J. Tokyo Med. Univ., **80**(2) : 102–108, 2022

Prevalence of toxin genes and clinical characteristics of *Clostridioides difficile* isolates in diarrheal samples among hospitalized patients at Tokyo Medical University Hospital

Yusuke WATANABE[1)2)], Takashi OKANDA[3)], Itaru NAKAMURA[2)], Hidehiro WATANABE[2)], Shigeki NAKAMURA[1)], Tetsuya MATSUMOTO[4)]

[1)]Department of Microbiology, Tokyo Medical University
[2)]Department of Infection Prevention and Control, Tokyo Medical University Hospital
[3)]Department of Microbiology, St. Marianna University School of Medicine
[4)]Department of Infectious Diseases, International University of Health and Welfare

Abstract

【Background】 *Clostridioides difficile* infection (CDI) is a major cause of antibiotic-associated diarrhea in hospitalized patients and in patients residing in long-term care facilities. The epidemiology, including the prevalence of binary toxin (CDT^+) producing strains, known as hypervirulent, and the clinical characteristics of CDI are less well known in Japan than in the United States and Europe. Here, we investigate the prevalence of toxin-producing genes of clinical isolates by polymerase chain reaction (PCR) and the clinical features of patients with CDI at our hospital over a 6-year period.

【Methods】 As part of a retrospective study at Tokyo Medical University Hospital, *C. difficile* strains were isolated from the stool of patients with CDI and positive glutamate dehydrogenase (GDH) antigen results and/or positive toxin results between June 2010 and May 2016. We investigated the prevalence of toxin-producing genes, their clinical characteristics, laboratory findings, severity, recurrence, all-cause mortality within 14 days, and cure rate for each antibiotic used.

【Results】 A total of 49 *C. difficile* strains were analyzed, including 46 strains that were GDH antigen positive. A total of 25 strains (51.0%) were toxin A positive, toxin B positive, and CDT negative ($A^+B^+CDT^-$), 16 (32.7%) were $A^-B^+CDT^-$, 7 (14.3%) were $A^-B^-CDT^-$, and 1 (2.0%) was $A^+B^+CDT^+$. Thirty-two cases were used for the subsequent analysis while excluding 7 cases in which non-toxin-producing strains were isolated, 5 cases for which clinical information was not available, and 5 cases in which CDI was not diagnosed. The mean patient age was 63.1 ± 22.3 and 82.0 ± 6.5 years among the group of patients who survived (surviving-patients group) and the group of patients that died (dead-patients group), respectively ($p = 0.196$). Sex, underlying diseases, antibiotics and clinical data were similar among the surviving-patients group and dead-patients group. Nine $A^+B^+CDT^-$ strains (52.9%), ten $A^-B^+CDT^-$ strains (71.4%), and one $A^+B^+CDT^+$ strain (100.0%) caused severe CDI cases ($p = 0.582$), respectively. Only one recurrent CDI case was recorded in one $A^-B^+CDT^-$ case (3.1%) ($p = 0.323$). All-cause mortality within 14 days occurred in two $A^+B^+CDT^+$ cases (11.7%) and one $A^-B^+CDT^-$ case (7.1%) ($p = 0.605$). The clinical cure rates were 50.0% in the MTZ and 85.7% in the VCM among mild cases and 62.5% in the MTZ and 66.7% in the VCM among severe cases, respectively, excluding two cases of unclear severity of CDI and one case of no treatment.

【Conclusions】 The prevalence of $A^+B^+CDT^-$, $A^-B^+CDT^-$, $A^-B^-CDT^-$, and $A^+B^+CDT^+$ strains of *C. difficile* in this Japanese cohort study were 51.0%, 32.7%, 14.3%, and 2.0%, respectively. The severity, recurrence, and mortality rates were similar in our study, irrespective of the presence or absence of toxin A or toxin B.

Received October 28, 2021, Accepted January 20, 2022
Key words : *Clostridioides difficile*, *Clostridioides difficile* infection, toxin A, toxin B, binary toxin, metronidazole, vancomycin
Corresponding author : Tetsuya MATSUMOTO, M.D., Ph.D., Department of Infectious Diseases, International University of Health and Welfare, Narita, Japan
TEL : 0476-35-5600 FAX : 2476-20-7702 E-mail : tetsuya.m@iuhw.ac.jp

Introduction

Clostridioides difficile, a spore-forming anaerobic gram-positive bacillus, is a major cause of diarrhea in hospitalized patients and in patients residing in long-term care facilities[1)2)]. *Clostridioides difficile* infection (CDI) is generally mediated by toxins A and B. The strains producing binary toxins [*C. difficile* transferase (CDT)] have been reported to be hypervirulent and associated with fulminant cases. According to a surveillance study conducted in 2011, the estimated numbers of incident cases, cases of first recurrence, and deaths linked to CDI cases in the United States amounted to 453,000, 83,000, and 29,300, respectively[3)]. Moreover, CDT^+ strains, such as restriction endonuclease analysis group BI, North American pulsed-field gel electrophoresis type NAP1, and polymerase-chain-reaction (PCR) ribotype 027, have been associated with high mortality rates and more severe CDI[2)4)]. Patients with CDT^+ status experience higher case-fatality rates, irrespective of the PCR ribotype[5)]. The effects of CDT^+ rely on two components, which act cooperatively to intoxicate cells, and result in the collapse of the actin cytoskeleton[6)]. North American and European outbreaks caused by CDT^+ strains, including PCR ribotype 027, remain a worldwide concern[7)]. However, epidemiological reporting of CDI in Japan is lacking due to an insufficient surveillance system. Although a systematic review suggested that the incidence, prevalence, and recurrence rates of CDI in Japan were 0.8 to 4.71/10,000 patient-days, 0.3 to 5.5/1,000 patients, and 3.3% to 27.3%[8)], respectively, few studies assessing CDI severity according to variations in the toxins produced have been published. Most *C. difficile* strains are toxin A positive, toxin B positive, and CDT^- ($A^+B^+CDT^-$), and CDT^+ strains seem as well to be particularly rare (0%-6.8%) in Japan[8)]. In this study, we investigated the proportions of strains with toxin A, toxin B, and CDT positivity and negativity, respectively, by clinical sampling, and assessed the severity, recurrence rate, all-cause mortality within 14 days, and clinical cure rate of CDI.

Methods

We conducted a retrospective analysis study in Japan, which included patients with initial and recurrent CDI identified at Tokyo Medical University Hospital (TMUH) (n = 1,015 beds) between June 2010 and May 2016. A total of 49 *C. difficile* strains were cultured from the stool of patients with CDI and a positive stool test result for *C. difficile* glutamate dehydrogenase (GDH) antigen and/or toxin using the C. DIFF QUIK CHEK COMPLETE® test (TechLab, Inc., Blacksburg, VA, USA), TOX A/B QUIK CHEK® test (TechLab, Inc., Blacksburg, VA, USA) or GE test immunochromato-CD GDH/TOX「Nissui」(Nissui Pharmaceutical Co., Ltd., Ueno, Tokyo, Japan). Toxin-producing genes were analyzed by real-time polymerase chain reaction (qPCR). Deoxyribonucleic Acid (DNA) was extracted using CellEase Bacteria II (Biocosm Inc. Amagasaki, Japan) followed by the detection of *tpi* (triose phosphate isomerase), *tcdA/B* (C. difficile toxin A/B), and cdtB (binary toxin B) genes by qPCR (Thermal Cycler Dice® Real-time System Lite, Takara Bio Inc. Kusatsu, Japan) CDI PCR mixtures were prepared by mixing each of primer pairs, SYBR® *Premix* Ex Taq (Perfect Real Time, Takara Bio. Inc.), distilled water, and sample DNA. qPCR was performed under the following conditions : denaturation at 95℃ for 30 seconds, followed by 40 cycles of 95℃ for 5s and 60℃ for 30 seconds[9)]. CDI was defined as diarrhea (at least three unformed bowel movements [types from 5 to 7 on the Bristol stool scale] within 24 h). Severe CDI was defined as a Zar score of at least two points (1-8 points possible). Recurrent CDI was defined as a new episode of CDI after clinical cure of the baseline episode, which was in turn defined as the absence of diarrhea for 2 consecutive days following completion of antibiotic therapy administered for a minimum of 16 days. The clinical cure rates in patients requiring a change of antibiotics during treatment were analyzed according to their initial treatment group, i.e., the metronidazole (MTZ) or vancomycin (VCM) group. Initial treatment was decided by each patient's primary physician, and the treatment medicines were MTZ and VCM ; fidaxomicin (FDX) was not available as an option. The physicians of the Department of Infection Prevention and Control advised the primary doctors where appropriate. After data collection, the following patient information was analyzed : age, sex, underlying diseases, severity of the disease, preantibiotic and/or preantifungal administration, proton pump inhibitor or histamine-2-receptor antagonist use, clinical symptoms, laboratory findings, clinical cure rate, recurrence rate, and rate of all-cause mortality within 14 days. In this study, we did not analyze the clinical characteristics of CDI cases without any toxins. Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows, version 24 (IBM Corporation, Armonk, NY, USA). Categorical variables were evaluated with the chi-squared test, the Fisher's exact test or the Kruskal-Wallis test. Continuous variables were evaluated using the two-sample t-test. Normality was assessed with the Shapiro-Wilk test. All p-values of 0.05 or less were considered to be statistically significant. This study was approved by the TMUH institutional review board (approval no. SH3641) ; after obtaining this approval, we communicated the details of this study to patients through the TMUH homepage, thus allowing them to either consent or refuse participation in this study.

Results

A total of 49 strains of *C. difficile* were analyzed. Of these, 46 strains were glutamate dehydrogenase (GDH) antigen positive, 25 (51.0%) were $A^+B^+CDT^-$, 16 (32.6%) were $A^-B^+CDT^-$, 7 (14.2%) were $A^-B^-CDT^-$, and 1 (2.0%) was $A^+B^+CDT^+$. Ultimately, among the 49 strains, 7 were excluded because they failed to produce any toxins. In the 42 remaining strains, 5 originated from patients with no related clinical information and 5 did not cause CDI. Consequently, we obtained clinical data from 32 cases. The 32 clinical characteristics of patients at the time of enrollment are shown in Table 1. The mean patient age was 63.1 ± 22.3 years among surviving-patients group and 82.0 ± 6.5 years among the dead-patients group (p = 0.690). There were 20 men (70.0%) among the surviving-patients group and 2 men (66.7%) among the dead-patients group. Underlying diseases in the surviving-patients group included human immunodeficiency virus (HIV) (6.9%), solid cancer (3.4%) hematologic malignancy (13.8%), steroid use (6.9%), chronic kidney disease (CKD) (13.8%), neutropenia (6.9%), and diabetes mellitus (DM) (27.6%). Underlying diseases in the dead-patients group included HIV (0.0%), solid cancer (0.0%) hematologic malignancy (33.3%), steroid use (33.3%), CKD (0.0%), neutropenia (0.0%), and DM (0.0%). Although the penicillins and cephalosporins with/without β-lactamase inhibitor had previously been administered to 22 patients (75.9%) in the surviving-patients group and 3 patients (100.0%) in dead-patients group, no differences were found among the two groups (p = 0.464, 0.356), respectively. Some fluoroquinolone and Vancomycin/Daptomycin/Linezolid had previously been administered among the surviving-patients group and the dead-patients group (p = 0.660, 0.660). Cases with a white blood cell count of greater than 15,000/μL, albumin level of less than 2.5 g/dL and temperature of greater than 38.3℃ were similarly found among the surviving-patients group and dead-patients group (p = 0.184, 0.420, 0.590). We studied the association between toxin type, CDI severity, recurrence, and all-cause mortality among the 32 included CDI cases (Table 2). No significant differences were observed among toxin types in severity,

Table 1. Characteristics of patients in the surviving-patients and dead-patients groups for *C. difficile* infections

Characteristics	Surviving-patients, n = 29 (%)	Dead-patients, n = 3(%)	P value
male	20 (70.0)	2 (66.7)	0.690
Age, mean years (standard deviation)	63.1 ± 22.3	82.0 ± 6.5	0.196
Underlying disease			
Diabetes mellitus	8 (27.6)	0 (0.0)	0.408
Neutropenia	2 (6.9)	0 (0.0)	0.340
Chronic kidney disease	4 (13.8)	0 (0.0)	0.660
Solid cancer	1 (3.4)	0 (0.0)	0.906
Hematologic malignancy	4 (13.8)	1 (33.3)	0.410
HIV	2 (6.9)	0 (0.0)	0.737
Steroid use	2 (6.9)	1 (33.3)	0.340
Proton pump inhibitor or H2 blocker	12 (41.4)	2 (66.7)	0.644
Antibiotics[a]			
Penicillin/cephalosporin without β-lactamase inhibitor	22 (75.9)	3 (100.0)	0.464
Penicillin/cephalosporin with β-lactamase inhibitor	18 (62.1)	1 (33.3)	0.356
Carbapenem	7 (24.1)	2 (66.7)	0.184
Fluoroquinolone	4 (13.8)	0 (0.0)	0.660
Vancomycin/Daptomycin/Linezolid	4 (13.8)	0 (0.0)	0.660
Clinical data[b]			
WBC > 15,000/μL	7 (24.1)	2 (66.7)	0.184
Albumin < 2.5 g/dL	11 (37.9)	2 (66.7)	0.420
Temperature > 38.3℃	5 (17.2)	1 (33.3)	0.590

[a] Within 12 weeks before diagnosis of *C. difficile* infection, [b] At time of *C. difficile* infection diagnosis

Table 2. Association among toxin type, CDI severity, recurrence, and mortality

Type of toxin	Total *n* (%)	$A^+ B^+ CDT^-$ *n* (%)	$A^- B^+ CDT^-$ *n* (%)	$A^+ B^+ CDT^+$ *n* (%)	*P* value
Overall	32	17	14	1	
Age > 60 years	22 (68.7)	11 (64.7)	11 (78.5)	0 (0.0)	0.329
Temperature > 38.3℃	5 (15.6)	3 (17.6)	2 (14.2)	0 (0.0)	0.597
WBC > 15,000/μL	9 (28.1)	6 (35.2)	2 (14.2)	1 (100.0)	0.180
Alb < 2.5 g/dL	13 (40.6)	7 (41.1)	5 (35.7)	1 (100.0)	0.756
Mild	10 (31.2)	7 (41.1)	3 (21.4)	0 (0.0)	
Severe	20 (62.5)	9 (52.9)	10 (71.4)	1 (100.0)	0.582
Unclear	2 (6.2)	1 (5.8)	1 (7.1)	0 (0.0)	
Recurrent CDI	1 (3.1)	0 (0.0)	1 (7.1)	0 (0.0)	0.542
All-cause mortality within 14 days	3 (9.3)	2 (11.7)	1 (7.1)	0 (0.0)	0.575

Table 3. Clinical cure rate of *C. difficile* infection according to severity and initial treatment

Severity	No. of patients clinically cured/ no. of patients' initial treatment (%)		OR (95% CI)	*P* value
	MTZ	VCM		
Mild	1/2 (50.0)	6/7 (85.7)	6.00 (0.18-196.28)	0.41
Severe	5/8 (62.5)	8/12 (66.7)	1.20 (0.18-7.77)	0.61
All	6/10 (66.6%)	14/19 (73.6%)	1.87 (0.37-9.49)	0.36

recurrence and all-cause mortality within 14 days (p = 0.582, 0.323, 0.605). Recurrence was found in only one case, and mortality from all-cause within 14 days occurred in three cases. We also identified that the single $A^+B^+CDT^+$ strain triggered toxic megacolon, which presented as fulminant colitis. In fact, this patient required a temporary colostomy, and VCM was administered via colostomy in concert with the administration of a VCM enema and oral MTZ and VCM medications. We studied the clinical cure rate in the MTZ and VCM initial treatment groups between mild and severe CDI cases (Table 3), and found that the clinical cure rates among severe cases were 62.5% and 66.7%, respectively. Furthermore, the clinical cure rates among mild cases were 50.0% in the MTZ group and 85.7% in the VCM group. From 29 cases, we excluded 2 cases of unknown severity and 1 case of no treatment, and the clinical cure rate of MTZ used as initial therapy was similar to the clinical cure rate of VCM used as initial therapy for CDI. Four cases of severe CDI in the MTZ group improved after switching from MTZ to VCM. One case of severe CDI in the VCM group required additional immunoglobulin. Finally, one case was a fulminant case caused by the documented $A^+B^+CDT^+$ strain.

Discussion

A 2011 surveillance study in the United States reported that NAP1 CDT^+ strains totaled 18.8% of community-associated CDI cases and 30.7% of healthcare-associated CDI cases, respectively[3]. A European surveillance study in 2013 reported that CDT genes were present in 38% of isolates[10]. In contrast, there are few CDT^+ cases in Asia. PCR ribotype 027 that is a hypervirulent strain was found in only 0.7% of cases in Hong Kong[11]. A systematic review from Japan suggested that CDT^+ strains were the cause of 0% to 6.8% of cases, and that hypervirulent ribotypes 027 and 078 were rare[8]. To the best of our knowledge, only 3 Japanese cases of CDI were caused by the PCR ribotype 027 strain until 2021[12-14]. Meanwhile, CDI cases caused by CDT^+ strains other than PCR ribotypes 027 or 078 are sporadic in Japan[15)16]. Because binary toxin could not be checked by commercial based test in Japan, hypervirulent strains may be underdiagnosed. We would be remiss if we failed to highlight this threat.

In this study, the rates of recurrence and all-cause mortality within 14 days were also similar between the A^+B^+ and A^-B^+ strains (Table 2). Toxin A leads to

inflammation, increased mucosal permeability, fluid secretion, and tissue damage. Toxin B has pathogenicity provided that prior intestinal damage is present during administration with toxin A. Therefore, toxins A and B may act synergistically[17]. However, detection of toxin A and/or B in clinical research is not attributed to the development of severe CDI or mortality[2)18)]. However, the sample sizes of both mild and severe CDI groups were small. The recurrence rate of Japanese CDI was lower than that of the United States, China and European countries[19]. Mikamo et al. discussed the rarity of hypervirulent strain of *C. difficile* in Japan as one of the reasons of low recurrent rate[20]. Because there was only 1 case of CDT^+ in our study, the recurrent rate might be low. Consequently, it is difficult to predict the severity of CDI according to the presence of toxin A and/or B based on this study. We judged the severity of CDI with Zar score which consisted of 4 variables, including age, in this study. The mean age was 70.0 years in the severe CDI group and 53.3 years in the mild CDI group. The patients of the severe CDI group were older than the mild CDI group. The preferred initial treatment for CDI in the United States is FDX, with VCM being the alternative option[21]. MTZ is an alternative for nonsevere CDI if both FDX and VCM are unavailable. Due to unavailability of FDX during the study period, FDX was not administered. The first choice of treatment for nonsevere CDI in Japan is MTZ rather than VCM[22]. The clinical cure rate of MTZ as the initial treatment for mild CDI was not different from that of VCM in this study (Table 3). Four of seven cases treated initially with MTZ required a switch to VCM. One patient undertaking this change experienced recurrence, and another patient died within 14 days from initial treatment. Consequently, clinicians should be extremely careful when treating severe CDI with MTZ. Although IDSA guidelines recommended FDX for initial CDI because FDX had lower recurrence rates[21], FDX is not recommended for initial CDI in Japan because Japanese date of FDX for CDI is lacking[22]. The recurrence rate was only 3.1% in our study. There is lacking evidence that FDX for initial CDI will be first-line therapy in Japan. Our study has certain limitations. First, this study was a single-center retrospective observational study, and thus, the number of included cases was small. Although the prevalence of hypervirulent strains of *C. difficile* in Japan is low, this may increase in the future, and a systematic surveillance system for CDT^+ strains is now required. Second, we did not investigate all ribotypes because this study aims to determine whether CDT has a clinically important impact that lead to severe CDI, recurrence and high mortality. However, the single CDT^+ strain identified in this study was PCR ribotype 027. Third, we collected clinical data from CDI cases from strains identified at the Department of Microbiology at Tokyo Medical University, and there is a possibility that a strain of CDT^+ was missing from these records. Fourth, we could not follow up after discharge from hospital. Therefore, the recurrence rate might be underrepresented.

Conclusions

The prevalence of $A^+B^+CDT^-$, $A^-B^+CDT^-$, $A^-B^-CDT^-$, and $A^+B^+CDT^+$ strains of *C. difficile* in this Japanese cohort study were 51.0%, 32.7%, 14.3%, and 2.0%, respectively. The severity, recurrence, and mortality rates were found to be similar in our study irrespective of the presence or absence of toxin A or toxin B. The clinical cure rates of MTZ and VCM used as the initial treatment were also similar.

Conflicts of interest

None to declare

References

1) Giancola SE, Williams RJ, 2nd, Gentry CA: Prevalence of the Clostridium difficile BI/NAP1/027 strain across the United States Veterans Health Administration. Clin Microbiol Infect **24**(8): 877-881, 2018
2) Rao K, Micic D, Natarajan M, Winter S, Kiel MJ, Walk ST, Santhosh K, Mogle JA, Galecki AT, LeBar W, Higgins PD, Young VB, Aronoff DM: Clostridium difficile ribotype 027: relationship to age, detectability of toxins A or B in stool with rapid testing, severe infection, and mortality. Clin Infect Dis **61**(2): 233-241, 2015
3) Banaei N, Anikst V, Schroeder LF: Burden of Clostridium difficile infection in the United States. In: N Engl J Med. United States, 2015: 2368-2369
4) Leffler DA, Lamont JT: Clostridium difficile infection. N Engl J Med **372**(16): 1539-1548, 2015
5) Bacci S, Mølbak K, Kjeldsen MK, Olsen KE: Binary toxin and death after Clostridium difficile infection. Emerg Infect Dis **17**(6): 976-982, 2011
6) Cowardin CA, Buonomo EL, Saleh MM, Wilson MG, Burgess SL, Kuehne SA, Schwan C, Eichhoff AM, Koch-Nolte F, Lyras D, Aktories K, Minton NP, Petri WA Jr: The binary toxin CDT enhances Clostridium difficile virulence by suppressing protective colonic eosinophilia. Nat Microbiol **1**(8): 16108, 2016
7) Clements AC, Magalhães RJ, Tatem AJ, Paterson DL, Riley TV: Clostridium difficile PCR ribotype 027: assessing the risks of further worldwide spread. Lancet Infect Dis **10**(6): 395-404, 2010
8) Riley TV, Kimura T: The Epidemiology of Clostridium difficile Infection in Japan: A Systematic Review. Infect Dis Ther **7**(1): 39-70, 2018
9) Okanda T, Mitsutake H, Aso R, Sekizawa R, Takemura H, Matsumoto T, Nakamura S: Rapid detection

assay of toxigenic Clostridioides difficile through PathOC RightGene, a novel high-speed polymerase chain reaction device. Diagn Microbiol Infect Dis **99**(2) : 115247, 2021

10) van Dorp SM, Kinross P, Gastmeier P, Behnke M, Kola A, Delmée M, Pavelkovich A, Mentula S, Barbut F, Hajdu A, Ingebretsen A, Pituch H, Macovei IS, Jovanović M, Wiuff C, Schmid D, Olsen KE, Wilcox MH, Suetens C, Kuijper EJ : Standardised surveillance of Clostridium difficile infection in European acute care hospitals : a pilot study, 2013. Euro Surveill **21**(29), 2016

11) Chow VCY, Kwong TNY, So EWM, Ho YII, Wong SH, Lai RWM, Chan RCY : Surveillance of antibiotic resistance among common Clostridium difficile ribotypes in Hong Kong. Sci Rep **7**(1) : 17218, 2017

12) Nishimura S, Kou T, Kato H, Watanabe M, Uno S, Senoh M, Fukuda T, Hata A, Yazumi S : Fulminant pseudomembranous colitis caused by Clostridium difficile PCR ribotype 027 in a healthy young woman in Japan. J Infect Chemother **20**(11) : 729-731, 2014

13) Nakamura I, Yamaguchi T, Tsukimori A, Sato A, Fukushima S, Mizuno Y, Matsumoto T : Fulminant colitis from Clostridium difficile infection, the epidemic strain ribotype 027, in Japan. J Infect Chemother **20**(6) : 380-383, 2014

14) Hiraki M, Suzuki R, Tanaka N, Fukunaga H, Kinoshita Y, Kimura H, Tsutsui S, Murata M, Morita S : Community-acquired fulminant Clostridioides (Clostridium) difficile infection by ribotype 027 isolate in Japan : a case report. Surg Case Rep **7**(1) : 137, 2021

15) Oguri N, Sakuraba A, Morikubo H, Kikuchi O, Sato T, Tokunaga S, Minowa S, Ikezaki O, Mitsui T, Miura M, Saito D, Hayashida M, Mori H, Osaki T, Kamiya S, Senoh M, Kato H, Hisamatsu T : Community-acquired fulminant colitis caused by binary toxin-producing Clostridium difficile in Japan. Clin J Gastroenterol **12**(4) : 325-329, 2019

16) Tagashira Y, Kato H, Senoh M, Nakamura A : Two cases of fulminant colitis due to binary toxin-positive Clostridium difficile that are not PCR ribotype 027 or type 078. J Med Microbiol **62**(Pt 9) : 1486-1489, 2013

17) Chandrasekaran R, Lacy DB : The role of toxins in Clostridium difficile infection. FEMS Microbiol Rev **41**(6) : 723-750, 2017

18) Tokimatsu I, Shigemura K, Osawa K, Kinugawa S, Kitagawa K, Nakanishi N, Yoshida H, Arakawa S, Fujisawa M : Molecular epidemiologic study of Clostridium difficile infections in university hospitals : Results of a nationwide study in Japan. J Infect Chemother **24**(8) : 641-647, 2018

19) Finn E, Andersson FL, Madin-Warburton M : Burden of Clostridioides difficile infection (CDI) - a systematic review of the epidemiology of primary and recurrent CDI. BMC Infect Dis **21**(1) : 456, 2021

20) Mikamo H, Kondo T, Okuyama K, Marcella SW, Ruzicka DJ : Incidence of and risk factors for recurrent Clostridioidesdifficile infection in Japan using a claims database : A retrospective cohort study. Anaerobe **61** : 102139, 2020

21) Johnson S, Lavergne V, Skinner AM, Gonzales-Luna AJ, Garey KW, Kelly CP, Wilcox MH : Clinical Practice Guideline by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) : 2021 Focused Update Guidelines on Management of Clostridioides difficile Infection in Adults. Clin Infect Dis, 2021

22) Kunishima H, Ohge H, Suzuki H, Nakamura A, Matsumoto K, Mikamo H, Mori N, Morinaga Y, Yanagihara K, Yamagishi Y, Yoshizawa S : The Japanese Clinical Practice Guidelines for Management of *Clostridioides* (*Clostridium*) *difficile* Infections. Japanese Journal of Chemotherapy **68** : 1-107, 2018

東京医科大学病院の入院症例の下痢検体から分離された *Clostridioides difficile* における毒素産生遺伝子保有状況と臨床的特徴の検討

渡邊裕介[1)2)]　大神田敬[3)]　中村造[2)]
渡邉秀裕[2)]　中村茂樹[1)]　松本哲哉[4)]

[1)]東京医科大学微生物学分野
[2)]東京医科大学病院感染制御部
[3)]聖マリアンナ医科大学微生物学講座
[4)]国際医療福祉大学社会医学研究分野感染症学

【要旨】【はじめに】*Clostridioides difficile* infection（CDI）は、医療関連下痢症の重要な原因菌である。強毒株として知られるバイナリートキシンの産生株の日本での病原性に関してのデータは限定的である。我々は、東京医科大学病院で CDI 症例から分離された *C. difficile* の、PCR 検査による毒素産生遺伝子の保有状況と臨床的特徴について検討した。

【対象と方法】東京医科大学病院での後ろ向き研究として、2010 年 6 月から 2016 年 5 月の間に CDI 症例の便から分離された GDH 抗原陽性またはトキシン陽性株を用いた。毒素産生遺伝子、患者の臨床的特徴、血液検査所見、重症度、再発、14 日死亡、使用した各抗菌薬の治癒率を調査した。

【結果】*C. difficile* の 49 株を解析し、46 株が GDH 抗原陽性であった。毒素産生遺伝子の保有状況は、toxin A 陽性かつ toxin B 陽性かつ Binary toxin 陰性（$A^+B^+CDT^-$）25 株（51.0%）、toxinA 陰性かつ toxinB 陽性かつ Binary toxin 陰性（$A^-B^+CDT^-$）16 株（32.7%）、toxinA 陰性かつ toxinB 陰性かつ Binary toxin 陰性（$A^-B^-CDT^-$）7 株（14.3%）、toxinA 陽性かつ toxinB 陽性かつ Binary toxin 陽性（$A^+B^+CDT^+$）1 株（2.0%）であった。毒素非産生株 7 例、臨床情報が不十分な 5 例、非 CDI 5 例を除外した 32 例の臨床的特徴を検討した。平均年齢は生存例で 63.1±22.3 歳、重症例で 82.0±6.5 歳であった（$p = 0.196$）。性別、基礎疾患、先行抗菌薬、臨床データは生存群と死亡群で似ていた。$A^+B^+CDT^-$ は 9 例（52.9%）、$A^-B^+CDT^-$ は 10 例（71.4%）、$A^+B^+CDT^+$ は 1 例（100%）が重症例だった。再発性 CDI は $A^-B^+CDT^-$ の 1 例（3.1%）に生じた。14 日死亡は 2 例（11.7%）の $A^-B^+CDT^-$、1 例（7.1%）の $A^+B^+CDT^-$ で生じた。臨床的治癒の割合は、軽症例では MTZ を投与した 50% と VCM を投与した 85.7%、重症例では MTZ を投与した 62.5%、VCM を投与した 66.7% で認めた。

【結論】本研究における *C. difficile* のトキシン陽性率は $A^+B^+CDT^-$ が 51.0%、$A^-B^+CDT^-$ が 32.7%、$A^-B^-CDT^-$ が 14.3%、$A^+B^+CDT^+$ が 2.0% であった。重症度、再発率、14 日死亡は toxin A または toxin B の有無に関わらず同様であった。

〈キーワード〉　*Clostridioides difficile*、*Clostridioides difficile* 感染症、Binary toxin、メトロニダゾール、バンコマイシン