Official journal of the Spanish Society of Chemotherapy

Original

Revista Española de Quimioterapia
doi:10.37201/req/098.2023

Desiree Gijón[1,2*]
María García-Castillo[1,2*]
María del Carmen Fernández-López[3]
Germán Bou[2,3]
María Siller[4]
Jorge Calvo-Montes[2,4]
Cristina Pitart[5]
Jordi Vila[2,5]
Nuria Torno[6]
Concepción Gimeno[6]
Hugo Cruz[7]
Helena Ramos[7]
Xavier Mulet[2,8]
Antonio Oliver[2,8]
Patricia Ruiz-Garbajosa[1,2]
Rafael Canton[1,2]

In vitro activity of cefiderocol and other newly approved antimicrobials against multi-drug resistant Gram-negative pathogens recovered in intensive care units in Spain and Portugal

[1]Servicio de Microbiología. Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain
[2]CIBER en Enfermedades Infecciosas, Instituto de Salud Carlos III, Madrid, Spain
[3]Laboratorio de Microbiología. Complejo Hospitalario Universitario A Coruña, La Coruña, Spain
[4]Servicio de Microbiología. Hospital Universitario Marqués de Valdecilla, and Instituto de Investigación Sanitaria Marqués de Valdecilla (IDIVAL), Santander, Spain
[5]Laboratorio de Microbiología. Hospital Clinic i Provincial de Barcelona, Barcelona. Facultad de Medicina, Universidad de Barcelona. Instituto de Salud Global, Spain
[6]Servicio de Microbiología. Consorcio Hospital General Universitario de Valencia, Universidad de Valencia.Valencia, Spain
[7]Serviço de Microbiologia. Centro Hospitalar Univiersitário de Santo António. Porto, Portugal
[8]Servicio de Microbiología. Hospital Universitario Son Espases, Palma de Mallorca, Spain

Article history
Received: 4 August 2023; Revision Requested: 7 September 2023; Revision Received: 13 September 2023; Accepted: 19 September 2023; Published: 26 October 2023

ABSTRACT

Introduction. The antimicrobial resistance is a significant public health threat, particularly for healthcare-associated infections caused by carbapenem-resistant Gram-negative pathogens which are increasingly reported worldwide. The aim of this study was to provide data on the in vitro antimicrobial activity of cefiderocol and that of commercially available comparator antibiotics against a defined collection of recent clinical multi-drug resistant (MDR) microorganisms, including carbapenem resistant Gram-negative bacteria collected from different regions in Spain and Portugal.

Material and methods. A total of 477 clinical isolates of Enterobacterales, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* were prospectively (n=265) and retrospectively (n=212) included (2016-2019). Susceptibility testing was performed using standard broad microdilution and results were interpreted using CLSI-2021 and EUCAST-2021 criteria.

Results. Overall, cefiderocol showed a good activity against Enterobacterales isolates, being 99.5% susceptible by CLSI and 94.5% by EUCAST criteria. It also demonstrated excellent activity against *P. aeruginosa* and *S. maltophilia* isolates, all being susceptible to this compound considering CLSI breakpoints. Regarding *A. baumannii* (n=64), only one isolate was resistant to cefiderocol.

Conclusions. Our results are in agreement with other studies performed outside Spain and Portugal highlighting its excellent activity against MDR gram-negative bacteria. Cefiderocol is a therapeutic alternative to those available for the treatment of infections caused by these MDR bacteria.

Keywords: cefiderocol, siderophore cephalosporin, carbapenemase, multi-drug resistant, Gram-negative pathogens

Correspondence:
Rafael Cantón. Servicio de Microbiología, Hospital Universitario Ramón. Carretera de Colmenar Km 9,1. 28034-Madrid. Spain.
E-mail: rafael.canton@salud.madrid.org

*Both authors have equally contributed to this study.

Actividad *in vitro* de cefiderocol y otros antimicrobianos recientemente aprobados frente a gramnegativos multirresistentes aislados en unidades de cuidados intensivos en España y Portugal

RESUMEN

Introducción. La resistencia a los antimicrobianos constituye una importante amenaza para la salud pública, especialmente en el caso de las infecciones relacionadas con la asistencia sanitaria causadas por patógenos gramnegativos resistentes a los carbapenémicos, las cuales están aumentando en todo el mundo. El objetivo de este estudio fue proporcionar datos sobre la actividad antimicrobiana *in vitro* de cefiderocol y la de antibióticos comparadores disponibles en el arsenal terapéutico frente a una colección definida de microorganismos multirresistentes (MDR) obtenidos de muestras clínicas, incluidas bacterias gramnegativas resistentes a

carbapenemas procedentes de diferentes regiones de España y Portugal.

Material y métodos. Se recogieron un total de 477 aislados clínicos de Enterobacterales, *Pseudomonas aeruginosa, Acinetobacter baumannii* y *Stenotrophomonas maltophilia* de forma prospectiva (n=265) y retrospectiva (n=212) (2016-2019). El estudio de sensibilidad se realizó por microdilución standard y los resultados se analizaron empleando criterios del CLSI de 2021 y de EUCAST de 2021.

Resultados. En general, cefiderocol demostró una buena actividad frente a aislados de Enterobacterales, siendo 99,5% sensible según criterios del CLSI y 94,5% según los de EUCAST. Cefiderocol demostró una excelente actividad frente a aislados de *P. aeruginosa y S. maltophilia*, siendo todos ellos sensibles a este compuesto considerando los puntos de corte del CLSI. En relación a *A. baumannii* (n=64), sólo un aislado fue resistente a cefiderocol.

Conclusiones. Nuestros resultados concuerdan con los de otros estudios realizados fuera de España y Portugal en los que se destaca la excelente actividad de cefiderocol frente a bacterias gramnegativas MDR. Cefiderocol constituye una alternativa terapéutica a las disponibles en el tratamiento de las infecciones causadas por estos microorganismos.

Palabras clave: cefiderocol, cefalosporina siderófora, carbapenemasa, multirresistente, patógeno Gram-negativo.

INTRODUCTION

The emergence of antimicrobial resistance is a significant public health threat, particularly for healthcare-associated infections caused by carbapenem-resistant Gram-negative pathogens. These have been increasingly reported worldwide [1]. Cefiderocol (S-649266), which has been developed by Shionogi & Co. Ltd, is a novel siderophore cephalosporin targeting Gram-negative bacteria, including strains with carbapenem resistance [2,3]. The structural characteristics of cefiderocol show similarity to both ceftazidime and cefepime, which enable cefiderocol to withstand hydrolysis by β-lactamases. The unique chemical component is the addition of a catechol moiety on the C-3 side chain, which chelates iron and mimics naturally occurring siderophore molecules. Following the chelation of iron, cefiderocol is actively transported across the outer membrane of the bacterial cell to the periplasmic space via specialized iron transporter channels. Furthermore, cefiderocol has demonstrated structural stability against hydrolysis by both serine- and metallo-β-lactamases, including clinically relevant carbapenemases such as *Klebsiella pneumoniae* carbapenemases (KPCs), oxacillin carbapenemase-48 (OXA-48), and New Delhi metallo-β-lactamases (NDMs) [4,5]. Few resistance rates have been communicated to cefiderocol and exceptionally cefiderocol resistant isolates have been involved in hospital outbreaks [6,7].

The aim of this study was to provide data on the in vitro antimicrobial activity of cefiderocol and that of commercially available comparator antibiotics against a defined collection of recent clinical multi-drug resistant (MDR), including carbapenem-resistant Gram-negative bacteria both prospectively and retrospectively collected from different regions in Spain and Portugal. These two countries have increasing rates of carbapenem resistance in Gram-negative bacteria according to different surveillance studies [8–10].

MATERIAL AND METHODS

Study design and hospital participants. This is a multicentre study designed to provide data on the in vitro antimicrobial activity of cefiderocol and comparators against a collection of Gram-negative isolates both retrospective and prospective collected in different Spanish and Portuguese Hospitals. The participant laboratories were located in Spain (Complejo Hospitalario A Coruña, A Coruña; Hospital Universitario Marqués de Valdecilla, Santander; Hospital Clinic, Barcelona; Consorcio Hospital General Universitario, Valencia; Hospital Universitario Son Espases. Palma de Mallorca, Hospital Universitario Ramón y Cajal, Madrid) and Portugal (Centro Hospitalar Universitário de Santo António, Porto). Hospital Universitario Ramón y Cajal was also the central testing laboratory.

The study included two parts. The first one included a prospective collection of 400 contemporary clinical isolates recovered from February 2019 to December 2019. Each hospital collected a maximum of 50 consecutive non-replicate isolates of clinical origin: 20 Enterobacterales, 15 *Pseudomonas aeruginosa*, 10 *Acinetobacter baumannii* and 5 *Stenotrophomonas maltophilia*. The second one included a retrospective collection of 320 MDR isolates recovered from January 2016 to January 2019. Each hospital selected 40 clinical MDR isolates following this scheme: 18 Enterobacterales, 12 *P. aeruginosa*, 8 *A. baumannii* and 2 *S. maltophilia*). All isolates were recovered from significant clinical samples from patients admitted in Intensive Care Units (ICU) with intra-abdominal (IAI), urinary tract (UTI), low respiratory tract (LRTI), and skin and skin structure (SSSI) infections or bacteraemia. Isolates from urine and respiratory tracts were excluded if they were considered as colonizing bacteria. Isolates were identified to the species level using routine local laboratory procedures and were confirmed by MALDI TOF mass spectrometry (Bruker Daltonics, Germany) at central testing laboratory.

The study was approved by the Ethical Committee of the central testing laboratory (Reference 056/19).

Antimicrobial susceptibility testing. Antimicrobial susceptibility of cefiderocol and comparators were tested by using standard broth microdilution method according to International Standard ISO 20776-2 (ISO 20776-2:2021) in pre-prepared frozen 96-well microtiter plates supplied by IHMA Inc. (Schaumburg, IL, US). The antimicrobials and the tested concentration ranges (in mg/L) were as follows: cefiderocol (CFDC, 0.03-64), cefepime (FEP, 0.12-16), ceftazidime-avibactam (CZA, 0.125-16), ceftolozano-tazobactam (C/T, 0.125-16), meropenem (MEM, 0.06-16), meropenem-vaborbactam (MEV, 0.06-16), imipenem-relebactam (IMR, 0.03-64), aztreonam-avibactam

Table 1 Resistance mechanisms of the isolates

Microorganisms (no. of isolates)	Phenotypic resistance mechanism	Number of isolates
Enterobacterales (220)	ESBL	31
	Carbapenemase	25
	ESBL+ Carbapenemase	71
	MDR	93
P. aeruginosa (159)	Carbapenemase	41
	ESBL+ Carbapenemase	2
	MDR	96
	XDR	10
A. baumannii (64)	Carbapenemase (OXA-23)	9
	MDR	55
S. maltophilia (34)	Wild-type intrinsic resistant	30
	Wild-type intrinsic resistant + cotrimoxazole resistance	4

ESBL: extended-spectrum beta-lactamases. MDR (multi-drug resistant) and XDR (extremely-drug resistant) were defined following Magiorakos criteria [29]

(AZA, 0.125-16), ciprofloxacin (CIP, 0.06-8), minocycline (MINO, 0.25-8), tigecycline (TGC, 0.125-4), trimethoprim-sulfamethoxazole (SXT, 0.25-8) and colistin (CST, 0.12-8). Categorical clinical interpretations were performed in agreement with EUCAST 2021 [11] and CLSI 2021 [12] criteria. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were use as quality control for antimicrobial susceptibility testing.

Resistance mechanisms characterization. Mechanism of resistance, including extended spectrum beta-lactamases (ESBLs) and carbapenemases, were characterized in each participant laboratory both using phenotypic and genotypic techniques. These included disk diffusion assays, PCR and sequencing and whole genome sequencing [13–16].

RESULTS

Bacterial isolates and patient demographic data. A total of 477 clinical isolates of Enterobacterales (n=220, 46.1%), *P. aeruginosa* (n=159, 33.3%), *A. baumannii* (n=64, 13.4%), and *S. maltophilia* (n=34, 7.1%) were prospectively (265 isolates) and retrospectively (212 isolates) collected. Enterobacterales included 116 *Klebsiella* spp., 53 *E. coli*, 30 *Enterobacter cloacae* complex, 7 *Citrobacter* spp, 8 *Serratia marcescens*, 4 *Proteus* spp, 1 *Morganella morganii*, and 1 *Raoutella planticola*. All isolates were obtained from 477 patients, being the majority males (77.6%, n=370) and the mean age of all included patients was 64 years (range, 1 to 99 years). The most frequent infection was LRTI (36%, n=172) followed by bacteraemia (25.8%, n=123), UTI (21.6%, n=103), IAI (11.7%, n=56) and SSSI (0.5%, n=23).

Resistance mechanisms. Resistance mechanisms of the studied isolates breakdown in different species is shown in Table 1. Due to different methods used at local level to characterized the resistance mechanisms by different participants' centres, only phenotypic information was finally recorded.

Antimicrobial susceptibility. MIC and cumulative MIC distributions of different antimicrobial tested are shown in Table 2 to Table 5. Overall, cefiderocol showed a good activity ($MIC_{50/90}$ 0.25/2 mg/L) against Enterobacterales isolates, being 99.5% susceptible by CLSI and 94.5% by EUCAST. Altogether, cefiderocol demonstrated excellent activity ($MIC_{50/90}$ 0.12/2 mg/L for *E. coli* and 0.25/2 mg/L for *Klebsiella* spp.); being 100% susceptible by CLSI against all 53 isolates of *E. coli* and all 116 isolates of *Klebsiella* spp. Figures when using EUCAST breakpoints were 96.2% and 96.5%, respectively. Other antimicrobials with high activity were ceftazidime-avibactam ($MIC_{50/90}$ ≤0.12/≤0.12 mg/L for *E. coli* and ≤0.12/8 mg/L for *Klebsiella* spp.; 98.1/98.1% and 94/94% susceptible by CLSI and EUCAST, respectively) and meropenem-vaborbactam ($MIC_{50/90}$ ≤0.06/0.5 mg/L for *E. coli* and $MIC_{50/90}$ ≤0.06/0.5 mg/L for *Klebsiella* spp; 98.1/96.2% and 93.1/88.8% susceptible by CLSI and EUCAST, respectively).

On the other hand, cefiderocol showed also high activity ($MIC_{50/90}$ 1/4 mg/L) against *E. cloacae* isolates, being 96.7% susceptible by CLSI. Only one *E. cloacae* isolate was resistant to cefiderocol based on CLSI breakpoints (MIC 8 mg/L). However, based on EUCAST breakpoints, 80% of *E. cloacae* isolates were susceptible to cefiderocol, being 6 isolates resistant to cefiderocol. Finally, regarding other Enterobacterales (including 7 *Citrobacter* spp, 8 *Serratia marcescens*, 4 *Proteus* spp, 1 *Morganella morganii*, and 1 *Raoutella planticola*), cefiderocol showed a very good activity ($MIC_{50/90}$ 0.06/1 mg/L) with 100% of susceptibility based on CLSI and EUCAST breakpoints.

Table 2 **Distribution and cumulative MIC distribution for 220 Enterobacterales.**

Antimicrobial	No. of isolates (cumulative percentage) inhibited at MIC (mg/L):										
	≤0.03	0.06 or ≤0.06	0.12 or ≤0.12	0.25	0.5	1	2	4	8 or >4	16 or >8	>16 or 32
CFDC	34 (15.4)	26 (27.3)	22 (37.3)	**45 (57.7)**	27 (70)	23 (80.5)	31 (94.5)	11 (99.5)	1 (100)		
FEP			57 (25.9)	9 (30)	4 (31.8)	4 (33.6)	5 (35.9)	12 (41.4)	11 (46.4)	**24 (57.3)**	94 (100)
CZA			**151 (68.6)**	15 (75.5)	12 (80.9)	6 (83.6)	3 (85)	3 (86.4)	9 (90.4)	3 (91.8)	18 (100)
C/T			32 (14.5)	34 (30)	24 (40.9)	17 (48.6)	**16 (56.8)**	6 (59.5)	8 (63.2)	18 (71.4)	65 (100)
MEM		89 (40.4)	11 (45.4)	7 (48.6)	**14 (55)**	27 (67.3)	15 (74.1)	9 (78.2)	14 (84.5)	13 (90.4)	21 (100)
MEV		**114 (51.8)**	5 (54.1)	8 (57.7)	19 (66.4)	25 (77.7)	15 (84.5)	5 (86.8)	10 (91.4)	7 (94.5)	12 (100)
IMR	1 (0.5)	5 (3.2)	78 (38.7)	**26 (50.5)**	20 (59.6)	30 (73.3)	20 (82.4)	6 (85.1)	14 (91.5)	7 (94.6)	12 (100)
AZA			**148 (67.3)**	18 (75.4)	17 (83.2)	12 (88.6)	5 (91)	0 (91)	2 (91.8)	2 (92.7)	16 (100)
CIP		54 (24.5)	13 (30.5)	6 (33.2)	8 (36.8)	3 (38.2)	9 (42.3)	4 (44.1)	7 (47.3)	**116 (100)**	
TGC			26 (11.8)	40 (30)	**45 (50.4)**	46 (70.8)	29 (84)	19 (92.7)	15 (100)		
CST			25 (11.36)	**86 (50.4)**	63 (79.1)	10 (83.6)	2 (84.5)	1 (85)	4 (86.8)	29 (100)	

Cefiderocol (CFDC), Cefepime (FEP), Ceftazidime-avibactam (CZA), Ceftolozane-tazobactam (C/T), Meropenem (MEM), Meropenem-vaborbactam (MEV), Imipenem-relebactam (IMR), Aztreonam-avibactam (AZA), Ciprofloxacin (CIP), Tigecycline (TGC), Colistin (CST). Bold letter: MIC_{50} and Bold letter underlined: MIC_{90}

Cefiderocol demonstrated excellent activity against *P. aeruginosa* and *S. maltophilia* isolates ($MIC_{50/90}$ 0.12/0.5 mg/L and 0.12/0.25 mg/L, respectively). All these isolates were susceptible to cefiderocol considering CLSI breakpoints. Regarding *A. baumannii* (n=64), all tested isolates but one was susceptible to cefiderocol ($MIC_{50/90}$ 0.12/1 mg/L).

Susceptibility to cefiderocol varied between centers, lowest being 86.5% (center named F) and highest being 100% (centers named B and E) in Enterobacterales isolates. However, in *P. aeruginosa* isolates, when considering EUCAST breakpoints, cefiderocol showed 100% susceptibility in 6/7 centers and in one of them a susceptibility of 96.3% was observed (Table 6).

On the other hand, using CLSI breakpoints, a susceptibility of 100% was obtained in all centers for *P. aeruginosa* and *S. maltophilia* isolates. Regarding Enterobacterales, cefiderocol showed a 97.4% susceptibility in center named A; in *A. baumannii* isolates susceptibility was 94.5% in center named D (Table 6 and 7).

In addition, when comparing MIC values of cefiderocol with the comparators in meropenem susceptible and resistant Enterobacterales isolates, a low resistant percentage for cefiderocol (8.8%) was observed (Table 1S, supplementary material). Likewise, when comparing MIC value of cefiderocol with the comparators in meropenem susceptible and resistant *P. aeruginosa* isolates, we did not observe any isolate resistant to cefiderocol within meropenem resistant isolates (Tables 2S, supplementary material).

DISCUSSION

Carbapenem resistant bacteria have become an important public health concern due to their rapid spread worldwide. Their surveillance and study are a priority not only to the challenged of their detection and control, but also because of the significant therapeutic limitations they represent and the need to positioning new antimicrobials [17–19]. In order to help in prioritizing the research and development of new antimicrobial treatments the World Health Organization published a priority pathogens list [20]. Thus, new antimicrobials have being developed and marketed against MDR microorganisms, such as ceftazidime-avibactam, ceftolozane-tazobactam, imipenem-relebactam, meropenem-vaborbactam, plazomizin and cefiderocol among others [21–23]. In our study, cefiderocol showed an excellent activity in all studied isolates with MIC_{90} values ranging from 0.25 mg/L in *S. maltophilia* to 2 mg/L in Enterobacterales. Nevertheless, when considering *E. cloacae* alone MIC_{90} value was one 4 mg/L, one dilution higher. In addition, cefiderocol susceptibility rates using CLSI breakpoints ranged from 98.4% in *A. baumannii* (only one isolate was resistant) to 100% in *P. aeruginosa* and *S. maltophilia*. The corresponding figure for Enterobacterales was 99.5%, and only one *E. cloacae* resistant isolate to cefiderocol was observed in this bacterial group (MIC 8 mg/L).

On the other hand, overall susceptibility (susceptible plus intermediate or susceptible standard dose plus susceptible increased exposure) to novel beta-lactam/beta-lactamase inhibitor combinations such as imipenem-relebactam, meropenem-vaborbactam, ceftazidime-avibactam or aztreonam-avibactam in Enterobacterales was much lower than that

Table 3 **Distribution and cumulative MIC distribution for the 159 *P. aeruginosa* isolates.**

Antimicrobial	No. of isolates (cumulative percentage) inhibited at MIC (mg/L):										
	≤0.03	0.06 or ≤0.06	0.12 or ≤0.12	0.25 or ≤0.25	0.5	1	2	4	8 or >4	16 or >8	>16 or 32
CFDC	16 (10.1)	46 (38.9)	**40 (64.1)**	34 (85.5)	9 (91,2)	4 (93.7)	9 (99.3)	1 (100)			
FEP	3 (1.9)	0 (1.9)	8 (6.9)	26 (23.2)	28 (40.8)	**40 (66)**	18 (77.3)	6 (81.1)	4 (83.6)	7 (88)	19 (100)
CZA		5 (3.1)	8 (8.2)	17 (18.8)	11 (25.8)	14 (34.6)	13 (42.8)	7 (47.2)	**26 (63.5)**	29 (81.7)	29 (100)
C/T		5 (3.1)	8 (8.2)	15 (17.6)	10 (23.9)	19 (35.8)	17 (46.5)	5 (49.7)	**29 (67.9)**	28 (85.5)	23 (100)
MEM			7 (4.4)	2 (5.6)	3 (7.5)	7 (11.9)	30 (30.8)	**43 (57.8)**	37 (81.1)	10 (87.4)	20 (100)
MEV			5 (3.1)	3 (5)	3 (6.9)	3 (8.2)	2 (10.1)	1 (10.7)	7 (15.1)	42 (41.5)	**93 (100)**
IMR			3 (1.9)	19 (13.8)	42 (40.2)	**31 (59.7)**	21 (72.9)	1 (73.6)	8 (78.6)	2 (79.9)	32 (100)
AZA				2 (1.2)	2 (2.5)	11 (9.4)	24 (24.5)	19 (36.5)	**24 (51.6)**	42 (78)	35 (100)
CIP			7 (4.4)	39 (28.9)	**77 (77.3)**	27 (94.3)	3 (96.2)	1 (96.8)	2 (98.1)	3 (100)	
TGC		17 (10.7)	23 (25.2)	15 (34.6)	11 (41.5)	9 (47.1)	**14 (55.9)**	3 (57.9)	5 (61)	62 (100)	
CST			2 (1.26)	1 (1.9)	1 (2.5)	8 (7.5)	6 (11.3)	31 (30.8)	**110 (100)**		

Cefiderocol (CFDC), Cefepime (FEP), Ceftazidime-avibactam (CZA), Ceftolozane-tazobactam (C/T), Meropenem (MEM), Meropenem-vaborbactam (MEV), Imipenem-relebactam (IMR), Aztreonam-avibactam (AZA), Ciprofloxacin (CIP), Tigecycline (TGC), Colistin (CST). Bold letter: MIC_{50} and Bold letter underlined: MIC_{90}

Table 4 **Distribution and cumulative MIC distribution for the 64 *Acinetobacter baumannii* isolates. Numbers in black corresponded to MIC_{50} value and the underlying to MIC_{90}**

Antimicrobial	≤0.03	0.06 or ≤0.06	0.12 or ≤0.12	0.25 or ≤0.25	0.5	1	2 or ≤2	4	8 or >4	16 or >8	>16 or 32	64	>64
CFDC	5 (7.8)	10 (23.4)	**18 (51.5)**	19 (81.2)	5 (89.1)	5 (96,8)	1 (98.4)	0 (98.4)	1 (100)				
IMR								1 (1.5)	1 (3.1)	4 (9.3)	58 (100)		
MEM			1 (1.5)	0 (1.5)	6 (10.9)	7 (21.8)	6 (31.2)	5 (39.1)	2 (42.2)	0 (42.2)	37 (100)		
MEV			1 (1.5)	4 (7.8)	5 (15.6)	6 (25)	8 (37.5)	2 (40.6)	1 (42.2)	1 (43.7)	36 (100)		
CZA					1 (1.5)	1 (3.1)	0 (3.1)	4 (9.3)	7 (20.3)	14 (42.2)	37 (100)		
FEP			1 (1.5)	0 (1.5)	0 (1.5)	2 (4.7)	8 (17.2)	12 (35.9)	4 (42.2)	3 (46.8)	34 (100)		
CST				11 (17.2)	**27 (59.4)**	16 (84.3)	6 (93.7)	0 (93.7)	0 (93.7)	4 (100)			
CIP			14 (21.8)	7 (32.8)	4 (39.1)	2 (42)	0 (42)	0 (42)	0 (42)	37 (100)			
TGC			13 (20.3)	8 (32.8)	6 (42.2)	4 (48.4)	**22 (82.8)**	10 (98.4)	1(100)				
MIN				24 (37.5)	7 (48.4)	**1 (50)**	3 (54.7)	3 (59.4)	6 (68.7)	20 (100)			
STX				24 (37.5)	4 (43.7)	2 (46.9)	**6 (56.2)**	0 (56.2)	1 (57.8)	27 (100)			
SAM							11 (17.2)	16 (42.2)	**11 (59.4)**	13 (79.7)	4 (85.9)	0 (85.9)	9 (100)

Cefiderocol (CFDC), Imipenem-relebactam (IMR), Meropenm (MEM), Meropenem-vaborbactam (MEV), Ceftazidime-avibactam (CZA),), Cefepime (FEP), Colistin (CST), Ciprofloxacin (CIP), Tigecycline (TGC), Minocycline (MINO), Trimethoprim-sulfamethoxazole (SXT), Ampicillin-Sulbactam (SAM). Bold letter: MIC_{50} and Bold letter underlined: MIC_{90}

Table 5 **Distribution and cumulative MIC distribution for the 34 *Stenotrophomonas maltophilia* isolates**

Antimicrobial	No. of isolates (cumulative percentage) inhibited at MIC (mg/L):												
	≤0.03	0.06	0.12 or ≤0.12	0.25 or ≤0.25	0.5	1	2 or ≤2	4	8 or >4	16 or >8	>16 or 32	64	>64
CFDC	10 (29.4)	5 (44.1)	**9 (70.6)**	7 (91.2)	1 (94.1)	1 (97.1)	1 (100)						
IMR											**34 (100)**		
MEM											**34 (100)**		
MEV											**34 (100)**		
CZA						2 (5.8)	3(14.7)	0 (14.7)	3 (23.5)	3 (32.3)	**23 (100)**		
FEP								2 (5.8)	0 (5.8)	5 (20.6)	**27 (100)**		
CST				2 (5.8)	2 (11.7)	7 (32.3)	**7 (52.9)**	5 (67.6)	3 (76.5)	**8 (100)**			
CIP					4 (11.7)	6 (29.4)	**11 (61.7)**	3 (70.5)	5 (85,3)	**5 (100)**			
TGC				2 (5.8)	5 (20.6)	**14 (61.7)**	11 (94.1)	2 (100)					
MIN			3 (8.8)	0 (8.8)	**17 (58.8)**	9 (85.3)	1 (88.2)	1 (91.2)	1 (94.1)	2 (100)			
STX				**21 (61.7)**	4 (73.5)	0 (73.5)	5 (88.2)	1 (91.2)	0 (91.2)	3 (100)			
SAM											1 (2.9)	4 (14.7)	**29 (100)**

Cefiderocol (CFDC), Imipenem-relebactam (IMR), Meropenm (MEM), Meropenem-vaborbactam (MEV), Ceftazidime-avibactam (CZA),), Cefepime (FEP), Colistin (CST), Ciprofloxacin (CIP), Tigecycline (TGC), Minocycline (MINO), Trimethoprim-sulfamethoxazole (SXT), Ampicillin-Sulbactam (SAM). Bold letter: MIC_{50} and Bold letter underlined: MIC_{90}

Table 6 **Comparative clinical categorization of Enterobacterales and *P. aeruginosa* to cefiderocol in different participating centers**

Center (n[a] of isolates)	Cefiderocol (EUCAST breakpoints)				Cefiderocol (CLSI breakpoints)					
	Enterobacterales		*P. aeruginosa*		Enterobacterales			*P. aeruginosa*		
	S ≤ 2	R > 2	S ≤ 2	R > 2	S ≤ 4	I = 8	R ≥ 16	S ≤ 4	I = 8	R ≥ 16
A (n=66)	37 (94.9%)	2 (5.1%)	27 (100%)	0	38 (97.4%)	1 (2.56%)	0	27 (100%)	0	0
B (n=22)	12 (100%)	0 (0%)	10 (100%)	0 (0%)	12 (100%)	0	0	10 (100%)	0	0
C (n=65)	36 (94.7%)	2 (5.3%)	26 (96.3%)	1 (3.7%)	38 (100%)	0	0	27 (100%)	0	0
D (n=65)	36 (94.9%)	2 (5.3%)	27 (100%)	0 (0%)	38 (100%)	0	0	27 (100%)	0	0
E (n=61)	36 (100%)	0 (0%)	25 (100%)	0 (0%)	36 (100%)	0	0	25 (100%)	0	0
F (n=65)	32 (86.5%)	5 (13.5%)	28 (100%)	0 (0%)	37 (100%)	0	0	28 (100%)	0	0
G (n=35)	19(95%)	1 (5%)	15 (100%)	0 (0%)	20 (100%)	0	0	15 (100%)	0	0

A: central testing laboratory and collecting center, Spain; B-F: collecting centers, Spain; G: collecting center, Portugal.
S= susceptible; I = susceptible, increased exposure (EUCAST) and intermediate (CLSI); R = resistant

of cefiderocol with range from 90.5% for ceftazidime-avibactam to 82.7% for imipenem-relebactam. In *P. aeruginosa*, overall susceptibility (susceptible plus intermediate) to ceftolozane-tazobactam and ceftazidime-avibactam reached 78.6% and 86.1%, respectively. For colistin, this value was 96.2%. Finally, in *A. baumannii*, the overall susceptibility (susceptible plus intermediate) of the comparators ranged from 39.1% for meropenem and 68.8% for minocycline; for *S. maltophilia* these values were 88.2% for trimethoprim-sulfamethoxazole and 94.1% for minocycline.

Kazmierczak *et al.* [24] tested the activity of cefiderocol against carbapenem-non-susceptible Gram negative bacilli, their MIC_{50} and MIC_{90} values were comparable to those of ceftazidime-avibactam against metallo-beta-lactamase-negative Enterobacterales isolates and superior to all tested comparators against carbapenemase producing Enterobacterales (NDM- and VIM-positive isolates). Subsequently, Karlowsky *et al.* demonstrated an in vitro activity of cefiderocol superior to ceftazidime-avibactam, ceftolozane-tazobactam and cefepime against Enterobacterales, *P. aeruginosa* and *A. baumannii*

Table 7 **Antimicrobial activity of cefiderocol per center using CLSI breakpoints**

Center	Cefiderocol (CLSI breakpoints)							
	Acinetobacter baumannii				*Stenotrophomonas maltophilia*			
	S ≤ 4	I =8	R ≥16	Total (%)	S ≤ 4	I =8	R ≥16	Total (%)
A (n=15)	12 (100%)	0	0	12 (100%)	3 (100%)	0	0	3 (100%)
B (n=9)	4 (100%)	0	0	4 (100%)	5 (100%)	0	0	5 (100%)
C (n=11)	4 (100%)	0	0	4 (100%)	7 (100%)	0	0	7 (100%)
D (n=25)	17 (94,5%)	1 (5,5%)	0	18 (100%)	7 (100%)	0	0	7 (100%)
E (n=16)	13 (100%)	0	0	13 (100%)	3 (100%)	0	0	3 (100%)
F (n=12)	8 (100%)	0	0	8 (100%)	4 (100%)	0	0	4 (100%)
G (n=10)	5 (100%)	0	0	5 (100%)	5 (100%)	0	0	5 (100%)

S= susceptible; I = intermediate; R = resistant

isolates [25]. Furthermore, Cercenado *et al.* demonstrated a potent in vitro activity of cefiderocol against Gram-negative bacilli isolates in different types of infection. Regarding Enterobacterales, susceptibility to cedfiderocol was better (p <0.01) than that of ceftolozane-tazobactam and colistin but similar to meropenem and ceftazidime-avibactam. Moreover, in non-fermenting isolates, susceptibility to cefiderocol was significantly better than all comparators (p <0.01) [26]. Our results are in agreement with those obtained in these and other studies [2,27,28] highlighting its activity against MDR *P. aeruginosa*, *A. baumannii* and *S. maltophilia*.

Our study has some limitations, particularly those related to the investigation of the resistance mechanisms as per protocol it was locally performed with different phenotypic and molecular methods and techniques. Nevertheless, the study has a microbiological and clinical relevance as it provides valuable information of antimicrobial susceptibility of new antimicrobials against MDR isolates recovered in patients admitted at ICUs in Spain and Portugal. Both countries have an increased prevalence of these isolates [8–10]. In this gram-negative challenging collection, cefiderocol shows an excellent activity being a therapeutic alternative to those available for treatment of infections caused by MDR bacteria.

In conclusion, cefiderocol shows an excellent *in vitro* activity against both susceptible and non-susceptible Gram-negative microorganisms being a therapeutic alternative to those available for treatment of infections caused by MDR bacteria.

FUNDING

This study was funded by Shionogi Spain.

AO, GB, RC, and JV receive overarching funding for research by Plan Nacional de I+D+I, Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Ciencia, Innovación y Universidades, CIBERINFEC (21/13/00099, 21/13/0055, 21/13/00084, 21/13/00081) co-financed by the European Development Regional Fund 'A way to achieve Europe', Operative Program Intelligence Growth 2014–2020.

TRANSPARENCY DECLARATIONS

RC has participated in an educational program sponsored by GSK, Menarini, MSD and Shionogi. RC has research grants funded by MSD, Shionogi and Venatorx.

AO has participated in an educational program sponsored by MSD Pfizer and Shionogi. AO has research grants funded by MSD and Shionogi.

All other authors declare no conflicts of interest.

REFERENCES

1. WHO Regional Office for Europe/European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2022 – 2020 data. Copenhagen: WHO Regional Office for Europe. 2022.
2. Zhanel GG, Golden AR, Zelenitsky S, Wiebe K, Lawrence CK, Adam HJ, et al. Cefiderocol: A Siderophore Cephalosporin with Activity Against Carbapenem-Resistant and Multidrug-Resistant Gram-Negative Bacilli. Drugs. 2019;79(3):271–89. doi: 10.1007/S40265-019-1055-2.
3. Ito A, Nishikawa T, Matsumoto S, Yoshizawa H, Sato T, Nakamura R, et al. Siderophore Cephalosporin Cefiderocol Utilizes Ferric Iron Transporter Systems for Antibacterial Activity against *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2016;60(12):7396–401. doi: 10.1128/AAC.01405-16.
4. Kohira N, West J, Ito A, Ito-Horiyama T, Nakamura R, Sato T, et al. In Vitro Antimicrobial Activity of a Siderophore Cephalosporin, S-649266, against *Enterobacteriaceae* Clinical Isolates, Including Carbapenem-Resistant Strains. Antimicrob Agents Chemother. 2015;60(2):729–34. doi: 10.1128/AAC.01695-15.

5. Ito-Horiyama T, Ishii Y, Ito A, Sato T, Nakamura R, Fukuhara N, et al. Stability of Novel Siderophore Cephalosporin S-649266 against Clinically Relevant Carbapenemases. Antimicrob Agents Chemother. 2016;60(7):4384–6. doi: 10.1128/AAC.03098-15.
6. Cordero DG, Castillo-Polo JA, Ruiz-Garbajosa P, Canton R. Antibacterial spectrum of cefiderocol. Rev Esp Quimioter. 2022;35 Suppl 2(Suppl 2):20–7. doi: 10.37201/REQ/S02.03.2022.
7. Castillo-Polo JA, Hernández-García M, Morosini MI, Pérez-Viso B, Soriano C, De Pablo R, et al. Outbreak by KPC-62-producing ST307 *Klebsiella pneumoniae* isolates resistant to ceftazidime/avibactam and cefiderocol in a university hospital in Madrid, Spain. J Antimicrob Chemother. 2023;78(5). doi: 10.1093/JAC/DKAD086.
8. García-Fernández S, García-Castillo M, Bou G, Calvo J, Cercenado E, Delgado M, et al. Activity of ceftolozane/tazobactam against *Pseudomonas aeruginosa* and *Enterobacterales* isolates recovered from intensive care unit patients in Spain: The SUPERIOR multicentre study. Int J Antimicrob Agents. 2019;53(5):682–8. doi: 10.1016/J.IJANTIMICAG.2019.02.004.
9. García-Fernández S, García-Castillo M, Melo-Cristino J, Pinto MF, Gonçalves E, Alves V, et al. In vitro activity of ceftolozane-tazobactam against *Enterobacterales* and *Pseudomonas aeruginosa* causing urinary, intra-abdominal and lower respiratory tract infections in intensive care units in Portugal: The STEP multicenter study. Int J Antimicrob Agents. 2020;55(3). doi: 10.1016/J.IJANTIMICAG.2020.105887.
10. Hernández-Garciá M, Garciá-Castillo M, Garciá-Fernández S, Melo-Cristino J, Pinto MF, Gonçalves E, et al. Distinct epidemiology and resistance mechanisms affecting ceftolozane/tazobactam in *Pseudomonas aeruginosa* isolates recovered from ICU patients in Spain and Portugal depicted by WGS. J Antimicrob Chemother. 2021;76(2):370–9. doi: 10.1093/JAC/DKAA430.
11. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1. The European Committee on Antimicrobial Susceptibility Testing. 2021. Available from: http://www.eucast.org
12. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100. Clinical and Laboratory Standards Institute. 2021.
13. Ruiz-Garbajosa P, Hernández-García M, Beatobe L, Tato M, Méndez MI, Grandal M, et al. A single-day point-prevalence study of faecal carriers in long-term care hospitals in Madrid (Spain) depicts a complex clonal and polyclonal dissemination of carbapenemase-producing *Enterobacteriaceae*. J Antimicrob Chemother. 2016;71(2):348–52. doi: 10.1093/jac/dkv355.
14. Tato M, Ruiz-Garbajosa P, Traczewski M, Dodgson A, McEwan A, Humphries R, et al. Multisite Evaluation of Cepheid Xpert Carba-R Assay for Detection of Carbapenemase-Producing Organisms in Rectal Swabs. J Clin Microbiol. 2016;54(7):1814–9. doi: 10.1128/JCM.00341-16.
15. Hernández-García M, García-Castillo M, Bou G, Cercenado E, Delgado-Valverde M, Oliver A, et al. Imipenem-Relebactam Susceptibility in *Enterobacterales* Isolates Recovered from ICU Patients from Spain and Portugal (SUPERIOR and STEP Studies). Microbiol Spectr. 2022;10(5). doi: 10.1128/SPECTRUM.02927-22.
16. Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V, et al. Identification and screening of carbapenemase-producing *Enterobacteriaceae*. 2012. doi: 10.1111/j.1469-0691.2012.03815.x.
17. Miller S, Humphries RM. Clinical laboratory detection of carbapenem-resistant and carbapenemase-producing *Enterobacteriaceae*. Expert Rev Anti Infect Ther. 2016;14(8):705–17. doi: 10.1080/14787210.2016.1206815.
18. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3):318–27. doi: 10.1016/S1473-3099(17)30753-3.
19. French CE, Coope C, Conway L, Higgins JPT, McCulloch J, Okoli G, et al. Control of carbapenemase-producing *Enterobacteriaceae* outbreaks in acute settings: an evidence review. J Hosp Infect. 2017;95(1):3–45. doi: 10.1016/J.JHIN.2016.10.006.
20. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. World Heal Organ. 2017; Available from: http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf.
21. Wright H, Bonomo RA, Paterson DL. New agents for the treatment of infections with Gram-negative bacteria: restoring the miracle or false dawn? Clin Microbiol Infect. 2017;23(10):704–12. doi: 10.1016/J.CMI.2017.09.001.
22. Doi Y. Treatment Options for Carbapenem-resistant Gram-negative Bacterial Infections. Clin Infect Dis. 2019;69(Suppl 7):S565–75. doi: 10.1093/CID/CIZ830.
23. Kish T. New Antibiotics in Development Target Highly Resistant Gram-Negative Organisms. P T. 2018;43(2):116–20.
24. Kazmierczak KM, Tsuji M, Wise MG, Hackel M, Yamano Y, Echols R, et al. In vitro activity of cefiderocol, a siderophore cephalosporin, against a recent collection of clinically relevant carbapenem-non-susceptible Gram-negative bacilli, including serine carbapenemase- and metallo-ą-lactamase-producing isolates (SIDERO-WT-2014 Study). Int J Antimicrob Agents. 2019;53(2):177–84. doi: 10.1016/J.IJANTIMICAG.2018.10.007.
25. Karlowsky JA, Hackel MA, Tsuji M, Yamano Y, Echols R, Sahm DF. In Vitro Activity of Cefiderocol, a Siderophore Cephalosporin, Against Gram-Negative Bacilli Isolated by Clinical Laboratories in North America and Europe in 2015-2016: SIDERO-WT-2015. Int J Antimicrob Agents. 2019;53(4):456–66. doi: 10.1016/J.IJANTIMICAG.2018.11.007.
26. Cercenado E, Cardenoso L, Penin R, Longshaw C, Henriksen AS, Pascual A. In vitro activity of cefiderocol and comparators against isolates of Gram-negative bacterial pathogens from a range of infection sources: SIDERO-WT-2014-2018 studies in Spain. J Glob Antimicrob Resist. 2021;26:292–300. doi: 10.1016/J.JGAR.2021.06.011.
27. Yamano Y. In Vitro Activity of Cefiderocol Against a Broad Range of Clinically Important Gram-negative Bacteria. Clin Infect Dis. 2019;69(Suppl 7):S544–51. doi: 10.1093/CID/CIZ827.
28. Hackel MA, Tsuji M, Yamano Y, Echols R, Karlowsky JA, Sahm DF.

In Vitro Activity of the Siderophore Cephalosporin, Cefiderocol, against Carbapenem-Nonsusceptible and Multidrug-Resistant Isolates of Gram-Negative Bacilli Collected Worldwide in 2014 to 2016. Antimicrob Agents Chemother. 2018;62(2). doi: 10.1128/AAC.01968-17.

29. Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81. Doi: 10.1111/j.1469-0691.2011.03570.xd