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Molecular epidemiological analyses of *Clostridioides difficile* isolates in a university hospital in Japan

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ABSTRACT

Background: We performed molecular epidemiological analyses of *Clostridioides difficile* isolates in a university hospital in Japan to reveal the risk of *C. difficile* infection.
Methods: Cultured isolates from 919 stool samples from 869 patients obtained from July 2015 to August 2016 were subjected to toxin gene detection, ribotyping, multilocus sequence typing, antimicrobial susceptibility testing, and quantitative real-time polymerase chain reaction testing for *C. difficile* toxin gene expression.
Results: Of the 919 stool samples from 869 patients, *C. difficile* was isolated from 153 samples (16.6%), of which 49 (32%) and 104 (68%) were from patients with and without *C. difficile* infection, respectively. Analyses showed genetic diversity, with ST8 and ST17 strains of healthcare-associated infections, some of which caused *C. difficile* infections. There was no significant difference in the transcription levels of *C. difficile* toxin genes between isolates from patients with and without *C. difficile* infection.
Conclusions: Major Japanese clonal strains, ST8 and ST17, have been in the hospital environment for a long time and cause healthcare-associated *C. difficile* infections. The C. *difficile* toxin genes were transcribed in the isolates from both patients with and without *C. difficile* infection but were no significant relationship with the development of *C. difficile* infection.

1. Introduction

The estimated annual incidence of *Clostridioides difficile* infection (CDI) is 453,000 in the United States [1]. It has been reported that 15%–25% of diarrhoea cases observed in connection with the use of antibiotics (antibiotic-associated diarrhoea) are caused by *C. difficile* [2]. Additionally, 50%–70% of enteritis cases and 90% of pseudomembranous enterocolitis cases are caused by this bacterium, and it is considered that the frequency of CDI is high during antibiotic administration or within 10 weeks after the completion of administration. Therefore, it is suspected that this bacterium causes a$^{\text{ntibiotic-associated diarrhea}}$ under such circumstances. Even if *C. difficile* exists in the digestive tract of healthy people, it does not necessarily exert pathogenicity; CDI is believed to develop in the presence of risk factors because 2%–3% of healthy adults and 20%–50% of hospitalised patients are asymptomatic carriers of *C. difficile*

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in their digestive tracts [3]. In addition, the highly pathogenic *C. difficile* strain BI/NAP1/027, which produces toxin A, toxin B, and a binary toxin (CDT), was first detected in North America [2]. This strain has caused outbreaks in hospitals and spread rapidly in the United States. Nineteen cases of infection by ribotype 027 were reported in Asia in November 2015 [4], and cases have been reported subsequently [5]. All cases in Japan were sporadic, including three cases occurring in one hospital ward and two cases identified in a single city. Although CDI has become a global threat, few clinical settings conduct bacterial culture tests for CDI in Japan, and epidemiological data and information, including susceptibility to antimicrobials, toxin gene profile, ribotyping, and sequence type (ST), are limited. Thus, the collection of this information is important for revealing the risk of CDI in Japan. In this study, we conducted molecular epidemiological analyses of 153 *C. difficile* isolates obtained from stool cultures of hospitalised patients in one medical facility. We also examined the transcription levels of toxin genes in some isolates from both patients with and without CDI.

2. Methods

2.1. Sample collection, bacterial culture, and bacterial DNA extraction

A total of 869 patients were assessed for intestinal tract CDI using stool culture between July 2015 to August 2016 from Toho University Ohashi Medical Center. The stool samples were cultured anaerobically for 48 h on cycloserine-cefoxitin mannitol agar (CCMA) plates (Nissui, Tokyo, Japan). A colony grown on a CCMA plate was purified on the same agar plate, and a single colony was picked up using a toothpick and suspended in 200 μl of 10% Chelex (Bio-Rad, Hercules, CA, USA). After boiling at 95 °C for 15 min and centrifugation at 15,000 rpm for 10 min, the supernatant was used as the DNA template for polymerase chain reaction (PCR) test. CDI episodes were defined according to the SHEA/IDSA guidelines. Diagnosis of CDI was made if the patient had diarrhoea 48 h after admission, more than three times a day, and toxin positivity.

2.2. Detection of the toxin genes

Detection of toxin genes, namely *tcdA*, *tcdB*, *cdtA*, and *cdtB*, and determination of species by 16S rDNA were performed using 5-plex PCR test as described by Persson et al. (Table S1) [6].

2.3. Antibiotic agents and agar dilution methods of antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of piperacillin/tazobactam (PIPC/TAZ), ceftriaxone (CTRX), meropenem (MEPM), clindamycin (CLDM), moxifloxacin (MFLX), metronidazole (MNZ), vancomycin (VCM), and fidaxomicin (FDX) were determined using agar dilution methods. The agar plates were incubated under anaerobic condition for 48 h. The clinical breakpoints for PIPC/TAZ, CTRX, CLDM, MEPM, MFLX, and MNZ used in this study were provided by the Clinical and Laboratory Standards Institute [7], and those for VCM were obtained from the European Committee on Antimicrobial Susceptibility Testing [8]. The FDX breakpoint was obtained from unpublished data.

2.4. PCR ribotyping

A QIAamp DNA Mini kit (QIAGEN, Netherlands) was used for DNA extraction. PCR ribotyping was performed as described in previous studies [9]. Briefly, 16S rRNA primers and 23S rRNA primers were used for PCR (Table S1). PCR ribotype profiles were analysed using the FPQuest software (Bio-Rad, Hercules, CA, USA).

2.5. Multilocus sequence typing (MLST)

MLST analysis was performed on isolates that exhibited similar ribotypes. The PCR test targeted seven housekeeping genes (*adk*, *atpA*, *dxr*, *glyA*, *recA*, *sodA*, and *tpi*) (Table S1) [10]. The DNA sequences of the seven genes were submitted to the MLST database (http://pubmlst.org/clostridiumdifficile) to obtain the sequence type (ST).

2.6. qRT-PCR

Total RNA was extracted using the FastRNA Red Kit (MP Biomedicals, CA, USA). qRT-PCR was performed as described in previous studies (Table S1) [11].

C. difficile isolates were inoculated in 10 mL of brain heart infusion (BHI) broth (Becton Dickinson and Company, MD, USA) and cells were grown anaerobically at 37 °C for 18 h. Cells were harvested by centrifugation, and total RNA was prepared according to the manufacturer's protocol. RNA for qRT-PCR was extracted after 18 h of growth in BHI broth in an anaerobic chamber. Five hundred microliters of culture were added to 1 mL of RNA Protect Bacteria Reagent in the anaerobic chamber and centrifuged at 5000×*g* for 10 min at 4 °C. The concentration of RNA samples was determined by NanoDrop (Thermo Fisher Scientific, MA, USA) and then diluted to 0.1 μg/mL for cDNA synthesis. cDNA was synthesised using the PrimeScript RT reagent kit (Takara Bio Inc., Japan) and was used for qRT-PCR with SYBR Premix Ex Taq kit (Tli RNase H Plus) (Takara Bio Inc., Japan) and Applied Biosystems 7500 Fast Real-time PCR system (Applied Biosystems, CA, USA).

2.7. Data analysis

To assess the risk factors for CDI, medical records of 49 patients with CDI and 104 patients without CDI were analysed using the Statistical Package for Social Sciences (IBM Corporation, NY, USA) version 23.0, and Microsoft Excel 2012. Fisher's exact test, Cochran-Armitage test, and χ^2 tests were used to examine the incidence of CDI with sex, age, and toxin gene profile, respectively. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Sample collection

A total of 869 patients were assessed for intestinal tract CDI using stool culture. *C. difficile* was isolated from 153 of 919 (16.6%) stool samples obtained from hospitalised patients; among them, 74 were males (48.4%) and 79 were females (51.6%) ($p = 0.227$). There was no difference in the isolation rates of *C. difficile* between sexes. However, the CDI rate was significantly high in the older adult population (age ≥65 years).

3.2. CD toxin gene profiles

The CD toxin gene profiles and the number of isolates were shown in Table 1. Forty-nine of 153 isolates (32%) were obtained from patients who were diagnosed with CDI according to the criteria of the IDSA/SHEA guidelines [3]. The toxin gene profiles of the 49 isolates from patients with CDI were $A^+B^+CDT^-$ (47 isolates, 95.9%) and $A^+B^+CDT^+$ (2 isolates, 4.1%), whereas the toxin gene profiles of the 104 isolates from patients without CDI were $A^-B^-CDT^-$ (49 isolates, 47.1%), $A^+B^+CDT^-$ (52 isolates, 50.0%), $A^+B^+CDT^+$ (1 isolate, 1.0%), $A^-B^+CDT^-$ (1 isolate, 1.0%), and $A^-B^+CDT^+$ (1 isolate, 1.0%). The definitive diagnostic criteria state that the presence of two toxin genes (*tcdA* and *tcdB*) was essential, but not sufficient for the development of CDI. Our results showed that about half of the *tcdA*- and *tcdB*-positive strains did not develop CDI, and the prevalence of binary toxin (CDT) was very low (0.03%, 4/153).

3.3. Antimicrobial susceptibility test

The results of the antimicrobial susceptibility tests are presented in Table 2. The MIC_{90} values for MNZ, VCM, and FDX were 0.5 mg/L, 1 mg/L, and 0.5 mg/L, respectively. *C. difficile* isolates showed good susceptibility to conventional anti-*C. difficile* drugs (MNZ and VCM), as well as to the newly approved FDX, while many isolates showed reduced susceptibility to CLDM and MFLX. There was no significant difference observed in the MIC_{90} of antimicrobials used, except for MFLX, between isolates from patients with and without CDI.

3.4. Ribotyping and MLST

Ribotyping analysis was performed on 130 isolates (Fig. 1). Although five strains, namely ATCC9689 (ribotype 001), BAA-1870 (ribotype 027), ATCC 700057 (ribotype 038), ATCC433593 (ribotype 060), and BAA-1875 (ribotype 078), were used as reference strains of ribotypes, no isolate exhibited the same pattern as the reference strains. Isolates, which were suspected to be nosocomial infections by ribotyping three groups (groupA, groupB, and groupC), were examined for MLST. The same ST was assigned to the isolates exhibiting similar or identical ribotype profiles, suggesting that multiple nosocomial infections by *C. difficile* strains (such as

Table 1
Clinical features of patients with Clostridioides difficile infection.

		CDI patient (n = 49)	non-CDI patient (n = 104)	*P*-value
Sex				
	Male	20 (40.8%)	54 (51.9%)	0.227[a]
	Female	29 (59.2%)	50 (48.1%)	
Age				
	Under 2 years	1 (2.0%)	13 (12.5%)	0.043[b]
	3–17 years	1 (2.0%)	8 (7.7%)	
	18–64 years	6 (12.2%)	17 (16.4%)	
	Over 65 years	41 (83.8%)	66 (63.4%)	
Toxin genes (A/B/C)				
	−/−/−	0 (0)	49 (47.1%)	<0.001[c]
	+/+/−	47 (95.9%)	52 (50.0%)	
	−/+/−	0 (0)	1 (1.0%)	
	+/+/+	2 (4.1%)	1 (1.0%)	
	−/+/+	0 (0)	1 (1.0%)	

[a] Fisher's exact test.
[b] Cochran-Armitage test was used to examine the trend of incidence of CDI with age.
[c] $\chi 2$ test.

Table 2
Antimicrobial susceptibility of Clostridioides difficile strain.

Agent	Patients type	n	MIC range (mg/L)	MIC50 (mg/L)	MIC90 (mg/L)	Clinical breakpoints (mg/L) S	I	R	S, n (%)	I, n (%)	R, n (%)
MNZ[a]	CDI	49	≦0.016–1	0.25	0.5	≦2	–	> 2	49 (100)	0 (0)	0 (0)
	Non-CDI	104	0.031–1	0.5	1				104 (100)	0 (0)	0 (0)
	total	153	≦0.016–1	0.5	1				153(100)	0 (0)	0 (0)
VCM[b]	CDI	49	0.125–4	0.5	2	≦2	–	> 2	48 (98)	0 (0)	1 (2)
	Non-CDI	104	0.125–2	0.5	1				104 (100)	0 (0)	0 (0)
	total	153	0.125–4	0.5	1				153 (99)	0 (0)	1 (1)
FDX	CDI	49	≦0.016–2	0.125	1	–	–	–	–	–	–
	Non-CDI	104	≦0.016–1	0.125	1				–	–	–
	total	153	≦0.016–2	0.125	1				–	–	–
PIPC/TAZ[a]	CDI	49	4–16	8	16	≦32	64	≧128	49 (100)	0 (0)	0 (0)
	Non-CDI	104	4–16	8	16				104 (100)	0 (0)	0 (0)
	total	153	4–16	8	16				154 (100)	0 (0)	0 (0)
CLDM[a]	CDI	49	2-≧64	≧64	≧64	≦2	4	≧8	2 (4)	0 (0)	47 (96)
	Non-CDI	104	≦0.5-≧64	16	≧64				0 (0)	11 (11)	93 (84)
	total	153	≦0.5-≧64	32	≧64				2 (1)	11 (7)	140 (92)
MFLX[a]	CDI	49	1–32	16	32	≦2	4	≧8	17 (35)	0 (0)	32 (65)
	Non-CDI	104	1-≧64	16	32				70 (67)	1 (1)	33 (32)
	total	153	1-≧64	2	32				87 (57)	1 (1)	65 (42)
MEPM[a]	CDI	49	1–16	4	4	≦4	8	≧16	44 (90)	5 (10)	0 (0)
	Non-CDI	104	≦0.25–8	4	4				98 (94)	5 (5)	1 (1)
	total	153	≦0.25–16	4	4				140 (92)	10 (7)	1 (1)

VAN, vancomycin; MNZ, metronidazole; FDX, fidaxomicin; CLDM, clindamycin; MEPM, meropenem; MFLX, moxifloxacin; PIPC/TAZ, piperacillin/tazobactam.
S, susceptible; I, intermediate; R, resistant.
n, The number of isolates.
[a] Breakpoints are those recommended for anaerobes by CLSI [7].
[b] Breakpoints are those recommended by EUCAST [8].

the major Japanese clonal strains, ST8 and ST17) occurred among the inpatients at different wards during this period in the hospital (Fig. 2).

3.5. Expression of CD toxin genes

The transcription levels of the toxin genes (*tcdA* and *tcdB*) were examined by qRT-PCR. Isolates 59, 65, 68, 73, and 121 (toxic gene profile of $A^+B^+CDT^-$) from patients with CDI, isolates 11, 40, 89, and 100 (toxic gene profile of $A^+B^+CDT^-$) from patients without CDI, and reference strain ATCC70057 were used to estimate the expression of toxin genes at the transcriptional level. The results are shown in Fig. 3. Toxin genes in isolates from patients without CDI were transcribed as much as those in isolates from patients with CDI, which were considered to cause CDI. In addition, there was no relationship between the expression levels of the toxin genes and ST. These results reveal the absence of a clear causal relationship between the transcription levels of toxin genes and the onset of CDI.

4. Discussion

Although there was no difference in infection rates of *C. difficile* among males and females, 52.9% of *C. difficile* carriers without CDI were colonized by *C. difficile* carrying the toxin genes. A report from France showed that 53.2% (42/79) of *C. difficile* carriers without CDI were colonized by *C. difficile* with the toxin gene [12], while in Taiwan 52.9% (72/136) of *C. difficile* carriers without CDI were colonized by *C. difficile* with the toxin gene [13]. Few data are available on *C. difficile* carriers in Japan, so additional studies are needed to investigate the prevalence of toxin positivity among *C.difficile* carriers. This suggests that *C. difficile* remains a resident bacterium without CDI development and may be able to cause CDI in the future.

People older than 65 years are considered to have a 10-fold higher risk of CDI development than younger people in terms of onset and severity. In this study, as in previous studies, CDI occurred most frequently in patients older than 65 years (41/49, 83.8%), who had an approximately 20-fold higher risk than patients younger than 17 years. Risk factors for CDI include old age and colonisation by *C. difficile* as a resident bacterium among new-borns and infants.

Many ribotypes were observed, but most strains did not show any significant level of relatedness, indicating that most isolates were carried in the hospital by patients (Fig. 1). However, the groups of strains shown in groupA, groupB, and groupC had identical or very similar profiles, suggesting that they belong to the same ribotype and are suspected to be healthcare-associated infections (HAI), especially in cases where they were isolated from different wards in the hospital.

MLST analysis was performed on three groups of isolates with identical or very similar ribotypes. Although HAI with specific clones of ST8 and ST17 were observed in several departments, internationally prevalent clones were not observed (Fig. 2). However, ST8 and ST17 strains were isolated over some time in mixed wards, although HAI were suspected in different departments. From the results of

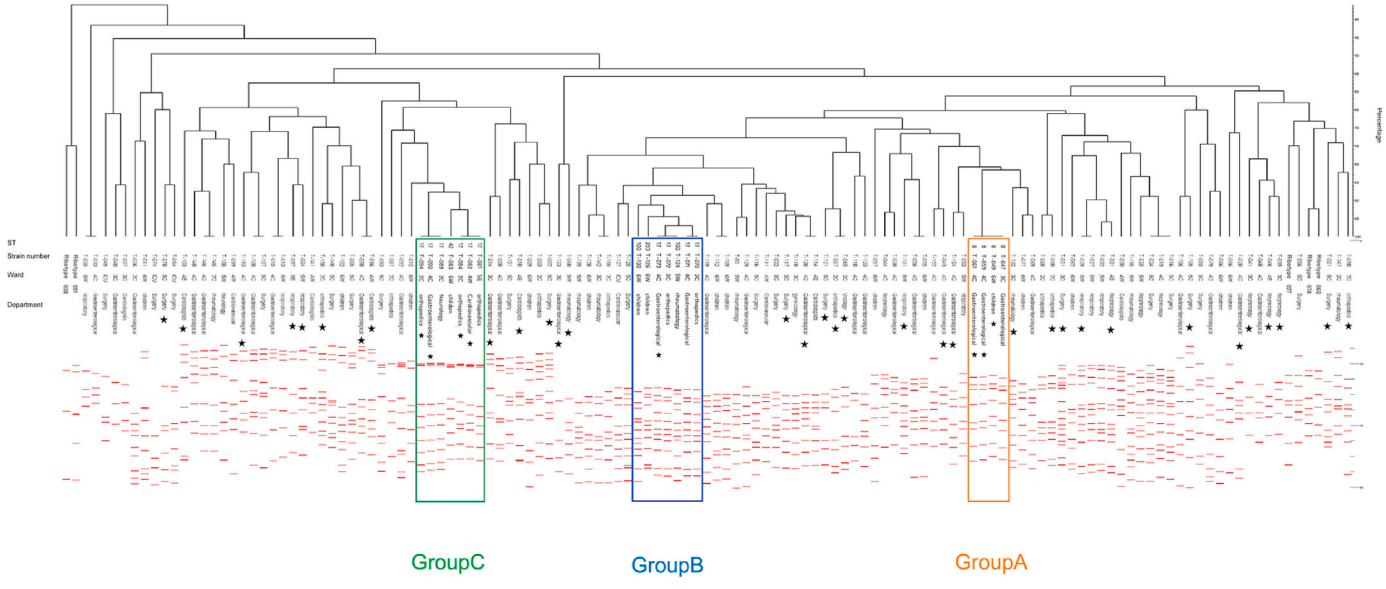

Fig. 1. Phylogenetic analysis of ribotyping profiles of *C. difficile* strains
The dendrogram was constructed based on similarity and clustering with Pearson correlation using FPQuest software (Bio-Rad). Strain numbers, wards, and medical departments are indicated below the dendrogram. Squares indicate the groups of strains exhibiting similar profiles of ribotyping, and their sequence types are indicated between the dendrogram and strain number. The star mark indicates a strain of *C. difficile* causing infection among patients.

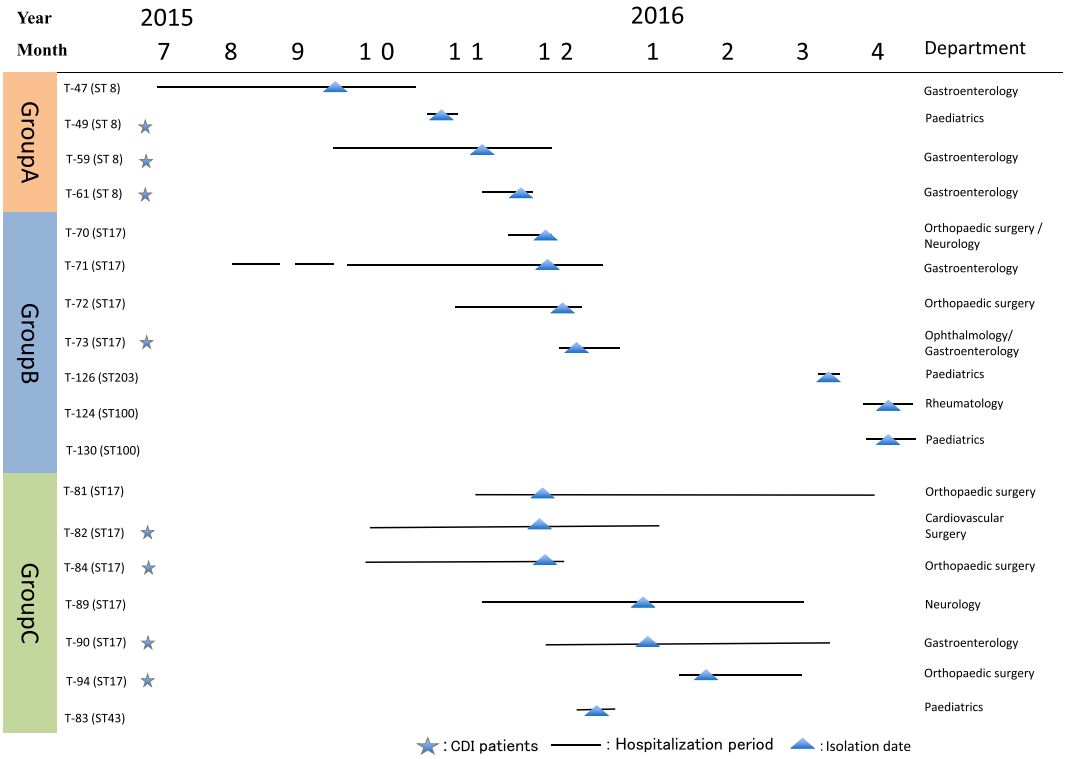

Fig. 2. Time series of *C. difficile* isolates with similar ribotypes
Isolation of *C. difficile* strains with similar ribotypes from inpatients. Group A, Group B and Group C isolates, which were categorized the similar ribotypes (same sequence types) as shown in Fig. 1, were selected representatively and inpatient data (hospitalization periods, isolation date, and departments) are shown. Group A strains were isolated from inpatients in the same ward (physically same floor); Group B and Group C strains were isolated from inpatients in multiple wards (physically different floors), respectively.

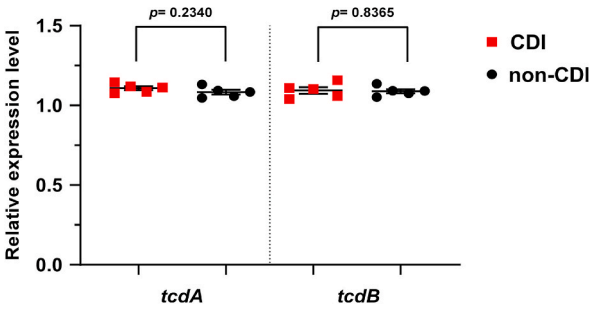

Fig. 3. Transcription levels of toxin genes (*tcdA* and *tcdB*) in isolates from patients with and without CDI
Transcription levels of the toxin genes were expressed as a ratio to that of 16S rRNA. The results were obtained from experiments with triplicate samples.
The dot mark indicates the transcription level of the toxin, and the horizontal bar indicates the
mean and variance values. Red squares indicate the expression levels of toxin genes T-59 (ST8), T-65 (ST17), T-68 (ST41), T-73 (ST17), and T-121 (ST100) in isolates from patients with CDI. Black circles indicate the expression levels of T-11 (ST2), T-40 (ST8), T-89 (ST17), and T-100 (ST17) toxin genes in isolates from patients without CDI and the reference strain ATCC 70057.

this study, STs with similar ribotypes but completely different sequences were also assigned. Therefore, it is necessary to use not only ribotyping but also other methods, such as MLST and Pulsed-field gel electrophoresis, to confirm that they are the same strain. According to a report by Kuwata et al. [14], the most frequently identified ST among 121 strains isolated from a university hospital in Japan was ST17 (21.8%). Moreover, ST8 and ST17 were closely related strains in the eBURST analysis (https://www.mlst.net/eburst/). In addition to this report, other studies reported that ST17, ST8, and ST2 were the dominant STs [15,16]. Taken together, these results suggest that strains with these STs had spread across Japan, and strains with such STs might have some advantages in adapting to the environment in a hospital. Even in the three groups of isolates with similar ribotypes, there was some association between

infection and department, or length of hospital stay for patients with isolates, suggesting contamination in the nosocomial infection in the hospital (Fig. 2). In particular, the group A was more likely to have had ST8 transmission between patients on the same ward. Patients in group B and C with ST17 strain also had a history of admission to a surgical ward or Intensive Care Unit. Gilboa et al. report that the rooms of *C. difficile* carriers and those of patients with active CDI were equally contaminated and significantly more so than non-carrier rooms [17]. Therefore, it is essential not only to check for the presence of diarrheal symptoms of CDI but also to control toxigenic *C. difficile* carriers and ensure environmental disinfection. Although many ribotypes were observed in this study, the CDT-positive BI/NAP1/027 strain was not found.

The only anti-C. *difficile* drugs used in Japan are MNZ and VCM. Despite the prevalence of CDI, we believe that drug resistance of the bacterium has not yet become a problem. However, in recent years, MNZ-resistant *C. difficile* has been reported abroad [18], and it is necessary to monitor its potential emergence in Japan soon as the incidence rate of CDI is rising. Although non-sensitive strains with MICs of 4 mg/L have been isolated overseas [19], VCM-resistant *C. difficile* has not been reported in Japan. In the present study, one strain exhibiting low susceptibility to VCM (MIC = 4 mg/L) was also isolated. Although it was an $A^{-}B^{-}C^{-}$ strain and was not considered problematic in therapy, it indicated the necessity of surveillance of drug resistance in *C. difficile.* FDX showed the strongest activity against *C. difficile.* In a study from another Japanese group with 100 *C. difficile* strain isolates from a university hospital in 2011 and 2012 [20], it was reported that MIC of FDX ranged from 0.03 to 0.5 mg/L, with an MIC_{90} of 0.25 mg/L. In this study, with 153 *C. difficile* strain isolates obtained in 2015 and 2016, a comparable result was observed, indicating that FDX was still effective. Although FDX was approved in Japan in 2018, careful use and surveillance are important to prevent the spread of resistant *C. difficile* in the future. The anti-*C. difficile* drugs MNZ and VCM also showed good susceptibility. However, due to the presence of one strain with low susceptibility to VCM (MIC = 4 mg/L), it may be necessary to pay attention to future trends.

Although four strains were determined to be CDT-positive by PCR, their ribotypes were not 027. In addition, BI/NAP1/027 strains are usually fluoroquinolone resistant but the CDT-positive strains in this study were sensitive to MFLX, a fluoroquinolone. In summary, although the four strains were not examined by pulsed-field gel electrophoresis, they were suggested to be different from the virulent BI/NAP1/027 strain. It is known that ribotype does not directly indicate the virulence level of the bacterium, and previous studies [21] have reported that ribotype 027/078, a virulent strain, is less toxic than other ribotypes. A virulent BI/NAP1/027 strain, one of the ribotype 027 strains, produces more toxin because of elevated levels of transcription of toxin genes (*tcdA* and *tcdB*) by the deletion of the negative regulator *tcdC* [22]. The BI/NAP1/027 strain is believed to exhibit high virulence because of the elevated expression of both genes in addition to the binary toxin. Toxin is a major virulence factor in the onset of CDI, and increased toxin production is expected to cause CDI. The regulation of toxin production is controlled by several factors, *tcdC* is one such factor [23,24]. Although toxin gene transcription could be an important virulence factor, quantitative analyses of toxin production or toxin gene transcription have not been performed. In this study, for the first time, we examined the transcription levels of toxin genes in clinical isolates from patients with and without CDI (Fig. 3). We also analysed the transcription levels of toxin genes in ST8 and ST17 strains suspected of HAI. The results of this study showed that the same levels of toxin gene transcription were observed in clinical isolates from patients with and without CDI. CDI did not consistently develop in patients infected with *C. difficile* with the same toxin gene profile and similar transcription levels. One *C. difficile* strain that had toxin genes ($A^{+}B^{+}$) caused CDI, but another that also had both toxin genes did not, although both pairs of toxin genes were transcribed at the same level. However, toxin gene expression in our *in vitro* experiment might be different from that in the patient's colon. As mentioned above, many factors are involved in the regulation of toxin expression. It is quite likely that differences in the environment could result in differences in the expression of toxin genes. Therefore, the possibility that isolates from patients with CDI could produce more toxins than those from patients without CDI *in vivo* is not excluded. The CDI risk could not be assessed by the amount of toxin expression in this study, and further mutant analysis of virulence factors of this bacterium and animal model of CDI are needed to reveal the mechanism.

5. Conclusions

Awareness and understanding of CDI are limited in Japan compared with those in other countries. There are increasing concerns about the influx of virulent strains, such as BI/NAP1/027, by travellers and migrants, and infection by 078 strains from meats and pets to humans. However, these strains were not detected in the hospital. Therefore, continuous and extensive national surveillance of this bacterium is needed in Japan. The results of this study suggest that more intensive infection control measures and prudent management of diarrhoea stools and human waste, regardless of the presence of CDI, are necessary to prevent stealthy nosocomial infections of *C. difficile* strains in the hospital.

Declarations

Author contributions

Yukitaka Ito: Conceived and designed the experiments; Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. </p>

Koichi Tanimoto, Haruyoshi Tomita: Conceived and designed the experiments; Performed the experiments. </p>;

Masanobu Otsuka, Masato Ota, Mieko Yoshida: Contributed reagents, materials, analysis tools or data. </p>

Naoko Chiba, Yusuke Hashimoto, Takahiro Nomura: Performed the experiments. </p>.

Data availability statement

Data included in article/supp. material/referenced in article.

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Ethical approval

This study was approved by the Ethical Committee of Toho University Ohashi Medical Center, Meguro, Tokyo, Japan (No. 27-3). Informed consent has been obtained from all individuals included in this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix B. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e20167.

Abbreviations

CDI	*Clostridioides difficile* infection
IDSA	Infectious diseases society of America
MIC	Minimum inhibitory concentrations
ST	Sequence type

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