non-*C. difficile* AAD. Over 90 % of the patients with CDI or non-*C. difficile* AAD were cured. Two patients had CDI recurrence. Ribotype H was the dominant (18.8 %) genotype, followed by ribotype 012 and ribotype 017. *C. difficile* plays a significant role in AAD in our setting in China. Because the severity of diarrhea ranges from mild to moderate, it is difficult for Chinese clinicians to identify CDI from AAD patients, therefore CDI should be included in the routine differential diagnoses for hospitalized patients presenting with AAD.

Correspondence to: H. H. Huang, drhaihuihuang@163.com.

Conflict of interest None declared.

Introduction

Antibiotic-associated diarrhea (AAD) is described as diarrhea that occurs in conjunction with antibiotic administration and cannot be explained by another diagnosis. AAD can range from a mild, self-limiting illness to more serious and progressive disease such as pseudomembranous colitis (PMC) [1]. AAD results from overgrowth of the intestinal mucosa by pathogenic microorganisms after antibiotic treatment; however, it may also occur in response to a reduction in the concentration of fecal flora. This can lead to a decrease in carbohydrate metabolism which in turn causes osmotic diarrhea and a decreased rate of primary bile acid breakdown [2].

Clostridium difficile has been linked to AAD since 1977. However, since the early 2000s, the incidence and severity of *C. difficile* infection (CDI) have increased dramatically in North America and Europe [3, 4]. *C. difficile* is the most important infectious cause of AAD, accounting for 10–30 % of all cases; when considering severe cases of AAD such as documented antibiotic-associated PMC, 90–100 % are attributed to CDI [2, 5]. Although other organisms, including *Clostridium perfringens*, *Staphylococcus aureus* and *Klebsiella oxytoca*, may cause AAD, they are not common [6].

European guidelines for treatment of CDI recommend that patients with mild-to-moderate CDI should be treated with metronidazole and patients with severe CDI should be treated with vancomycin; anti-peristaltic agents are contraindicated [4]. In contrast, for the treatment of AAD from other causes, anti-peristaltic agents are often used and metronidazole or vancomycin is not indicated [5]. Therefore it is important for clinicians to correctly diagnose a CDI case, not only for successful treatment, but also to decrease unnecessary use of metronidzole and/or vancomycin.

Although CDI is recognized as a major epidemic organism in North America and Europe, data from China remain limited. A prospective, observational study was conducted to evaluate the clinical characteristics of CDI in patients with AAD at University Hospital Huashan, a 1,216-bed hospital from August 1, 2012 to July 31, 2013 and to raise awareness of this disease in China.

Materials and methods

Definitions

Diarrhea was defined as three or more loose stools (corresponding to Bristol stool chart types 5–7) in≥24 h or more frequently than is normal for the individual case. A case of AAD was defined as otherwise unexplained diarrhea in a hospitalized patient occurring in association with the administration of antibiotics [1]. A case-patient with CDI was defined as a positive toxigenic *C. difficile* culture from a diarrhea hospitalized patient, or PMC diagnosed during enteroscopy. We defined diarrhea as severe when it occurred with one or more of the following: visible blood in the stool, fever (T> 38.0 °C) and leukocytosis (>12.0×10^9/l), hypoalbuminemia (<20 g/l) or PMC. A complicated course of CDI was defined as either an admission to the intensive care unit (ICU), a surgical intervention in association with CDI or death within one month of diarrhea onset. Mortality was considered

to be attributable to CDI when a patient died during admission, partly due to the consequences of CDI [7]. Patients were followed until hospital discharge. Two episodes in the same patient were considered different events if they occurred ≥8 weeks apart.

Patients

Eligibility criteria included being a hospitalized patient aged ≥18 years with acute diarrhea, and receiving antibiotic treatment ≥4 weeks before the onset of diarrhea. Patients with chronic diarrhea or who had history of using a laxative within 3 days preceding diarrhea onset were excluded. The study was approved by the Institutional Review Board at Fudan University Hospital Huashan.

C. difficile isolates

All fecal specimens from qualified patients were cultured on selective cycloserine–cefoxitin–fructose agar plates (Oxoid, Basingstoke, UK) and incubated in an anaerobic chamber (Ruskinn Technology Limited, Bridgend, UK) at 37ºC for 72 h. *C. difficile* colonies were identified on the basis of their typical morphology on agar plates, Gram stain and Rapid ID 32A identification test strips (BioMérieux, Marcy l'Etoile, France). The *tcdA* gene was detected by conventional PCR [8]. Multiplex real-time PCR to detect *tcdB* and *cdtA* genes was performed using the Cepheid Xpert™ *C. difficile* assay (Cepheid, Sunny Vale, CA, USA). Toxin B was confirmed by the cytotoxicity neutralization assay (Techlab, Blacksburg, VA, USA) with Vero cell lines. Strains were characterized further by ribotyping [9] and multilocus sequence typing (MLST) (http://pubmlst.org/cdifficile/) [10].

Clinical characteristics

A questionnaire was completed for each patient. The following data were collected: demographic data (e.g., age, gender, ward, community versus hospital acquisition, comorbidity), presumed risk factors in the 4 weeks before the onset of diarrhea (e.g., antimicrobial treatment, chemotherapy, naso-gastric intubation, surgery), biological parameters (e.g., albumin, white blood cell count [WBC]), and clinical course (e.g., severity, clinical and laboratory findings, treatment, and outcome) [11, 12].

Statistical analysis

SPSS version 16.0 (SPSS, Cary, NC, USA) was used for statistical analyses. To preserve the assumption of the independence of the observations, only the first episode of CDI for an individual patient was included in the analysis. The chi-square or Fisher's exact test was applied to compare categorical variables. The level of statistical significance was defined as $p<0.05$. Predictors of CDI were investigated using a logistic regression analysis. Age, sex and location were adjusted for multivariate analysis. Odds ratios and 95 % confidence intervals were used to quantify the strength of these associations.

Results

During the one-year study period, 46,558 patients were discharged from the University Hospital Huashan; 20,437 patients had received at least one dose of antibiotic. AAD developed in 1.0 % (206/20,437) of the treated patients and toxigenic *C. difficile* was

isolated from 30.6 % (63/206) of patients with AAD. The frequency of AAD was highest in the intensive care unit (ICU) (10.7 %, 56/525) (Fig. 1); however, the proportion of CDI in AAD cases was highest in the geriatric unit (38 %, 11/29). In total, 65 *C. difficile* isolates (63 from the first episode and two from recurrence) were recovered but one was not retrievable upon subculture. Eighteen different ribotypes were identified; ribotype H (ST-81, 18.8 %) was the most prevalent, followed by 012 (ST-54, 14.1 %) and 017 (ST-37, 12.5 %). None of the isolates belonged to ribotype 027 (ST-1) or 078 (ST-11).

Clinical characteristics and risk factors

Clinical characteristics and risk factors for CDI and non*C. difficile* AAD are summarized in Table 1. The mean age for CDI and non-*C. difficile* AAD case-patients were 66±17 and 62±20 years old, respectively. Most of the case-patients were male. Diabetes mellitus and malignancy were the most common underlying diseases in both groups. Beta-lactamase inhibitors were the most commonly prescribed class of antibiotic in both groups of patients (44.4 and 55.2 %, respectively). Carbapenems were prescribed significantly more frequently in case-patients with CDI than those with non*C. difficile* AAD (42.9 % vs.28.0 %, p=0.036). After univariate analysis and using a multivariate logistic regression model, only the use of carbapenems was found to be significantly associated with increased risk of CDI (RR, 2.31; 95 % CI, 1.22–4.38; p=0.011).

Clinical course

Neither fever (44.4 % vs. 33.6 %), abdominal pain (11.1 % vs. 7.7 %), nor WBC count showed significant differences between CDI and non-*C. difficile* AAD case-patients (Table 1). The results of diarrheal stool examinations were similar in both groups as well. Among patients who had received a stool examination, WBC were present in 10 % (5/50) and 4.0 % (4/99) diarrheal stool samples with CDI and non-*C. difficile* AAD (p=0.385), respectively. Red blood cells (RBC) were present in 4 % (2/50) and 1.0 % (1/99) of diarrheal stool samples of patients from both groups (p=0.220), respectively. Only one patient presented with PMC. The severity of other patients with CDI or non-*C. difficile* AAD ranged from mild to moderate diarrhea.

Once diagnosed as AAD, clinicians withdrew the implicated antibiotic from the treatment regime of the majority of patients (Table 2). Twenty-three of the 63 case-patients with CDI (36.5 %) received specific empirical treatment. Metronidazole was used in 10 (15.9 %) case-patients, but was changed to oral vancomycin in four case-patients because of a lack of clinical response at 10.5±6.6 days of treatment. Oral vancomycin was initially used in 13 case-patients. Among patients with non-*C. difficile* AAD, only five patients received vancomycin and three patients received metronidazole respectively (p<0.001). Almost 40 % of patients received probiotics in both groups, including *Bacillus bifidus*, *Lactobacillus acidophilus* and *Enterococcus* spp. In addition, more case-patients with CDI received berberine, a traditional Chinese medicine, than patients with non-*C. difficile* AAD (39.7 % vs. 17.5 %, p=0.001). In total, 85.7 % (54/63) and 92.3 % (132/ 143) of patients with CDI or non-*C. difficile* AAD, respectively, were cured. Two case-patients experienced one episode of CDI recurrence. Three patients died, one in the CDI group and two patients in the non-*C. difficile* AAD group. However, AAD did not contribute to these deaths.

Discussion

Diarrhea is one of the most frequent side effects of antibiotic therapy in hospitalized patients, occurring in 5–25 % of all patients [13]. The frequency of AAD depends on the antibiotics used for treatment as well as host-specific factors. In Sweden, Wiström et al. [14] reported the frequency of AAD varied from 1.8 to 6.9 % in participating centers in a large prospective study. The percentage of AAD cases reported in China is higher. Chen et al. [15] reported the frequency of AAD at 9.3 % (78/842) among patients hospitalized in a Chinese medical unit from 2002 to 2005, and Wang et al. [16] reported that the frequency of AAD among patients hospitalized in a Chinese digestive unit was 6.7 % (26/423) in 2007. In the present study, of the 20,437 antibiotic-treated patients, only 1.0 % developed AAD. One reason that the percentage of cases in this study with AAD is lower than expected might be the number of treated patients from surgical wards is more than two times that from medical wards (13,684 vs. 6,228). Most of the patients from the surgical wards received antibiotics for peri-operative prophylaxis only. In addition, clindamycin, which is related to a higher frequency of diarrhea, has not been used in the University Hospital Huashan setting since 2009.

C. difficile has been reported to account for approximately 20–30 % of all cases of AAD and is responsible for most antibiotic-related colitis (AAC) [2, 5]. As the incidence of CDI has increased in North America and Europe, some Chinese clinicians have expressed concern about the situation in China. Chen et al. [17] tested 1,845 diarrheal stool samples submitted to the clinical microbiology laboratory of a tertiary hospital in China and 161 (8.7 %) toxigenic *C. difficile* isolates were recovered between 2009 and 2011. Similarly, Hawkey et al. examined fecal samples from 70 hospital patients with diarrhea who were receiving or had received antibiotics within the previous six weeks in a separate Chinese tertiary hospital; 21 (30 %) *C. difficile* isolates were recovered [18]. In the present study, the CDI was detected in 30.6 % of 206 patients with AAD, a result consistent with Hawkey et al.

Patients who are likely to develop clinically significant AAD are at high risk for CDI. Antibiotic use itself is the most important risk factor for the development of CDI. Virtually all antibiotics have been implicated in CDI and AAD. Clindamycin, cephalosporins, and extended-spectrum penicillins are the antibiotics most frequently associated with CDI, although they also cause diarrhea that is unrelated to superinfection with this organism. Recently, fluoroquinolones such as moxifloxacin have been implicated as a common cause of CDI [5, 19–21]. In contrast, erythromycin acts as a motilin-receptor agonist and accelerates the rate of gastric emptying. This scenario is more likely to result in non-*C. difficile* AAD [6]. In the present study clindamycin was not used and only two patients with non-*C. difficile* AAD had previously received azithromycin. Compared with non-*C. difficile* AAD, only previous use of a carbapenem antibiotic was determined to be an independent risk factor for development of CDI. Carbapenems have the broadest spectrum of activity within the β-lactam class of antibiotics, and therefore it is reasonable to assume that they have a stronger propensity to disrupt normal intestinal microflora. Asha et al. [6] reported that despite the use of different control groups, symptomatic patients without infected AAD or asymptomatic hospitalized patients, the risk factors for CDI identified in their study were broadly similar. However, other well-known risk factors for CDI including being elderly,

impaired immune status, length of hospital stay, infected roommates, and nasogastric tube feeding [20, 21] were not identified as risk factors in the present study. This may be because with the exception of a single PMC patient, the severity of CDI was similar to that of non-*C. difficile* AAD, ranging from mild to moderate diarrhea. It is also possible that due to the low frequency of AAD and CDI cases, the methods used in this study were not sensitive enough to identify additional risk factors.

Clinical presentation has been used to distinguish between CDI and AAD due to other causes. Typically, other causes of AAD result in less severe disease than CDI. Patients with CDI may have evidence of colitis with abdominal cramps, low-grade fever, and fecal leukocytes; however, patients with diarrhea from other causes usually present with moderately severe diarrhea without evidence of colitis. Complications reported for CDI include hypoalbuminemia, anasarca, and toxic megacolon; non-CDI AAD is generally uncomplicated, with occasional cases of dehydration [2, 5, 19]. In the present study, no significant difference in clinical presentation was found between the two groups. This finding is consistent with previous studies in China [15, 17, 22] and helps to explain why CDI has been seriously underestimated in China as well. Clinicians do not effectively diagnose CDI based on the more mild presentation.

According to the guidelines published by the United States and European countries, metronidazole and vancomycin are recommended for treatment of CDI [4, 23]. Resolution of AAD symptoms other than *C. difficile* can usually be achieved through discontinuation of the offending antibiotic [5, 19]. In the present study 36.5 % of the patients with CDI received metronidazole and/or vancomycin compared to 5.6 % patients with non-*C. difficile* AAD. The reasons could be the clinical manifestation of CDI and AAD were similar in the present study and a lack of routine testing for *C. difficile* in clinical laboratory. Therefore it is difficult for clinicians to identify a CDI case from AAD. Berberine, a traditional plant alkaloid isolated from many kinds of plants such as *Hydrastis canadensis*, *Berberis aristata* and *Coptis chinensis*, has been used in treatment of gastroenteritis, abdominal pain and diarrhea in Ayurvedic, Chinese and Middle Eastern folk medicine for centuries [24]. It has been shown to have anti-inflammatory and antimicrobial properties [24, 25]. In addition, it is thought to be poorly absorbed through the gut wall and therefore is present in high concentrations in the stool [26]. In the present study, berberine was prescribed in 39.7 % and 17.5 % of patients with CDI and non-*C. difficile* AAD, with cure rates of 84 % and 92.0 %, respectively. A meta-analysis concluded that the therapeutic effects of pure traditional Chinese medicine (TCM) or a combination of TCM and Western medicine for treatment of AAD were superior to the effects of Western medicine alone [27]. Shu et al. [28] reported the inhibition of expression of *C. difficile* toxin genes was a potential pharmacological pathway of TCM for the treatment of CDI. Further studies are clearly required.

Our previous studies [9, 22] reported that *C. difficile* ribotype 017 was the dominant clone in University Hospital Huashan between 2007 and 2009. However, in the present study another clone, ribotype H (ST-81, 18.8 %) became dominant, followed by ribotype 012 (ST-54, 14.1 %). Ribotype 017 (ST-37, 12.5 %) had been displaced to third most prevalent. In China, Yan et al. in Beijing [29] reported ST-37 (24.0 %), ST-35 (15.4 %) and ST-54 (11.5 %) while Chen et al. in Hangzhou [17] reported ST-54 (23.0 %), ST-35 (19.3 %) and ST-37 (10.0 %)

as the top three prevalent genotypes, respectively. This diversity is likely due to the different geographical location. Recovery of *C. difficile* ribotype 027 is rare in Asia. At University Hospital Huashan, ribotype 027 has not been recovered. However, in late 2013, Wang et al. [30] reported the identification of a *C. difficile* ribotype 027 isolate in mainland China for the first time. Since this hypervirulent strain has spread rapidly in North America and Europe and is associated with many outbreaks, Chinese health workers must become highly vigilant.

A limitation of this study is that University Hospital Huashan is a tertiary care hospital, and thus receives many referrals, including patients from all over China. This fact makes it very difficult to perform follow-up studies with the patients. Therefore the recurrence rate is likely to be an underestimate.

In conclusion, *C. difficile* plays an important role in AAD in China. It is difficult for clinicians to identify CDI cases based on clinical presentation of AAD patients since the severity of disease ranges from mild to moderate diarrhea. Therefore, *C. difficile* infections should be included in the routine differential diagnoses for hospitalized patients with diarrhea, especially AAD. With the recent discovery of the first ribotype 027 cases in China, national surveillance is crucial to monitor the incidence, identify populations at risk, and characterize the molecular epidemiology of the strains causing CDI.

Acknowledgments

This work was supported by a grant from the National Natural Science Foundation of China (no. 30973594 and no. 81101292) and Shanghai Pujiang Programme (no. 10PJ1401800). The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

References

1. Beaugerie L, Petit JC. Microbial-gut interactions in health and disease. Antibiot Assoc diarrhoea Best Pract Res Clin Gastroenterol. 2004; 18(2):337–352.
2. Varughese CA, Vakil NH, Phillips KM. Antibiotic-associated diarrhea: a refresher on causes and possible prevention with probiotics-continuing education article. J Pharm Pract. 2013; 26(5):476–482. [PubMed: 24064436]
3. McDonald LC, Coignard B, Dubberke E, Song X, Horan T, Kutty PK. Ad Hoc Clostridium difficile Surveillance Working Group. Recommendations for surveillance of *Clostridium difficile*–associated disease. Infect Control Hosp Epidemiol. 2007; 28(2):140–145. [PubMed: 17265394]
4. Debast SB, Bauer MP, Kuijper EJ. Committee. European society of clinical microbiology and infectious diseases : update of the treatment guidance document for *Clostridium difficile* infection. Clin Microbiol Infect. 2014; 20(2):1–26. [PubMed: 24118601]
5. Bartlett JG. Antibiotic-associated diarrhea. N Engl J Med. 2002; 346(5):334–339. [PubMed: 11821511]
6. Asha NJ, Tompkins D, Wilcox MH. Comparative analysis of prevalence, risk factors, and molecular epidemiology of antibiotic-associated diarrhea due to Clostridium difficile, Clostridium perfringens, and Staphylococcus aureus. J Clin Microbiol. 2006; 44(8):2785–2791. [PubMed: 16891493]
7. Bauer MP, Kuijper EJ, van Dissel JT. European Society of Clinical Microbiology and Infectious Diseases. European society of clinical microbiology and infectious diseases (ESCMID): treatment guidance document for *clostridium difficile* infection (CDI). Clin Microbiol Infect. 2009; 15(12): 1067–1079. [PubMed: 19929973]

8. Kato H, Kato N, Watanabe K, Iwai N, Nakamura H, Yamamoto T, Suzuki K, Kim SM, Chong Y, Wasito EB. Identification of toxin A-negative, toxin B-positive Clostridium difficile by PCR. J Clin Microbiol. 1998; 36(8):2178–2182. [PubMed: 9665986]
9. Huang H, Weintraub A, Fang H, Wu S, Zhang Y, Nord CE. Antimicrobial susceptibility and heteroresistance in Chinese Clostridium difficile strains. Anaerobe. 2010; 16(6):633–635. [PubMed: 20849968]
10. Stabler RA, Dawson LF, Valiente E, Cairns MD, Martin MJ, Donahue EH, Riley TV, Songer JG, Kuijper EJ, Dingle KE, Wren BW. Macro and micro diversity of Clostridium difficile isolates from diverse sources and geographical locations. PLoS ONE. 2012; 7(3):e31559. [PubMed: 22396735]
11. Loo VG, Bourgault AM, Poirier L, Lamothe F, Michaud S, Turgeon N, Toye B, Beaudoin A, Frost EH, Gilca R, Brassard P, Dendukuri N, Béliveau C, Oughton M, Brukner I, Dascal A. Host and pathogen factors for Clostridium difficile infection and colonization. N Engl J Med. 2011; 365(18):1693–1703. [PubMed: 22047560]
12. Rodríguez-Pardo D, Almirante B, Bartolomé RM, Pomar V, Mirelis B, Navarro F, Soriano A, Sorlí L, Martínez-Montauti J, Molins MT, Lung M, Vila J, Pahissa A. Barcelona Clostridium difficile Study Group. Epidemiology of *Clostridium difficile* infection and risk factors for unfavorable clinical outcomes: results of a hospital-based study in Barcelona, Spain. J Clin Microbiol. 2013; 51(5):1465–1473. [PubMed: 23447638]
13. Bergogne-Bérézin E. Treatment and prevention of antibiotic associated diarrhea. Int J Antimicrob Agents. 2000; 16(4):521–526. [PubMed: 11118872]
14. Wiström J, Norrby SR, Myhre EB, Eriksson S, Granström G, Lagergren L, Englund G, Nord CE, Svenungsson B. Frequency of antibiotic-associated diarrhoea in 2462 antibiotic-treated hospitalized patients: a prospective study. J Antimicrob Chemother. 2001; 47(1):43–50. [PubMed: 11152430]
15. Chen J, Guo X. Clinical characteristics, control and prevention of antibiotic—associated diarrhea. World Chin J Digestol. 2006; 14(9):927–929.
16. Wang Y, Lin J. Clinical analysis of antibiotic associated diarrhea. Chin J Health Lab Technol. 2008; 18(7):1393–1394.
17. Chen YB, Gu SL, Wei ZQ, Shen P, Kong HS, Yang Q, Li LJ. Molecular epidemiology of Clostridium difficile in a tertiary hospital of China. J Med Microbiol. 2014; 63(Pt 4):562–569. [PubMed: 24344206]
18. Hawkey PM, Marriott C, Liu WE, Jian ZJ, Gao Q, Ling TK, Chow V, So E, Chan R, Hardy K, Xu L, Manzoor S. Molecular epidemiology of Clostridium difficile infection in a major Chinese hospital: an underrecognized problem in Asia? J Clin Microbiol. 2013; 51(10):3308–3313. [PubMed: 23903542]
19. Cote GA, Buchman AL. Antibiotic-associated diarrhoea. Expert Opin Drug Saf. 2006; 5(3):361–372. [PubMed: 16610966]
20. Hensgens MP, Goorhuis A, van Kinschot CM, Crobach MJ, Harmanus C, Kuijper EJ. Clostridium difficile infection in an endemic setting in the Netherlands. Eur J Clin Microbiol Infect Dis. 2011; 30(4):587–93. [PubMed: 21194003]
21. Vesteinsdottir I, Gudlaugsdottir S, Einarsdottir R, Kalaitzakis E, Sigurdardottir O, Bjornsson ES. Risk factors for Clostridium difficile toxin-positive diarrhea: a population-based prospective case–control study. Eur J Clin Microbiol Infect Dis. 2012; 31(10):2601–10. [PubMed: 22441775]
22. Huang H, Wu S, Wang M, Zhang Y, Fang H, Palmgren AC, Weintraub A, Nord CE. Molecular and clinical characteristics of Clostridium difficile infection in a University Hospital in Shanghai, China. Clin Infect Dis. 2008; 47(12):1606–1608. [PubMed: 19025371]
23. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, McFarland LV, Mellow M, Zuckerbraun BS. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. Am J Gastroenterol. 2013; 108(4):478–498. [PubMed: 23439232]
24. Tillhon M, Guaman Ortiz LM, Lombardi P, Scovassi AI. Berberine: new perspectives for old remedies. Biochem Pharmacol. 2012; 84(10):1260–1267. [PubMed: 22842630]
25. Zhang Y, Wang X, Liu L, Cai N, Li Q, Wu K. The anti-diarrhea mechanisms of berberine in diarrhea disease. Shaanxi Med J. 2010; 39(1):6–8.

26. Chen CM, Chang HC. Determination of berberine in plasma, urine and bile by high-performance liquid chromatography. J Chromatogr B Biomed Appl. 1995; 665(1):117–123. [PubMed: 7795781]

27. He C, Gao P. Meta-analysis of antibiotic-associated diarrhea treated by traditional Chinese medicine. Chin J TCM WM Crit Care. 2010; 17(2):69–72.

28. Shu Q, Peng S, Feng Y, Zhang Z, Xie Y, Zhou S. The influences of Chinese herbal compounds on in vitro expression of toxic genes *tcd*A/*tcd*B of *Clostridium difficile*. Chin J Microecol. 2013; 25(4):373–375. 380.

29. Yan Q, Zhang J, Chen C, Zhou H, Du P, Cui Z, Cen R, Liu L, Li W, Cao B, Lu J, Cheng Y. Multilocus sequence typing (MLST) analysis of 104 *Clostridium difficile* strains isolated from China. Epidemiol Infect. 2013; 141(1):195–199. [PubMed: 22475233]

30. Wang P, Zhou Y, Wang Z, Xie S, Chen Y, Jiang B, Zhang T, Lin M, Li R, Tan J. Identification of *Clostridium difficile* Ribotype 027 for the first time in mainland China. Infect Control Hosp Epidemiol. 2014; 35(1):95–98. [PubMed: 24334809]

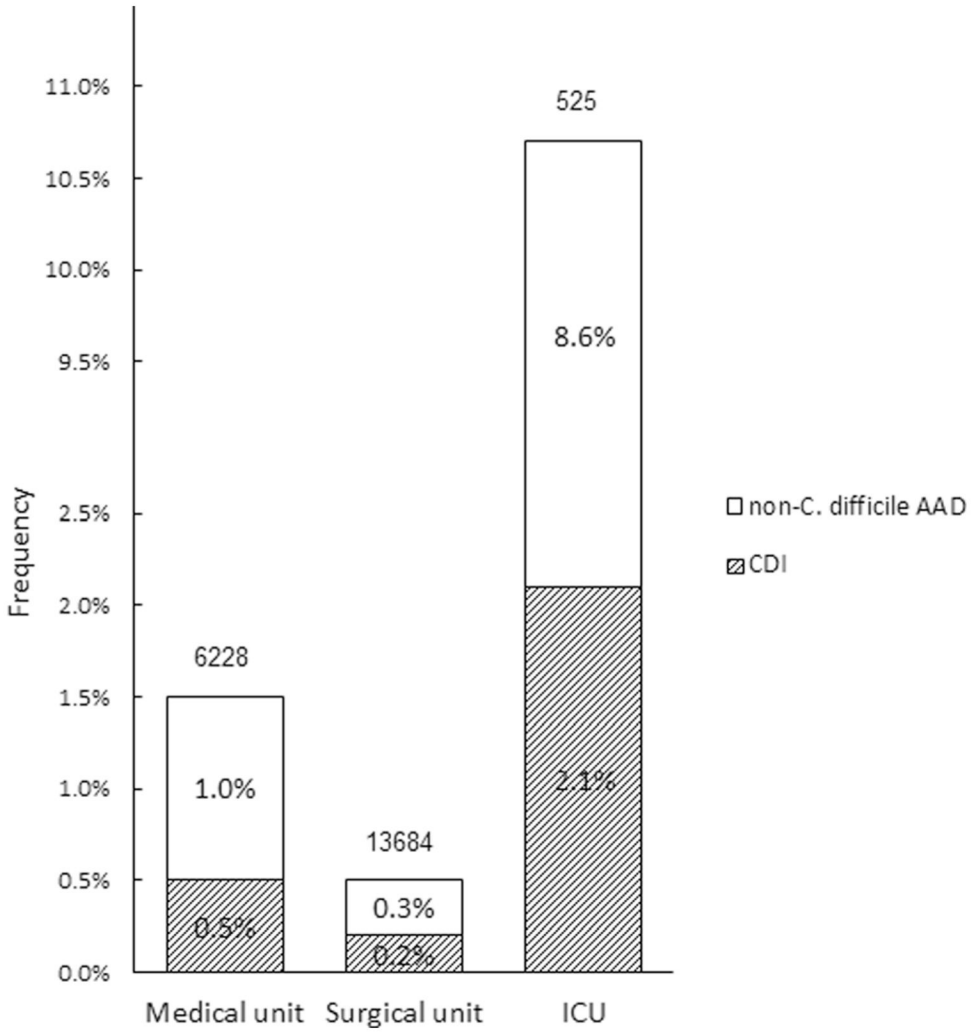

Fig. 1.

Frequency of *Clostridium difficile* infection (CDI) and non*C. difficile* antibiotic-associated (AAD) diarrhea in hospitalized patients who had received prior antibiotic therapy

Table 1

Univariate and multivariate analyses of development of *Clostridium difficile* infection (CDI) compared with patients with non-*Clostridium difficile* antibiotic-associated diarrhea (non-*C. difficile* AAD)

Characteristic	CDI patients (*N*=63) *n* (%)	Non-*C. difficile* AAD patients (*N*=143) *n* (%)	Crude odds ratio (95 % CI)	Adjusted odds ratio (95 % CI)
Age≥65 years	30 (47.6)	59 (41.3)	1.29 (0.71–2.35)	1.17 (0.64–2.16)
Male sex	39 (61.9)	89 (62.2)	0.99 (0.54–1.82)	0.97 (0.51–1.83)
Major comorbidities				
Chronic heart failure	2 (3.2)	0	NA	NA
Diabetes mellitus	28 (44.4)	59 (41.3)	1.14 (0.63–2.07)	1.16 (0.62–2.15)
Gastrointestinal disease	26 (41.3)	48 (33.6)	1.31 (0.71–2.40)	0.95 (0.44–2.03)
Malignancy	26 (41.3)	61 (42.7)	0.94 (0.52–1.72)	0.86 (0.45–1.63)
Predisposing factors 4 weeks preceding diagnosis				
Prior hospitalization	29 (46.0)	59 (41.3)	1.21 (0.67–2.21)	1.14 (0.61–2.13)
Length of stay (≥7 days)	55 (87.3)	124 (86.7)	1.05 (0.43–2.55)	1.02 (0.41–2.53)
Proton pump inhibitors	45 (71.4)	102 (71.3)	1.00 (0.52–1.94)	1.00 (0.51–1.97)
Immunosuppressive agents	5 (7.9)	8 (5.6)	1.45 (0.46–4.64)	1.33 (0.41–4.34)
Nasogastric tube	38 (60.3)	84 (58.7)	1.07 (0.58–1.95)	1.45 (0.74–2.86)
Mechanical ventilation	9 (14.3)	23 (16.1)	0.87 (0.38–2.00)	1.51 (0.56–4.04)
Dialysis	1 (1.6)	3 (2.1)	0.75 (0.08–7.38)	0.70 (0.07–7.08)
Abdominal surgery	21 (33.3)	46 (32.2)	1.45 (NA)	6.71 (NA)
Antimicrobial treatment				
No. of antibiotics (≥3)	27 (42.9)	61 (42.7)	1.01 (0.55–1.84)	1.11 (0.60–2.06)
Aminoglycosides	14 (22.2)	28 (19.6)	1.17 (0.57–2.42)	1.37 (0.64–2.92)
Beta-lactamase inhibitor	28 (44.4)	79 (55.2)	0.65 (0.36–1.18)	0.77 (0.41–1.44)
Carbapenems	27 (42.9)	36 (25.2)	2.23 (1.19–4.17) [a]	2.31 (1.22–4.38)[a]
Cephalosporins				
1st and 2nd generation	18 (28.6)	37 (25.9)	1.15 (0.59–2.22)	1.16 (0.57–2.37)
3rd and 4th generation	18 (28.6)	33 (23.1)	1.33 (0.68–2.61)	1.17 (0.59–2.33)
Fluoroquinolones	9 (14.3)	29 (20.3)	0.66 (0.29–1.48)	0.62 (0.27–1.42)
Metronidazole	8 (12.7)	15 (10.5)	1.24 (0.50–3.10)	1.01 (0.39–2.60)
Vancomycin	11 (17.5)	27 (18.9)	0.91 (0.42–1.97)	1.06 (0.48–2.35)
Biological markers at diagnosis				
WBC count >9.5×10^9/L	29 (46.0)	54 (37.8)	1.36 (0.75–2.48)	1.69 (0.90–3.19)
Neutropenia (>75 %)	35 (55.6)	76 (53.1)	1.13 (0.62–2.07)	1.40 (0.74–2.66)
Hypoalbuminemia (<35 g/L)	35 (55.6)	74 (51.7)	1.17 (0.64–2.11)	1.37 (0.73–2.57)
Serum creatinine >195 μmol/L	3 (4.8)	7 (4.9)	0.96 (0.24–3.86)	1.07 (0.26–4.46)

[a] Significant difference ($p<0.05$)

Table 2

Treatment of patients with *Clostridium difficile* infection (CDI) and non-*Clostridium difficile* antibiotic-associated diarrhea (non-*C. difficile* AAD)

Treatment	No. (%) of patients		Chi-square value	*P*
	CDI	Non–*C. difficile* AAD		
Discontinuation of implicated antibiotic	15 (23.8)	14 (9.8)	7.106	0.008
Metronidazole and/or vancomycin	23 (36.5)	8 (5.6)	32.692	0.000
Biotherapy	24 (38.1)	60 (42.0)	0.270	0.603
Biotherapy alone	7 (11.1)	31 (21.7)	3.246	0.072
Berberine	25 (39.7)	25 (17.5)	11.726	0.001
Berberine alone	7 (11.1)	10 (7.0)	0.980	0.322