DECREASE OF CEFIDEROCOL IN VITRO ACTIVITY AGAINST CARBAPENEMASES-PRODUCING GRAM NEGATIVE BACTERIA

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INTRODUCTION

Infections caused by carbapenem-resistant Enterobacterales and other difficult-to-treat Gram-Negative bacteria are a major global health threat. Cefiderocol is a new cephalosporin approved for the treatment of these bacterial infections. This drug uses a "trojan-horse approach" to enter into the periplasmic space of Gram-negative bacteria, via bacterial siderophore iron uptake system and outer membrane porin channels. *In vitro* susceptibility testing of Cefiderocol is performed using iron-depleted cation-adjusted Mueller–Hinton broth, recommended by EUCAST. EUCAST breakpoints bring to 2 different categories for Enterobacterales and Cefiderocol: susceptible if MIC is ≤2 mg/L and resistant if MIC is >2 mg/L.
Aim of this work was assaying different methods to test Cefiderocol susceptibility on carbapenemases-producing Gram negative bacteria.

METHODS

From January to April 2022, at Papa Giovanni XXII Hospital (Bergamo, Italy), carbapenemase-producing Gram negative bacteria (n=27) were found.
All isolates were identified as *E. cloacae complex* using MALDI-ToF technology (Vitek MS, bioMérieux) and/or by Vitek2 System (bioMérieux) and confirmed by sequencing as *E. hormaechei*. Carbapenemase resistance mechanisms were recognised with an immunochromatographic method (O.K.N.V.I. RESIST-5, CORIS, BioConcept) directly from colonies. Firstly, the 27 bacterial isolates were tested against Cefiderocol by disk diffusion agar method, plating a 0.5 McF suspension on Mueller-Hinton Agar (MH Agar, bioMérieux) and using a Cefiderocol disk (30 mcg, Liofilchem). In order to retest the results obtained, in a second time, we subcultivated the resistant strains (starting from frozen strains) on a MacConkey Agar plate (McK Agar, bioMérieux), placing a disk of meropenem (10 mcg, Oxoid) on the massive part of the growth, to ensure the maintenance of the carabapenemases production. Subsequently, a further subculture on Columbia agar + 5% horse blood plate (COH, bioMérieux) of the colonies grown around the meropenem halo was performed, always placing a meropenem disk on the massive part. The colonies around the meropenem disk were picked to make a 0.5 McF suspension, plated on MH Agar and a Cefiderocol disk was used, with the same method of routinary work. As well as disk diffusion, also for the execution of the broth microdilution (ComASp@ Cefiderocol 0.008-128, Liofilchelm), we started from frozen strains in the same time: the isolates were subcultered on McK Agar with meropenem disk and then on Columbia agar + 5% horse blood plate, again with a meropenem disk. The broth microdilution was performed starting from the colonies developed at the edge of the meropenem halo. The interpretation of Cefiderocol susceptibility tests here reported is based on EUCAST breakpoints. To verify the decrease of Cefiderocol *in vitro* activity, some strains were tested after “induction”: after subculture on McK Agar with Cefiderocol disk and then on Columbia agar + 5% horse blood plate, again with a Cefidercol disk. The broth microdilution was performed starting from the colonies developed at the edge of the Cefiderocol halo.

RESULTS

Of the 27 carbapenemase-producing Gram negative bacteria, 15 had human origin whereas 12 were collected from hospital environment (Table 1 summarizes the species identification). Regarding the carbapenemases typing, all patients isolates produced VIM-type-metallo-β-lactamases. Most of non-human isolates were VIM-positive (10/12), in one case we found two different carbapenemases (KPC+VIM) and in another case a KPC-producer. As described before, resistant isolates (21/27), obtained with disk diffusion method performed during routine work, were retested to confirm the results: 20/27 strains reconfirmed as resistant, with a diameter between 19 and 21 mm (see table 1). We considered these 20 strains as resistant, despite some diameters were inside the ATU, as described on EUCAST warning. During this replication of the test by agar diffusion technique, we observed for the first time the growth of colonies inside the inhibition zone of Cefiderocol disk in 6 strains (see Table 2). Then, the sensitivity to Cefiderocol was tested with broth microdilution method, choosing 26 strains: the 20 strains resulting resistant with the Kirby Bauer repetition and the 6 “IC” strains isolated from internal halo. The MICs of the 20 strains were distributed as indicated in Table 1. On the contrary, MICs of the 6 strains were higher, because ranged from 32 to >128 mcg/mL (Table 2, MICs in the first column): these strains were put in contact with the Cefiderocol 30 mcg/mL disk twice (the first time when tested routinely and the second time when the test was repeated). Broth microdilution method was applied picking the colonies directly from the internal zone of the inhibition halo of Cefiderocol. Then, we repeated broth microdilution method from 6 frozen strains (isolated from internal halo of Cefiderocol disk). The increase of MICs was confirmed: MICs ranged from 16 to >128 mcg/mL (Table 2). In Table 2, we also report the MICs of original strains and the MICs derived from the repetition of broth microdilution from frozen original isolates: the strains were passed on plates without putting Cefiderocol disk on the massive growth of the plate: MICs were reconfirmed.

Sample	Isolation site	Resistance mechanisms	Identification	1st test (mm)	1st test S/I/R	2nd test (mm)	2nd test S/I/R	MIC (µg/ml)	S/I/R (MIC method)
8035850639	blood	VIM	*E. hormaechei*	19	R	19	R	2	S
8040878325	lung	VIM	*E. hormaechei*	19	R	19	R	2	S
8035851496	blood	VIM	*E. hormaechei*	18	R	19	R	2	S
8040881617	blood	VIM	*E. hormaechei*	21	R	19	R	4	R
8040882505	lung	VIM	*E. hormaechei*	21	R	21	R	2	S
8040884607	survelliance colture	VIM	*E. hormaechei*	18	R	19	R	2	S
8040885080	survelliance colture	VIM	*E. hormaechei*	19	R	19	R	2	S
8035857311	blood	VIM	*E. hormaechei*	19	R	19	R	2	S
8040889939	survelliance colture	VIM	*E. hormaechei*	20	R	20	R	2	S
8040889943	survelliance colture	VIM	*E. hormaechei*	20	R	19	R	2	S
8040887707	lung	VIM	*E. hormaechei*	19	R	20	R	1	S
8040887700	survelliance colture	VIM	*E. hormaechei*	20	R	19	R	4	R
8040891730	survelliance colture	VIM	*E. hormaechei*	20	R	19	R	2	S
8040898152	survelliance colture	VIM	*E. hormaechei*	20	R	19	R	4	R
8040898538	survelliance colture	VIM	*K. aerogenes*	20	R	20	R	4	R
8040024194	hospital environment	VIM	*C. freundii*	26	S	nt	nt	nt	nt
8040024195	hospital environment	VIM	*C. freundii*	31	S	nt	nt	nt	nt
8040024196/1	hospital environment	VIM+KPC	*E. hormaechei*	19	R	20	R	2	S
8040024196/2	hospital environment	VIM	*Pseudomonas spp*	30	S	nt	nt	nt	nt
8040024201	hospital environment	VIM	*E. hormaechei*	21	R	19	R	2	S
8040024204	hospital environment	VIM	*E. hormaechei*	21	R	19	R	2	S
8040024206/2	hospital environment	VIM	*P. mendocina*	34	S	nt	nt	nt	nt
8040024206/1	hospital environment	VIM	*E. hormaechei*	14	R	20	R	0,125	S
8040024207	hospital environment	VIM	*P. mendocina*	37	S	nt	nt	nt	nt
8040024208	hospital environment	VIM	*E. hormaechei*	21	R	20	R	1	S
8040024210	hospital environment	KPC	*K. pneumoniae*	31	S	nt	nt	nt	nt
8040024232	hospital environment	VIM	*E. hormaechei*	21	R	24	S	nt	nt
ATCC 25922			*E. coli*	27	S	30	S	0,25	S

Table 1. Cefiderocol disk diffusion (mm) and MICs results (µg/ml) of the 27 carbapenemases-producing Gram-negative isolates (nt; not tested)

				Colonies inside halo (IC)		Original strains	
Sample	Isolation site	Resistance mechanisms	Identification	MICs (µg/ml)	MICs from frozen strains after induction	Original strains MICs (µg/ml)	Repetition from frozen strains MICs (µg/ml)
8040881617 IC	blood	VIM	*E. hormaechei*	64	64	4	4
8040889939 IC	survelliance colture	VIM	*E. hormaechei*	32	16	2	4
8040898152 IC	survelliance colture	VIM	*E. hormaechei*	128	64	4	4
8040024208 IC	hospital environment	VIM	*E. hormaechei*	32	16	1	2
8040887700 IC	survelliance colture	VIM	*E. hormaechei*	128	128	4	4
8040898538 IC	survelliance colture	VIM	*K. aerogenes*	>128	>128	4	4
ATCC 25922			*E. coli*	0.5			/

Table 2. MICs results (µg/ml) of the 6 strains growing inside the halo of Cefiderocol disk (IC, Inside Colonies) compared to MICs of the original strains

CONCLUSIONS

Cefiderocol is a very good agent for the treatment of Gram negative bacterial infections with limited therapeutic options. The rapid decrease of Cefiderocol in vitro activity was confirmed during the repetition of the broth microdilution methods after “induction”: if we tested MICs of the colonies grown inside the inhibition halo of Cefiderocol, we observed a considerable increase of the MIC values. *In vivo* Cefiderocol resistance is now rare, nevertheless Klein et al. 2022 reported the development of high-level Cefiderocol resistance in *E. cloacae* during after 21 days therapy, due to mutations of the CirA siderophore receptor. They didn’t know if the propensity for resistance acquisition was related to the genre *Enterobacter* or to the presence of carbapenemases. We observed the acquisition of *in vitro* resistance to Cefiderocol in *E. hormaechei (E. cloacae complex)*. In conclusion, we demonstrated the necessity of using microdilution method with iron-depleted cation-adjusted Mueller–Hinton broth to test Cefiderocol susceptibility, to avoid problems related to the induction of resistance seen if KB was used. We also confirmed the rapid increase of Cefiderocol in vitro resistance, when the isolates were put in contact with Cefiderocol disk.

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