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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

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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 break-

points. 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

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

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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
<i>Enterobacterales</i> (220)	ESBL	31
	Carbapenemase	25
	ESBL+ Carbapenemase	71
	MDR	93
<i>P. aeruginosa</i> (159)	Carbapenemase	41
	ESBL+ Carbapenemase	2
	MDR	96
	XDR	10
<i>A. baumannii</i> (64)	Carbapenemase (OXA-23)	9
	MDR	55
<i>S. maltophilia</i> (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 (MI-NO, 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 used 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 *Raoultella 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 characterize 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 *Raoultella 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: MIC₅₀ and Bold letter underlined: MIC₉₀

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 been 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 MIC₉₀ values ranging from 0.25 mg/L in *S. maltophilia* to 2 mg/L in Enterobacterales. Nevertheless, when considering *E. cloacae* alone MIC₉₀ 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

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)	<u>9 (91.2)</u>	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)	<u>19 (100)</u>
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)	<u>29 (100)</u>
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)	<u>23 (100)</u>
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)	<u>20 (100)</u>
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)	<u>32 (100)</u>
AZA				2 (1.2)	2 (2.5)	11 (9.4)	24 (24.5)	19 (36.5)	24 (51.6)	42 (78)	<u>35 (100)</u>
CIP			7 (4.4)	39 (28.9)	77 (77.3)	<u>27 (94.3)</u>	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)	<u>62 (100)</u>	
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: MIC₅₀ and Bold letter underlined: MIC₉₀

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)	<u>5 (96.8)</u>	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)	<u>6 (93.7)</u>	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)	<u>10 (98.4)</u>	1 (100)				
MIN				24 (37.5)	7 (48.4)	1 (50)	3 (54.7)	3 (59.4)	6 (68.7)	<u>20 (100)</u>			
STX				24 (37.5)	4 (43.7)	2 (46.9)	6 (56.2)	0 (56.2)	1 (57.8)	<u>27 (100)</u>			
SAM							11 (17.2)	16 (42.2)	11 (59.4)	13 (79.7)	4 (85.9)	0 (85.9)	<u>9 (100)</u>

Cefiderocol (CFDC), Imipenem-relebactam (IMR), Meropenem (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: MIC₅₀ and Bold letter underlined: MIC₉₀

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: MIC₅₀ and Bold letter underlined: MIC₉₀

Center (n° of isolates)	Cefiderocol (EUCAST breakpoints)				Cefiderocol (CLSI breakpoints)					
	Enterobacterales		<i>P. aeruginosa</i>		Enterobacterales			<i>P. aeruginosa</i>		
	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 MIC₅₀ and MIC₉₀ 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)							
	<i>Acinetobacter baumannii</i>				<i>Stenotrophomonas maltophilia</i>			
	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 cefiderocol 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.

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