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

**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 ≥28 mg/L) was more common among RT078 lineage (75.9%, 44/58 vs 6.0%, 47/784; *P* <0.001). Also reduced tigecycline susceptibility (MIC ≥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

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**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%. 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.\(^1\) 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 \(cdtB\) and truncated \(cdtA\) 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).\(^11\) 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), \(cdtA\) (encoding toxin A), \(cdtB\) (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 Institute (CLSI) guidelines. The MICs were determined by the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

**Results**

**Comparison of antimicrobial susceptibilities**

The MICs of metronidazole and vancomycin of the 349 isolates were determined by the agar dilution method according to the CLSI guidelines. The MICs were determined by the agar dilution method according to the CLSI guidelines.

**Discussion**

**Conclusions**

This study demonstrated that the prevalence of metronidazole- or vancomycin-resistant \(C.\) difficile isolates in Taiwan was not high. The prevalence of metronidazole- or vancomycin-resistant \(C.\) difficile isolates in Taiwan was not high.

**Acknowledgment**

This study was supported by grants from the Ministry of Science and Technology (MOST 104-2320-B-002-048-MY3, 104-2622-E-002-012-CC2, 105-2314-B-002-271-MY3, 105-2314-B-002-272-MY3, 106-2314-B-002-226-MY3) and the Ministry of Health and Welfare (MOHW105-TD-C-111-004 to T-C Hung and MOHW106-TD-C-111-004).

**Competing interests**

The authors have no conflicts of interest to declare.

**Consent**

Informed consent was obtained from all participating patients.

**Author contributions**

T-C Hung was responsible for study concept and design, data collection, and writing of the manuscript. Y-H Huang, Y-C Lin, and J-Y Lin were responsible for data collection and analysis. J-Y Lin and J-F Lin were responsible for critical revision of the manuscript. All authors read and approved the final manuscript.

**References**


Institute (CLSI) guidelines (M11-A8).\(^\text{15}\) Supplemented \textit{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\(^\circ\)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 \(\geq 32\) and \(\geq 16\) mg/L, respectively, according to CLSI 2012.\(^\text{15}\) Quality control strains included \textit{Bacteroides fragilis} ATCC 25285, \textit{C. difficile} ATCC 700057, \textit{Staphylococcus aureus} ATCC 29213, and \textit{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 \(\geq 8\) and \(\geq 0.5\) mg/L, respectively, according to a previous report\(^\text{11}\) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) version 6.0.\(^\text{16}\)

### Statistical analysis

Statistical analysis was performed using the statistical software, SPSS, version 13.0. The \(\chi^2\)-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 patient-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.

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

<table>
<thead>
<tr>
<th>Toxin genes and RTs</th>
<th>Total (n=842)</th>
<th>Hospital A (n=214)</th>
<th>Hospital B (n=87)</th>
<th>Hospital C (n=147)</th>
<th>Hospital D (n=289)</th>
<th>Hospital E (n=105)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tcdA+/tcdB+</td>
<td>749</td>
<td>204</td>
<td>85</td>
<td>121</td>
<td>265</td>
<td>74</td>
</tr>
<tr>
<td>cdtA+/cdtB+</td>
<td>82 (10.9)</td>
<td>21 (10.3)</td>
<td>5 (5.9)</td>
<td>19 (15.7)</td>
<td>32 (12.1)</td>
<td>5 (6.8)</td>
</tr>
<tr>
<td>tcdC deletion</td>
<td>76 (10.1)</td>
<td>20 (9.8)</td>
<td>5 (5.9)</td>
<td>18 (14.9)</td>
<td>29 (10.9)</td>
<td>4 (5.4)</td>
</tr>
<tr>
<td>RT027 lineage</td>
<td>13 (1.7)</td>
<td>2 (1.0)</td>
<td>1 (1.2)</td>
<td>5 (4.1)</td>
<td>5 (1.9)</td>
<td>0</td>
</tr>
<tr>
<td>RT027</td>
<td>8 (1.1)</td>
<td>1 (0.5)</td>
<td>0</td>
<td>4 (3.3)</td>
<td>3 (1.1)</td>
<td>0</td>
</tr>
<tr>
<td>RT034</td>
<td>4 (0.5)</td>
<td>1 (0.5)</td>
<td>1 (1.2)</td>
<td>1 (0.8)</td>
<td>1 (0.4)</td>
<td>0</td>
</tr>
<tr>
<td>RT075</td>
<td>1 (0.1)</td>
<td>0</td>
<td>0</td>
<td>1 (0.4)</td>
<td>1 (0.4)</td>
<td>0</td>
</tr>
<tr>
<td>RT078 lineage</td>
<td>58 (7.7)</td>
<td>16 (7.8)</td>
<td>4 (4.7)</td>
<td>13 (10.7)</td>
<td>21 (7.9)</td>
<td>4 (5.4)</td>
</tr>
<tr>
<td>RT033</td>
<td>2 (0.3)</td>
<td>2 (1.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RT078</td>
<td>11 (1.5)</td>
<td>1 (0.5)</td>
<td>1 (1.2)</td>
<td>8 (6.6)</td>
<td>1 (0.4)</td>
<td>0</td>
</tr>
<tr>
<td>RT126</td>
<td>23 (3.1)</td>
<td>11 (5.4)</td>
<td>3 (3.5)</td>
<td>2 (1.7)</td>
<td>7 (2.6)</td>
<td>0</td>
</tr>
<tr>
<td>RT127</td>
<td>22 (2.9)</td>
<td>2 (1.0)</td>
<td>0</td>
<td>3 (2.5)</td>
<td>13 (4.9)</td>
<td>4 (5.4)</td>
</tr>
<tr>
<td>tcdA−/tcdB+</td>
<td>93</td>
<td>10</td>
<td>2</td>
<td>26</td>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>RT017</td>
<td>84 (90.3)</td>
<td>8 (80.0)</td>
<td>2 (100)</td>
<td>25 (96.2)</td>
<td>22 (91.7)</td>
<td>27 (87.1)</td>
</tr>
</tbody>
</table>

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-toxigenic 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-toxigenic 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

<table>
<thead>
<tr>
<th>Hospital (number of isolates tested)</th>
<th>Metronidazole MICs (mg/L)</th>
<th>Resistance, %</th>
<th>Vancomycin MICs (mg/L)</th>
<th>Resistance, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>A (310)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (181)</td>
<td>≤0.0625 to &gt;32</td>
<td>0.5</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>C (151)</td>
<td>≤0.0625 to &gt;32</td>
<td>0.5</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>D (308)</td>
<td>≤0.0625 to &gt;32</td>
<td>0.5</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>E (162)</td>
<td>≤0.0625 to &gt;32</td>
<td>0.25</td>
<td>1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

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

Abbreviations: MIC<sub>50</sub>, minimum inhibitory concentration required to inhibit 50% of bacteria; MIC<sub>90</sub>, 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

<table>
<thead>
<tr>
<th>Hospital (number of isolates tested)</th>
<th>Doxycycline MICs (mg/L)</th>
<th>Resistance, %</th>
<th>Tigecycline MICs (mg/L)</th>
<th>Resistance, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>A (310)</td>
<td>≤0.0625 to &gt;32</td>
<td>≤0.0625</td>
<td>4</td>
<td>7.7</td>
</tr>
<tr>
<td>B (181)</td>
<td>≤0.0625 to 32</td>
<td>0.125</td>
<td>8</td>
<td>11.0</td>
</tr>
<tr>
<td>C (151)</td>
<td>≤0.0625 to &gt;32</td>
<td>≤0.0625</td>
<td>4</td>
<td>9.4</td>
</tr>
<tr>
<td>D (308)</td>
<td>≤0.0625 to &gt;32</td>
<td>≤0.0625</td>
<td>4</td>
<td>9.8</td>
</tr>
<tr>
<td>E (162)</td>
<td>≤0.0625 to 32</td>
<td>0.25</td>
<td>16</td>
<td>17.3</td>
</tr>
</tbody>
</table>

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

Abbreviations: MIC<sub>50</sub>, minimum inhibitory concentration required to inhibit 50% of bacteria; MIC<sub>90</sub>, minimum inhibitory concentration required to inhibit 90% of bacteria.

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

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Toxicogenic isolates (n=842)</th>
<th>Non-toxigenic isolates (n=270)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC range, (mg/L)</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>Metronidazole*</td>
<td>≤0.125 to &gt;32</td>
<td>0.5</td>
</tr>
<tr>
<td>Vancomycin*</td>
<td>≤0.0625 to &gt;8</td>
<td>0.5</td>
</tr>
<tr>
<td>Doxycycline**</td>
<td>≤0.0625 to &gt;32</td>
<td>≤0.0625</td>
</tr>
<tr>
<td>Tigecycline***</td>
<td>≤0.0625 to &gt;32</td>
<td>≤0.0625</td>
</tr>
</tbody>
</table>

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

Abbreviations: MIC<sub>50</sub>, minimum inhibitory concentration required to inhibit 50% of bacteria; MIC<sub>90</sub>, minimum inhibitory concentration required to inhibit 90% of bacteria.

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Abbreviations:
- *Resistance is defined as the breakpoints in Clinical and Laboratory Standards Institute Guidelines.*
- MIC breakpoints for resistance to doxycycline and tigecycline are defined as ≥8 and ≥0.5 mg/L, respectively.
- Notes on antimicrobial agents and MIC values are provided as necessary.
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 RT078 lineage isolates (such as RT126 and 127) C. difficile in 3 major cities in Taiwan were RT078 and RT027 lineages. cdtA deletion, cdtB tcdC isolates with, and from 5 hospitals each hospital, the dominant ribotypes among C. difficile pattern, ribotype or antimicrobial susceptibility varied in study hospitals. These data suggest the substantial, although 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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