In Vitro Activity of Cefiderocol and Comparator Agents against Molecularly Characterized Carbapenem-resistant *Enterobacterales* Clinical Isolates Causing Infection in United States Hospitals (2020)

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Introduction

- Cefiderocol represents a new addition to the antimicrobial armamentarium for the treatment of serious infections caused by Gram-negative bacteria.
- This molecule is a novel injectable siderophore cephalosporin, which hijacks the bacterial iron transport machinery to facilitate cell entry and achieve high periplasmic concentrations.
- This siderophore cephalosporin has broad *in vitro* activity against Gram-negative bacteria, including multidrug resistant (MDR) organisms like carbapenem resistant *Enterobacterales* (CRE), carbapenem resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.
  - In addition, cefiderocol remains stable to hydrolysis in the presence of serine β-lactamases (ESBLs, KPC and OXA-type carbapenemases) and metallo-β-lactamases.
- Cefiderocol was approved by the Food and Drug Administration (FDA) for the treatment of complicated urinary tract infections and nosocomial pneumonia based on clinical trials demonstrating noninferiority to comparator agents.
- The activities of cefiderocol and comparator agents were analyzed against *Enterobacterales*, including molecularly characterized CRE, as a part of the SENTRY Antimicrobial Surveillance Program for 2020 in the USA.

Materials and Methods

Bacterial organisms

- This study comprises a collection of 4,043 *Enterobacterales* collected from clinical specimens from patients hospitalized in 31 medical centers in 9 US Census Divisions. Only consecutive isolates (1 per patient infection episode) responsible for documented infections according to local criteria were included.
- Bacterial identification was confirmed by standard algorithms supported by MALDI-TOF (Bruker Daltonics, Bremen, Germany).

Susceptibility testing

- Isolates were tested for susceptibility by broth microdilution following the Clinical and Laboratory Standards Institute (CLSI) M07 (2018) guidelines.
- Frozen-form broth microdilution panels were manufactured by JMI Laboratories (North Liberty, IA, USA) and contained cation-adjusted Mueller-Hinton broth (CA-MHB) for comparator agents. Cefiderocol susceptibility testing used broth microdilution panels containing iron-depleted media as per the CLSI guidelines.
- Quality assurance was performed by sterility checks, colony counts, and testing CLSI-recommended quality control reference strains. MIC interpretations were performed using CLSI breakpoints for comparators and FDA/CLSI breakpoints for cefiderocol (≤4/8/≥16 μg/mL for susceptible, intermediate, and resistant). Imipenem-relebactam MIC interpretations used FDA breakpoints.
- Isolates displaying MIC values ≥4 μg/mL for imipenem (excluded for *Proteus mirabilis, P. penneri* and indole-positive *Proteus*) or meropenem were subjected to genome sequencing and screening of β-lactamase genes.

Screening of β-lactamase genes

- Selected isolates had total genomic DNA extracted by the fully automated Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor (Cleveland, OH, USA), which was used as input material for library construction.
- DNA libraries were prepared using the Nextera™ library construction protocol (Illumina, San Diego, CA, USA) following the manufacturer's instructions and were sequenced on MiSeq Sequencer platforms at JMI Laboratories.
- FASTQ format sequencing files for each sample set were assembled independently using *de novo* assembler SPAdes 3.11.0. An in-house software was applied to align the assembled sequences against a comprehensive in-house database containing known β-lactamase genes. This database was originally based on the NCBI Bacterial Antimicrobial Resistance Reference Gene Database (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA313047).

Results

- A total of 36 (0.9%) CRE isolates were detected, which were represented by 8 *Enterobacterales* species and most isolates carried bla_{KPC} (75.0%; 27/36) (Table 1).
- A small number of *Enterobacterales* (11.1%; 4/36) carried carbapenemase genes other than bla_{KPC}, as follows: 1 each of bla_{NDM-1}, bla_{NDM-5}, $bla_{OXA-232}$, and bla_{SME-2}.
  - Five (13.9%) *Enterobacterales* isolates did not carry any known carbapenemases.
- Cefiderocol inhibited all but 8 *Enterobacterales* at ≤4 μg/mL (99.8% susceptible) (Table 1 and Figure 1).
  - A single isolate (non-CRE) showed a resistant phenotype to cefiderocol (MIC, 32 μg/mL).
  - Among CRE isolates, all but 1 *K. pneumoniae* were inhibited by cefiderocol at ≤4 μg/mL (97.2% susceptible).
  - This single CRE isolate showed a cefiderocol MIC of 8 μg/mL and carried CTX-M-15, OXA-1, SHV-168, and TEM-1.
- In general, cefiderocol, imipenem-relebactam, meropenem-vaborbactam, ceftazidime-avibactam, piperacillin-tazobactam and carbapenems were active (92.2% susceptible) against non-CRE isolates (Table 2).
- Cefiderocol ($MIC_{50/90}$, 0.5/4 μg/mL; 97.2% susceptible) and ceftazidime-avibactam ($MIC_{50/90}$, 1/8 μg/mL; 94.4% susceptible) were the most active agents against CRE (Table 2).
  - All newest agents (i.e. cefiderocol, imipenem-relebactam, meropenem-vaborbactam, ceftazidime-avibactam) were active (100% susceptible) against the KPC subset.
- Finally, cefiderocol (MIC, 0.5-8 μg/mL) was the most active agent against non-carbapenemase CRE and CRE isolates carrying genes other than bla_{KPC} (Table 2).

Conclusions

- The cefiderocol activity was potent and consistent across *Enterobacterales* with different phenotypes and genotypes.
- Cefiderocol also demonstrated an *in vitro* activity advantage against non-carbapenemase CRE and isolates carrying genes other than bla_{KPC}, where approved β-lactam/β-lactamase inhibitor combinations showed limited activity.
- These data confirm cefiderocol as an important option for the treatment of serious infections caused by *Enterobacterales* and resistant subsets in patients hospitalized in US medical centers.

Acknowledgements

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Figure 1. Cumulative MIC distribution of cefiderocol against CRE and non-CRE subsets from the USA

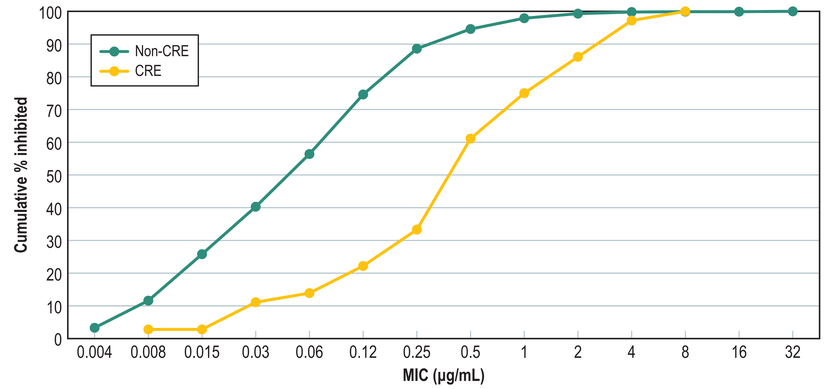

Table 1. MIC distribution of cefiderocol obtained against *Enterobacterales* and resistant subsets from the USA

Organism/ Organism group (no. of isolates)	No. and cumulative % of isolates inhibited at MIC (μg/mL) of:														MIC$_{50}$	MIC$_{90}$
	≤0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32		
Enterobacterales (4,053)	131 (3.2)	337 (11.5)	569 (25.6)	585 (40.0)	649 (56.0)	735 (74.2)	565 (88.1)	253 (94.3)	134 (97.7)	63 (99.2)	24 (99.8)	7 (>99.9)	0 (>99.9)	1 (100.0)	0.06	0.5
Non-CRE[a] (4,017)	131 (3.3)	336 (11.6)	569 (25.8)	582 (40.3)	648 (56.4)	732 (74.6)	561 (88.6)	243 (94.6)	129 (97.9)	59 (99.3)	20 (99.8)	6 (>99.9)	0 (>99.9)	1 (100.0)	0.06	0.5
CRE[a] (36)		1 (2.8)	0 (2.8)	3 (11.1)	1 (13.9)	3 (22.2)	4 (33.3)	10 (61.1)	5 (75.0)	4 (86.1)	4 (97.2)	1 (100.0)			0.5	4
KPC[b] (27)		1 (3.7)	0 (3.7)	3 (14.8)	1 (18.5)	3 (29.6)	4 (44.4)	6 (66.7)	5 (85.2)	3 (96.3)	1 (100.0)				0.5	2
Other[c] (4)								2 (50.0)	0 (50.0)	0 (50.0)	2 (100.0)				-	-
No-carbapenemases[d] (5)								2 (40.0)	0 (40.0)	1 (60.0)	1 (80.0)	1 (100.0)			-	-

[a] Non-CRE, includes isolates with imipenem and/or meropenem MIC ≤2 μg/mL; CRE, defined as isolates with imipenem (excluded for *P. mirabilis, P. penneri* and indole-positive *Proteus*) and/or meropenem MIC ≥4 μg/mL and includes *Citrobacter freundii* (3), *Enterobacter cloacae* (3), *Escherichia coli* (2), *Klebsiella aerogenes* (2), *K. oxytoca* (4), *K. pneumoniae* (18), *Serratia marcescens* (2), *Raoultella ornithinolytica* (2)
[b] Includes 12 isolates carrying bla_{KPC-2} and 15 bla_{KPC-3}
[c] Includes 1 isolate each of bla_{NDM-1} (MIC, 4 μg/mL), bla_{NDM-5} (MIC, 4 μg/mL), $bla_{OXA-232}$ (MIC, 0.5 μg/mL), and bla_{SME-2} (MIC, 0.5 μg/mL)
[d] Includes 5 CRE with no known carbapenemase genes detected

Table 2. Antimicrobial activity of cefiderocol tested against *Enterobacterales* and resistant subsets from the USA

Antimicrobial agent	MIC (μg/mL)			CLSI[a]		
	50%	90%	Range	%S	%I	%R
Non-CRE (4,017)						
Cefiderocol[b]	0.06	0.5	≤0.004 to 32	99.8	0.1	<0.1
Imipenem-relebactam[b]	0.12	0.5	≤0.03 to 8	99.9	0.1	0.0
Meropenem-vaborbactam	0.03	0.06	≤0.015 to 0.5	100.0	0.0	0.0
Ceftazidime-avibactam	0.12	0.25	≤0.015 to 8	100.0	—	0.0
Piperacillin-tazobactam	2	16	≤0.06 to >128	92.2	3.9	3.8
Aztreonam	0.12	>16	≤0.03 to >16	84.5	1.7	13.7
Ceftriaxone	≤0.06	>8	≤0.06 to >8	81.7	0.7	17.5
Ceftazidime	0.25	16	0.03 to >32	85.2	2.0	12.7
Cefepime	0.06	8	≤0.03 to >32	88.3	2.4	9.3
Meropenem	0.03	0.06	≤0.015 to 2	99.9	0.1	0.0
Imipenem	≤0.12	1	≤0.12 to 8	94.4	4.7	0.8
Ciprofloxacin	0.03	>4	≤0.008 to >4	77.9	3.4	18.7
Levofloxacin	0.06	8	≤0.015 to >32	80.3	3.2	16.5
Amikacin	2	4	≤0.25 to >32	99.6	0.3	0.1
Gentamicin	0.5	2	≤0.12 to >16	91.4	0.7	7.9
CRE (36)						
Cefiderocol[b]	0.5	4	0.008 to 8	97.2	2.8	0.0
Imipenem-relebactam[b]	0.12	4	0.06 to >8	80.6	5.6	13.9
Meropenem-vaborbactam	0.03	8	≤0.015 to >8	83.3	8.3	8.3
Ceftazidime-avibactam	1	8	≤0.015 to >32	94.4	—	5.6
Meropenem	8	>32	1 to >32	2.8	13.9	83.3
Imipenem	8	>8	4 to >8	0.0	0.0	100.0
KPC (27)						
Cefiderocol[b]	0.5	2	0.008 to 4	100.0	0.0	0.0
Imipenem-relebactam[b]	0.12	0.25	0.06 to 1	100.0	0.0	0.0
Meropenem-vaborbactam	0.03	1	≤0.015 to 2	100.0	0.0	0.0
Ceftazidime-avibactam	0.5	2	≤0.015 to 4	100.0	—	0.0
Meropenem	8	>32	1 to >32	3.7	14.8	81.5
Imipenem	8	>8	4 to >8	0.0	0.0	100.0
CRE Non-KPC (9)						
Cefiderocol[b]	2	—	0.5 to 8	88.9	11.1	0.0
Imipenem-relebactam[b]	4	—	0.12 to >8	22.2	22.2	55.6
Meropenem-vaborbactam	8	—	0.03 to >8	33.3	33.3	33.3
Ceftazidime-avibactam	2	—	0.25 to >32	77.8	—	22.2
Meropenem	16	—	2 to >32	0.0	11.1	88.9
Imipenem	>8	—	4 to >8	0.0	0.0	100.0

CRE, carbapenem resistant *Enterobacterales*; KPC, *Klebsiella pneumoniae* carbapenemase.
[a] Criteria as published by CLSI (2021) unless otherwise indicated; "—", breakpoint not available.
[b] Using FDA breakpoints. Imipenem-relebactam breakpoints applied to organisms other than *Morganella* spp., *Proteus* spp., and *Providencia* spp.

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