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In vitro activity of imipenem/relebactam against Gram-negative clinical isolates in two Spanish tertiary hospitals

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ABSTRACT

Objetive. The aim of this study was to analyze the activity of the imipenem-relebactam combination (IMI/REL) against a collection of multidrug-resist Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* clinical isolates.

Material and methods. The study was conducted in two tertiary hospitals in Spain and included 192 clinical isolates of these 3 genera (139 resistant and 53 susceptible to IMI). The MICs for IMI with and without REL (at a fixed concentration of 4 mg/L) were determined by a standard broth microdilution method according to international recommendations.

Results. All IMI-susceptible *E. coli* strains were also susceptible to IMI/REL. Enterobacterales resistant to IMI due to the production of carbapenemases, the MIC_{50} and MIC_{90} decreased from 64/256 with IMI to 8/64 mg/L with IMI/REL. This high activity was principally detected among isolates with KPC enzymes. Enterobacterales with class B carbapenemases, *P. aeruginosa* carrying VIM carbapenemase and *A. baumannii* strains showed no changes on IMI MIC_{50} or MIC_{90} after adding REL. Among *P. aeruginosa* strains without carbapenemase the MIC for IMI/REL was reduced between 1 to 5 dilutions.

Conclusions. IMI/REL showed high activity against the strains that carry *Klebsiella pneumoniae* carbapenemase (KPC) and against carbapenem-resistant *P. aeruginosa* unrelated to the VIM enzyme, mainly AmpC beta lactamase associated with impermeability. Against strains carrying oxacillinase 48 (OXA-48) associated with extended-spectrum beta-lactamase (ESBL), IMI/REL presented activity only slightly better than IMI and had no beneficial effect superior to IMI against *A. baumannii.*

Keywords: Imipenem-relebactam, *Pseudomonas aeruginosa*, Carbapenem-resistant Enterobacterales, Klebsiella pneumoniae carbapenemase, oxacillinase 48, Extended-spectrum beta-lactamase.

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Actividad *in vitro* de imipenem/relebactam frente aislados clínicos de gramnegativos en dos hospitales terciarios españoles

RESUMEN

Objetivo. El objetivo de este estudio fue analizar la actividad de la combinación imipenem-relebactam (IMI/REL) frente a una colección de aislados clínicos multirresistentes de Enterobacterales, *Pseudomonas aeruginosa* y *Acinetobacter baumannii.*

Material y métodos. El estudio se realizó en dos hospitales terciarios de España e incluyó 192 aislados clínicos de estos 3 géneros (139 resistentes y 53 susceptibles a IMI). Las CMI para IMI con y sin REL (a una concentración fija de 4 mg/L) se determinaron por un método estándar de microdilución en caldo según las recomendaciones internacionales.

Resultados. Todas las cepas de *E. coli* sensibles a IMI fueron también sensibles a IMI/REL. En el caso de las enterobacterias resistentes a IMI debido a la producción de carbapenemasas, la CMI_{50} y la CMI_{90} disminuyeron de 64/256 con IMI a 8/64 con IMI/REL. Esta elevada actividad se detectó principalmente entre los aislados con enzimas KPC. Las enterobacterias con carbapenemasas de clase B, *P. aeruginosa* portadora de carbapenemasas VIM y las cepas de *A. baumannii* no mostraron cambios en la CMI_{50} ni en la CMI_{90} tras añadir REL. Entre las cepas de *P aeruginosa* sin carbapenemasas, la CMI para IMI/REL se redujo entre 1 y 5 diluciones.

Conclusiones. IMI/REL mostró una elevada actividad frente a las) y frente a *Pseudomonas aeruginosa* resistente a carbapenemasas no relacionadas con la enzima VIM, principalmente AmpC beta lactamasa asociada a la impermeabilidad. Contra las cepas portadoras de oxacilinasa 48 (OXA-48) asociadas a betalactamasas de espectro extendido (ESBL), IMI/ cepas portadoras de *Klebsiella pneumoniae* carbapenemasa (KPC). REL presentó una actividad sólo ligeramente mejor que

IMI y no tuvo un efecto beneficioso superior a IMI contra *A. baumannii*.

Palabras clave: Imipenem, Imipenem-relebactam, *Pseudomonas aeruginosa*, Enterobacteriaceae resistentes a los carbapenémicos, beta-lactamasa de espectro extendido.

INTRODUCTION

The continuing emergence of antibiotic resistance in the community and hospitals is considered a major public health threat [1, 2]. This resistance is mainly due to the presence of carbapenemase enzymes and the addition of other resistance mechanisms frequently related with the external membrane's permeability [1].

To avoid this problem, various combinations of beta-lactam antibiotics and beta-lactamase inhibitors have been developed. Relebactam (REL) is a new inhibitor of class A, C and certain D beta-lactamases (not active against OXA-48 beta-lactamase), which in combination with imipenem (IMI) has recently been approved by the European Medicines Agency (EMA) and the Food and Drug Administration (US FDA). IMI/REL combination can restore carbapenem's activity when resistance is due class A or certain type D carbapenemases, either alone or in combination with other resistance mechanisms, mainly extended-spectrum beta-lactamases (ESBL) and AmpC beta-lactamases [3, 4].

The aim of this study was to evaluate the *in vitro* activity of IMI/REL against 192 clinical isolates (139 resistant and 53 susceptible to imipenem) from the collections of the Department of Microbiology of Hospital Clínico San Carlos de Madrid and Clínica Universitaria de Navarra.

MATERIAL AND METHODS

Strains were selected according to their resistance mechanisms including the most common of them. Table 1 shows the distribution of these isolates according to their predominant resistance mechanism. The resistance genes were either previously characterized [5,6] or detected using polymerase chain reaction with specific primers. Isolates were subcultivated at least twice in Mueller-Hinton agar before proceeding to minimum inhibitory concentration (MIC) determination. IMI and REL were supplied by Merck Sharp and & Dohme. The stock solutions of the compounds were prepared and stored in aliquots at -80 ºC until use. No aliquot was refrozen once unfrozen. The MICs for IMI with and without REL (at a fixed concentration of 4 mg/L) were determined by a standard broth microdilution method [7]. The MIC ranges used for IMI were 512 to 0.5 mg/L for resistant strains and 8 mg/L to 0.0625 mg/L for susceptible strains. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were included as quality controls in each MIC assay. MICs were measured using broth microdilution according to international recommendations [8].

Table 1 Characterization of the tested strains

Microorganism	Enzyme	n
Imipenem-susceptible *Escherichia coli* (n = 49)		
	TEM	8
	CTXM 9	11
	CTXM 15	14
	SHV	5
	AmpC	10
	AmpC + CTXM 9	1
Imipenem-resistant Enterobacterales (n = 50)		
Klebsiella pneumoniae		30
	KPC	14
	OXA 48	8
	OXA 48 + ESBL	7
	VIM	1
Enterobacter cloacae		13
	KPC	5
	OXA 48	4
	VIM	4
Serratia marcescens	VIM	2
Citrobacter freundii	VIM	2
Klebsiella variicola	VIM	1
Klebsiella oxytoca	VIM	1
Escherichia coli	NDM	1
Acinetobacter baumannii (n = 50)		
	OXA23	10
	OXA24+OXA58	1
	OXA24	14
	OXA51+ISAba1	11
	OXA58	14
Pseudomonas aeruginosa (n = 43)		
	OprD ↓ +/- ↑AmpC +/-↑MexAB	20
	VIM	19
	KPC	4

RESULTS

As expected, all IMI-susceptible *E. coli* strains were also susceptible to IMI/REL. The MIC_{50} remained at 0.125 mg/L in both cases, and the MIC_{90} decreased 1 dilution, from 0.25 mg/L for IMI to 0.125 for IMI/REL.

In the Enterobacterales resistant to IMI due to the production of carbapenemases, the MIC_{50} and MIC_{90} decreased from 64/256 with IMI to 8/64 with IMI/REL. This high activity was principally detected among isolates with KPC enzymes. In these, the MIC values decreased at least 5 dilutions in up to 63.16% (12/19) of the strains. For the 12 Enterobacterales with class B carbapenemases, there was no differences between the MIC with IMI and those with IMI/REL. In the same way, 19 *P.*

Table 2 **MIC distribution of imipenem-resistant strains.**

Group of organism/ Enzyme	N	Imipenem MIC (mg/L) N (%)				Imipenem/relebactam MIC (mg/L) N (%)			
		≤2	≤4	≤8	≥16	≤2	≤4	≤8	≥16
Enterobacterales (n=49)									
KPC	19				19 (100)	9 (47.4)	15 (78.9)	17 (89.5)	2 (10.5)
OXA 48 + ESBL	7				6 (85.7)	2 (28.6)	3 (42.9)	7 (100)	
OXA-48	12				12 (100)				9 (75)
VIM / NDM	11 / 1				12 (100)				12 (100)
Acinetobacter baumannii (n=50)		4 (8)	4 (8)	5 (10)	45 (90)	4 (8)	4 (8)	6 (12)	44 (88)
Pseudomonas aeruginosa (n=43)									
NO carbapenamase	20	1 (5)	1 (5)	10 (50)	10 (50)	19 (95)	19 (95)	20 (100)	
VIM	19		2 (10.5)	2 (10.5)	17 (89.5)	1 (5.3)	2 (10.5)	4 (21,1)	
KPC	4				4 (100)	4 (100)			

aeruginosa carrying VIM carbapenemase and all *A. baumannii* strains, showed no changes in the MIC_{50} and MIC_{90} (64 – 256 mg/L, and 16 – 128 mg/L respectively). In *P. aeruginosa* isolates without carbapenemase (20/43), the MIC for IMI/REL was reduced between 1 to 5 dilutions. The group with carbapenemases obtained a reduction of 4 dilutions for the 4 KPC-carrying strains, obtaining MIC≤2 in all strains (4/4). The distribution of MIC values is shown in Table 2.

DISCUSSION

As expected, the addition of REL to IMI can improve the MIC of IMI in KPC carbapenemase-producing Enterobacterales, reaching up to 7 dilutions of difference [9-12]. For *P. aeruginosa* resistant to IMI due to reduced porin expression, associated or not with the overexpression of AmpC enzymes and efflux pumps, IMI/REL showed high activity. These results are in agreement with previous published studies [9- 11]. In our study, IMI/REL decreases MIC values 4 dilutions in *P. aeruginosa* strains with KPC enzymes, similarly to that observed in Enterobacterales.

Our results show that OXA-48 carrier strains were resistant to IMI/REL in almost 90% of cases. The same results were obtained by other authors [12]. The difference observed in the MIC reduction between OXA-48-carrying Enterobacterales and those that also carry an ESBL suggest the presence of impermeability resistance mechanisms in the last ones. Other studies with strains resistant to IMI for the association of ESBL and/or AmpC with impermeability showed that IMI/REL reduced the MIC to IMI susceptibility values [9, 10, 13]. Finally, the *Acinetobacter baumannii* strains showed no improvement when adding REL to IMI, showing the lack of activity of REL on class D enzymes characteristic of *A. baumannii* [10].

This analysis may be subject to some limitations. Due to the low stability of imipenem, the concentration of active drug in the titrated substance used in the experiments could be less than the expected. This fact could justify an increase in the MICs obtained, especially in the case of those carriers of carbapenemases of the KPC family compared with previous published data [9- 3]. In addition, the selection of the strains was based on the mechanisms of resistance instead of frequency of appearance in clinical samples being not representative of the local susceptibility of these 3 groups.

IMI/REL shows high activity against KPC carrier strains. This, together with its effective inhibition of other high-dispersion broad-spectrum beta-lactamases such as ESBL and AmpC, as well as the combination's low susceptibility to active efflux pumps, makes this combination an alternative treatment for infections caused by KPC carbapenemase-carrying Enterobacterales with or without other associated mechanisms of beta-lactam resistance. The study conducted by Haidar et al. [14] showed that IMI/REL also maintains its activity in strains with KPC-2 and KPC-3 mutations, associated with resistance to another new combination: ceftazidime/avibactam. Similarly, IMI/REL appears to be a good candidate for those medical centers that, in addition to the above-mentioned problem with KPC carbapenemases, have multidrug-resistant *P. aeruginosa* that do not carry VIM carbapenemases.

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None to declare

CONFLICT OF INTEREST

Authors declare no conflict of interest

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