

Nationwide surveillance of ribotypes and antimicrobial susceptibilities of toxigenic *Clostridium difficile* isolates with an emphasis on reduced doxycycline and tigecycline susceptibilities among ribotype 078 lineage isolates in Taiwan

Yuan-Pin Hung,^{1-3,*} Pei-Jane Tsai,^{4,*} Yuan-Ti Lee,^{5,6} Hung-Jen Tang,⁷ Hsiao-Ju Lin,¹⁻³ Hsiu-Chuan Liu,¹ Jen-Chieh Lee,² Bo-Yang Tsai,⁴ Po-Ren Hsueh,⁸ Wen-Chien Ko^{2,9,10}

¹Department of Internal Medicine, Tainan Hospital, Ministry of Health and Welfare, Tainan, Taiwan; ²Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan; ³Graduate Institute of Clinical Medicine, National Cheng Kung University, Tainan, Taiwan; ⁴Department of Medical Laboratory Science and Biotechnology, College of Medicine, National Cheng Kung University, Tainan, Taiwan; ⁵Institute of Medicine and School of Medicine, Chung Shan Medical University, Taichung, Taiwan; ⁶Division of Infectious Diseases, Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan; ⁷Department of Internal Medicine, Chi-Mei Hospital, Tainan, Taiwan; ⁸Departments of Laboratory Medicine and Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan; ⁹Center of Infection Control, National Cheng Kung University Hospital, Tainan, Taiwan; ¹⁰Department of Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan

*These authors contributed equally to this work

Correspondence: Po-Ren Hsueh
Departments of Laboratory Medicine and Internal Medicine, National Taiwan University Hospital, No. 7, Chung-Shan South Road, Taipei 100, Taiwan
Email hsporen@ntu.edu.tw

Wen-Chien Ko
Department of Internal Medicine, National Cheng Kung University Hospital, No. 138, Sheng Li Road, Tainan 704, Taiwan
Email winston3415@gmail.com

Objectives: The information of antimicrobial susceptibility, toxin gene, and ribotype distribution of toxigenic *Clostridium difficile* isolates in Taiwan remain limited.

Patients and methods: The study was conducted from January 2015 to December 2016 in 5 hospitals in Taiwan. Adults aged ≥ 20 years with a hospital stay for >5 days were included, and those with colectomy or intestinal infection due to other enteropathogens were excluded. Multiplex PCR was used to detect *tcdA*, *tcdB*, *cdtA*, *cdtB*, and *tcdC* deletions, and antimicrobial susceptibility for metronidazole, vancomycin, doxycycline, and tigecycline was investigated. Ribotypes of those isolates with *tcdC* deletion and *tcdA*+/*tcdB*+ were determined.

Results: Of 1112 *C. difficile* isolates collected from adults at 5 hospitals, 842 were toxigenic, including 749 (89.0%) *tcdA*+/*tcdB*+ isolates and 93 (11.0%) *tcdA*-/*tcdB*+. Of the toxigenic isolates, 76 (9.0%) had a *tcdC* deletion and were *cdtA*+/*cdtB*+, indicative of hypervirulence, and RT078 lineage, including RT126, RT127, and RT078, predominated ($n=53$, 76.3%). Similar to the susceptibility data in Asia countries, metronidazole or vancomycin resistance was rare, noted in 1.2% or 2.1%, respectively. Reduced doxycycline susceptibility (minimum inhibitory concentration [MIC] of ≥ 8 mg/L) was more common among RT078 lineage than non-RT078 lineage (75.9%, 44/58 vs 6.0%, 47/784; $P<0.001$). Also reduced tigecycline susceptibility (MIC ≥ 0.125 mg/L) was more common among RT078 lineage (20.7%, 12/58 vs 6.5%, 51/784; $P<0.001$).

Conclusion: In Taiwan, toxigenic *C. difficile* isolates remain susceptible to metronidazole and vancomycin. RT078 lineage predominated among toxigenic isolates with *cdtA*, *cdtB*, and *tcdC* deletion, and more often had reduced doxycycline and tigecycline susceptibility than the isolates other than RT078 lineage.

Keywords: MIC, metronidazole, vancomycin, RT126, RT127

Introduction

Clostridium difficile is the primary cause of nosocomial antibiotic-associated diarrhea, with clinical symptoms ranging from mild diarrhea to pseudomembranous colitis or toxic megacolon, with a mortality rate of up to 25%–40%.¹ An epidemic *C. difficile* strain circulating in western countries had been assigned to the North American pulse-field type 1 (NAP1), restriction endonuclease analysis group BI, and polymerase

chain reaction (PCR) ribotype 027 (sometimes referred to as BI/NAP1/027) has 3 important microbiological characters: increased production of toxin A and B (in conjunction with *tcdC* deletion), fluoroquinolone resistance, and production of binary toxin (encoded by *cdtA* and *cdtB*).² Toxigenic *C. difficile* isolates with *tcdC* deletion, *cdtA*, and *cdtB* have been found in Taiwan.^{3–7} Three cluster cases presenting infectious diarrhea with multiple recurrences due to *C. difficile* RT126, belonging to RT078 lineage, were reported in 2014.³ In the following year, 3 cases infected by RT027 were reported in Taiwan, with clinical manifestations of pseudomembranous colitis, toxic megacolon or bowel perforation.^{4–6} In addition to the RT027 and RT078 lineage isolates, toxigenic *C. difficile* isolates harboring *tcdB* and truncated *tcdA* were predominant, accounting for 43.3% of infections in a district hospital in Taiwan.⁷ *C. difficile* RT027 and RT078 isolates were reported sporadically in Mainland China.⁸ Information about the toxin genes or ribotype distribution of toxigenic or hypervirulent *C. difficile* isolates in Taiwan has not been reported.

Some metronidazole- or vancomycin-resistant *C. difficile* isolates were observed in Taiwan. Among 403 non-duplicate isolates of *C. difficile* from 3 teaching hospitals in Taiwan between 2005 and 2010, all isolates were susceptible to metronidazole, but 2 had reduced susceptibility to vancomycin (minimum inhibitory concentration [MIC], 4 mg/L).⁹ In a retrospective study in a medical center in northern Taiwan, 2 (1.8%) isolates in 2003 showed resistance to metronidazole (MIC >32 mg/L) and 5 (4.5%) obtained in 2003 (n=1), 2006 (n=1), and 2007 (n=3) showed resistance to vancomycin (MIC >2 mg/L).¹⁰ Doxycycline and tigecycline had been suggested as potential alternatives for *C. difficile* infection (CDI).^{9,11} Tigecycline was in vitro highly active against an earlier collection, between 2001 and 2009, of clinical *C. difficile* isolates in Taiwan.¹² In the present study, we conducted a multicenter and prospective study to investigate the distribution of toxin genes, ribotypes, and antimicrobial susceptibilities among clinical *C. difficile* isolates from 2015 to 2016 in Taiwan.

Patients and methods

Hospital settings

This study was conducted in the National Taiwan University Hospital (Hospital A: a medical center in northern Taiwan), Chung Shan Medical University Hospital (B: a medical center in central Taiwan), Chi-Mei Hospital (C: a medical center in southern Taiwan), National Cheng Kung University Hospital (D: a medical center in southern Taiwan), and

Tainan Hospital, Ministry of Health and Welfare (E: a district hospital in southern Taiwan), as noted in Figure 1.

C. difficile isolates collected from participating hospitals

This study was conducted from January 2015 to December 2016, and only patients aged at least 20 years were included. In routine clinical care, stool samples from those with suspected CDI were tested by GeneXpert *C. difficile* PCR assay (Cepheid, CA, USA) in the Hospital A, BD GeneOhm™ Cdiff PCR assay (BD Diagnostics, San Diego, CA, USA) in the Hospital B, C, and D, or C. Diff Quik Chek Complete (Alere, Waltham, MA, USA) in the Hospital E. If positive, stools were plated on cycloserine-cefoxitin-fructose agar and incubated anaerobically for 24–48 h. During the study period, fecal samples or *C. difficile* isolates in the Hospital A, B, C, and E, were submitted to the Hospital D for cultures and further tests. For surveillance purposes, the isolate collected >60 days after the initial one was considered a new and distinct one and those collected between 14 and 60 days after the initial isolate were regarded as recurrent ones.¹³ The isolates obtained within 14 days after the initial isolate were excluded.

Multiplex PCRs for toxin genes and PCR ribotyping

C. difficile isolates were sub-cultured on cycloserine-cefoxitin fructose selective plates and incubated anaerobically for 24–48 h. The colony with suspected morphology of *C. difficile* from the culture plate was selected for further identification and studies. A multiplex PCR detecting 16S ribosomal RNA, *tpi* (encoding triose phosphate isomerase, used as a *C. difficile*-specific marker), *tcdA* (encoding toxin A), *tcdB* (encoding toxin B), *cdtA* and *cdtB* (encoding binary toxin), and *tcdC* deletion, and PCR ribotyping were performed at the Hospital D, as previously described.¹⁴ Briefly, after PCR amplification, the samples were concentrated using the Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, New Taipei, Taiwan) and separated with the QIAxcel capillary electrophoresis system (Qiagen, Hilden, Germany) using the “OM500” method and QX Alignment Marker 15 bp/3 kb (Qiagen, Hilden, Germany). The PCR ribotypes were confirmed by the WEBRIBO database (<http://webribo.ages.at>).

Antimicrobial susceptibility testing

MICs were determined by the agar dilution method according to the Clinical and Laboratory Standards

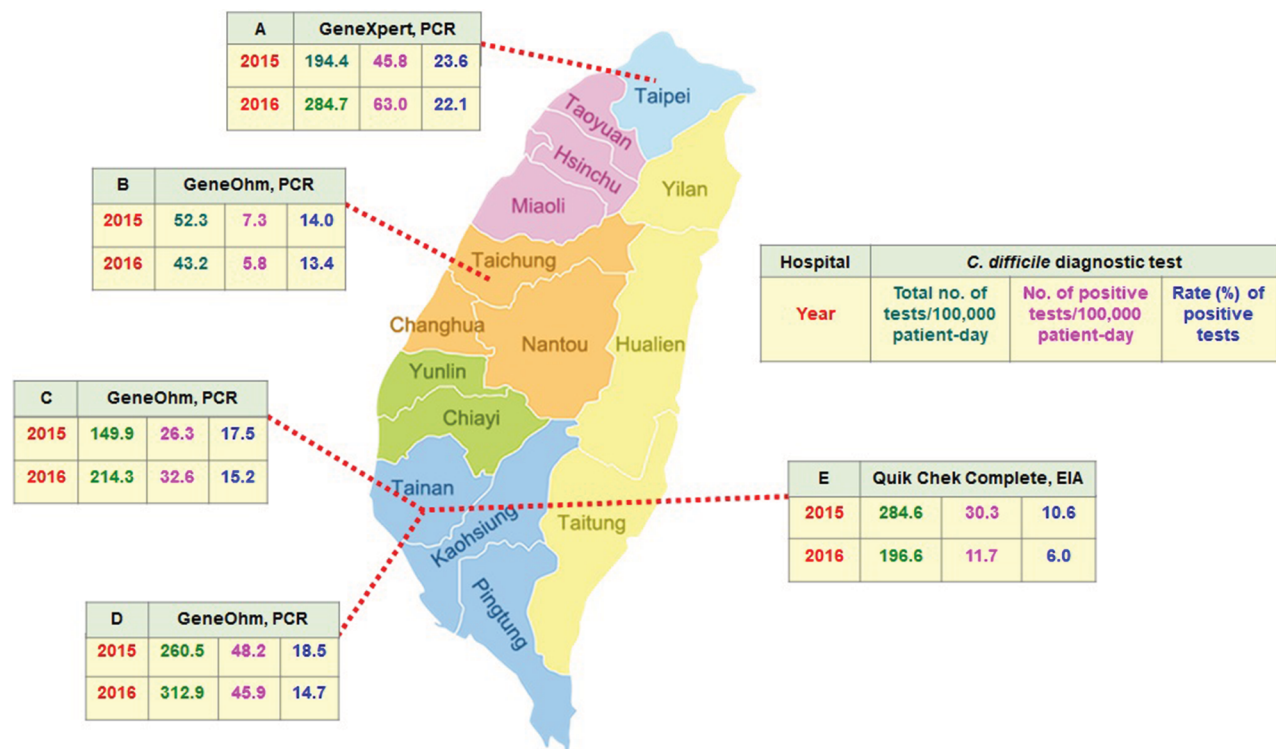


Figure 1 Geographic distribution and laboratory burden of *Clostridium difficile* tests, expressed as total test numbers per 100,000 patient-days, positive test numbers per 100,000 patient-days, and positive rates of the 5 participating hospitals in 2015 and 2016.

Abbreviations: PCR, polymerase chain reaction; EIA, enzyme immunoassay.

Institute (CLSI) guidelines (M11-A8).¹⁵ Supplemented *Brucella* agar deeps were obtained from Anaerobe Systems (Morgan Hill, CA, USA). Frozen defibrinated sheep blood (Hema Resources Inc., Aurora, OR, USA) was thawed to produce laked blood. On the day of testing, laked blood and antimicrobial agents were added to tubes of molten agar before pouring into agar dilution plates. The tested isolates were applied to the plates using a Steers multipronged inoculator for a final concentration of $\sim 10^5$ colony forming unit/spot. After 44 h of incubation at 36°C in an anaerobic chamber incubator, the plates were examined for bacterial growth and the MICs interpreted. Metronidazole and vancomycin were tested at concentrations of 0.03 to 512 mg/L, and MIC breakpoints for resistance are defined as ≥ 32 and ≥ 16 mg/L, respectively, according to CLSI 2012.¹⁵ Quality control strains included *Bacteroides fragilis* ATCC 25285, *C. difficile* ATCC 700057, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212. The MIC was defined as the lowest concentration that yielded no visible growth or a marked change/reduction in growth compared to the growth controls. MIC breakpoints for

resistance to doxycycline and tigecycline are defined as ≥ 8 and ≥ 0.5 mg/L, respectively, according to a previous report¹¹ and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) version 6.0.¹⁶

Statistical analysis

Statistical analysis was performed using the statistical software, SPSS, version 13.0. The χ^2 -test or Fisher's test was used for categorical variables, and a 2-tailed *P*-value of < 0.05 was considered statistically significant.

Ethical approval

The study was approved by the Institutional Review Board in each hospital: A (National Taiwan University Hospital Research Ethics Committee: 201412190RIND), B (Institutional Review Board, Chung Shan Medical University Hospital: CS14179), C (Institutional Review Board, Chi Mei Medical Center: 10402-006), D and E (Institutional Review Board, National Cheng Kung University Hospital: B-ER-103-098). The written informed consents were waived because of the retrospective studies of clinical isolates without interventions on the patients.

Results

Laboratory burden of *C. difficile* tests in study hospitals

In the 2-year period, the incidence of *C. difficile* tests performed in 5 hospitals varied greatly, ranging from 53.3 to 284.6 tests per 100,000 patient-days in 2015 and 43.2–312.9 tests per 100,000 patients-days in 2016. With a positivity rate of 10.6%–23.6% in 2015 and 6.0%–22.1% in 2016, the incidence of positive *C. difficile* tests ranged from 7.3 to 48.3 tests per 100,000 patient-days in 2015 and 5.8 to 63.0 tests per 100,000 patient-days in 2016 (Figure 1).

Ribotypes of *C. difficile* isolates

A total of 1112 *C. difficile* isolates were obtained from 1262 fecal samples, and 842 (75.7%) were toxigenic. By the surveillance definition, the recurrent rate of CDI was 4.6% (39/842). The numbers of toxigenic *C. difficile* isolates collected in Hospitals A–E were 214, 87, 147, 289, and 105, respectively (Table 1). Of 842 toxigenic isolates, the vast majority (749, 89.0%) had *tcdA* and *tcdB* (i.e., *tcdA*/*tcdB*+), and 93 (11.0%) were *tcdA*-/*tcdB*+. Of the *tcdA*/*tcdB*+ isolates, 10.9% (82/842) possessed *cdtA* and *cdtB*, and 76 (9.0%) also had *tcdC* deletion. Among participating hospitals, *tcdC* deletion was most often found in 18 (12.2%) isolates of 147 toxigenic isolates from Hospital C. By contrast, 3.8%–10.0% of toxigenic isolates in other 4 hospitals had a *tcdC* deletion. Two major lineages of 76 isolates with *tcdC* deletion were RT078 (58, 76.3%) and RT027 lineages (13, 17.1%) (Table 1). Major ribotypes of 58 RT078 lineage isolates included RT126 (39.7%,

23), RT127 (37.9%, 22), and RT078 (19.0%, 11). Of 13 RT027 lineage isolates, 8 belonged to RT027, 4 RT034, and 1 RT075 (Figure 2). Among 749 toxigenic isolates with *tcdA* and *tcdB*, the percentages of RT027 or RT078 lineage isolates were not significantly different, ranging from 0% to 4.1% ($P=0.22$) or 4.7%–10.7% ($P=0.29$), respectively, in five hospitals. As for 93 *tcdA*-/*tcdB*+ isolates which accounted for 10.0% of *tcdB*+ toxigenic isolates, 89.2% were RT017.

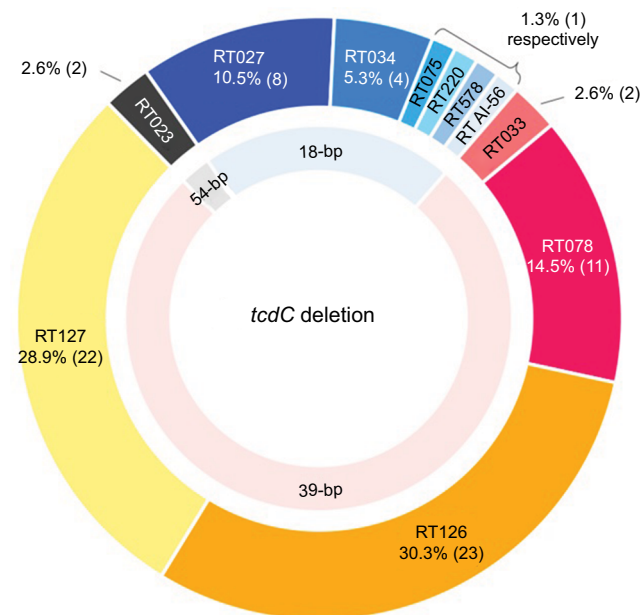


Figure 2 Ribotype distribution of 76 *Clostridium difficile* isolates with *cdtA* and *cdtB*, in which there were 3 types of *tcdC* deletion, 18-bp, 39-bp, and 54-bp deletion. **Abbreviation:** bp, base pair.

Table 1 RTs of *tcdB*-positive *Clostridium difficile* isolates from 5 hospitals in Taiwan

Toxin genes and RTs	No. (%) of isolates with indicated toxin genotypes and RTs					
	Total (n=842)	Hospital A (n=214)	Hospital B (n=87)	Hospital C (n=147)	Hospital D (n=289)	Hospital E (n=105)
<i>tcdA</i> / <i>tcdB</i> +	749	204	85	121	265	74
<i>cdtA</i> / <i>cdtB</i> +	82 (10.9)	21 (10.3)	5 (5.9)	19 (15.7)	32 (12.1)	5 (6.8)
<i>tcdC</i> deletion	76 (10.1)	20 (9.8)	5 (5.9)	18 (14.9)	29 (10.9)	4 (5.4)
RT027 lineage	13 (1.7)	2 (1.0)	1 (1.2)	5 (4.1)	5 (1.9)	0
RT027	8 (1.1)	1 (0.5)	0	4 (3.3)	3 (1.1)	0
RT034	4 (0.5)	1 (0.5)	1 (1.2)	1 (0.8)	1 (0.4)	0
RT075	1 (0.1)	0	0	0	1 (0.4)	0
RT078 lineage	58 (7.7)	16 (7.8)	4 (4.7)	13 (10.7)	21 (7.9)	4 (5.4)
RT033	2 (0.3)	2 (1.0)	0	0	0	0
RT078	11 (1.5)	1 (0.5)	1 (1.2)	8 (6.6)	1 (0.4)	0
RT126	23 (3.1)	11 (5.4)	3 (3.5)	2 (1.7)	7 (2.6)	0
RT127	22 (2.9)	2 (1.0)	0	3 (2.5)	13 (4.9)	4 (5.4)
<i>tcdA</i> -/ <i>tcdB</i> +	93	10	2	26	24	31
RT017	84 (90.3)	8 (80.0)	2 (100)	25 (96.2)	22 (91.7)	27 (87.1)

Abbreviation: RT, ribotype.

Susceptibility data of *C. difficile* isolates in Taiwan

Metronidazole MICs of the 1112 *C. difficile* isolates ranged from <0.125 to >32 mg/L, and metronidazole resistance rate varied among hospitals, ranging from 0.6% (Hospital E) to 3.3% (Hospital C). Vancomycin MICs ranged from <0.0625 to >8 mg/L, and vancomycin resistance rate ranged from 1.1% (Hospital B) to 4.6% (Hospital C) (Table 2). As for tigecycline, MIC values ranged from ≤0.0625 to >32 mg/L, and resistance to tigecycline (arbitrarily defined as MIC ≥0.5 mg/L) was noted in 0.6%–3.2% of the isolates in 5 hospitals. In contrast, the MIC range of doxycycline was between ≤0.0625 and >32 mg/L, and doxycycline resistance (defined as doxycycline MIC ≥8 mg/L) was present in 7.7%–17.3% of

the isolates from different hospitals (Table 3). However, doxycycline and tigecycline MICs of <0.0625 mg/L were noted in 51.4% and 92.3%, respectively, of all *C. difficile* isolates.

Overall, 1.2%, 2.1%, and 1.9% of toxigenic *C. difficile* isolates were resistant to metronidazole, vancomycin, and tigecycline, respectively, and the corresponding figure for non-toxicogenic isolates was 1.5%, 1.1%, and 2.2%, respectively (Table 4). However, doxycycline resistance was observed in 10.7% and 10.7% of toxigenic and non-toxicogenic isolates, respectively.

Compared with toxigenic isolates of non-RT078 lineage, more toxigenic RT078 lineage isolates exhibited reduced susceptibility to doxycycline (MIC >8 mg/L: 75.9%, 44/58 vs 6.0%, 47/784; $P < 0.001$) and tigecycline (MIC >0.125 mg/L: 20.7%, 12/58 vs 6.5%, 51/784; $P < 0.001$).

Table 2 Antimicrobial susceptibilities of metronidazole and vancomycin in 1112 *Clostridium difficile* isolates from 5 hospitals*

Hospital (number of isolates tested)	Metronidazole MICs (mg/L)			Resistance, %	Vancomycin MICs (mg/L)			Resistance, %
	Range	MIC ₅₀	MIC ₉₀		Range	MIC ₅₀	MIC ₉₀	
A (310)	≤0.125 to >32	0.5	1	1.0	≤0.0625 to >8	0.5	1	1.6
B (181)	≤0.125 to >32	0.25	1	1.1	≤0.0625 to 4	0.5	1	1.1
C (151)	≤0.125 to >32	0.5	1	3.3	≤0.0625 to >8	0.5	1	4.6
D (308)	≤0.125 to >32	0.5	1	1.0	≤0.0625 to >8	0.5	2	1.3
E (162)	≤0.125 to >32	0.25	1	0.6	≤0.0625 to >8	0.5	1	1.9

Note: *Resistance is defined as the breakpoints in Clinical and Laboratory Standards Institute Guidelines.¹⁵

Abbreviations: MIC50, minimum inhibitory concentration required to inhibit 50% of bacteria; MIC90, minimum inhibitory concentration required to inhibit 90% of bacteria.

Table 3 Antimicrobial susceptibilities of doxycycline and tigecycline in 1112 *Clostridium difficile* isolates from 5 hospitals*

Hospital (number of isolates tested)	Doxycycline MICs (mg/L)			Resistance, %	Tigecycline MICs (mg/L)			Resistance, %
	Range	MIC ₅₀	MIC ₉₀		Range	MIC ₅₀	MIC ₉₀	
A (310)	≤0.0625 to >32	≤0.0625	4	7.7	≤0.0625 to 1	≤0.0625	≤0.0625	3.2
B (181)	≤0.0625 to 32	0.125	8	11.0	≤0.0625 to 0.5	≤0.0625	0.125	1.7
C (151)	≤0.0625 to >32	≤0.0625	4	11.9	≤0.0625 to >32	≤0.0625	≤0.0625	1.3
D (308)	≤0.0625 to >32	≤0.0625	4	9.4	≤0.0625 to >32	≤0.0625	≤0.0625	1.9
E (162)	≤0.0625 to 32	0.25	16	17.3	≤0.0625 to 1	≤0.0625	≤0.0625	0.6

Note: *MIC breakpoints for resistance to doxycycline and tigecycline are defined as ≥8 and ≥0.5 mg/L, respectively.^{11,16}

Abbreviations: MIC50, minimum inhibitory concentration required to inhibit 50% of bacteria; MIC90, minimum inhibitory concentration required to inhibit 90% of bacteria.

Table 4 Antimicrobial susceptibilities of metronidazole, vancomycin, doxycycline, and tigecycline among toxigenic and non-toxicogenic *Clostridium difficile* isolates collected from 5 hospitals

Antimicrobial agents	Toxigenic isolates (n=842)				Non-toxicogenic isolates (n=270)			
	MIC range, (mg/L)	MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)	Resistance, %	MIC range, (mg/L)	MIC ₅₀ (mg/L)	MIC ₉₀ (mg/L)	Resistance, %
Metronidazole*	≤0.125 to >32	0.5	1	1.2	≤0.125 to >32	0.25	1	1.5
Vancomycin*	≤0.0625 to >8	0.5	2	2.1	≤0.0625 to >8	0.5	1	1.1
Doxycycline**	≤0.0625 to >32	≤0.0625	8	10.8	≤0.0625 to 32	0.25	8	10.7
Tigecycline**	≤0.0625 to >32	≤0.0625	≤0.0625	1.9	≤0.0625 to 1	≤0.0625	≤0.0625	2.2

Notes: *Resistance is defined as the breakpoints in Clinical and Laboratory Standards Institute Guidelines.¹⁵ **MIC breakpoints for resistance to doxycycline and tigecycline are defined as ≥8 and ≥0.5 mg/L, respectively.^{11,16}

Abbreviations: MIC50, minimum inhibitory concentration required to inhibit 50% of bacteria; MIC90, minimum inhibitory concentration required to inhibit 90% of bacteria.

Discussion

The incidence of CDI was reported as 45 and 42.6/100,000 patient-days in 2 retrospective studies in Hospitals A¹⁷ and D,¹⁸ respectively, and 42.4/100,000 patient-day in a prospective study in Hospital E.¹⁹ However, the present study reported the laboratory burden of *C. difficile* tests, either PCR or enzyme immunoassay, ranging from 43.2 to 312.8 tests/100,000 patient-days, which may be related to the real burden of CDIs or the clinical awareness of CDIs among attending physicians. The incidence of positive *C. difficile* tests ranged from 5.8 to 63.0 tests/100,000 patient-day and the positive test rate ranged from 6.0% to 23.6% among study hospitals. These data suggest the substantial, although geographically diverse, disease burden of CDIs in Taiwan.

In our study, although the distribution of the toxin gene pattern, ribotype or antimicrobial susceptibility varied in each hospital, the dominant ribotypes among *C. difficile* isolates with *tcdC* deletion, *cdtA*, and *cdtB* from 5 hospitals in 3 major cities in Taiwan were RT078 and RT027 lineages. *C. difficile* RT078 lineage isolates (such as RT126 and 127) have been linked to animal reserves or contact²⁰ and zoonotic potential of RT078 lineage in Taiwan warrants more investigations. The emergence of severe colitis due to *C. difficile* RT027 and RT078 in Taiwan and Asia indicates the need for comprehensive surveillance of CDI and molecular typing of clinical *C. difficile* isolates in the continent with a significant burden of multidrug-resistant organisms.

Metronidazole and vancomycin are regarded as the primary therapy options for CDI, depending on the severity of disease.² Among a collection of >800 clinical toxigenic *C. difficile* isolates in our study, 1.2% or 2.1% were resistant to metronidazole or vancomycin, respectively. The published literature also indicates that antimicrobial resistance to 2 commonly prescribed antimicrobial agents was rare among clinical *C. difficile* isolates from several southeastern and northeastern Asian countries.

Lower MICs were noted for doxycycline and tigecycline, which were suggested as alternative choices for CDI.^{9,11} In a study for each day of doxycycline receipt, the CDI rate was 27% lower than that of no doxycycline therapy (hazard ratio, 0.73; 95% CI: 0.56–0.96).²¹ Successful treatment with CDI using tigecycline-based combination regimens was reported.²² A previous study reported that in clinical isolates in Taiwan, the in vitro susceptibility rate of *C. difficile* to tigecycline was high.¹² Both doxycycline and tigecycline revealed relatively low MICs among *C. difficile* isolates, except in some RT078 lineage isolates in the study. Resistance to one or more of drugs tetracycline, moxifloxacin, clindamycin, or erythromycin

has been reported in 26.9% (46) of 171 *C. difficile* isolates in Australia, predominantly RT126 and 078.²³ The clinical utility of doxycycline or tigecycline alone or in combination as therapeutic alternatives for CDI warrants further investigations.

Limitations

There were some limitations in our study. First, because the protocols or clinical algorithms to harvest *C. difficile* isolates from stool samples varied, we cannot compare the CDI incidence among study hospitals. Second, clinical information was not available to be correlated with the distribution of toxin genes, ribotypes, and antimicrobial susceptibilities. The clinical implications of predominant ribotypes among *C. difficile* isolates with *tcdC* deletion, *cdtA*, and *cdtB* or antimicrobial resistance in Taiwan warrant further studies. Third, the ribotypes of more than 700 *tcdA*+/*tcdB*+/*cdtA*-/*cdtB*- *C. difficile* isolates were not investigated, and the likelihood of the existence of other predominant ribotypes in Taiwan cannot be excluded. Finally, no local information of antibiotic consumption in pig feed was available, so the potential linkage between higher prevalence of reduced susceptibility to doxycycline and tigecycline in RT078 isolates and agricultural antibiotic use remains obscure.

Conclusion

The dominant ribotypes among *C. difficile* isolates with *tcdC* deletion, *cdtA*, and *cdtB* were RT078 and RT027 lineages. Metronidazole or vancomycin resistance remained rare in toxigenic *C. difficile* isolates, but reduced susceptibility to doxycycline and tigecycline was more common among RT078 than non-RT078 lineage isolates.

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Disclosure

The authors report no conflicts of interest in this work.

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