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Cefiderocol, the first catechol-cephalosporin

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Antibacterial spectrum of cefiderocol

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ABSTRACT

Cefiderocol, a siderophore catechol cephalosporin, recently introduced in the market has been developed to enhance the *in vitro* activity of extended spectrum cephalosporins and to avoid resistance mechanisms affecting cephalosporins and carbapenems. The *in vitro* study of cefiderocol in the laboratory requires iron depleted media when MIC values are determined by broth microdilution. Disk diffusion presents good correlation with MIC values. In surveillance studies and in clinical trials it has been demonstrated excellent activity against Gram-negatives, including carbapenemase producers and non-fermenters such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*. Few cefiderocol resistant isolates have been found in surveillance studies. Resistance mechanisms are not directly associated with porin deficiency and or efflux pumps. On the contrary, they are related with gene mutations affecting iron transporters, AmpC mutations in the omega loop and with certain beta-lactamases such us KPC-variants determining also ceftazidime-avibactam resistance, certain infrequent extended-spectrum betalactamases (PER, BEL) and metallo-beta-lactamases (certain NDM variants and SPM enzyme).

Keywords: Cefiderocol, antimicrobial activity, surveillance, breakpoints

INTRODUCTION

The World Health Organisation has warned that antimicrobial resistance is one of the most important threats to humanity. It has also indicated that several actions are urgently needed to address the problem of bacterial resistance and that new antimicrobials need to be developed [1,2]. In recent years, the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have granted marketing authorisation for several antimicrobials [3]. The latter include beta-lactam combinations with beta-lactamase inhibitors and a new class of cephalosporins, represented by cefiderocol. The originality of this cephalosporin is that it has a catechol group in its structure that favours its penetration into the bacteria, as it competes with the transport of iron. This unique mechanism of entry into the bacterial cell has been described as "Trojan horse" [4,5]. Cefiderocol also contains in its structure radicals present in ceftazidime and cefepime cephalosporins which make this drug particularly active against Gram-negative bacilli, including non-fermenters. These radicals doubly favour its enhanced intrinsic activity compared to other extended-spectrum cephalosporins by facilitating penetration through porins and its resistance to a large number of beta-lactamases [6,7]. Due to these characteristics, the arrival at PBPs, the site of action of beta-lactams, is very efficient, which makes it active even in most of the carbapenem resistant and carbapenemase-producing microorganisms.

In this paper we review the activity of cefiderocol on Gram-negative microorganisms with information obtained from isolates obtained in cefiderocol clinical trials and epidemiological surveillance studies. We also include methodological aspects in the determination of cefiderocol susceptibility, including clinical breakpoints interpretation and published data related to the potential mechanisms of resistance to this antimicrobial.

TECHNICAL ASPECT IN THE STUDY OF *IN VITRO* ACTIVITY OF CEFIDEROCOL

Cefiderocol, as a siderophore cephalosporin, needs active iron transporters to enter the periplasm and access to the PBPs. These transporters are upregulated under iron-depleted conditions as it would happen *in vivo*, which is considered advantageous for the antibiotic activity [8]. Because of this, iron

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concentrations in antimicrobial susceptibility testing media need special consideration when cefiderocol is tested in order to mimic *in vivo* conditions and accurately predict clinical efficacy [4]. Moreover, resistance to cefiderocol has been already described [10-13] and should be accurately detected in the laboratory.

Broth microdilution and disk diffusion techniques have been used to determine the *in vitro* activity of cefiderocol. MIC obtained by agar dilution method do not match with those obtained by broth microdilution and it is not a recommended technique for this compound [14]. Other techniques, such as gradient diffusion strips, are also now available but experience is limited and manufactures only recommend it for *Pseudomonas aeruginosa* isolates and no other non-fermentative rods or *Enterobacterales* [4]. Nevertheless, a recent study used cefiderocol MIC strips (Liofilchem, IT) in comparison with MIC obtained in iron-depleted broth (reference method) and disk diffusion in a collection of carbapenemase producing *Enterobacterales* [15]. The conclusion was that MIC strip should be avoided in these isolates due to the high number of discrepancies (only 64% of categorical agreement and 94.9% of very major errors due to critical underestimation of MICs), which were highly reproducible.

The inclusion of cefiderocol in panels used in automatic system is still waiting due to the fact of technical challenges of cefiderocol testing.

Broth microdilution. Standard cation-adjusted Mueller-Hinton broth (CAMHB) is not a medium controlled for iron concentration and this may vary among the different manufacturers. Some studies referred by the Clinical and Laboratory Standards Institute (CLSI) guidelines already demonstrated that MICs were higher when standard CAMHB was used, compared to those obtained with iron-depleted CAMHB (ID-CAMHB) [16]. These results are supported by the idea that iron transport, as well as the uptake of cefiderocol, are increased in low iron-concentration conditions.

A study demonstrated reproducibility of the ID-CAMHB in broth microdilution technique by testing 19 clinical isolates of Gram-negative bacilli (including 9 *Acinetobacter baumannii* isolates) over 10 replicates in CAMHB from 3 different manufacturers. More than 95% of MIC results were within one doubling dilution when analysed by individual medium lot. Besides this, when all medium lots were combined, 92.2% of MIC results were within one doubling dilution and 99.8% within two dilutions [17]. Thus, iron depletion is necessary to accurately perform MIC testing and to use this data to predict *in vivo* efficacy of cefiderocol. Moreover, MICs determined under these conditions have been proved to be reproducible and correlate with *in vivo* activity in animal models [18].

Following CLSI guidelines, the solvent and diluent required to prepare the medium for broth microdilution is a solution of 0.85% to 0.9% NaCl. To prepare the ID-CAMHB, both the European Committee of Antimicrobial Susceptibility testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) recommendations use chelation with a resin to remove the polyvalent metal cations in the medium with a final iron content below 0.03 mg/L. After that, the resin is filtered out and the non-iron cations are re-added to concentrations of 20–25 mg/L of calcium, 10–12.5 mg/L of magnesium, and 0.5–1.0 mg/L of zinc; all the reagents should have a low content of iron. The pH should be checked after the chelation and the addition of cations and adjusted if required. The rest of the procedure is like the susceptibility testing of other cephalosporins [16,19].

To read the MIC values, the MIC of cefiderocol corresponds to the first well in which a button of <1 mm or a faint turbidity can be observed, with the positive control showing a strong growth (button of >2 mm or heavy turbidity) [19]. In some organisms such as *Acinetobacter* spp., a trailing has been reported, where up to 30% of isolates demonstrated such effect [4]. The MIC should then be read as the first well with a significant reduction of growth, ignoring buttons <1 mm and faint turbidity comparted with the control growth [17].

Disk diffusion. To determine the susceptibility by the disk diffusion technique, standard recommendations for non-fastidious organisms should be followed using a cefiderocol 30-mg disk. In contrast to broth microdilution, this method has been developed to be performed on regular unsupplemented Mueller-Hinton agar (MHA), since only small variations in the zone diameters were found when MHA with different concentrations of iron (0.03 to 10 mg/L) were tested [4]. Although it may vary among different manufacturers, the medium usually contains around 0.5 mg/L of iron. It is thought iron to be bound in the agar, simulating iron-depleted conditions without interfering with the results [20]. Regarding reading of inhibition zones and the interpretation of the results, some colonies may be found within inhibition zone and need to be taken into consideration. Zone diameters should be measured, therefore, as the inner zone without bacterial growth. [4]

BREAKPOINTS AND EPIDEMIOLOGICAL CUT-OFF VALUES OF CEFIDEROCOL

The clinical breakpoints for cefiderocol have been established by both EUCAST and CLSI [15,21]. In the first case, they are those listed in the summary of product characteristics of the EMA (SmPC) [21]. In the United States, the FDA and the United States Committee on Antimicrobial Susceptibility Testing (USCAST) have also published clinical breakpoints that differ in some cases from those defined by CLSI (Table 1) [23,24]. In the case of EUCAST, the susceptible breakpoints are one dilution lower than CLSI. This decision was based on the PK/PD analysis which is explained in their rational document [25]. EUCAST also does not recognize a "susceptible, increased exposure" (I) ("intermediate" in CLSI terms) category as the marketing authorization only includes a single dose (2 g/8 h over 3 hours of infusion). Moreover, EUCAST, unlike CLSI, have not yet stablished clinical breakpoint for *Acinetobacter* spp. and *Stenotrophomonas malthophilia* due to the lack of clinical data to correlate outcomes with MIC values. In the future, real life

Table 1 **Clinical breakpoints for cefiderocol published by breakpoint committees and/or regulatory agencies in 2022**

Microorganisms and non-species related PK/PD breakpoints	EUCAST - EMA					USCAST				CLSI				FDA			
	MIC, mg/L		Inhibition zone diameter, mm[a]			MIC, mg/L		Inhibition zone diameter, mm[a]		MIC, mg/L		inhibition zone diameter, mm		MIC, mg/L		inhibition zone diameter, mm[a]	
	≤S	>R	≥S	<R	ATU[b]	≤S	≥R	≥S	≤R	≤S	≥R	≥S	≤R	≤S	≥R	≥S	≤R
Enterobacterales	2	2	22	22	18-22	2[c] (4)[d]	4[c] (8)[d]	-	-	4	16	16	8	4	16	16	8
Pseudomonas aeruginosa	2	2	22	22	14-22	2[c] (4)[d]	4[c] (8)[d]	-	-	4	16	18	12	1	4	22	12
Acinetobacter spp.	IE[e]	IE	-[f]	-[f]	-	IE	IE			4	16	15	-[g]	1	4	19	11
Stenotrophomonas maltophilia	IE	IE	-[h]	-[h]	-	IE	IE	-	-	1[i]	-	15[i]	-	-	-	-	-
PK/PD	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a]30-µg disk content; [b]ATU: area of technical uncertainty; [c]breakpoints for pneumonia; [d]breakpoints for non-pneumonia; [e]IE: insufficient evidence; [f]Zone diameters of ≥17 mm for the cefiderocol 30-µg disk correspond to MIC values below the PK-PD breakpoint of S ≤ 2 mg/L; [g]Disk diffusion diameters ≤14 mm should not be interpreted or reported because zone diameters ≤14 mm occur with resistant, intermediate and susceptible isolates. For isolates with zone diameters ≤14 mm, do not report cefiderocol without performing an MIC test; [h]Zone diameters of ≥20 mm for the cefiderocol 30-µg disk correspond to MIC values below the PK-PD breakpoint of S ≤ 2 mg/L; [i]Breakpoints are based on PK/PD properties, and limited clinical data.

studies will help to define these breakpoints. In the absence of them, PK/PD breakpoints have been defined, which can help to take decisions of the use of this drug when other therapeutic alternatives are not available [21]. To note that, USCAST is the only breakpoint committee that discriminates breakpoints for pneumonia and non-pneumonia infections being one-fold dilution lower in the former than in the later.

The epidemiological cut off values (ECOFF) of cefiderocol have been recently published but to a low number of species due to the technical particularities that arise when MIC values are determined [25]. These values have been established following EUCAST guidelines. Tentative ECOFFs (TECOFF, based in 3-4 MIC distributions) for *Escherichia coli*, *Klebsiella pneumoniae* and *P. aeruginosa* are 0.25 mg/L, 0.125 mg/L and 0.5 mg/L, respectively. For *A. baumannii* and *S. maltophilia* ECOFFs (based in at least 5 MIC distributions) are 0.25 mg/L and 0.06 mg/L, respectively.

Disk diffusion breakpoints are also included in table 1. EUCAST includes for *Enterobacterales* and *Pseudomonas* spp. an area of technical uncertainty (ATU) when interpreting disk diffusion susceptibility due to difficulties in correlating inhibition zones with MIC values at the wild-type end of the population. In this case, it is recommended to establish susceptibility to cefiderocol by calculating and interpreting MIC values.

IN VITRO ACTIVITY OF CEFIDEROCOL IN SURVEILLANCE STUDIES

The *in vitro* activity of cefiderocol has been studied both nationally and internationally. Among the international studies, we highlight SIDERO-WT-2014, SIDERO-WT-2015 and the studies carried out by different investigators [27-29].

The SIDERO-WT-2014 [28] study includes meropenem- and colistin-resistant *Enterobacterales* isolates and meropenem-resistant *P. aeruginosa* and *A. baumannii* isolates from both the United States and Europe. These isolates were also screened for the presence of genes encoding beta-lactamases, loss of porins and resistance to colistin mediated by plasmids, in order to later define the spectrum of cefiderocol activity against these challenging Gram-negative isolates. Most meropenem-resistant *Enterobacterales* carried carbapenemases, being KPC-type the most frequent enzyme. *P. aeruginosa* isolates from the United States did not carry acquired beta-lactamases, while 16% of the isolates from Europe carried VIM-, IMP- or GES-carbapenemase. Regarding *A. baumannii* isolates, the most common carbapenemase in both regions was OXA-23 followed by OXA-24, however, OXA-58 was only detected in Europe. In the collection of meropenem-resistant isolates, the MIC of cefiderocol ranged between 0.002 mg/L and 64 mg/L. A total of 97.7% of isolates tested had cefiderocol MIC values ≤4 mg/L, including isolates producing KPC, IMP, VIM and OXA-48 enzymes. In these carbapenemases producing isolates, 99.6% of them were inhibited with MIC values of cefiderocol ≤8 mg/L. In meropenem-resistant *Enterobacterales* isolates, the MIC_{90} value was 4 mg/L compared to MIC_{90} values ≥64 mg/L for meropenem, ceftazidime, ceftolozane-tazobactam and ceftazidime-avibactam, and ≥8 mg/L for ciprofloxacin and colistin. Ceftazidime-avibactam showed MIC values equal to or slightly better than cefiderocol in isolates producing KPC-, OXA-types, and those meropenem-resistant without carbapenemase, however, unlike cefiderocol, ceftazidime-avibactam was not active against isolates producing VIM and IMP-enzymes. On the other hand, no correlation was observed between cefiderocol MICs and the presence of different combinations of intact and disrupted porin genes. Regarding *P. aeruginosa* isolates, the MIC_{90} value was 1 mg/L compared with MIC_{90} values of ≥32 mg/L for meropenem, cefepime, ceftazidime-avibactam, and ceftolozane-tazobactam and >8 mg/L for ciprofloxacin. With the exception of colistin, the comparator agents showed

reduced activity against the GES and MBL producing isolates. Finally, the MIC_{90} value in meropenem-resistant *A. baumannii* isolates was 1 mg/L. As in *P. aeruginosa*, in *A. baumannii* isolates, both carbapenemase-producers and non-producers, meropenem, cefepime and ciprofloxacin showed reduced activity in comparison with cefiderocol. In addition, a total of 136 colistin-resistant *Enterobacterales* were screened for the presence of the transmissible colistin resistance determinant *mcr*-1 gene. Most of these isolates (n = 101) were susceptible to meropenem and 35 of them produced different carbapenemases. The MIC_{90} value of cefiderocol for these isolates was 2 mg/L. In summary, results of the SIDERO-WT-2014 surveillance program demonstrate the potent *in vitro* activity of cefiderocol against meropenem-resistant *Enterobacterales*, *P. aeruginosa* and *A. baumannii* isolates. Cefiderocol activity was comparable to that of ceftazidime-avibactam against MBL-negative *Enterobacterales* isolates but superior to all the comparator agents against NDM- and VIM-positive isolates. Furthermore, cefiderocol was also active against colistin-resistant *Enterobacterales*, including those carrying the transmissible colistin resistance determinant *mcr*-1.

Data generated during the second year of this global surveillance initiative for cefiderocol is included in the SIDERO-WT-2015 study [29]. During this period, isolates of *Enterobacterales*, *P. aeruginosa*, *A. baumannii*, *S. maltophilia* and *Burkholderia cepacia* complex were collected. Results of this study support those obtained in the previous year demonstrating an *in vitro* activity of cefiderocol superior to ceftazidime-avibactam, ceftolozane-tazobactam and cefepime against of *Enterobacterales*, *P. aeruginosa* and *A. baumannii* isolates. Regarding *S. maltophilia* and *B. cepacia* complex, 99.4% and 94.4% respectively, showed cefiderocol MIC values ≤4 mg/L. It should be noted that there was no cross-resistance between cefiderocol and colistin. This study concludes that cefiderocol is a good therapeutic option in patients infected with multidrug-resistant Gram-negative bacilli due to its demonstrated activity against carbapenem-resistant Gram-negative isolates and MDR phenotypes, its stability to hydrolysis by different beta-lactamases and its activity against bacteria resistant to carbapenems by other resistance mechanisms.

At the international level, Hackel *et al.* [27] also demonstrated that cefiderocol is a more potent antimicrobial than cefepime, ceftazidime-avibactam and ceftolozane-tazobactam. The study included 1,022 meropenem-resistant *Enterobacterales* isolates collected between 2014 and 2016 by medical center laboratories in 52 countries (24 in Europe, 10 in Latin America, 2 in North America, 8 in Asia, 3 in the South Pacific, 2 in Africa and 3 in Middle East). The MIC_{90} value for cefiderocol was 4 mg/L with MIC ranges between 0.004 and 32 mg/L (97% of the isolates had MIC values ≤ 4 mg/L) [27]. Results of other studies are in agreement with those mentioned above, cefiderocol has excellent *in vitro* activity (MIC_{90} values ≤1 mg/L) against problematic isolates such as KPC- and MBL-producing *Enterobacterales* (including NDM-1 enzymes) and ESBL producers [30]. Regarding non-fermenting Gram-negative bacteria, Ito *et al.* obtained MIC_{90} values of 2 mg/L in *A. baumannii* isolates, 1 mg/L for *P. aeruginosa* and 0.5 mg/L for *S. maltophilia* isolates. These results also demonstrate the potent *in vitro* activity of cefiderocol against non-fermenters, with MIC_{90} values significantly lower than those obtained for ceftazidime, meropenem, levofloxacin, cefepime and piperacillin-tazobactam. Cefiderocol was also active against *A. baumannii*, including those isolates resistant to carbapenems [31].

At the national level, studies have also been published about the *in vitro* activity of cefiderocol in Spain, showing that it is a good therapeutic option for the treatment of infections caused by MDR bacteria. Thus, Cercenado *et al.* [32] recently published the subset of Spanish isolates from the SIDERO-WT-2014-2018 study, demonstrating that cefiderocol showed potent *in vitro* activity against Gram-negative bacilli isolated in different types of infection. Furthermore, a significant percentage of isolates ($p < 0.01$) were susceptible to cefiderocol. Susceptibility to cefiderocol in *Enterobacterales* was significantly better ($p < 0.01$) than ceftolozane-tazobactam and colistin but similar to meropenem and ceftazidime-avibactam, while susceptibility to cefiderocol in non-fermenting isolates was significantly better than all comparators ($p < 0.01$). It should be noted that cefiderocol activity was significantly better than all comparators against isolates from patients with nosocomial pneumonia.

In Greece, a country with high resistance rates, Falagas *et al.* [33] studied the *in vitro* activity of cefiderocol in carbapenem-resistant isolates and compared it with that of commercially available antibiotics. Cefiderocol demonstrated potent *in vitro* activity with MIC_{90} values ≤1 mg/L for all groups of microorganisms. However, MIC_{90} of cefiderocol was lower in non-fermenters than for *Enterobacterales*. In addition, they observed minor differences in MIC values according to specific resistance mechanisms.

Ballesté-Delpierre *et al.* [34] tested a diverse collection of *A. baumannii* clinical isolates, including Spanish one. The most active antimicrobials against this collection were colistin and cefiderocol, with 12.38% and 21.23% of non-susceptibility, respectively. Interestingly, a high proportion of multidrug-resistant (76.7%) and carbapenem-resistant (75.3%) *A. baumannii* isolates remained susceptible to cefiderocol, which was clearly superior to novel beta-lactam-beta-lactamase inhibitor combinations, including ceftazidime-avibactam, imipenem-relebactam and meropenem-vaborbactam. Cefiderocol-non susceptible isolates were more frequently observed among meropenem-resistant isolates, but could not be associated with any particular resistance mechanism or clonal lineage.

A recent publication including isolates collected from the United States and Europe collected as part of the SENTRY study in 2020, showed 99.8% *Enterobacterales* susceptibility to cefiderocol, with similar values (98.2%) in the subset of carbapenem resistant isolates [34]. In *P. aeruginosa* isolates, cefiderocol was the most active antimicrobial (99.6% susceptible). In XDR isolates cefiderocol susceptibility was very high

Table 2 Cefiderocol resistance mechanisms

Microorganisms	Cefiderocol MIC (MIC or range) (mg/L)	Resistance mechanism	Country (Year of publication)	Reference
K. pneumoniae	16 - >32	Mutation of two-component regulation system (BaeSR and OmpR/EnvZ). Mutation of *exbD* (accessory protein related to iron transport)	Japan (2020)	44
K. pneumoniae *E. coli* *E. cloacae*	4 - >32	KPC β-lactamase mutants	France (2021)	49
K. pneumoniae	8	KPC β-lactamase mutant (KPC-31)	Italy (2021)	50
E. cloacae	>16	AmpC R2 loop deletion	USA (2020)	10
E. cloacae	≥256	Mutations in *cir*A gene	Germany (2021)	13
P. aeruginosa	8	Mutations in *pir*A and deletion in piuA	USA (2021)	43
A. baumannii *B. multivorans* *P. aeruginosa* *S. maltophilia*	>4	PER and NDM β-lactamase Disruption of iron transport genes (*piu*A, *pir*A and *fiu*A)	Russia, Turkey and USA (2020)	45
A.baumannii	≥32	Loss of *pir*A and *piu*A	USA (2020)	46

(97.3%) compare with meropenem (only 7.4%). In this collection *Acinetobacter* spp and *S. maltophilia* susceptibility to cefiderocol was 97.7% and 97.9%, respectively [35].

CEFIDEROCOL RESISTANCE MECHANISMS

Mechanisms of resistance to cefiderocol are being described and different reviews include subheading of this emergence [4,36-40]. Nevertheless, and according with surveillance studies and clinical trials, prevalence of cefiderocol non susceptible or resistant isolates remains very low and their clinical implications remains to be clarified [41]. Table 2 summarized resistance mechanisms described to cefiderocol in different species. These mechanisms are complex and normally, they do not involve a single gene. Cefiderocol resistance has been described in *in vitro* mutants, in isolates recovered from surveillance studies and in clinical cases involving difficult to treat pathogens.

In carbapenemase producing microorganisms, it has been shown that the loss of Omp35 and Omp36 porins in *K. pneumoniae* isolates as well as the overproduction of MexA-MexB-OprM efflux pumps in *P. aeruginosa* isolates do not have a significant impact on cefiderocol activity [31]. On the contrary, mutants in TonB dependent iron transporter pathway might affect cefiderocol susceptibility [42]. This mechanism of resistance involves potential defects in the inner membrane proteins (TonB-ExbB-ExbD) and/or the corresponding two-component regulator systems that affect the necessary energy for the iron transportation and hence for cefiderocol [43].

The implication of iron transport pathway in cefiderocol resistance have been studied in *K. pneumoniae* isolates but also in *P. aeruginosa*, *A. baumannii* and other Gram-negative non-fermentative rods. In that sense, Yamano *et al.* [44] suggests the mutation of two-component regulation systems (BaeSR and OmpR/EnvZ) and iron transport-related proteins as a possible resistance mechanism involved *in vitro* cefiderocol resistant mutants of *K. pneumoniae* isolates. Moreover, in SIDERO-WT clinical studies, some isolates of different species (128 *A. baumannii*, 22 *Enterobacterales*, 7 *Burkholderia multivorans*, 2 *P. aeruginosa* and 2 *S. maltophilia*) with cefiderocol MICs >4 mg/L were found. Yamano *et al.* [45] performed molecular characterization of isolates with MICs >4 mg/L from these studies. They observed that PER and NDM enzymes (*bla*-PER were found in *A. baumannii* and *bla*NDM were found in *K. pneumonia*e and *A. baumannii*) could increase cefiderocol MIC values as well as disruption of iron transport genes (*piu*A, *pir*A and *fiu*A). Similarly, cefiderocol resistance (MIC ≥32 mg/L) have been described in *A. baumannii* isolates due to the loss of *pir*A and *piu*A genes which are two TonB-dependent receptors involved in the transport of siderophores or vitamin B12 in Gram-negative organisms, as well as carbohydrates, thiamine, and cations [46].

In *P. aeruginosa*, it has been also shown that certain mutations in the omega loop of the AmpC beta-lactamase can determine resistance to both ceftolozane-tazobactam and ceftazidime-avibactam but also reduced susceptibility to cefiderocol and increased susceptibility to imipenem-relebactam [47]. In *P. aeruginosa* PA01, *in vitro* inactivation of *piuA* (a gene encoding drug import channel) determined to a 16-fold increase

in cefiderocol MIC (0.5 to 8 mg/L). This increase was reverted with complementation experiments using a plasmid containing the *pirA* gene [48].

Within the clinical cases, in Germany, a 58-years-old male patient developed cefiderocol resistance within 3 weeks after therapy with cefiderocol in monotherapy. This MIC increase was observed in NDM- and OXA-48 producing *Enterobacter cloacae* and was caused by mutations of the *cir*A siderophore receptor during cefiderocol treatment [13]. Emergence of resistance can be also present in isolates recovered in patients with now previous treatment with cefiderocol. An elegant report published from USA by Streling *et al* [43] showed development of a cefiderocol non-susceptible *P. aeruginosa* isolate in a patient with previous treatment with different antimicrobials, including ceftazidime-avibactam. Resistance was due to mutations in major iron transport pathways previously associated with cefiderocol uptake.

In addition, cross-resistance, both to ceftazidime-avibactam and cefiderocol have been reported [49] using *in vitro* KPC beta-lactamase ceftazidime-avibactam-resistant mutants. This study evaluated the impact of these mutations on cefiderocol MICs, so that, in 76% of the KPC mutants studied, cefiderocol MIC increased compared to the wild isolate. This resistance mechanism was also observed in Pisa (Italy) in clinical isolates, where a KPC-31-producing *K. pneumoniae* was isolated from a 68-years-old male patient 7 days after ceftazidime-avibactam discontinuation [50]. Moreover, Poirel *et al* [51] recently confirmed that some KPC-3 mutants that confer resistance to ceftazidime-avibactam might also affect cefiderocol. This occurs with KPC-41 and to a lesser extend with KPC-50.

Apart from KPC carbapenemase variants affecting ceftazidime-avibactam susceptibility, some clavulanic acid inhibited extended spectrum beta-lactamases (ESBLs), such as PER, BEL and some SHV derivatives (i.e. SHV-12) might increase cefiderocol MIC values. This is also the case for some metallo-beta-lactamase (MBL) variants, including NDM and SPM. In both cases, ESBLs and MBL, the increase in MIC is more evident in *P. aeruginosa* than in *E. coli* or *K. pneumoniae* [52].

Other mechanism described that confers resistance to ceftazidime-avibactam and cefiderocol is AmpC R2 loop deletion in *E. cloacae*, which was isolated from a hospitalized patient with ventilator-associated pneumonia. The whole-genome sequencing of this isolate identified an alanine-proline deletion (A294_P295del) and a leucine-to-valine substitution (L296V) in the *amp*C gene. In the other hand, functional genome cloning of *E. cloacae* was performed obtaining several *E. coli* transformants; ceftazidime-avibactam and cefiderocol MICs of *E. coli* in which deletion was reverted by site-directed mutagenesis were both 0.5 mg/L suggesting the contribution of the R2 loop deletion to the ceftazidime-avibactam and cefiderocol MICs increase [10].

CONCLUSIONS

Cefiderocol is a new cephalosporin with a unique mechanism of action in which it also enters through the bacterial wall using the iron transport pathway. This fact determines that the *in vitro* study of cefiderocol by broth microdilution must be performed with the usual Mueller-Hinton medium, but depleted in iron so that MIC values are reproducible. Disk diffusion uses standard Mueller-Hinton agar. Surveillance studies indicate that it is one of the most active antimicrobials with a profile that includes *Enterobacterales*, including carbapenemase producers, *P. aeruginosa, Acinetobacter* spp, and other non-fermenters such as *S. maltophilia*. Isolates with impaired sensitivity or resistance to cefiderocol have been described in which the most common mechanism is disruption of the iron transport system, resulting in the loss of all or part of the advantage of cefiderocol entry via this route. Other situations in which higher MICs to cefiderocol may occur are in isolates expressing KPC variants that confer resistance to ceftazidime-avibactam or certain infrequent ESBL, or metallo-beta-lactamases, particularly in *P. aeruginosa*. However, in epidemiological surveillance studies and clinical trials such isolates are rare.

CONFLICT OF INTEREST

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