Molecular characterisation of, and antimicrobial resistance in, *Clostridioides difficile* from Thailand, 2017-2018

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Running title: *Clostridioides difficile* in Thailand

Synopsis

Background: Antimicrobial resistance (AMR) plays an important role in the pathogenesis and spread of *Clostridioides difficile* infection (CDI). Many antimicrobials, such as fluoroquinolones, have been associated with outbreaks of CDI globally.

Objectives: This study aimed to characterise AMR among clinical *C. difficile* strains in Thailand, a country where the use of antimicrobials remains inadequately regulated.

Methods: Stool samples were screened for *tcdB* and positives were cultured. *C. difficile* isolates were characterised by toxin profiling and PCR ribotyping. Antimicrobial susceptibility testing was performed using an agar incorporation method, and whole-genome sequencing and AMR genotyping performed on a subset of strains.

Results: A total of 321 *C. difficile* strains were isolated from 326 stool samples. The most common toxigenic ribotype (RT) was RT 017 (18%), followed by RTs 014 (12%) and 020 (7%). There was a high resistance prevalence (≥ 10%) to clindamycin, erythromycin, moxifloxacin and rifaximin, and resistance prevalence was greatest among RT 017 strains. AMR genotyping revealed a significant correlation between resistance genotype and phenotype for moxifloxacin and rifampicin. The presence of *erm*-class genes was associated with high-level clindamycin and erythromycin resistance. Point substitutions on the penicillin-binding proteins (PBP1 and PBP3) were not sufficient to confer meropenem resistance, however, a Y721S substitution in PBP3 was associated with a slight increase in meropenem MIC. No resistance to metronidazole, vancomycin or fidaxomicin was observed.

Conclusion: There was a large proportion of *C. difficile* RT 017 in Thailand and significant AMR among these strains. The concordance between AMR phenotype and genotype was significant.

Introduction

Clostridioides (*Clostridium*) *difficile* is a major cause of antimicrobial-associated diarrhoea globally (1). *C. difficile* infection (CDI) is mediated by toxins and, so far, there have been three different major toxins identified: toxin A (TcdA), toxin B (TcdB) and binary toxin (*C. difficile* transferase, CDT). The genes encoding TcdA and TcdB are located on the 19.6 kb pathogenicity locus (PaLoc) (2) and the genes for CDT are located in a different part of the chromosome, the CDT locus (3). In non-toxigenic *C. difficile* (NTCD), the PaLoc is replaced by a fixed 115 bp locus (2). The toxin genes (*tcdA*, *tcdB*, *cdtA* and *cdtB*) can be detected by PCR (4, 5), however, some *C. difficile* strains, such as *C. difficile* ribotype (RT) 017, have a deletion at the repeating region of the *tcdA* gene, resulting in a truncated and non-functional toxin A (6). Several methods have been developed to detect this deletion (7, 8).

C. difficile can be separated into different RTs by amplifying the intergenic spacer region between the 16S and 23S rRNA genes (9). This classification method has been used worldwide due to its simplicity and high discriminating power (10). Important *C. difficile* RTs include *C. difficile* RT 027, an A+B+CDT+ strain associated with outbreaks of severe CDI in North America and Europe in the early 2000s (11), *C. difficile* RT 078, another A+B+CDT+ strain associated with zoonotic disease (12), and *C. difficile* RT 017, a *tcdA*-negative (A-B+CDT-) strain associated with global outbreaks since 1995 (6).

Although resistance to antimicrobials used for the treatment of CDI is relatively rare (13), resistance to other commonly used antimicrobials plays an important role in the pathogenesis of CDI and the spread of *C. difficile*. Resistance to clindamycin, new generation fluoroquinolones, rifampicin and tetracycline has been associated with outbreaks of CDI (14), and these antimicrobials are also associated with an increased risk of developing CDI in general (15). Strict regulation of antimicrobials can be a successful measure to control CDI. In the US, such regulation resulted in a significant decrease in CDI cases and CDI-related deaths (16). Fluoroquinolone regulation in Australia is also associated with a relatively low prevalence of fluoroquinolone-resistant organisms (17), including *C. difficile* (18).

In previous studies, the epidemiology of CDI in Thailand has been characterised by a high prevalence of A-B+CDT- and NTCD, and an absence of A+B+CDT+ strains (19-21). *C. difficile* strains isolated in Thailand, especially *C. difficile* RT 017, were resistant to many antimicrobial groups, reflecting the use and misuse of these antimicrobials in the country (22). This study provides an update on the characterisation and antimicrobial susceptibility of *C. difficile* isolated from a tertiary hospital in Bangkok, Thailand.

Materials and Methods

Isolation and characterisation of *C. difficile*

The study included 326 diarrhoeal stools samples collected from patients being treated at Siriraj Hospital, a large teaching hospital in Bangkok, Thailand, during 2017 – 2018. All stool samples were screened for the presence of the *tcdB* gene using the BD Max Cdiff assay (Becton Dickinson, US) as a part of routine investigations at Siriraj Hospital, and stools that were *tcdB* positive were then sent to a reference laboratory in Perth, Western Australia, for further investigation.

At the reference laboratory, stool samples were processed as previously described, including enrichment culture in cooked meat broth supplemented with gentamicin, cefoxitin and cycloserine (23). Characterisation of *C. difficile* was performed by PCR ribotyping and toxin gene profiling. PCR ribotyping was performed using the method described by Stubbs *et al* (9). The banding patterns were compared to a local database consisting of more than 80 internationally recognised RTs, including 15 reference RTs from the European Centre for Disease Prevention and Control. Patterns that did not match strains in the database were given an internal nomenclature. Detection of *tcdA* and *tcdB,* and the binary toxin genes (*cdtA* and *cdtB*), was performed using the methods described by Kato

81 *et al* (7) and Stubbs *et al* (5), respectively. All NTCD isolates in this study were confirmed as such by
82 the absence of the PaLoc using the method described by Braun *et al* (*lok* PCR) (2).

83 All stool samples were screened also for colonisation with multiple *C. difficile* strains. Briefly,
84 DNA extraction was performed on all enrichment broths. DNA was then screened with either *tcdB* (7)
85 or *lok* PCR (2), based on the toxin profile of the first *C. difficile* strain isolated from the specimen (2,
86 7). For example, a specimen previously positive for toxigenic *C. difficile* (TCD) was screened with
87 *lok* PCR for NTCD and vice versa. All screening-positive broths were re-cultured and the second
88 *C. difficile* strain characterised as described above.

89 Antimicrobial susceptibility testing

90 Antimicrobial susceptibility testing (AST) was performed using the agar incorporation method as
91 described by the Clinical and Laboratory Standards Institute (CLSI) against nine antimicrobials listed in
92 **Table 1** (24). *C. difficile* ATCC 700057, *Bacteroides fragilis* ATCC 25285, *Eubacterium lentum* ATCC
93 43055 and *B. thetaiotamicron* ATCC 29741 were included as control strains. Susceptibility results were
94 interpreted using the minimal inhibitory concentration (MIC) breakpoints listed in **Table 1** (24-28).
95 *C. difficile* strains that were resistant to at least three different antimicrobial classes were classified as
96 multidrug-resistant (MDR). Resistance to clindamycin and erythromycin was considered as resistance
97 to a single class (macrolide-lincosamide-streptogramin B; MLS_B).

98 **Table 1** – List of antimicrobials, test ranges and susceptibility breakpoints used in this study.

Antimicrobial	Test range (mg/l)	Breakpoint (mg/l)			Reference
		S	I	R	
Clindamycin	0.008 – 256	≤ 2	4	≥ 8	(24)
Erythromycin	0.06 – 256	-	-	> 8	(25)
Moxifloxacin	0.06 – 64	≤ 2	4	≥ 8	(24)
Amoxicillin/clavulanate	0.015 – 16	≤ 4	8	≥ 16	(24)
Meropenem	0.12 – 16	≤ 4	8	≥ 16	(24)
Rifaximin	0.008 – 64	-	-	> 32	(26)
Metronidazole	0.008 – 4	≤ 2	-	> 2	(27)
Vancomycin	0.03 – 4	≤ 2	-	> 2	(27)
Fidaxomicin	0.002 – 2	-	-	> 1	(28)

99

100 Whole-genome sequencing, high-resolution typing and antimicrobial resistance characterisation

101 A subset of *C. difficile* strains (n = 37) was selected for whole-genome sequencing (WGS) to explore
102 possible antimicrobial resistance (AMR) genotypes. Genomic DNA was extracted, sequenced on an
103 Illumina HiSeq platform which generated 150 bp pair-end reads with a median coverage of 73X and
104 characterised by multi-locus sequence typing (MLST) as previously described (29). Clade assignment
105 of a newly defined sequence type (ST) was confirmed by comparing the average nucleotide identity
106 (ANI) with *C. difficile* strains 630 (clade 1, GenBank accession AM180355) and R20291 (clade 2,
107 GenBank accession FN545816) using FastANI (30). Known accessory AMR genes were identified by
108 interrogating the read files with SRST2 version 0.2.0 against ARGannot database version 3 (31, 32).
109 Draft annotated genomes were interrogated on Artemis version 17.0.1 to look for additional accessory
110 genes (33). The genomes were interrogated also for the presence of known point substitutions
111 associated with resistance to carbapenems (substitution in penicillin-binding proteins PBP1 and PBP3),
112 fluoroquinolones (substitution in GyrA and GyrB subunit of the gyrase enzyme) and rifaximin
113 (substitution in RpoB enzyme) (34, 35). The genotypes were then compared with phenotypic
114 susceptibility data. All sequence data generated in this study have been submitted to the European
115 Nucleotide Archive under BioProject PRJEB40974, sample accessions ERS5247348 – ERS5247384.
116 Details of the genomes are available in the **Supplementary Table S1**.

117 Statistical analysis

118 All statistical analyses were performed using online tools by Social Science Statistics available at
119 https://www.socscistatistics.com/. A p-value ≤ 0.05 was considered statistically significant.

120 Human research ethics approval

121 This study was approved by the Human Research Ethics Committee of The University of Western
122 Australia (reference file RA/4/20/4704) and the Siriraj Institutional Review Board (protocol number
123 061/2558 [EC1]).

124

125 **Results**

126 Characterisation of Thai *C. difficile*

127 A total of 296 *C. difficile* strains were initially isolated from the stool samples. Forty-four of the original
128 326 PCR positive stool samples were negative for TCD by culture; 30 contained no *C. difficile* while
129 from 14 only NTCD was cultured. The enrichment broths for these samples were re-screened for TCD
130 and were all negative by *tcdB* PCR. Another 25 strains were identified from the co-colonisation
131 screening process, yielding a total of 321 *C. difficile* strains. Of these, 221 (68.85%) were positive for
132 *tcdA* and *tcdB* (A+B+CDT-), 58 (18.07%) were positive for *tcdB* only and had a deletion in *tcdA* (A-
133 B+CDT-), three (0.93%) were positive for *tcdA*, *tcdB*, as well as *cdtA* and *cdtB* (A+B+CDT+) and 39 strains
134 (12.15%) were negative for all toxin genes (A-B-CDT-, NTCD).

135 The 321 *C. difficile* strains belonged to 63 different RTs, 19 of which were internationally
136 recognised. The remaining RTs were given internal nomenclature beginning with either "QX" or "KI".
137 The prevalence of the most common RTs is summarised in **Table 2**. The most common TCD strain was
138 *C. difficile* RT 017 (A-B+CDT-), followed by RTs 014 and 020 (both A+B+CDT-). The most common NTCD
139 was *C. difficile* RT 010.

140 Characterisation of a novel binary toxin-positive *C. difficile* isolate

141 One of the *C. difficile* strains isolated in this study was positive for all three toxin genes (A+B+CDT+)
142 and had a unique ribotyping pattern compared to the local reference library. According to the MLST
143 scheme, this isolate was characterised as the novel ST 692 within evolutionary clade 1. However,
144 pairwise ANI analysis showed that this strain was more closely related to *C. difficile* R20291 (clade 2,
145 ANI = 99.17%) than *C. difficile* 630 (clade 1, ANI = 98.89%).

146 Antimicrobial susceptibility of Thai *C. difficile*

147 Overall antimicrobial susceptibility results are shown in **Table 3** and the MIC distribution of selected
148 six antimicrobial classes is displayed in **Figure 1**. Based on the MIC value, clindamycin-resistant
149 *C. difficile* strains could be divided into two groups: those with MIC ≥ 32 mg/l (n = 97) and those with
150 MIC < 32 mg/l (n = 166). There was a strong correlation between high-level clindamycin resistance and
151 erythromycin resistance: 95 strains (97.94%) that had clindamycin MIC ≥ 32 mg/l were also resistant
152 to erythromycin while only 16 strains (9.64%) in the other group were resistant to erythromycin
153 (Cohen's kappa = 0.857). There was also a clear separation in MIC value between strains with and
154 without rifaximin resistance. The separation was less clear for moxifloxacin and was not observed in
155 amoxicillin/clavulanate and meropenem.

156 **Table 2** – Ribotypes of 321 *C. difficile* strains from Thailand, by toxin profile.

Toxin Profile	Ribotype	Number	% of toxigenic strains	% of all
A+B+CDT-	**Total**	**221**	**78%**	**69%**
	014	40	14%	12%
	020	24	9%	7%
	046	18	6%	6%
	QX517	17	6%	5%
	297	13	5%	4%
	QX026	11	4%	3%
	043	10	4%	3%
	Others	88	31%	27%
A-B+CDT-	**Total**	**58**	**21%**	**18%**
	017	58	21%	18%
A+B+CDT+	**Total**	**3**	**1%**	**1%**
	078	1	<1%	<1%
	QX273	1	<1%	<1%
	KI008	1	<1%	<1%
A-B-CDT-	**Total**	**39**	-	**12%**
	010	7	-	2%
	QX002	5	-	2%
	009	4	-	1%
	QX011	4	-	1%
	Others	19	-	6%

157 Note: RTs designated with "QX" and "KI" were RTs that did not match the internationally recognised
158 RTs in the database and were given internal nomenclature.

159

160 **Table 3** – Antimicrobial susceptibility of 321 *C. difficile* strains from Thailand.

Antimicrobial	MIC range (mg/l)	MIC_{50} (mg/l)	MIC_{90} (mg/l)	Susceptibility (%)		
				S	I	R
Clindamycin	0.5 – >256	8	>256	26 (8%)	32 (10%)	263 (82%)
Erythromycin	0.5 – >256	2	>256	-	-	109 (34%)
Moxifloxacin	0.5 – >64	2	32	242 (75%)	3 (1%)	76 (24%)
Amoxicillin/clavulanate	0.25 – 8	0.5	1	320 (>99%)	1 (<1%)	0
Meropenem	1 – 16	4	8	280 (87%)	39 (12%)	2 (1%)
Rifaximin	≤0.008 – >64	0.03	2	-	-	31 (10%)
Metronidazole	0.06 – 1	0.25	0.25	321 (100%)	-	0
Vancomycin	0.5 – 2	1	2	321 (100%)	-	0
Fidaxomicin	0.03 – 0.25	0.125	0.25	-	-	0

161

When classified by toxin gene profiles, resistance to clindamycin, erythromycin, moxifloxacin and rifaximin were more prevalent among A-B+CDT- *C. difficile*, all of which belonged to RT 017, than A+B+CDT- and NTCD (**Figure 1**). A total of 29 (9.03%) *C. difficile* isolates were MDR, 26 (8.10%) of which were *C. difficile* RT 017 (A-B+CDT-). The remaining isolates were NTCD (n=2) and A+B+CDT- *C. difficile* (n=1). All MDR isolates were resistant to the MLS_B group (both clindamycin and erythromycin), moxifloxacin and rifaximin. One MDR isolate was also resistant to meropenem (A-B+CDI-, RT 017, MIC = 16 mg/l).

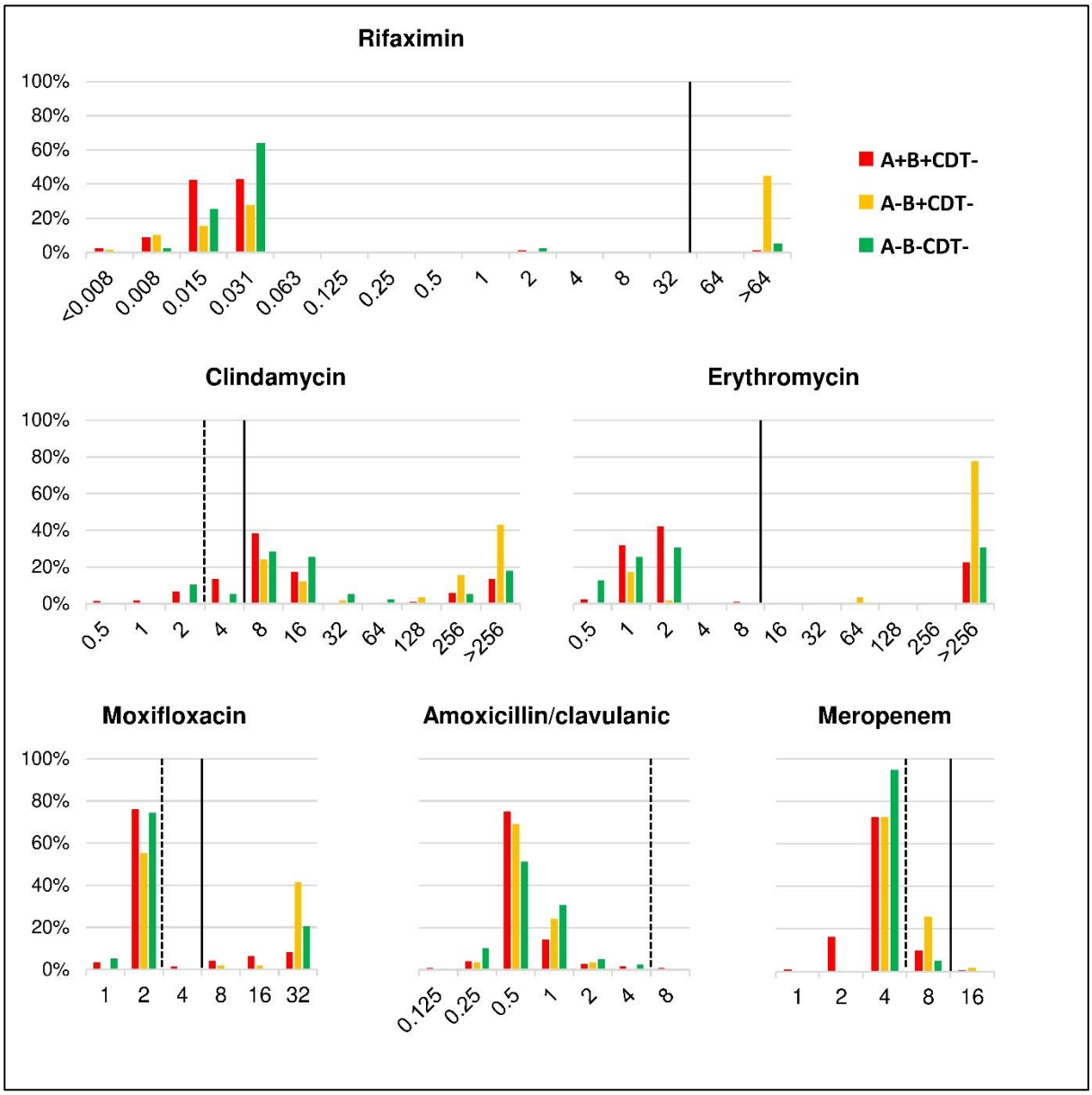

Figure 1 – MIC distribution for six antimicrobials against 321 strains of *C. difficile* in Thailand. *C. difficile* strains were classified according to their toxin gene profiles: A+B+CDT-, red; A-B+CDT-, yellow; A-B-CDT-, green. The number of A+B+CDT+ *C. difficile* strains was low (n = 3) and these were excluded. Breakpoints for intermediate resistance (I) and resistance (R) are shown by broken and solid lines, respectively.

176 Genotypic resistance determinants in Thai *C. difficile*

177 A summary of MIC values and genotypic resistance determinants of 37 sequenced *C. difficile* strains is
178 available in **Supplementary Table S2**. Of these strains, 31 had high-level resistance to clindamycin: 23
179 strains carried an *erm*(B) gene, five carried an *erm*(G) gene and three carried a gene encoding an rRNA
180 adenine N(6)-methyltransferase family protein [GenBank accession WP_002571018.1], the closest
181 match to which in the CARD database was the Erm(42) protein [39% identity, E-value = 1.19E-53] (36).
182 Of the 23 *erm*(B)-positive strains, 19 carried the *erm*(B) gene on transposon Tn*6194* (82.61%), while
183 the other four (17.39%) carried the gene on Tn*6189*. No *erm*-class genes were identified among strains
184 with low-level clindamycin resistance. The concordance between the presence of *erm*-class gene and
185 high-level clindamycin resistance was 91.89%. The concordance increased to 100% when the
186 uncharacterised *erm*-class gene was included. A gene encoding a macrolide efflux protein [accession
187 WP_009271522.1] was identified in two strains with high-level erythromycin resistance (MIC > 256
188 mg/l). No significant genotypic resistance determinants were identified in strains with low-level
189 clindamycin resistance.

190 A T82I substitution in GyrA was found in 23 strains and a D426V substitution in GyrB was found
191 in one strain with a moxifloxacin MIC of 16 – 32 mg/l. No known point substitutions were found in the
192 remaining 14 strains, 13 of which were moxifloxacin-susceptible; one had low-level moxifloxacin
193 resistance (MIC 8 mg/l) [97.37% concordance]. H502N and R505K substitutions in RpoB were found in
194 all 23 rifaximin-resistant strains and none of the susceptible strains [100% concordance].

195 Twelve strains had an A555T substitution in penicillin-binding protein 1 (PBP1) and another
196 seven had a Y721S substitution in PBP3. A multiple linear regression analysis was performed to assess
197 the association between the presence of these substitutions and the MIC values for meropenem. The
198 Y721S substitution on the PBP3 was associated with an increase in meropenem MIC (adjusted R^2 =
199 0.516, t = 5.521, p < 0.0001), while the A555T substitution on the PBP1 was not associated with the
200 change in meropenem MIC (t = -1.127, p = 0.268).

201

202 **Discussion**

203 This study provides an update on the molecular epidemiology and antimicrobial susceptibility of
204 *C. difficile* strains circulating in Thailand. It also explores the genomic basis of important AMR in these
205 strains. As the focus was on patients suspected of having CDI and not on the prevalence of *C. difficile*
206 in the general population, the stool samples included in this study were first screened for the *tcdB*
207 gene by PCR before culture. Thus the overall prevalence of TCD was higher than previous studies in
208 Thailand (19-21), however, the common strains found were similar. The majority of A+B+CDT- strains
209 belonged to *C. difficile* RT 014/020 group, all A-B+CDT- strains belonged to *C. difficile* RT 017 and most
210 NTCD belonged to *C. difficile* RTs 009, 010 and 039. Three binary toxin-positive isolates were found in
211 this study, one of which was *C. difficile* RT 078. The epidemic *C. difficile* RT 027 remained absent in
212 Thailand despite its successful spread in other regions (37).

213 Why *C. difficile* RT 027 has failed to spread and to establish in Thailand, and Southeast Asia in
214 general, remains unknown. One possible reason is that the successful spread of this RT was mainly
215 due to its resistance to fluoroquinolones which provided an advantage over other less resistant RTs
216 (38). Although there is high consumption of fluoroquinolones, such as levofloxacin, in the country (39),
217 Thailand already harbours *C. difficile* RT 017, another epidemic RT with a high prevalence of resistance
218 to fluoroquinolones, as well as other antimicrobials (14). Thus, it may have been difficult for *C. difficile*
219 RT 027 to compete with this local RT compared to other regions.

220 Though *C. difficile* RT 027 was not identified in this study, a possible relative of this
221 hypervirulent strain was identified (ST 692). The MLST result for this strain was unusual, as it was
222 classified into clade 1 despite containing binary toxin genes in a complete CDT locus. The presence of

223 binary toxin genes is a feature common in *C. difficile* clades 2 and 5 but rare in clade 1 (40). Thus, an
224 ANI analysis was performed, which indicated that this newly characterised strain was more related to
225 clade 2 than clade 1 *C. difficile*, as expected from the toxin gene profile. Clades 1 and 2 *C. difficile* are
226 closely related and share a large proportion of housekeeping gene alleles used in the MLST scheme.
227 As a result, it may be difficult to properly discriminate these two clades by this method. The use of ANI
228 analysis, which involves the whole genome rather than a specific set of housekeeping genes, can help
229 in the correct classification of some borderline strains as shown in a previous study (40). According to
230 the ANI analysis, it is likely that this newly described *C. difficile* ST 692 belongs to clade 2 and is related
231 to *C. difficile* RT 027.

232 A discordance between culture results and the result of a conventional real-time *tcdB* PCR was
233 observed in 44 stool samples. The false-positive rate of the real-time PCR method (13.50%) was
234 comparable to the previous report comparing *tcdB* PCR with a similar enrichment culture method but
235 without the colonisation screening step (23). This suggests that the additional screening step does not
236 significantly increase the yield of the culture method, although it may help identify stool samples with
237 multiple *C. difficile* strains. This false-positive rate also highlights the importance of patient clinical
238 data or additional tests to improve the accuracy of CDI diagnosis. In the latest guidelines for the
239 treatment and diagnosis of CDI, *tcdB* PCR is no longer recommended as a stand-alone test unless the
240 patient has symptoms suggestive of CDI (41).

241 AMR in *C. difficile* affects both the pathogenesis and treatment of CDI. To cause the disease,
242 *C. difficile* must tolerate the presence of antimicrobials in the intestinal lumen while the microbiota
243 perishes (14). Many successful *C. difficile* lineages have indeed been characterised with increased
244 resistance to at least one major drug group (14). In this study, *C. difficile* RT 017, which was the most
245 common RT, had greater resistance to MLS_B (both clindamycin and erythromycin), moxifloxacin and
246 rifaximin than other RTs. It was also the most common MDR *C. difficile* strain in this study. *C. difficile*
247 RT 017 has been reported also to be the most prevalent RT with significant resistance to many
248 antimicrobials in other parts of Thailand (21). This particular RT has been associated with resistance
249 to at least six antimicrobial groups (14), which may have accounted for its successful global spread (6).
250 As regulation of antimicrobial use has reduced the impact of *C. difficile* in many countries (16, 18), a
251 similar approach should effective for the control CDI in Thailand.

252 All *erm*(B)-positive *C. difficile* strains carried the gene on two well-characterised *erm*(B)-
253 positive transposons: Tn*6189* and Tn*6194*, the latter being found also in *C. difficile* M68, a *C. difficile*
254 RT 017 strain widely used as a reference in genomic studies (14). Tn*6194*, the most prevalent
255 transposon in this study, is capable of inter-species transfer, most notably between *C. difficile* and
256 *Enterococcus faecalis* (42). This emphasises another aspect of AMR in *C. difficile*; its possible role as a
257 reservoir of AMR genes for other pathogenic bacteria residing in the colon.

258 Previously, it has been reported that concordance between the presence of the *erm*(B) gene
259 and the MLS_B resistance phenotype was low (43). This is likely due to the presence of multiple
260 resistance mechanisms. In a previous study, however, the *erm*(B) gene was found in all *C. difficile*
261 isolates with high-level resistance to both clindamycin and erythromycin, hinting that the gene may
262 be associated with high-level MLS_B resistance (44). We also observed separation between *C. difficile*
263 strains with high-level and low-level clindamycin and erythromycin resistance (**Figure 1**). Upon
264 genomic analysis of a subset of strains, we found a correlation between the presence of an *erm*-class
265 gene (*erm*(B), *erm*(G) and another possible *erm*-class gene) with high-level resistance to clindamycin.
266 Many of these strains also had high-level erythromycin resistance, supporting the previous study (44).
267 A gene encoding a macrolide efflux protein was identified also in strains with high-level erythromycin
268 resistance and not in erythromycin-susceptible strains, suggesting an association between the
269 presence of this efflux protein and phenotypic erythromycin resistance, although the function of this
270 gene was not characterised. Resistance determinants were not identified among strains with low-level
271 clindamycin resistance, however, this underestimation is likely irrelevant, as the median clindamycin

MIC in this population (8 mg/l) remained significantly lower than the clindamycin level in stools (approximately 240 mg/g of stool) (45). Besides MLS_B, a separation between strains resistant and susceptible to rifaximin and fluoroquinolones was observed (**Figure 1**). The concordance between resistant phenotype and known genotype was also high, similar to a previous study (43).

Compared to the study at the same hospital in 2015, there was no difference in the overall resistance prevalence (25), however, there was a slight increase in meropenem MICs and the emergence of carbapenem resistance. In a previous study, high-level imipenem resistance was associated with point substitutions on PBP1 and PBP3 (35). It appears that these substitutions only confer high-level resistance to imipenem and not to meropenem, although the linear regression analysis suggests that the Y721S substitution on PBP3 may have contributed to a slight increase in meropenem MIC in this study.

C. difficile remained susceptible to metronidazole, vancomycin and fidaxomicin, similar to the other parts of the world (46). Thus, these antimicrobials should remain an effective treatment for CDI. There was a slight increase in vancomycin MIC reaching the clinical breakpoint, which is consistent with a previous study (25), however, this should have little impact on the treatment of CDI given that the faecal vancomycin concentration remains far greater than the MIC (>2,000 mg/l vs 2 mg/l) (47). The increase in vancomycin MIC in this study is in contrast to other hospitals in Thailand and this could reflect the usage of vancomycin at the study site (21). The use of vancomycin can lead to the emergence of vancomycin-resistant *Enterococcus* spp., which can have a devastating effect on patients (48, 49). Therefore, the use of vancomycin should be carefully monitored.

Conclusion

A-B+CDT- and NTCD were prevalent in Thailand. Few binary toxin-positive strains (A+B+CDT+) were identified; one belonged to a known epidemic lineage and another was a novel strain related to *C. difficile* RT 027. The most common RT in this study was *C. difficile* RT 017 (A-B+CDT-), a large proportion of which was resistant to MLS_B, moxifloxacin and rifaximin. Many strains were also MDR. Such resistance may have played a role in the success of *C. difficile* RT 017 in Thailand. There was a strong concordance between the presence of *erm*-class genes and high-level clindamycin resistance, as well as significant concordance between point substitutions on gyrase subunits and RpoB protein with fluoroquinolone and rifaximin resistance, respectively. Resistance to antimicrobials for treatment of CDI was not detected.

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Transparency declaration

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