Journal Pre-proof

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Highlight

- Cefiderocol (CFDC) is a novel siderophore cephalosporin for injection.
- β-lactamases contributed to non-susceptibility to CFDC.
- Multiple factors including NDM and PER could be related to CFDC non-susceptibility.

Reduced susceptibility mechanism to cefiderocol, a siderophore cephalosporin, among clinical isolates from global surveillance program (SIDERO-WT-2014)

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Short Communications

Highlight

- Cefiderocol (CFDC) is a novel siderophore cephalosporin for injection.
- β-lactamases contributed to non-susceptibility to CFDC.
- Multiple factors including NDM and PER could be related to CFDC non-susceptibility.

Abstract

- Objectives

To investigate possible mechanistic factors to explain cefiderocol (CFDC) non-susceptibility, we characterized 38 clinical isolates with cefiderocol MIC of >4 μg/mL from a multi-national surveillance study.

- Methods

The minimum inhibitory concentration (MIC) measurement in the presence of β-lactamase inhibitors and whole genome sequencing were performed.

- Results

The MIC decrease of CFDC by β-lactamase inhibitors were observed against all of the test isolates. Among 38 isolates, NDM or PER genes were observed in 5 and 25 isolates, respectively. No other β-lactamases responsible for high MIC were identified in other 8 isolates. MIC of CDFC against *Escherichia coli* isogenic strains introduced with NDM and PER β-lactamase increased by ≥ 16-fold, suggesting the contribution of NDM and PER to the non-susceptibility to CFDC. Against NDM producers, ≥ 8-fold MIC increase was observed only when both serine- and metallo-type beta-lactamase inhibitors were added. In addition, many of PER- or NDM-producers remained susceptible to CFDC. These results suggested that the presence of only NDM or PER would not lead to non-susceptibility to CFDC and that multiple factors would be related with CFDC resistance.

- Conclusions

Multiple factors including NDM and PER could be related to reduced susceptibility to CFDC.

Keywords: Cefiderocol, siderophore cephalosporin, Gram-negative bacilli, PER-type β-lactamase, cefiderocol non-susceptible

1. Introduction

The infections caused by carbapenem-resistant Gram-negative bacteria (CR-GNB) are major threats to public health [1, 2]. Therapeutic options available to treat patients with CR-GNB infections are currently limited as these isolates frequently acquire multi-drug resistance [2, 3]. Cefiderocol (formerly S-649266) is a novel siderophore cephalosporin for injection which has been approved by the US FDA for the treatment of complicated urinary tract infection in 2019. CFDC has two unique mechanisms to overcome carbapenem resistance. First, the catechol moiety of CFDC facilitates the efficient entry through the outer membrane of GNB using active iron transport systems [4]. Second, CFDC has been shown to be more stable *in vitro* than carbapenems to both serine- and metallo-type carbapenemases such as KPC-3, OXA-48, OXA-23, OXA-24/40, VIM-2, IMP-1, NDM-1, and L1 by enzymatic studies [5, 6]. Several multi-national surveillance studies demonstrated potent antimicrobial activity of CFDC against CR-GNB including Enterobacterales and non-fermenters such as *Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Stenotrophomonas maltophilia.* [7-9]. Among 9,205 Gram-negative bacteria from North America and Europe from a multi-national surveillance SIDERO-WT-2014 study, CFDC was shown to be active with MIC_{90} of ≤4 μg/mL against various carbapenemase-positive isolates such as KPC, OXA-48, OXA-23, OXA-24, IMP, VIM although MIC_{90} against NDM-positive isolates was 8 μg/mL [10]. Among these 9,205 isolates, 39 isolates had cefiderocol MIC of >4μg/mL, which was defined to be non-susceptible according to provisional break points by Clinical and Laboratory Standards Institute (CLSI) [11]. In this study, we characterized 38 isolates except for one *Burkholderia cepacia* isolate to identify possible mechanistic factors to explain CFDC non-susceptibility.

2. Materials and Methods

2.1 Bacterial isolates

Thirty-eight isolates included 9 Enterobacterales (6 *Klebsiella pneumoniae*, 2 *Serratia marcescens* and 1 *Klebsiella aerogenes*), 28 *Acinetobacter baumannii*, and 1 *Pseudomonas aeruginosa*. The Enterobacterales isolates were from Turkey (6), Italy (1), Germany (1), and United States (1). The *A. baumannii* isolates were from Russia (18), Turkey (6), United States (3), and Sweden (1). One *P. aeruginosa* isolate was from Canada.

Escherichia coli isogenic strains introducing each β-lactamase gene were constructed in this study. Amplification of DNA fragments of the β-lactamase genes were performed from first codon to stop codon by polymerase chain reaction (PCR). The fragments were introduced into multi-cloning site in pET9a (Invitrogen^{TM}). The constructed plasmid was introduced into *E. coli* BL21(DE3) (Invitrogen^{TM}), respectively. The DNA sequences of β-lactamase genes were referred to the database maintained by the United States National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

2.2 Antimicrobial susceptibility testing

MICs were determined by broth microdilution method using iron-depleted cation-adjusted Mueller Hinton broth for CFDC and cation-adjusted Mueller Hinton broth for meropenem (MEM) and ceftazidime (CAZ), according to CLSI [11, 12]. To investigate the effect of β-lactamase on activity of CFDC against these strains, MICs were determined with or without β-lactamase inhibitors such as 100 μg/mL of dipicolinic acid (DPA) (a metallo-β-lactamase inhibitor) and/or 4 μg/mL of avibactam (AVI) (a serine-β-lactamase inhibitor). Meropenem (MEM) was studied in parallel with the same combinations of AVI and/or DPA. In the case of *E. coli* isogenic mutants, test broth included 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG).

2.3 Whole genome sequencing

Genomic DNA from each bacterial sample was extracted from overnight cultures using the illustra bacteria genomicPrep Mini Spin Kit (Cytiva). DNA libraries were prepared using Nextera XT DNA Sample Prep Kit (Illumina) according to the manufacturer's instructions. Briefly, the Genomic DNA samples were fragmented and index-tagged to ligate into illumina adapters. Fragments were amplified by Veriti Thermal Cycler (Applied Biosystemss) and purified by Agencourt AMPure XP beads (Beckman coulter). The purified pooled DNA libraries were sequenced using the Illumina MiSeq system with 300-base paired-end reads. The raw FASTQ reads were first trimmed to quality score limit 0.05 (Q13) with maximum 2 ambiguous nucleotides and assembled into contigs for each test sample using CLC Genomics Workbench version 11.0 (Qiagen). To investigate β-lactamase genes, all contig datasets of test samples were loaded to Pipeline pilot version 9.5.0.831 (Dassault Systems Biovia), and subjected to BLASTn-based search against in-house β-lactamase gene database. The hit sequences were translated into amino acid sequences. Then, the amino acid sequences were used to identify the subtypes of β-lactamases.

2.4 Determination of multilocus sequencing typing (MLST) against A. baumannii

The *A. baumannii* MLST profiles were determined by comparison of 7 allele sequences from the public database [13]. The allele sequences of *A. baumannii* isolates were determined by blastn search for generated contigs using Pipeline pilot version 9.5.0.831 (Dassault Systemes Biovia).

3. Results and Discussion

Previously, we reported NDM-producing strains showed a MIC distribution of CFDC

with higher concentration range compared with other carbapenemase producers although CFDC showed MIC of ≤4 μg/mL against 64 to 87% of NDM-producers [10, 14]. Therefore, we categorized these CFDC non-susceptible isolates into NDM- and non-NDM-groups. Although several isolates showed CFDC MIC of ≤4 μg/mL, the effect of β-lactamase inhibitors was assessed by the resulting MICs with or without the β-lactamase inhibitors presented in Table 1.

Against 5 Enterobacterales isolates possessing the NDM gene, all of which are from Turkey, a >2-fold CFDC MIC decrease was not observed by the addition of either DPA or AVI except for 2 isolates showing a 4-fold decrease in the presence of DPA (MIC from 4 μg/mL to 1 μg/mL). On the other hand, MEM MIC of all isolates decreased >64-fold by the addition of only DPA. Interestingly, the CFDC MIC against all 5 isolates decreased 8- to 64-fold by the addition of both DPA and AVI to the susceptible level (≤0.5 μg/mL). These results clearly suggested that the non-susceptibility to CFDC of NDM-producing Enterobacterales isolates in this study was not only due to the NDM production and could be due to co-expression of multiple β-lactamases such as both metallo- and serine-type β-lactamases.

To clarify the responsible β-lactamases for CFDC non-susceptibility, the susceptibility was evaluated using *E. coli* isogenic strain introduced with possible β-lactamases (Table 2). *E. coli* isogenic strain introduced with NDM-1 showed MIC increase to CFDC and MEM by 64-fold and 256-fold although the MIC remained 4 or 8 μg/mL, respectively, suggesting NDM could be the responsible β-lactamase for non-susceptibility to CFDC and MEM. On the other hand, the CFDC MICs did not changed with the introduction of OXA-1, TEM-1, and CTX-M-15 which were present in the CFDC non-susceptible clinical isolates. However, a 4-fold elevation of CFDC MICs was observed by the introduction of some SHV-type β-lactamase (Table 2). These results suggest that the non-susceptibility to

CFDC of NDM-producing Enterobacterales isolates in this study could be due to co-expression of NDM and serine-type β-lactamases although we could not identify a specific serine-type β-lactamase responsible for CFDC non-susceptibility.

Against 32 of 33 non-NDM-possessing Enterobacterales, *A. baumannii*, and *P. aeruginosa* isolates, ≥8-fold decreases of CFDC MIC was observed by addition of AVI (Table 1). Even against the remaining 1 isolate, 4-fold MIC decrease was observed by the addition of AVI. The results suggest that a serine-type β-lactamase contributed to the increase MIC of CFDC. The whole genome sequencing showed these isolates could be categorized into two groups based on the presence of the PER gene; 25 isolates were PER-positive isolates and 8 isolates were PER-negative. All 25 PER-positive isolates were *A. baumannii* which are from Russia (18 isolates), Turkey (6 isolates), and Sweden (1 isolate). The MLST analysis based on Oxford scheme showed that these 25 *A. baumannii* isolates have closely related genetic backgrounds as they belong to 5 MLST groups (8 ST502, 6 ST558, 5 ST452, 4 ST450, and 2 ST493) and five of seven allele number (*cpn60*, *gdhB*, *gltA*, *recA*, and *rpoD*) were the same among these 5 MLST groups [15]. It was also interesting that 24 of 25 PER-positive isolates had CFDC MIC of >8 μg/mL although none of other 14 non-PER isolates including NDM-positive isolates had MICs ranging from 2-8 μg/mL (Table 1). Previously, Ito reported that CFDC showed good activity with MIC of ≤4 μg/mL against ≥90% of 47 PER-producing isolates which included only 4 *A. baumannii* strains [16]. The effect of PER-type β-lactamase against CFDC MIC was confirmed by MIC increases to CFDC and CAZ by 16-fold and >256-fold, respectively, due to the introduction of PER-1 gene into *E. coli* (Table 2). Considering that the degree of MIC increase by the introduction of PER-1 was smaller than by NDM-1, and that CFDC showed MIC of ≤4 μg/mL against ≥90% among 47 PER-producing isolates including 4 *A. baumannii* isolates [16], suggests that the presence of only PER-type β-lactamase might not result in high MIC of >8 μg/mL in PER-positive *A.*

baumannii. Although it has not been studied, additional factor(s) such as additional serine-type β-lactamase or outer membrane permeability related factors, which could be associated with species-type, might contribute to the high MICs to CFDC. As no common specific β-lactamases could be related to non-susceptibility to CFDC in 8 PER-negative isolates, further investigation will be needed.

4. Conclusion

β-lactamases were shown to contribute to CFDC non-susceptibility in all CFDC non-susceptible isolates with MIC of ≥ 4 μg/mL from multi-national surveillance study (SIDERO-WT-2014). The frequent identification of the NDM- and PER-producers suggested the contribution of these β-lactamases to CFDC non-susceptibility, however additional factor(s) such as the other β-lactamase seemed to be needed to produce high CFDC resistance. Overall, the presence of CFDC non-susceptibility (MIC > 4 μg/mL) remains very low (<1%) [7, 9]. Continuous observation is needed to identify the epidemiology and mechanism of CFDC resistance.

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Declaration

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Competing Interests

All authors are employees of Shionogi & Co., Ltd. or International Health Management Associates, Inc. The IHMA authors do not have personal financial interests in Shionogi & Co., Ltd.

Ethical Approval

Not required.

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Table 1 *In vitro* activity of CFDC against Gram-negative bacilli with CFDC MIC of ≥8 μg/mL from multi-national surveillance studies in 2014 (SIDERO-WT-2014)

NDM β-lactamase	Test strains		Characteristics	MIC (μg/mL) [a]							
				CFDC				MEM			
				- -	+DPA -	- +AVI	+DPA +AVI	- -	+DPA -	- +AVI	+DPA +AVI
NDM positive	*K. pneumoniae*	SR200277	NDM-1, CTX-M-15, TEM-1, OXA-1, SHV-group	4	2	2	0.25	> 32	0.5	32	0.125
	K. pneumoniae	SR200278	NDM-1, TEM-1, OXA-1, CTX-M-group, SHV-group	4	1	4	0.06	16	≤ 0.03	1	≤ 0.03
	K. pneumoniae	SR200279	NDM-1, CTX-M-15, TEM-1, OXA-1, SHV-group	4	1	4	0.5	> 32	0.5	> 32	0.125
	K. pneumoniae	SR200282	NDM-1, CTX-M-15, TEM-1, OXA-1, SHV-group	4	2	4	0.5	> 32	0.5	> 32	0.125
	K. pneumoniae	SR200283	NDM-1, CTX-M-15, TEM-1, OXA-1, SHV-group	4	4	2	0.5	> 32	0.5	32	0.06
NDM negative	*K. aerogenes*	SR200232	AmpC	4	4	≤ 0.03	≤ 0.03	0.125	0.125	≤ 0.03	≤ 0.03
	K. pneumoniae	SR200264	CTX-M-15, TEM-1, SHV-11, OXA-1	4	2	0.125	0.06	0.06	0.06	≤ 0.03	≤ 0.03
	S. marcescens	SR200217	TEM-10, AmpC	8	32	0.25	0.25	0.06	0.06	0.06	0.06
	S. marcescens	SR200218	SHV-12, AmpC	4	4	0.06	0.06	0.06	0.06	0.06	0.06
	A. baumannii	SR200179	PER-1, ADC-11, OXA-23, OXA-66	> 32	> 32	1	1	32	32	32	16
	A. baumannii	SR200180	PER-1, ADC-11, OXA-69	16	16	0.06	0.125	0.5	0.25	0.25	0.25
	A. baumannii	SR200181	PER-1, ADC-11, OXA-23, OXA-66	> 32	> 32	2	1	32	32	32	16
	A. baumannii	SR200182	PER-1, ADC-11, OXA-66, OXA-72	16	32	0.25	0.25	> 32	32	32	16
	A. baumannii	SR200183	PER-1, ADC-25, OXA-23, OXA-66	32	> 32	0.25	0.25	32	16	16	8
	A. baumannii	SR200184	PER-1, ADC-25, OXA-23, OXA-66	> 32	> 32	0.25	0.25	32	16	16	8
	A. baumannii	SR200185	PER-1, ADC-25, OXA-23, OXA-66	> 32	> 32	0.25	0.25	32	16	16	8
	A. baumannii	SR200186	PER-1, TEM-1, ADC-11, OXA-66	> 32	32	0.5	0.5	1	1	1	1
	A. baumannii	SR200187	PER-1, ADC-25, OXA-23, OXA-66	> 32	> 32	0.25	0.25	> 32	32	32	32
	A. baumannii	SR200188	PER-1, ADC-25, OXA-23, OXA-66	> 32	> 32	0.25	0.25	> 32	32	32	32
	A. baumannii	SR200189	PER-1, TEM-1, ADC-11, OXA-66	> 32	> 32	0.25	0.25	1	1	1	0.25
	A. baumannii	SR200191	PER-1, GES-11, ADC-11, OXA-66, OXA-72	16	32	0.125	0.125	32	32	32	16
	A. baumannii	SR200192	ADC-33, OXA-82	2	2	0.5	0.5	> 32	32	32	32
	A. baumannii	SR200193	PER-1, ADC-25, OXA-23, OXA-66	> 32	> 32	0.25	0.25	32	32	16	16
	A. baumannii	SR200194	PER-1, ADC-25, OXA-23, OXA-66	> 32	> 32	≤0.03	≤ 0.03	16	8	4	4
	A. baumannii	SR200195	PER-1, ADC-25, OXA-23, OXA-66	> 32	> 32	0.25	0.25	32	16	16	8

A. baumannii	SR200196	PER-1, ADC-25, OXA-23, OXA-66	> 32	32	0.25	0.25	32	16	16	8
A. baumannii	SR200197	PER-1, ADC-25, OXA-23, OXA-66	16	16	0.25	0.25	16	16	8	8
A. baumannii	SR200198	PER-1, ADC-11, OXA-66	8	16	0.25	0.5	2	1	2	1
A. baumannii	SR200200	ADC-33, OXA-82	4	2	0.5	0.5	> 32	32	32	32
A. baumannii	SR200201	PER-1, ADC-25, OXA-23, OXA-66	> 32	> 32	0.25	0.25	32	16	16	8
A. baumannii	SR200202	PER-1, ADC-25, OXA-23, OXA-66	32	> 32	0.25	0.25	32	32	16	16
A. baumannii	SR200203	PER-1, ADC-25, OXA-23, OXA-66	> 32	> 32	0.5	0.25	32	16	16	8
A. baumannii	SR200204	PER-1, ADC-11, OXA-66, OXA-72	> 32	> 32	0.5	0.25	32	32	16	16
A. baumannii	SR200205	PER-1, ADC-11, OXA-66, OXA-72	> 32	> 32	0.5	0.25	32	32	32	16
A. baumannii	SR200206	PER-1, ADC-11, OXA-66, OXA-72	> 32	> 32	0.5	0.5	32	32	32	16
A. baumannii	SR200207	PER-1, ADC-11, OXA-66, OXA-72	> 32	> 32	0.25	0.125	32	32	32	32
A. baumannii	SR200208	ADC-33, OXA-82	8	8	0.25	0.5	16	16	4	4
P. aeruginosa	SR200210	AmpC, OXA-50-like	2	2	0.25	0.25	1	1	0.125	0.125

[a] MICs were determined with or without β-lactamase inhibitors such as 100 μg/mL of dipicolinic acid (DPA) and 4 μg/mL of avibactam (AVI)

271 Table 2 *In vitro* activity of CFDC and reference compounds against *E. coli* isogenic mutants

Species	Test strains	MIC (μg/mL) in the presence of 1 mM IPTG		
		CFDC	CAZ	MEM
E. coli	BL21(DE3)/pET9a (vector control)	0.063-0.125	0.031-0.125	0.031-0.063
E. coli	BL21(DE3)/pET9a::TEM-1	0.063	0.031	0.031
E. coli	BL21(DE3)/pET9a::SHV-1	0.25	0.125	0.031
E. coli	BL21(DE3)/pET9a::SHV-3	0.125	0.125	0.031
E. coli	BL21(DE3)/pET9a::SHV-4	0.5	8	0.031
E. coli	BL21(DE3)/pET9a::SHV-5	0.25-0.5	8	0.031
E. coli	BL21(DE3)/pET9a::SHV-11	0.125	0.063	0.031
E. coli	BL21(DE3)/pET9a::SHV-12	0.5	4	0.063
E. coli	BL21(DE3)/pET9a::SHV-26	0.125	0.063	0.031
E. coli	BL21(DE3)/pET9a::SHV-28	0.125	0.125	0.031
E. coli	BL21(DE3)/pET9a::CTX-M-15	0.125	0.25	0.031
E. coli	BL21(DE3)/pET9a::CTX-M-27	0.5	2	0.063
E. coli	BL21(DE3)/pET9a::PER-1	1	>16	0.031
E. coli	BL21(DE3)/pET9a::KPC-2	0.125	1	2
E. coli	BL21(DE3)/pET9a::KPC-3	0.125	1	0.5
E. coli	BL21(DE3)/pET9a::NDM-1	4	>16	8
E. coli	BL21(DE3)/pET9a::ACT-1	0.125	0.25	0.063
E. coli	BL21(DE3)/pET9a::ACT-20-like (V68I,G91S)	0.125	0.125	0.063
E. coli	BL21(DE3)/pET9a::OXA-1	0.125	0.063	0.031
E. coli	BL21(DE3)/pET9a::OXA-23	0.125	0.125	0.125
E. coli	BL21(DE3)/pET9a::OXA-24	0.125	0.125	1

272

273

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