Antimicrobial susceptibility of *Clostridium difficile* isolated in Thailand

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Introduction

Antimicrobial exposure is the major risk factor for *Clostridium difficile* infection (CDI)[1]. In Thailand, many antimicrobials are traded as over-the-counter drugs. When coupled with a general lack of knowledge regarding the appropriate use of antimicrobials in the community, misuse is inevitable[2]. Paradoxically, treatment of CDI with antimicrobials remains the preferred option. Current knowledge regarding the antimicrobial susceptibility of Thai *C. difficile* isolates is limited to two studies published in the 1990s and one published in 2015.

Study Objective

In view of this lack of contemporary data and continual antimicrobial misuse[3], we investigated the antimicrobial susceptibility of recently isolated strains of *C. difficile* in Thailand.

Materials and methods

Collection, isolation and characterisation of *C. difficile*

The collection of 105 *C. difficile* isolates included in the study was sourced from a study of CDI in Thailand (Putsathit P, unpublished data) undertaken at Siriraj Hospital in Bangkok from April to June 2015. Toxigenic culture was performed on all faecal specimens as previously described[4]. A total of 38 different PCR ribotypes (RTs) were identified; 55.2% (58/105) were assigned to internationally recognised RTs while others were designated with an internal nomenclature, prefixed with QX. The top five RTs were 014/020 group (16.2%), 010 (11.4%), 017 (11.4%), 039 (8.6%) and 009 (5.7%). The majority of the isolates were non-toxigenic (62.9%; 66/105), while 25.7% (27/105) were toxin A and B genes positive (A+B+) and 11.4% (12/105) were A−B+. All A−B+ isolates belong to RT 017. None of the isolates carried binary toxin genes.

Minimum inhibitory concentration (MIC) determination using agar incorporation

MICs of metronidazole, vancomycin, amoxicillin/clavulanate, meropenem, fidaxomicin, rifaximin, clindamycin, erythromycin, ceftriaxone and moxifloxacin were determined by the agar incorporation method described by the Clinical and Laboratory Standards Institute (CLSI)[5]. The breakpoints for metronidazole and vancomycin were those recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) based on epidemiological cut-off values (ECOFFs) which distinguish wild-type isolates from those with reduced susceptibility[6]. The ECOFF value for fidaxomicin was recommended by the European Medicines Agency[7]. Currently, there are no published breakpoints for rifaximin. The clinical breakpoints used for other agents were those provided by CLSI[8].

Results and discussion

- All isolates were susceptible to metronidazole, vancomycin, amoxicillin/clavulanate, meropenem and fidaxomicin (Table 1).
- Fidaxomicin has recently been recommended as an alternative treatment for relapse CDI in adults[9]. Here, the *in vitro* activity of fidaxomicin was superior to vancomycin and metronidazole (Table 1).
- Rifaximin exhibited relatively large MIC range (0.008->16 mg/L) and was potent (MICs ≤0.03 mg/L) against 85.7% of the isolates. The MIC_{50} of rifaximin (0.015 mg/L) was the lowest of all antimicrobials tested (Table 1).
- RT 027 previously reported to have high rifaximin MICs was found to harbour a mutation in the *rpoB* gene[10]. Here, rifaximin MIC_{50}/MIC_{90} values for RT 017 A−B+ isolates (>16/>16) were higher than those of non-toxigenic and A+B+ isolates (0.015/0.03 in both groups) (Table 2), suggesting a possible alteration in *rpoB* and warrants further investigation.
- In Asia, RT 017 is highly prevalent and is known to harbour the *ermB* gene[11]. This observation supports the high level of resistance to clindamycin (73.3%) and erythromycin (35.2%) observed among Thai isolates (Table 1), particularly in RT 017 (66.7% and 83.3%, respectively) (Table 2).
- Compared to non-toxigenic and A+B+ isolates, RT 017 A−B+ isolates exhibited a higher level of resistance to erythromycin (40.9% and 0.0% vs. 83.3%, respectively) (Table 2).
- Resistance to ceftriaxone was observed in 23.8% of the isolates. A typical clustering around the breakpoint was observed (Table 1).
- Fluoroquinolones was reported as the top agents prescribed inappropriately to treat acute diarrhoea in adults in a 2011 study conducted in a large tertiary hospital in Bangkok[3]. It is therefore not surprising that resistance to moxifloxacin was observed in 21.0% of Thai isolates given the rapid rate that resistance to quinolones develops following exposure[12] (Table 1).
- Resistance to cephalosporin and fluoroquinolone was particularly pronounced among RT 017 A−B+ isolates (83.3% for both antibiotics), compared to non-toxigenic (19.7% and 18.2%, respectively) and A+B+ (7.4% and 0.0%, respectively) isolates (Table 2).
- The prevalence of multi-drug resistance, as defined by resistance to ≥3 antimicrobials tested, was 21.9% (23/105). Multi-resistance to clindamycin, erythromycin, ceftriaxone and moxifloxacin was observed only among RT 017 (*n*=7) and 039 (*n*=4) strains.

Table 1 Summary MIC data for 10 antimicrobials against 105 Thai *C. difficile* isolates.

Antibiotics	MIC Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Clinical Breakpoints			Susceptible		Intermediate		Resistant	
				S	I	R	N	%	N	%	N	%
MTZ	0.015-0.5	0.25	0.25	≤2	-	>2	105	100.0	0	0.0	0	0.0
VAN	0.06-2	1	2	≤2	-	>2	105	100.0	0	0.0	0	0.0
AMC	0.03-2	0.5	1	≤4	8	≥16	105	100.0	0	0.0	0	0.0
MEM	0.25-4	2	4	≤4	8	≥16	105	100.0	0	0.0	0	0.0
FDX	0.004-0.25	0.06	0.25	-	-	>1	105	100.0	0	0.0	0	0.0
RFX	0.008->16	0.015	>16	-	-	-	-	-	-	-	-	-
CLI	0.015->32	8	>32	≤2	4	≥8	17	16.2	11	10.5	77	73.3
ERY	0.12->256	2	>256	-	-	>8	-	-	-	-	37	35.2
CRO	8->128	35	128	≤16	32	≥64	16	15.2	64	61.0	25	23.8
MXF	0.12-32	2	16	≤2	4	≥8	82	78.1	1	1.0	22	21.0

MTZ, metronidazole; VAN, vancomycin; AMC, amoxicillin/clavulanate; MEM, meropenem; FDX, fidaxomicin; RFX, rifaximin; CLI, clindamycin; ERY, erythromycin; CRO, ceftriaxone; MXF, moxifloxacin.

Table 2 Summary MIC data for 5 antimicrobials and their susceptibility data against Thai *C. difficile* isolates by toxin gene profile.

Antibiotics	A−B− isolates (*n*=66)		A+B+ isolates (*n*=27)		RT017 A−B+ isolates (*n*=12)	
	MIC_{50}/MIC_{90}	NR (%)	MIC_{50}/MIC_{90}	NR (%)	MIC_{50}/MIC_{90}	NR (%)
RFX	0.015/0.03	-	0.015/0.03	-	>16/>16	-
CLI	8/>32	51 (77.3)	8/16	18 (66.7)	>32/>32	8 (66.7)
ERY	2/>256	27 (40.9)	2/2	0 (0.0)	>256/>256	10 (83.3)
CRO	32/64	13 (19.7)	32/32	2 (7.4)	128/>128	10 (83.3)
MXF	2/16	12 (18.2)	2/2	0 (0.0)	16/32	10 (83.3)

RFX, rifaximin; CLI, clindamycin; ERY, erythromycin; CRO, ceftriaxone; MXF, moxifloxacin; NR, number of resistant isolates.

Conclusions

- *C. difficile* in Thailand is characterised by a high level of multi-drug resistance. This includes resistance to fluoroquinolones which are frequently used to treat acute diarrhoeal disease.
- This finding emphasises the need for antimicrobial stewardship. Education plays a pivotal role in creating behavioural changes and healthcare professionals should be encouraged to educate their patients.
- Although toxigenic culture is not popular as a standalone diagnostic test, stool culturing should still be performed to enable surveillance of the ever-changing epidemiology of CDI and in particular the development of antimicrobial resistance.

Acknowledgements

We thank staffs in the Microbiology Departments at Siriraj Hospital, Bangkok for their assistance with sample collection. We are grateful to our colleagues at PathWest Media (Mt Claremont, Western Australia, Australia) for preparation of the testing media. The authors declare no conflict of interest.

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