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In Vitro and *In Vivo* Activity of Cefiderocol against *Stenotrophomonas maltophilia* Clinical Isolates

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ABSTRACT

Background : Cefiderocol (S-649266, CFDC) is a novel siderophore cephalosporin with activity against a wide variety of Gram-negative bacteria including carbapenem-resistant (CR) strains. Importantly, CFDC has a potent activity against not only Enterobacteriaceae but also non-fermenters including *Stenotrophomonas maltophilia*, an opportunistic pathogen with intrinsic resistance to carbapenem antibiotics. In this study, *in vitro* activity and *in vivo* efficacy of CFDC and comparators against *S. maltophilia* were determined.

Materials/Method : MICs of CFDC and comparators (trimethoprim/sulfamethoxazole (TMP/SMX), minocycline (MINO), tigecycline (TGC), ciprofloxacin(CPFX), cefepime (FPM), meropenem (MEM), and colistin (CST)) were determined by broth microdilution method as recommended by CLSI guidelines. The MIC against CFDC was determined using iron-depleted cation-adjusted Mueller-Hinton broth. In vivo efficacy of CFDC, and comparators FPM, ceftazidime/avibactam (CZA), MEM, and CST was evaluated using a neutropenic murine systemic infection model caused by strain SR21970. The 50% effective doses (ED_{50}s) were calculated by the logit method using the survival number at each dose 7 days after infection. *In vivo* efficacy of CFDC was further evaluated using a neutropenic murine lung infection model caused by five strains. The numbers of viable cells in lungs were determined 24 hrs after infection.

Results : MIC_{90} of CFDC and comparators against the 216 clinical isolates from global countries collected in SIDERO-CR 2014/2016 study are shown in the Table. CFDC, TMP/SMX, MINO, and TGC showed good activity with MIC_{90} of 0.5, 0.25/4.75, 1, and 2 µg/mL, respectively. CFDC inhibited growth of all tested strain at ≤1 µg/mL while 2 strains showed resistance to TMP/SMX. MIC of CFDC against *S. maltophilia* SR21970 was 0.125 µg/mL. MICs of FPM, CZA, MEM, and CST were ≥ 32 µg/mL. The ED_{50} of CFDC against *S. maltophilia* SR21970 was 1.17 mg/kg/dose. On the other hand, FPM, CZA, MEM/cilastatin, and CST were ineffective, showing that CFDC had potent *in vivo* efficacy against *S. maltophilia* strain which was resistant to other antibiotics.

Conclusion : CFDC showed potent *in vitro* activity against *S. maltophilia* including TMP/SMX resistant isolates. CFDC also showed potent *in vivo* efficacy reflecting *in vitro* activity against *S. maltophilia* in murine systemic infection model and murine lung infection model.

INTRODUCTION

Cefiderocol (S-649266, CFDC) is a novel siderophore cephalosporin with potent activity against a wide variety of Gram-negative bacteria including carbapenem-resistant strains (1, 2). In this study, we evaluated *in vitro* activity of CFDC and comparators against *S. maltophilia* collected from a global surveillance study of CR and MDR Gram negative clinical isolates. We also evaluated *in vivo* activity of CFDC against *S. maltophilia* in murine systemic infection model and murine lung infection model.

MATERIALS AND METHODS

Test organisms
All clinical isolates of *S. maltophilia* (216 strains) which were collected from global countries in the SIDERO-CR-2014/2016 study, were used. *S. maltophilia* SR21970, which was isolated in Japan, was also used in the *in vivo* systemic infection model.

Compounds
1) *In vitro* activity : CFDC, trimethoprim/sulfamethoxazole (TMP/SMX), minocycline (MINO), tigecycline (TGC), ciprofloxacin (CIP), cefepime (FPM), meropenem (MEM), and colistin(CST) were tested.
2) *In vivo* activity: CFDC, FPM, ceftazidime/avibactam(CZA), MEM, and CST were tested. Cilastatin was used in combination with MEM to prevent the hydrolysis by murine dihydropeptidase-I.

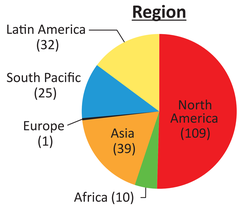
MIC determination
MICs were determined by broth microdilution methods according to the Clinical and Laboratories Standard Institute (CLSI) guidline (1, 2). CFDC was tested in iron-depleted cation adjusted Mueller-Hinton Broth (ID-CAMHB), following the method approved by the CLSI (3). The MIC of CFDC was read as the first drug well in which the growth is significantly reduced (i.e., a button of < 1 mm or light/faint turbidity) relative to the growth observed in the growth control.

Neutropenic murine systemic infection model
ICR male mice were rendered neutropenic by intraperitoneal injection of cyclophosphamide (Shionogi) with 150 and 100 mg/kg at 4 and 1 days before infection, respectively. Neutropenic mice were infected by intraperitoneal injection of 1.0 mL of bacterial suspension with 5% gastric mucin (MP Biomedicals, Inc.) (bacteria:mucin=1:9). The challenge dose were approximately 1.3×10^7 CFU/mouse. CFDC, FPM, CZA, MEM/CS were administered subcutaneously at 1, 3 and 5 hours (hr) after infection. CST was administered subcutaneously at 1 and 5 hours after infection. The 50% effective doses (ED_{50}s) of CFDC and comparators were calculated by the logit method using the survival numbers at each dose 7 days after infection. Windows SAS program (SAS® Version 9.4) was used for calculating ED_{50}s.

Neutropenic murine lung infection model
ICR male mice were rendered neutropenic by intraperitoneal injection of cyclophosphamide with 150 and 100 mg/kg at 4 and 1 days before infection, respectively. Neutropenic mice were anesthetized by intramuscular injection of the mixture of medetomidine (Nippon zenyaku kogyo, Japan), midazolam (Sandoz), butorphanol (Meiji seika pharma, Japan), and saline (0.75: 2.00: 2.50: 44.75 as volume), and then infected by intranasal instillation of 0.07 mL of bacterial suspension with 5% gastric mucin to the desired concentration (bacteria: mucin = 1: 9) in order to prevent rapid bacterial clearance. The challenge inocula were approximately from 2 - 4×10^5 CFU/mouse. Cefiderocol was administered subcutaneously at 2, 5, and 8 hr after infection. At 24 hr after infection, mice were euthanized and dissected to remove tissue. Lungs were excised, homogenized and serial dilution were plated on Drigalski improved medium (Eiken Chemical Co., Ltd., Japan) containing 1 mg/mL ammonium iron (III) citrate solution for bacterial counts.

RESULTS

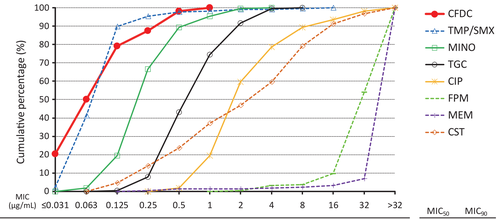
Figure 1. Source of 216 *S. maltophilia* strains from SIDERO-CR-2014/2016 study



North America	Canada (39), United States (70)
Africa	South Africa (10)
Asia	Taiwan (13), Japan (11), Korea (8), Thailand (6), Singapore (1)
Europe	United Kingdom (1)
South Pacific	Australia (19), New Zealand (4), Philippines (2)
Latin America	Mexico (11),Venezuela (4), Panama (4), Chile (7), Colombia (5), Puerto Rico (1)

- 83% of test strain were obtained from respiratory organs.

Figure 2. MIC Distribution against *S. maltophilia* (N=216)



MIC (µg/mL)	≤0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	>32	MIC_{50}	MIC_{90}
CFDC	44	64	63	18	23	4							0.063	0.5
* TMP/SMX	5	84	105	12	5	1	2		1	1			0.125	0.25
MINO		4	38	102	49	13	9	1					0.25	1
TGC			1	16	76	68	37	17	1				1	2
CIP					4	38	87	41	23	9	10	4	2	16
FPM							1	6	1	13	96	99	32	>32
MEM			1	2				1	1	2	8	201	>32	>32
CST		10	20	21	29	21	28	42	26	12	7		4	16

* TMP/SMX=1/19,Concentration of TMP was shown.

- MIC_{90} of CFDC was 0.5 µg/mL. MIC_{90} of TMP/SMX, MINO, and TGC were 0.25/4.75, 1, and 2 µg/mL, respectively. MIC_{90} of CIP, FPM, MEM, and CST were ≥ 16 µg/mL.
- CFDC inhibited growth of all tested strain at ≤ 1µg/mL.

Table 1. MIC Distribution of CFDC, TMP/SMX, MINO against test strains by region

		MIC (µg/mL) ≤0.031	0.063	0.13	0.25	0.5	1	2	4	8	16	32	64	>32
CFDC	North America	22	33	33	8	10	3							
	Africa	1	3	2	1	3								
	Asia	10	10	14	1	4								
	Europe			1										
	South Pacific	8	10	2	5									
	Latin America	3	8	11	3	6	1							
TMP/SMX	North America	2	44	51	6	5		1						
	Africa		3	6							1			
	Asia	2	8	25	4									
	Europe			1										
	South Pacific	1	8	13	1		1	1						
	Latin America		21	9	1					1				
MINO	North America		2	18	46	32	6	4	1					
	Africa			1	8			1						
	Asia		1	7	18	8	2	3						
	Europe				1									
	South Pacific		1	6	10	3	4	1						
	Latin America		6	19	6	1								

Susceptible | Intermidiate | Resistant

- Two TMP/SMX resistant strains were obtained from Latin America and Africa.

Table 2. MIC against TMP/SMX resistant *S. maltophilia*

Strain	Region	Body site distribution	MIC (µg/mL) CFDC	TMP/SMX	MINO	TGC	CIP	FPM	MEM	CST
1376660	Latin America: Venezuela	Bodily Fluids: Peritoneal	0.063	8/152	0.125	0.5	2	32	>32	8
1376469	Africa: South Africa	Respiratory: Endotracheal aspirate	0.125	16/304	2	4	32	32	>32	>32

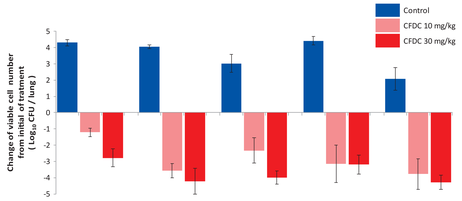
- CFDC was active with MIC of ≤ 0.125 µg/mL against *S. maltophilia* 1376660 and 1376469, which were resistant to TMP/SMX with MICs of 8/152 and 16 /304 µg/mL, respectively.

Table 3. Therapeutic efficacies against systemic infection model caused by *S. maltophilia* SR21970

Test and reference substances	ED_{50} (mg/kg/dose)	95% CI	MIC (µg/mL)
CFDC	1.17	0.0417-4.68	0.125
FPM	> 100	N.C.	> 32
CZA	> 100	N.C.	> 32/4
MEM/CS	> 100	N.C.	> 32
CST	> 10.0	N.C.	32

- CFDC was effective in murine systemic infection model, reflecting *in vitro* activity.
- Other comparators were not effective due to the high MIC.
- The antibacterial activity of TMP/SMX is depending on thymidine concentration. TMP/SMX could not be used in this study, because the thymidine concentration in murine is markedly higher than human or medium.

Figure 3. Therapeutic efficacy of CFDC in neutropenic murine lung infection model caused by *S. maltophilia*



	1373179	1400678	1476057	1371071	1444463
MIC of CFDC (µg/mL)	0.125	0.063	0.5	0.125	0.25

- MIC of CFDC against test strains were 0.063 –0.5 µg/mL.
- CFDC showed dose dependent bactericidal effect against all the 5 strains tested, and showed ≥2-log_{10} CFU/lung reduction at 10 mg/kg/dose.

CONCLUSIONS

1) CFDC showed potent antimicrobial activity against *S. maltophilia* including TMP/SMX resistant isolates.
2) CFDC showed potent *in vivo* efficacy reflecting in vitro activity against *S. maltophilia* in murine systemic and lung infection model.
3) CFDC is the first β-lactam antibiotic to show potent *in vivo* activity against *S. maltophilia*.

REFERENCES

1. Clinical and Laboratory Standards Institute (CLSI), 2015. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standards - Tenth Edition. CLSI document M07-A10 (ISBN 1-56238-988-2).
2. Clinical and Laboratory Standards Institute (CLSI), 2016. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI Document M100-S26 (ISBN 1-56238-923-8).
3. Ito A et al. Siderophore Cephalosporin Cefiderocol Utilizes Ferric Iron Transporter Systems for Antibacterial Activity against *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2016; 60:7396-7401.