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Comparative *in-vitro* activities of MCB3681 and 8 comparators against 200 *Clostridium difficile* isolates with known ribotypes and diverse geographical spread

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Background

Antimicrobial treatments for *C. difficile* infection (CDI) are limited to metronidazole (M), vancomycin (V) and fidaxomicin (FDX). Symptomatic recurrences are common following conventional antimicrobial treatment, therefore further therapeutic options are necessary. MCB3681 is a novel investigational quinolonyl-oxazolidinone antibacterial with good anti-*C. difficile* activity currently being assessed for intravenous treatment of CDI.[1] We determined *in vitro* activities of MCB3681 and 8 comparators against *C. difficile* isolates of known PCR ribotypes and diverse geographical spread.

Materials and methods

MICs of MCB3681, M, V, FDX, moxifloxacin (MXF), ciprofloxacin (CIP), clindamycin (CL), tigecycline (TG), linezolid (LZD) were determined for 200 *C. difficile* of prevalent or emerging/multi-resistant ribotypes (RT) sourced across Europe (The ClosER Study, with permission from Astellas Pharma Europe).[2] MICs were determined by Wilkins-Chalgren agar incorporation. CD were designated susceptible (S), intermediate (I) or resistant (R) according to breakpoints established according to CLSI or EUCAST data, where available, or published data.

Results

FDX and MCB3681 were the most active CD treatments tested (geometric means (GM) MIC=0.05 mg/L and 0.12mg/L, respectively). All CD were MCB3681 susceptible, including those CD with multidrug resistance phenotypes (including MXF-CIP-LZD resistance).

There was no resistance seen for MCB, FDX or TG and V resistance was rare (1.5%).

mg/L	M	V	FDX	MXF	CIP	CL	TG	LZD	MCB3681
GM MIC	0.33	1.02	0.05	5.87	66.27	16.17	0.05	5.16	0.12
MIC_{50}	0.25	1	0.06	2	64	16	0.06	4	0.125
MIC_{90}	1	2	0.125	32	256	128	0.06	8	0.25
%S	99	96	100	50.5	-	5.5	100	78.9	100
%I	1	2.5	-	1	-	29.5	-	-	-
%R	0	1.5	-	48	100	54	-	21.1	-
range	<0.1 25-4	0.5-8	0.004-0.25	1->64	8->128	1->64	0.03-0.125	2->64	0.008-0.5

Table 1. Susceptibility of 199 *C. difficile* isolates to MCB3681 and 8 comparators

	M	V	FDX	MXF	CIP	CL	TG	LZD	MCB3681
RT001	0.42	0.79	0.02	16.00	111.43	61.11	0.03	10.08	0.07
RT002	0.19	0.87	0.06	1.82	27.86	12.13	0.04	4.39	0.11
RT005	0.29	1.16	0.06	2.00	37.12	9.28	0.04	5.66	0.14
RT014	0.28	0.88	0.07	3.36	39.74	10.37	0.05	4.36	0.11
RT015	0.25	0.87	0.06	1.91	26.60	7.29	0.04	4.19	0.14
RT017	0.26	0.74	0.04	12.88	86.67	64.00	0.06	7.03	0.15
RT018	0.41	1.49	0.06	6.90	110.33	8.83	0.04	4.42	0.12
RT020	0.25	0.75	0.06	2.59	36.44	11.31	0.05	4.76	0.10
RT027	0.96	1.14	0.09	21.67	206.14	19.87	0.05	5.19	0.16
RT078	0.26	0.92	0.05	2.38	34.90	12.34	0.05	5.42	0.11
RT106	0.74	1.10	0.09	7.61	81.98	10.77	0.04	4.42	0.11
RT126	0.32	1.00	0.06	8.35	72.88	38.05	0.06	4.56	0.12
RT356	0.27	2.28	0.04	29.34	245.15	12.88	0.04	4.76	0.08
All isolates	0.33	1.02	0.05	5.87	66.27	16.17	0.05	5.16	0.12

Table **2 Geometric mean MICs of MCB3681 against prevalent and emerging European *C. difficile* PCR ribotypes**

Geometric mean MCB3681 MICs were **lowest** in **RTs 001 and 356** (0.007 and 0.008 mg/L, respectively),
Geometric mean MCB3681 MICs were **highest** in **RTs 017 and 027** (0.15 and 0.16 mg/L, respectively).

The **highest** MCB3681 MICs (0.5 mg/L) were observed in **CIP-MXF-LZD resistant RTs 017** (n=3) and **027** (n=2), but other CIP-MXF-LZD resistant CD showed no such elevated MCB3681 MICs. This may indicate that the multidrug resistance gene, *cfr*, (conferring oxazolidinone-quinolone resistance) is not linked to elevated MCB3681 MICs
Single isolates of RTs 005 and 018 also showed MCB3681 MICs of 0.5 mg/L

Ribotype	M	V	FDX	MXF	CIP	CLINDA	TIGE	LZD	MCB	CHL
001	0.25	0.5	0.015	32	128	128	0.03	32	0.0015	32
001	0.25	0.5	0.015	16	128	128	0.03	32	0.008	256
005	0.25	1	0.03	2	32	8	0.06	16	0.5	2
017	0.125	0.5	0.008	32	64	128	0.06	32	0.5	64
017	0.25	1	0.125	32	64	128	0.06	16	0.5	64
017	0.25	1	0.06	32	128	128	0.125	32	0.5	64
018	2	4	0.125	2	128	16	0.06	4	0.5	4
027	2	1	0.125	32	256	128	0.06	32	0.5	64
027	2	1	0.25	32	256	128	0.06	32	0.5	64

Table 3. MCB3681 MICs in *C. difficile* isolates with and without quinolone, linezolid and chloramphenicol resistance (highlighting indicates resistance)

MXF (48%) and/or CIP (100%) resistance was evident in all RTs.
High level resistance to both MXF (≥32 mg/L and CIP (≥128 mg/L) was prevalent in **RTs 001, 027 and 356**.
CL resistance (54%) was also evident in all RTs, but greatest in **RTs 001, 017 and 126** (GM MICs = 61.1, 64 and 38.1 mg/L, respectively).
Most (78.9%) CD were LZD susceptible (GM MIC=5.16 mg/L). **RTs 001 and 017** had the highest GM LZD MICs (10.1 and 7.0 mg/L, respectively).

Discussion

MCB3681 had good activity against CD from emerging/ prevalent European RTs, superior to M and V, with no evidence of resistance. MCB3681 was slightly (one titration step) less active than FDX. Rashid *et al*. reported similar *C. difficile* susceptibilities to MCB3681 in the range of 0.008-0.5 mg/L,[1]. However, MIC_{50} and MIC_{90} values (0.125 and 0.25 mg/L, respectively), were slightly higher than those reported previously (0.03 and 0.06 mg/L, respectively) and are similar to those recently described for cadazolid, another quinolonyl-oxazolidinone molecule.[3]

Quinolone/oxazolidinone resistance was not associated with increased MCB3681 MICs (Table 3**).**
Many isolates displaying either/both MXF or LZD resistance showed MCB3681 MICs towards the lower (0.008 mg/L), as well as the higher (0.5 mg/L) end of the range observed. Three RT017 and two RT027 isolates showed dual quinolone-oxazolidinone resistant phenotype, and also showed the highest MCB3681 MICs. We previously reported high level chloramphenicol resistance for these isolates.[2,4] This may indicate the presence of the multidrug resistance gene, *cfr.* Marin *et al*. reported LZD, chloramphenicol, erythromycin, and CL resistance associated with the presence of *cfr* in *C. difficile* RT017, 078 and 126 isolates.[10] However, we also found isolates with quinolone-oxazolidinone and chloramphenicol resistance that demonstrated very low MCB3681 MICs (0.008 mg/L), indicating that this phenotype (and possibly *cfr* genotype) is not linked to increased MCB3681 MICs.

Conclusions

MCB3681 had good activity against CD from emerging or prevalent European RTs, superior to M and V, with no evidence of resistance. The presence of quinolone and/or linezolid resistance did not influence MCB3681 MICs.

Acknowledgments

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