Poster 1455

IDWeek 2020
22–25 October, 2020

Potential Mechanisms of Cefiderocol MIC Increase in Enterobacterales in *In Vitro* Resistance Acquisition Studies

Y Yamano,[1] M Takemura,[1] R Nakamura,[2] R Echols,[3]

[1]Shionogi & Co. Ltd, Osaka, Japan; [2] Shionogi Techno Advance Research, Osaka, Japan; [3]Infectious Disease Drug Development Consulting LLC, Easton, CT, USA

Contact information:
Yoshinori Yamano
3-1-1, Futaba-cho, Toyonaka,
Osaka, 561-0825, Japan
Phone: +81-6-6331-6293
Fax: +81-6-6331-8612
Email: yoshinori.yamano@shionogi.co.jp

Abstract (Revised)

Background: Cefiderocol (CFDC) is a novel siderophore, iron-chelating cephalosporin, which is transported into bacteria via iron transporters. CFDC has potent *in vitro* and *in vivo* activity against all aerobic Gram-negative bacteria, including carbapenem-resistant strains. To date, clinical isolates with cefiderocol MIC >4 μg/mL have been found infrequently, in which the presence of a few β-lactamases or altered iron transport was found. We investigated potential new mechanisms causing CFDC MIC increases in non-clinical studies.

Methods: The mutation positions were determined by whole genome sequencing using four *K. pneumoniae* mutants including two KPC producers and one NDM producer that had shown CFDC MIC increases in previous *in vitro* resistance-acquisition studies. The mutant strains were obtained at the frequency of 10^{-7} to $<10^{-8}$ by spreading bacteria on standard Mueller–Hinton agar medium containing CFDC at concentrations of 10× MIC, with or without apo-transferrin (20 μg/mL). CFDC MIC was determined by broth microdilution using iron-depleted cation-adjusted Mueller-Hinton broth based on Clinical and Laboratory Standards Institute guidelines. The emergence of MIC increase mutants was also assessed by *in vitro* chemostat models under humanized plasma pharmacokinetic exposures of CFDC.

Results: The possible resistance mechanisms were investigated. Mutation of *baeS* or *envZ*, sensors of two-component regulation systems, were found in three or two mutants among the tested four isolates, respectively, and caused the MIC to increase 4- to 32-fold. The altered expression level of iron transporter genes by the *envZ* mutation could affect CFDC susceptibility, although the specific genes by the *baeS* mutation have not been identified. In addition, the mutation of *exbD*, an accessory protein related to iron transport, was identified in one case and caused the MIC increase of >8-fold. *In vitro* chemostat studies using two isolates (one NDM producer and one KPC producer) showed no resistance acquisition during 24-hour exposure.

Conclusions: The mutation of two-component regulation systems (BaeSR and OmpR/EnvZ) and iron transport-related proteins were shown to be possible mechanisms by which CFDC MIC increases, but these mutants did not appear under human PK exposures.

Introduction

- Cefiderocol (CFDC) is a novel siderophore cephalosporin with activity against a wide variety of Gram-negative bacteria, including carbapenem-resistant Enterobacterales and non-fermenters.
- CFDC has been approved in the USA for the treatment of patients with complicated urinary tract infections (cUTI) and hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia (HABP/VABP) caused by Gram-negative bacteria and in Europe for the treatment of infections due to Gram-negative pathogens with limited treatment options in 2019 [1, 2].
- The high CFDC MIC in the clinical isolates, which appeared infrequently (161 isolates with CFDC MIC of >4 μg/mL among 28,629 isolates) in the 3-years-concecutive multinational surveillance studies (SIDERO-WT) conducted between 2014 and 2017 was mainly due to the production of multiple β-lactamases such as PER or NDM [3, 4].
- The non-clinical studies showed the relationship with iron metabolism mutation and CFDC MIC increase. The deficiency of specific iron transporters (Fiu and Cir in case of *E. coli* and *K. pneumoniae*) have been shown to cause the CFDC MIC increase [5].
- In this study, we evaluated the molecular profile of the isolates of K. pneumoniae which showed high MIC to CFDC in the frequency of the resistance studies.

Table 1. Dosing regimen which were used to recreate humanized PK in chemostat models

Drugs	Dosing regimens used in this study
Cefiderocol	2 g, q8h, 3h-infusion
Ceftazidime/avibactam	ceftazidime 2 g/avibactam 0.5 g, q8h, 2h-infusion
Meropenem	1 g, q8h, 0.5h-infusion

Materials and Methods

Bacterial strains

- Four strains of *K. pneumoniae* were used in this study; ATCC 13883, two KPC producers (VA-361 and VA-384) and one NDM producer (NCTC 13443).

Isolation and characterization of the mutants with CFDC MIC increase

- The mutant strains were obtained by spreading the overnight culture of each strain on standard Mueller–Hinton agar medium containing CFDC at concentrations of 10× MIC, with or without apo-transferrin (20 μg/mL).
- The MICs were determined by broth microdilution methods recommended by Clinical and Laboratory Standards Institute guidelines. The CFDC MIC was determined in the iron-depleted cation-adjusted Mueller-Hinton broth [6].
- The mutation site was determined by whole genome sequencing.

***In vitro* chemostat studies**

- The bactericidal activity against 2 isolates (NCTC 13443 and VA-361), which showed high MIC in the mutant, under humanized PK exposure was evaluated using chemostat models. The PK profiles which occurred by the dosing regimen shown in **Table 1** were reproduced in this study.
- The bacterial suspension was incubated under the humanized PK exposure for 24 hours with the initial inoculum of 3×10^5 to 1×10^6 CFU/mL, and the viable cells were counted every 2 hours.

Results

- The mutant strains were obtained at the frequency of 10^{-7} to $<10^{-8}$ by spreading bacteria on standard Mueller–Hinton agar medium containing CFDC at concentrations of 10× MIC, with or without apo-transferrin (20 μg/mL). (**Table 2**).
- Against ATCC 13663, the 32-fold MIC increase was observed probably due to *baeS* mutation, but the mutant MIC was 2 μg/mL (**Table 3-1**).
- Against an NDM producer NCTC 13443, >8-fold MIC increase was observed, and the mutant MIC was high (>32 μg/mL). Variable gene mutation such as *baeS*, *exbD* and *yicM* in each mutant was observed (**Table 3-2**).
- Against 2 KPC producers VA-361 and VA-384, only ≤4-fold MIC increase was observed, and the mutant MIC was not so high (8 to 16 μg/mL). The gene mutation in *envZ*, *ompR* and *baeS* was observed in these mutants (**Table 3-3, 4**)
- Mutation of *baeS*, a sensor of two-component regulation systems, was observed in the 3 strains among 4 test strains. It is expected that the *baeS* mutation could affect the expression of various genes, but the specific CFDC MIC increase-related genes by the *baeS* mutation have not been identified (**Table 4**).
- Mutation of *envZ* or *ompR*, two-component regulation systems, was observed in 2 strains among 4 test strains. These mutations were reported to be contributed to the MIC increase of other catechol-substituted cephalosporin, which could be due to the altered expression level of iron transporter genes [8] (**Table 4**).
- Mutation of exbD, an accessory protein related to iron transport, was identified in one case and caused the MIC increase by >8-fold (**Table 4**).
- Against VA-361, bactericidal action without re-growth for 72 hours was observed under the human PK exposure of CFDC (Fig. 1). Against NCTC13443, no resistant colonies were detected for 72 hours although re-growth was observed (Fig. 2)

Table 2. Frequency of resistance to CFDC in *K. pneumoniae*

	Frequency of resistance appearance on MHA	
	without apo-T	with apo-T
K. pneumoniae ATCC 13883	$<1 \times 10^{-8}$	$<1 \times 10^{-8}$
K. pneumoniae NCTC 13443 (NDM producer)	Not determined	4×10^{-7}
K. pneumoniae VA-361 (KPC producer)	$<2.1 \times 10^{-8}$	1.9×10^{-7}
K. pneumoniae VA-384 (KPC producer)	1.3×10^{-7}	2.2×10^{-8}

Results

Table 3. Profile of the resistant mutants

1. *K. pneumoniae* ATCC 13883
   - BaeS (transcription regulator) mutation in all 5 isolates
     - Val295Gly mutation in all 5 isolates

	MIC (μg/mL) of						
	CFDC	CAZ	CFPM	MEPM	PIPC/TAZ	CAZ/AVI	CEF/TAZ
Parent	0.063	0.25	0.063	≤ 0.031	4	0.25	0.5
baeS Mutant	2	1	0.125 to 0.25	≤ 0.031	4 to 8	1 to 2	2

2. *K. pneumoniae* NCTC 13443 (NDM producer)
   - BaeS (transcription regulator) mutation in 2 of 5 isolates
     - Val295Gly or Thr 279 Pro mutation
   - ExbD (TonB-dependent energy transduction) mutation in 1 of 5 isolates
     - Leu49frame shift mutation
   - YicM (Unknown function) mutation in 2 of 5 isolates
     - Gly32Asp mutation in both isolates

	MIC (μg/mL) of						
	CFDC	CAZ	CFPM	MEPM	PIPC/TAZ	CAZ/AVI	CEF/TAZ
Parent	4	> 32	> 32	> 32	> 32	> 32	> 32
baeS mutant	>32	> 32	> 32	> 32	> 32	> 32	> 32
exbD mutant	>32	> 32	> 32	> 32	> 32	> 32	> 32
yicM mutant	>32	> 32	> 32	> 32	> 32	> 32	> 32

3. *K. pneumoniae* VA-361 (KPC producer)
   - EnvZ (transcription regulator) mutation in 3 of 4 isolates
     - Val124Gly or Val147Gly or Ile152Asp mutation
   - OmpR (transcription regulator) mutation in 1 of 4 isolates
     - Met62Arg mutation

	MIC (μg/mL) of						
	CFDC	CAZ	CFPM	MEPM	PIPC/TAZ	CAZ/AVI	CEF/TAZ
Parent	4	> 32	> 32	16	> 32	0.5	> 32
envZ mutant	4 to 16	> 32	> 32	16	> 32	2	> 32
ompR mutant	8	> 32	> 32	16	> 32	1	> 32

4. *K. pneumoniae* VA-384 (KPC producer)
   - EnvZ (transcription regulator) mutation in 4 of 5 isolates
     - Leu18 frameshift or Val54Gly or Val145Gly or Ile152Asn mutation
   - BaeS (transcription regulator) mutation in 1 of 5 isolates
     - Thr200Pro mutation

	MIC (μg/mL) of						
	CFDC	CAZ	CFPM	MEPM	PIPC/TAZ	CAZ/AVI	CEF/TAZ
Parent	4	> 32	> 32	>32	> 32	1	> 32
baeS mutant	16	> 32	> 32	16	> 32	2	> 32
envZ mutant	16	> 32	> 32	16	> 32	2	> 32

Table 4. Summary of the possible factors which might affect CFDC MIC increase

Mutated Genes	CFDC MIC increse	Possible fuction related with CFDC MIC increase
baeS	0.063 → 2	Two-component transcriptional regulator, which might affect the expression of a variety of genes related with envelope stress response pathway. The genes which affect cefiderocol activity under the cotrol by BaeS/R have not been identified.
	4 → >32	
	4 → 16	
ompR/envZ	4 → 16	Two-component transcriptional regulator which has been reported to affect the expression of iron transporters
	4 → 8	
	4 → 16	
exbD	4 → >32	TonB-dependent energy transduction system which has been reported to affect the ability of iron transporters

Fig. 1. *In vitro* bactericidal activity against *K. pneumoniae* VA-384 under human PK exposure

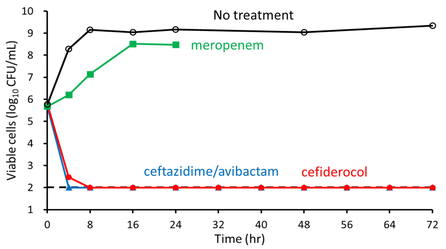
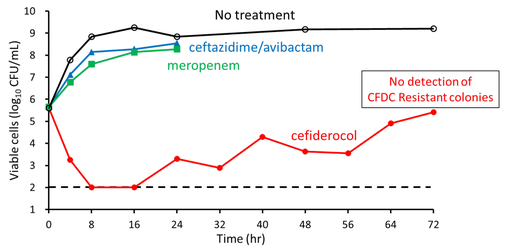


Fig. 2. *In vitro* bactericidal activity against *K. pneumoniae* NCTC 13443 under human PK exposure



Conclusions

- The mutation of two-component regulation systems (BaeSR and OmpR/EnvZ) and iron transport-related proteins were shown to be possible mechanisms causing CFDC MIC increases.
- The potential risk for the resistance acquisition to CFDC was not high against *K. pneumoniae* as no resistant mutants emerged under human PK exposure.

References

1. Fetroja [cefiderocol] for injection, for intravenous use. Prescribing Information. Shionogi Inc., Florham Park, NJ, USA. 2020.
2. Fetcroja [cefiderocol]. 1 g powder for concentrate for solution for infusion. Summary of Product Characteristics. Shionogi B.V., Kingsfordweg 151, 1043 GR, Amsterdam, Netherlands. 2020.
3. Hackel MA, et al. Antimicrob Agents Chemother 2018;62:pii:e00093-17.
4. Kohira N et al. J Glob Antimicrob Resist 2020; 22:738-741
5. Ito A et al. Antimicrob Agents Chemother 2017;61:pii: e01454-17.
6. CLSI. CLSI supplement M100: M100–S30. Wayne, PA: CLSI; 2020.
7. Gerken H et al. mbio 2020;11:pil:e01192-20