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Original Article

High *in vitro* activity of fidaxomicin against *Clostridium difficile* isolates from a university teaching hospital in China

Jing-Wei Cheng [a,b], Qi-Wen Yang [a,*], Meng Xiao [a], Shu-Ying Yu [a,b], Meng-Lan Zhou [a], Timothy Kudinha [c,d], Fanrong Kong [c], Jian-Wei Liao [e], Ying-Chun Xu [a,]**

[a] *Department of Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China*
[b] *Graduate School, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China*
[c] *The Charles Sturt University, Leeds Parade, Orange, New South Wales, Australia*
[d] *Centre for Infectious Diseases and Microbiology Laboratory Services, Westmead Hospital, Westmead, New South Wales, Australia*
[e] *ZheJiang Hisun Pharmaceutical Co. ltd, Taizhou, Zhejiang, China*

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Abstract *Background: Clostridium difficile* infection (CDI) is a significant cause of morbidity and mortality in both the acute care setting and the wider healthcare system. The purpose of this study was to evaluate the *in vitro* activity of fidaxomicin against *C. difficile* isolates from a university teaching hospital in China.
Methods: One hundred and one *C. difficile* isolates were collected and analyzed for toxin genes by multiplex PCR. The toxin gene positive strains were also typed by multilocus sequence typing (MLST) and PCR-ribotyping. The MICs of the isolates were determined against fidaxomicin, metronidazole, vancomycin, tigecycline and moxifloxacin, by the agar dilution method.
Results: All the 101 isolates exhibited low MICs to fidaxomicin (0.032–1 mg/L), metronidazole (0.125–1 mg/L), vancomycin (0.25–2 mg/L) and tigecycline (0.016–0.5 mg/L). Tigecycline showed the lowest geometric mean MIC value (0.041 mg/L), followed by fidaxomicin (0.227 mg/L), metronidazole (0.345 mg/L), and vancomycin (0.579 mg/L). About 35% of the strains (n = 35) were resistant to moxifloxacin, and the resistance rate to moxifloxacin for A–B+CDT– isolates (85.0%) was much higher than that of A+B+CDT– (15.7%) and A–B–CDT– (29.2%) isolates ($P < 0.001$). The MIC values of fidaxomicin, metronidazole, vancomycin and moxifloxacin against the 3 ST1 isolates were higher than for other STs. All the 28 moxifloxacin-resistant toxigenic isolates carried a mutation either in *gyrA* or/and *gyrB*.

* Corresponding author. Fax: +86 10 6915 9742.
** Corresponding author. Fax: +86 10 6915 9742.
E-mail addresses: yangqiwen81@163.com (Q.-W. Yang), xycpumch@139.com (Y.-C. Xu).

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Conclusion: Fidaxomicin exhibited high antimicrobial activity against all *C. difficile* isolates tested, which shows promise as a new drug for treating Chinese CDI patients.

Introduction

Clostridium difficile infection (CDI) is a significant cause of morbidity and mortality in both the acute care setting and the wider healthcare system.[1,2] The global increase in the incidence of CDI is partly driven by the emergence of hypervirulent *C. difficile* strain BI/NAP1/027 (restriction endonuclease analysis group BI, North American pulse-field type 1, PCR ribotype 027), placing a huge financial burden on health care systems worldwide.[3] In Asian countries, toxin A-negative toxin B positive (A−B+) strains are one of the major clones spreading in hospitals.[4] Furthermore, these strains show higher drug resistance rates compared to toxin A positive-toxin B positive (A+B+) strains for the antibiotics clindamycin, erythromycin, levofloxacin, rifampicin, rifaximin and tetracycline.[5,6]

In China, CDI may be an underestimated problem due to limited laboratory capacity and low awareness.[7–9] Metronidazole and vancomycin are the mainstay options for CDI treatment. However, until recently, metronidazole was the only oral antibiotic approved for the treatment of CDI in China. And up to date, there is no oral formulation of vancomycin for CDI treatment. Clinicians in China often empirically treat severe CDI patients orally using a mixture of vancomycin injection with saline or glucose solution, leading to an uncertain therapeutic effect. In addition, both metronidazole and vancomycin are associated with treatment failure in about 3%–18% of patients,[10] and disease recurrence in about 30%.[11,12] Therefore, developing and investigating new antibiotics for CDI treatment is of great importance.

Fidaxomicin is a new macrocyclic bactericidal antibiotic against *C. difficile* with a narrow spectrum activity against gram-positive anaerobes, leading to less disruption of the normal colonic anaerobic microflora.[13] This drug has recently been approved by the U.S. Federal Drug Administration and the European Medicines Agency for the treatment of CDI,[14] and may be an appropriate therapy for patients with severe or recurrent CDI.[15] However, little is known about the *in vitro* activity of fidaxomicin against *C. difficile* isolates from China. The aim of the present study was to investigate the *in vitro* activity of fidaxomicin and four other antimicrobial agents against recent clinical *C. difficile* isolates collected in Beijing, China.

Materials and methods

Bacterial isolates

A total of 101 non-duplicate *C. difficile* isolates, recovered from Peking Union Medical College Hospital (PUMCH) between August 2012 and July 2015, were included in the study. The isolates were consecutively collected from fecal specimens routinely sent to the clinical laboratory for *C. difficile* culture. Fresh fecal specimens were taken from patients aged ≥18 years with CDI symptoms, and for patients with multiple isolates, only the first isolate was included.

Toxin gene detection and multilocus sequence typing (MLST) analysis

DNA extraction and subsequent toxin gene detection was carried out as previously described.[16] Capillary sequencer-based PCR ribotyping was performed as described by Indra et al.[17] and ribotypes were assigned by querying the results against WEBRIBO database (https://webribo.ages.at/). Other unknown ribotypes were serially named by using a letter designation. MLST was performed by sequencing seven house-keeping gene loci (*adk, atpA, dxr, glyA, recA, sodA* and *tpi*) as previously described by Griffiths et al.,[18] and sequence types (STs) of *C. difficile* isolates were determined by querying on the official website (http://pubmlst.org/cdifficile/). Capillary sequencer-based PCR ribotyping and MLST were only performed on the toxigenic isolates.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (document M11-A8).[19] The following five antimicrobial agents were used: fidaxomicin, metronidazole, vancomycin, tigecycline and moxifloxacin. Fidaxomicin was obtained from ZheJiang Hisun Pharmaceutical CO.LTD (Taizhou, Zhejiang Province, China), and the remaining antimicrobial agents were obtained from National Institute for Food and Drug Control (Beijing, China). The resistance breakpoints for fidaxomicin have not been established, whilst the breakpoints for the remaining drugs were based on CLSI M100-S26 (metronidazole, ≥32 mg/L; moxifloxacin, ≥8 mg/L), EUCAST clinical breakpoints (vancomycin, >2 mg/L) or FDA document (tigecycline, ≥16 mg/L).[20,21] *Bacteroides fragilis* ATCC 25285 and *C. difficile* ATCC 700057 were used for quality control.

Resistance gene detection

The gyrase subunits *gyrA* and *gyrB*, responsible for fluoroquinolone resistance, were amplified and sequenced as previously reported by Huang et al.[22] The fluoroquinolone

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resistance gene was only detected in toxin gene positive and moxifloxacin resistant isolates.

Statistical analyses

Statistical analyses were performed using SPSS software (version 17.0, IBM, New York, USA). The chi-square test was applied to compare categorical variables. The level of statistical significance was defined as $P < 0.05$.

Results

Toxin gene detection

Of the 101 strains studied, 51 (50.5%) were *tcdA*-positive, *tcdB*-positive and *cdtA*/*cdtB*-negative (A+B+CDT−), 20 (19.8%) were *tcdA*-negative, *tcdB*-positive and *cdtA*/*cdtB*-negative (A−B+CDT−), 6 (5.9%) isolates were *tcdA*-positive, *tcdB*-positive and *cdtA*/*cdtB*-positive (A+B+CDT+), while the remaining 24 (23.8%) isolates were *tcdA*-negative, *tcdB*-negative and *cdtA*/*cdtB*-negative (A−B−CDT−).

MLST and PCR-ribotype

MLST was performed on the 77 toxigenic *C. difficile* isolates. Fifteen STs and 18 ribotypes (RTs) were identified, and ST 54/RT 012 was the most common (n = 13, 16.9%), followed by ST 37/RT 017 (n = 11, 14.3%), ST 3/RT 001 and ST 81/RT A (n = 9, 11.7% each). The relationship among the STs, PCR-ribotypes and toxin genes of the 77 toxigenic *C. difficile* isolates was shown in Table 1.

Table 1 Distribution of the STs, PCR-ribotypes and toxin genes of the 77 toxigenic *C. difficile* isolates.

MLST	PCR-ribotype	Toxin gene	Isolate number	%
54	012	A+B+CDT−	13	16.9
37	017	A−B+CDT−	11	14.2
3	001	A+B+CDT−	9	11.7
81	A	A−B+CDT−	9	11.7
2	014/020	A+B+CDT−	8	10.4
35	046	A+B+CDT−	6	7.8
8	002	A+B+CDT−	3	3.9
	B		1	1.3
	C		1	1.3
42	106	A+B+CDT−	4	5.2
1	027	A+B+CDT+	2	2.6
	D		1	1.3
5	063	A+B+CDT+	3	3.9
55	070	A+B+CDT−	2	2.6
91	E	A+B+CDT−	1	1.3
129	F	A+B+CDT−	1	1.3
286	G	A+B+CDT−	1	1.3
289	H	A+B+CDT−	1	1.3
Total			77	100

Antimicrobial susceptibility

All the 101 strains were sensitive to metronidazole, vancomycin and tigecycline, with MIC_{90} values of 0.5, 1 and 0.064 mg/L, respectively (Table 2). About 35% of the strains (n = 35) were resistant to moxifloxacin. Tigecycline showed the lowest geometric mean (GM) MIC value (0.041 mg/L), followed by fidaxomicin (0.227 mg/L), metronidazole (0.345 mg/L), vancomycin (0.579 mg/L), and moxifloxacin (3.22 mg/L). The rate of moxifloxacin resistance was much higher among A−B+CDT− isolates (85.0%) than among A+B+CDT− (15.7%) and A−B−CDT− (29.2%) isolates ($P < 0.001$). However, the GM MIC value of fidaxomicin against A−B+CDT− isolates (0.171 mg/L) was lower than that of A+B+CDT− (0.241 mg/L) and A−B−CDT− (0.258 mg/L) isolates (Table 2). Among the six A+B+CDT+ isolates, the MIC values for fidaxomicin, metronidazole, vancomycin and moxifloxacin against the 3 ST 1 isolates, were higher than that of the 3 ST 5 isolates (Table 3).

Genes and mutations conferring moxifloxacin resistance

In order to investigate the mechanism of moxifloxacin resistance, *gyrA* and *gyrB* genes were amplified and sequenced on the 28 toxin gene positive and moxifloxacin resistant isolates. All the isolates carried a mutation either in *gyrA* or/and *gyrB* (Table 4). Twelve isolates exhibited mutations in *gyrA* only, 2 in *gyrB* only, and the rest in both *gyrA* and *gyrB*. Overall, only one type of amino acid substitution in *gyrA* (Thr82→Ile) and three different types of substitutions in *gyrB* (Ser366→Ala, Asp426→Asn and Asp426→Val), were identified. Mutations in *gyrA* (Thr82→Ile) and *gyrB* (Ser366→Ala, Asp426→Val) were identified in 5 of the 7 ST 81/RT A isolates, while *gyrA* (Thr82→Ile) and *gyrB* (Ser366→Ala) were identified in 9 of the 10 ST 37/RT 017 isolates.

Discussion

While the developed world has seen a significant increase in the number of scientific articles on CDI, the developing world including China, still lags behind on this subject. This may be attributed to low awareness of the CDI problem and limited laboratory capacity and surveillance in developing countries.[7–9] Recently, our group proposed a practical workflow for CDI diagnosis, compared different molecular typing methods, and described the first two ribotype 027 isolates in Beijing.[5,9,16] In this study, we performed antimicrobial susceptibility tests on *C. difficile* isolates from one hospital in China, against fidaxomicin and four other antimicrobial agents, which may provide some foundations for clinical treatment of CDI.

According to the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) guidelines,[23] metronidazole is the drug of choice for the initial episode of mild-to-moderate CDI, while vancomycin is the drug of choice for an initial episode of severe CDI. In this study, all the isolates were sensitive to metronidazole and vancomycin. However, recurrence occurs in approximately 30% of cases treated with metronidazole or vancomycin,[11,12] and patients with at least one

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Table 2 MICs of fidaxomicin, metronidazole, vancomycin, tigecycline and moxifloxacin for 101 *C. difficile* isolates.

Toxin type (n)	Antimicrobial agent	MIC (mg/L)				R %
		Range	MIC_{50}	MIC_{90}	GM	
Overall total (101)	Fidaxomicin	0.032–1	0.25	0.5	0.227	–
	Metronidazole	0.125–1	0.25	0.5	0.345	0
	Vancomycin	0.25–2	0.5	1	0.579	0
	Tigecycline	0.016–0.5	0.032	0.064	0.041	0
	Moxifloxacin	0.5–64	2	16	3.22	34.7
A+B+CDT− (51)	Fidaxomicin	0.064–1	0.25	0.5	0.241	–
	Metronidazole	0.125–0.5	0.5	0.5	0.346	0
	Vancomycin	0.25–2	0.5	1	0.605	0
	Tigecycline	0.016–0.5	0.032	0.064	0.041	0
	Moxifloxacin	1–16	1	8	1.77	15.7
A−B+CDT− (20)	Fidaxomicin	0.032–0.5	0.25	0.5	0.171	–
	Metronidazole	0.125–1	0.25	1	0.354	0
	Vancomycin	0.25–1	0.5	1	0.518	0
	Tigecycline	0.032–0.064	0.032	0.064	0.042	0
	Moxifloxacin	1–64	16	64	17.753	85.0
A−B−CDT− (24)	Fidaxomicin	0.064–0.5	0.25	0.5	0.258	–
	Metronidazole	0.125–0.5	0.25	0.5	0.324	0
	Vancomycin	0.25–1	0.5	1	0.5	0
	Tigecycline	0.032–0.5	0.032	0.157	0.043	0
	Moxifloxacin	1–16	2	16	2.748	29.2

Table 3 MICs of fidaxomicin, metronidazole, vancomycin, tigecycline and moxifloxacin for 6 A+B+CDT+ *C. difficile* isolates.

Strain no.	MIC (mg/L)					MLST	PCR-ribotype
	Fidaxomicin	Metronidazole	Vancomycin	Tigecycline	Moxifloxacin		
40	0.25	1	2	0.016	32	1	D
301	1	1	1	0.032	16	1	027
235	1	1	2	0.032	16	1	027
2	0.125	0.125	0.5	0.032	1	5	063
5	0.25	0.125	0.5	0.032	1	5	063
196	0.064	0.125	0.5	0.032	2	5	063

episode of recurrent CDI have a 45%–65% chance of additional episodes.[24]

Some studies have indicated that fidaxomicin has similar clinical cure rates to vancomycin and lower recurrence rates for CDI treatment.[25,26] Our findings on the *in vitro* activity data for fidaxomicin, specifically the MICs, is similar with those published by the U.S-based national sentinel surveillance study and another different USA study.[27,28] In contrast to the above, the fidaxomicin MICs reported in the present study are higher than that of the recently published pan-European surveillance program, and the ones reported in Spain, Sweden, Hungary and Taiwan.[29–33] To date, fidaxomicin has not been approved by China Food and Drug Administration for the treatment of CDI, and more studies involving multicentres are needed to investigate the clinical treatment outcome for Chinese CDI patients.

In the present study, tigecycline exhibited good *in vitro* activity against different toxin genotypes of *C. difficile* isolates with the lowest MIC value compared to other antibiotics. Intravenous tigecycline has only been used in a small number of patients with severe CDI refractory to standard therapy.[34] According to the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines,[35] intravenous tigecycline is only recommended as salvage therapy. In the present study, A−B+CDT− strains, which are the most common *C. difficile* epidemic clones in China, Japan and Korea,[5,36,37] exhibited higher moxifloxacin drug resistance rates than A+B+CDT− and A−B−CDT− strains. In addition, the three ST 1 isolates showed higher MIC values for fidaxomicin, metronidazole, vancomycin and moxifloxacin, highlighting the significance of establishing a local surveillance network for CDI. However, it is worth mentioning that A−B−CDT− isolates have no pathogenic role in causing human disease, and treatment for such isolates in the stool of symptomatic patients is not suggested.

The *gyrA* and *gyrB* mutations were the principal mechanism conferring fluoroquinolone resistance in *C. difficile* in the present study. All the moxifloxacin-resistant isolates carried a mutation either in *gyrA* or *gyrB* or both. This finding is in agreement with those of two studies performed

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Table 4 Molecular characterization of the 28 moxifloxacin-resistant toxigenic *C. difficile* strains.

Isolate	Toxin genotype	MLST	PCR-ribotype	Moxifloxacin MIC (mg/L)	Amino acid substitution	
					GyrA	GyrB
40	A+B+CDT+	1	D	32	Thr82→Ile	
235	A+B+CDT+	1	027	16	Thr82→Ile	
301	A+B+CDT+	1	027	16	Thr82→Ile	
119	A+B+CDT−	3	001	16	Thr82→Ile	
232	A+B+CDT−	3	001	8		Asp426→Asn
603	A+B+CDT−	3	001	16	Thr82→Ile	
604	A+B+CDT−	8	002	8		Asp426→Asn
608	A+B+CDT−	8	002	8	Thr82→Ile	
612	A+B+CDT−	8	002	8	Thr82→Ile	
211	A+B+CDT−	35	046	16	Thr82→Ile	
308	A+B+CDT−	35	046	16	Thr82→Ile	
8	A−B+CDT−	37	017	16	Thr82→Ile	
77	A−B+CDT−	37	017	16	Thr82→Ile	Ser366→Ala
88	A−B+CDT−	37	017	16	Thr82→Ile	Ser366→Ala
268	A−B+CDT−	37	017	16	Thr82→Ile	Ser366→Ala
276	A−B+CDT−	37	017	16	Thr82→Ile	Ser366→Ala
303	A−B+CDT−	37	017	16	Thr82→Ile	Ser366→Ala
327	A−B+CDT−	37	017	16	Thr82→Ile	Ser366→Ala
377	A−B+CDT−	37	017	16	Thr82→Ile	Ser366→Ala
402	A−B+CDT−	37	017	16	Thr82→Ile	Ser366→Ala
610	A−B+CDT−	37	017	16	Thr82→Ile	Ser366→Ala
4	A−B+CDT−	81	A	64	Thr82→Ile	Ser366→Ala, Asp426→Val
10	A−B+CDT−	81	A	64	Thr82→Ile	
83	A−B+CDT−	81	A	64	Thr82→Ile	Ser366→Ala, Asp426→Val
96	A−B+CDT−	81	A	64	Thr82→Ile	
103	A−B+CDT−	81	A	64	Thr82→Ile	Ser366→Ala, Asp426→Val
150	A−B+CDT−	81	A	64	Thr82→Ile	Ser366→Ala, Asp426→Val
218	A−B+CDT−	81	A	64	Thr82→Ile	Ser366→Ala, Asp426→Val

by Huang, Dong et al. in Shanghai, China.[22,38] The *gyrA* substitution occurred more frequently than *gyrB*, and one type of substitution in *gyrA* (Thr82→Ile) was observed in the present investigation. Furthermore, double substitutions in *gyrB* were associated with a higher fluoroquinolone MIC (64 mg/L).

In conclusion, this study integrated the molecular epidemiology and antimicrobial activity of fidaxomicin against *C. difficile* isolates in mainland China. Fidaxomicin exhibited high antimicrobial activity against all *C. difficile* isolates tested, which shows promise as a new drug for treating Chinese CDI patients.

Conflicts of interest

All contributing authors declare no financial interests related to the material in the manuscript.

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