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Molecular Profile of β-Lactamase Genes and Siderophore-Dependent Iron Transporter Genes of Cefiderocol High MIC Isolates from SIDERO-WT Studies

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Abstract (Revised)

Background: Cefiderocol (CFDC) is a novel siderophore cephalosporin with efficacy against Gram-negative (GN) bacteria, including carbapenem-resistant Enterobacterales and non-glucose-fermenters such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. In consecutive multinational surveillance (SIDERO-WT) studies (SIDERO-WT-2014 to -2016 studies conducted from Nov 2014 to Oct 2017), CFDC demonstrated activity with minimum inhibitory concentrations (MICs) of ≤4 μg/mL against 99.4% of 28,629 GN clinical isolates. We conducted molecular characterization of 161 isolates with CFDC MICs >4 μg/mL from the SIDERO-WT studies.

Methods: A total of 161 isolates underwent whole genome sequencing by Illumina Hiseq. Analyses were done using the CLC genomics workbench (Qiagen) for possible resistance-related genes (e.g., β-lactamases, porin channels or penicillin-binding protein genes) and some TonB-dependent siderophore uptake receptor genes (*fiu*, *cir*, *piu*). Fiu and Cir in *Escherichia coli* and Piu in *P. aeruginosa* are the iron-siderophore transporters involved in CFDC uptake.

Results: Of 161 isolates with CFDC MIC >4 μg/mL, 128 were *A. baumannii*, 22 Enterobacterales, 7 *Burkholderia cepacia* complex, 2 *P. aeruginosa*, and 2 *Stenotrophomonas maltophilia*. Genes encoding bla_{PER}/bla_{VEB} extended-spectrum β-lactamases and NDM-type metallo-β-lactamases were detected in some isolates, but other β-lactamase genes (*bla*) were not shown to be linked to high CFDC MICs. bla_{PER}/bla_{VEB} were found only in *A. baumannii* and bla_{NDM} was found in *A. baumannii* and *Klebsiella pneumoniae*. In 128 *A. baumannii* isolates, 103 harbored bla_{PER} or bla_{VEB}, including bla_{PER} positive isolates from Russia (n=87) and Turkey (n=6) and 4 bla_{VEB} positive isolates from USA. Nine NDM-positive isolates (7 *K. pneumoniae*, 2 *A. baumannii*) were found. Disruption of iron transport genes was also detected in some isolates, including *piuA* (11 *A. baumannii*, 1 *P. aeruginosa*) and *fiuA* (4 *B. multivorans*, 1 *Proteus mirabilis*). No *cir* homologs were also found in 2 *B. multivorans* with *fiuA* disruption.

Conclusions: PER and NDM could reduce susceptibility to CFDC; such isolates have been seen in some countries. Iron transporter disruption was also observed in some isolates with high CFDC MICs, but not consistently; the contribution of these deficiencies in *A. baumannii* and *B. multivorans* requires further study.

Introduction

- Cefiderocol (CFDC) is a novel siderophore cephalosporin with activity against a wide variety of Gram-negative bacteria, including carbapenem-resistant Enterobacterales and glucose 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 [1, 2].
- We have conducted 3-years-concecutive multinational surveillance studies (SIDERO-WT-2014 to -2016) conducted between Nov 2014 to Oct 2017 to evaluate the *in vitro* activity of cefiderocol against clinical isolates collected in Europe and North America and to evaluate the carbapenemase profile of the subset of meropenem-non-susceptible isolates from the same studies [3].
- The elevated CFDC MIC (>4 μg/mL) of all 39 isolates observed in SIDERO-WT-2014 study were shown to be associated with multiple factors including the presence of specific β-lactamases, PER and NDM [4].
- Non-clinical studies suggested that the deficiency of specific outer membrane iron-siderophore transporters caused an MIC increase of 8- to 16-fold, suggesting these iron uptake transporters contributed to the penetration of CFDC through the outer membrane of Gram-negative bacteria. In *E. coli* and *K. pneumoniae*, Fiu and Cir were shown to be the primary iron transporters responsible for uptake of CFDC. In *P. aeruginosa*, Piu, a Fir homologue, was shown to be the major iron transporter of CFDC [5].
- In this study, we evaluated the molecular profile of the isolates which showed high MIC to CFDC in SIDERO-WT studies focusing on bla_{PER}, bla_{NDM} and *fiu* (*piu*) and *cir* iron transporters.

Materials and Methods

Bacterial strains and MIC determination

- The 161 isolates which showed CFDC MIC of >4 μg/mL, defined as non-susceptible based on CLSI investigational breakpoints, among a total of 28,629 isolates were used (19,119 Enterobacterales, 4,942 *Pseudomonas aeruginosa*, 3,231 *Acinetobacter baumannii* complex, 1,173 *Stenotrophomonas maltophilia* and 164 *Burkholeria cepacia* complex) from SIDERO-WT-2014 to -2016 studies. These isolates were collected from North America (United States and Canada) and European countries (Czech Republic, France, Germany, Greece, Hungary, Italy, Russia, Spain Sweden, Turkey and United Kingdom) from November 2014 to October 2017.
- MIC of CFDC was determined by International Health Management Associate (IHMA) using the broth microdilution in iron-depleted cation-adjusted Muller Hinton broth (ID-CAMHB) as recommended by CLSI [6].

Whole genome sequencing analysis

- Whole genome sequencing was conducted to evaluate the presence of bla_{PER}, bla_{NDM}, truncation of Piu, Fiu or Cir, and PBP 3 modification, which could be related with CFDC resistance, in the 161 isolates which showed CFDC MIC of >4 μg/mL.

Results

- CFDC showed the MIC of ≤4 μg/mL against >99% of a total of 28,629 isolates from SIDERO-WT-2014 to -2016 studies. A total of 161 isolates showed CFDC MIC of >4 μg/mL, defined as non-susceptibility to CFDC based on CLSI investigational breakpoint. (**Fig. 1**).
- Of these 161 isolates, 128 were *A. baumannii*, followed by 22 Enterobacterales, 7 *Burkholderia cepacia* complex, 2 *P. aeruginosa*, and 2 *Stenotrophomonas maltophilia*. The frequency of the isolate was high for *A. baumannii* complex and *B. cepacia* complex at 4 and 4.3%, respectively (**Table 1**).
- A large variation of the frequency of the high CFDC MIC isolates was observed among different countries. The frequency was the highest in Russia (5.7%), followed by Turkey (1.2%), Italy (0.5%), United Kingdom (0.3%) and United States (0.3%) (**Fig. 2**).
- Among a total of 161 isolates, bla_{PER} or bla_{VEB} was identified in 103 isolates, followed by 9 bla_{NDM}-positive isolates. In addition, the truncation of iron transporters Piu, Fiu or Cir was identified in 23 isolates, and 16 isolates among these 23 isolates had bla_{PER}, bla_{VEB} or bla_{NDM}. The modification of PBP 3, which is a target protein of CFDC, was identified in 3 isolates (**Fig. 3**).
- Many of the isolates with CFDC MIC of 32 to >256 μg/mL were bla_{PER} or bla_{VEB}-positive isolates, and the addition of serine-type β-lactamase inhibitors such as avibactam have been reported to decrease CFDC MIC significantly [4]. On the other hand, many of the isolates having bla_{NDM}, iron transporter truncation or PBP 3 modification showed CFDC MIC of 8 to 32 μg/mL, which is low-level resistant (**Fig. 4**).

Non-fermenters (Table 2)

- Of 139 non-fermenters, 128 isolates were *A. baumannii*, followed by 6 *Burkholderia multivorans*, each 2 of *P. aeruginosa* and *S. maltophilia* and 1 *B. cepacia*.
- Of 128 *A. baumannii*, 103 isolates had bla_{PER} or bla_{VEB} , and 86 of these 103 isolates were isolated in Russia. In addition, 19, 3 and 2 isolates had Piu disruption, PBP3 modification and bla_{NDM}, respectively although specific resistance determinants were not identified in other 17 isolates (Fig. 4).
- Of other 11 non-fermenter isolates, 4 isolates of *B. multivorans* had Fiu disruption, and 2 among these 4 isolates also had no Cir homologues.

Enterobacterales (Table 3)

- Of 22 Enterobacterales, 7 isolates were bla_{NDM} possessing *Klebsiella pneumoniae*, and 6 of these 7 isolates were isolated in Turkey. In addition, 1 isolate of *Proteus mirabilis* had Cir disruption.

References

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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 2017;61:pii:e00093-17.
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Results

Figure 1. MIC distribution of each bacterial species to cefiderocol in SIDERO-WT-2014 to -2016 studies

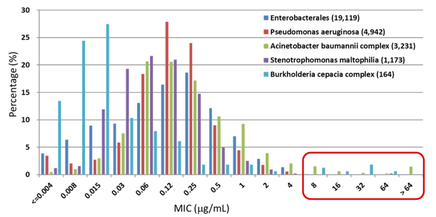

Table 1. Number and frequency of the CFDC high MIC isolates (MIC with ≥8 μg/mL)

Organisms (number of isolates)	Number of isolates with CFDC of ≥8 μg/mL			
	WT-2014	WT-2015	WT-2016	Total
Enterobacterales (19,119)	9	8	5	22 (0.12%)
P. aeruginosa (4,942)	1	1	0	2 (0.04%)
A. baumannii complex (3,231)	28	35	65	128 (4.0%)
S. maltophilia (1,173)	0	2	0	2 (0.17%)
B. cepacia complex (164)	1	5	1	7 (4.3%)
Total	39	51	71	161 (0.56%)

Figure 2. Distribution of CFDC high MIC isolates by country

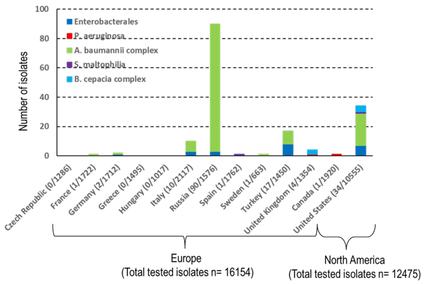

Figure 3. Frequency of the possible resistance mechanisms observed in the clinical isolates from SIDERO-WT studies

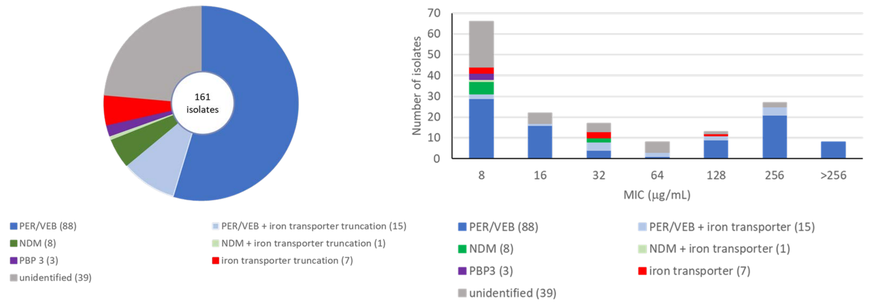

Figure 4. MIC of the CFDC high MIC isolates based on the possible resistance mechanisms

Table 2. Number and frequency of the CFDC high MIC isolates of each bacterial species with possible resistance mechanisms (Non-fermenters)

	bla_{PER} or bla_{VEB}		bla_{NDM}		Piu (Fiu) truncation	PBP3 modification	unidentified
	-	with Piu (Fiu) truncation	-	with Piu (Fiu) truncation			
A. baumannii (128)	88	15	1	1	3	3	17
B. multivorans (6)	-	-	-	-	4	-	2
P. aeruginosa (2)	-	-	-	-	-	-	2
S. maltophilia (2)	-	-	-	-	-	-	2
B. cepacia (1)	-	-	-	-	-	-	1

Russia	86
Turkey	6
USA	5
Italy	4
Germany	1
Sweden	1

Turkey	1
Italy	1

Table 3. Number and frequency of the CFDC high MIC isolates of each bacterial species with possible resistance mechanisms (Enterobacterales)

	bla_{PER} or bla_{VEB}		bla_{NDM}		Piu (Fiu) truncation	PBP3 modification	unidentified
	-	with Piu (Fiu) truncation	-	with Piu (Fiu) truncation			
K. pneumoniae (10)	-	-	7	-	-	-	3
S. marcescens (4)	-	-	-	-	-	-	4
E. cloacae (2)	-	-	-	-	-	-	2
K. aerogenes (2)	-	-	-	-	-	-	2
C. freundii (1)	-	-	-	-	-	-	1
C. koseri (1)	-	-	-	-	-	-	1
P. mirabilis (1)	-	-	-	-	1	-	0

Turkey	6
Russia	1

Conclusions

- *A. baumannii* possessing bla_{PER} or bla_{VEB} was observed most frequently from the clinical isolates with CFDC MIC of ≥8 μg/mL, many of which were from Russia, suggesting the possible contribution with beta-lactamase inhibitors to overcome the resistance.
- The NDM producers were also observed in *K. pneumoniae* and *A. baumannii* with CFDC MIC of ≥8 μg/mL, many of which were from Turkey.
- The truncation of iron transporters Fiu (Piu)/Cir, which could be related with CFDC penetration through the outer membrane, and modification of PBP3, a target protein of CFDC, was observed in some isolates of *A. baumannii* and *B. cepacia* complex.