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ORIGINAL ARTICLE

In vitro activity of imipenem-relebactam against non-MBL carbapenemase-producing *Klebsiella pneumoniae* isolated in Greek hospitals in 2015–2016

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Abstract

Relebactam is a β-lactamase inhibitor of class A and class C β-lactamases, including carbapenemases. We evaluated the ability of relebactam to restore imipenem susceptibility against a collection of *Klebsiella pneumoniae* isolates from Greek hospitals. We tested 314 non-MBL carbapenemase-producing *K. pneumoniae* consecutive clinical strains isolated from unique patients at 18 hospitals in Greece, between November 2014 and December 2016. Susceptibility testing of imipenem, imipenem-relebactam, meropenem, doripenem, gentamicin, and colistin was performed using broth microdilution. Additionally, MICs of ceftazidime-avibactam, fosfomycin, and tigecycline were determined by MIC Test Strips. MICs were interpreted per EUCAST breakpoints. Imipenem-relebactam MICs were interpreted using the breakpoints proposed for imipenem. Carbapenemase genes were detected using PCR. Whole genome sequencing was performed for selected isolates. Imipenem-relebactam inhibited 98.0% of the KPC-producing isolates at ≤ 2 mg/L ($MIC_{50}/_{90}$, 0.25/1 mg/L) and was considerably more active than imipenem ($MIC_{50}/_{90}$, 32/> 64 mg/L). Reduced activity of imipenem-relebactam was rarely detected (2%) and was associated with chromosomal factors (*ompK35* disruption and/or mutated *ompK36*). Only ceftazidime-avibactam showed in vitro activity comparable to imipenem-relebactam (99.6% susceptible). Relebactam provided only weak potentiation of imipenem activity against *K. pneumoniae* with class D OXA-48-like enzymes. Relebactam exhibited strong potential for restoring the in vitro activity of imipenem against KPC-producing *K. pneumoniae*, lowering the imipenem MIC_{50} and MIC_{90} from 32 to 0.25 mg/L, and from > 64 to 1 mg/L, respectively. Production of KPC carbapenemase represents the main cause of carbapenem resistance among *K. pneumoniae* in Greek hospitals (66.5%), and this carbapenemase appears to be very well inhibited by relebactam.

Keywords Relebactam · Carbapenemase · KPC · OXA-48 · *K. pneumoniae*

Introduction

Relebactam is a novel non-β-lactam, bicyclic diazabicyclooctane β-lactamase inhibitor that is structurally related to avibactam, differing by the addition of a piperidine ring to the 2-position carbonyl group.

Relebactam has been combined with imipenem-cilastatin, to restore imipenem's clinical activity against KPC-producing *K. pneumoniae*, other KPC-producing Enterobacteriaceae, and *Pseudomonas aeruginosa* demonstrating carbapenem resistance due to impermeability combined with AmpC expression [1, 2]. Both inhibitors display activity against class A β-lactamases, including KPC-type carbapenemases and class C β-lactamases (AmpC), but unlike avibactam, relebactam does not consistently inhibit isolates producing OXA-48 carbapenemases [2].

Unfortunately, ceftazidime-avibactam resistance has emerged [3, 4]. Little is known about the potential for relebactam to select for resistance; however, inactivation of

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the porin OmpK36 in *K. pneumoniae* has been reported to confer resistance to both imipenem-relebactam and meropenem-vaborbactam [5].

In this study, we determined the activity of imipenem combined with relebactam against *K. pneumoniae*–producing non-MBL carbapenemases isolated from hospitalized patients in 18 hospitals in six Greek cities [6, 7]. Isolates with elevated imipenem-relebactam MIC were further evaluated.

Material and methods

Clinical isolates

Carbapenem non-susceptible *K. pneumoniae* isolates, producing a non-MBL carbapenemase, were selected from two previous studies. The isolates were collected from unique patients hospitalized between November 2014 and December 2016 in 18 hospitals from six cities in Greece [6, 7].

Species identification and primary MIC determination was performed by Vitek®2 (bioMérieux, Marcy-l'Etoile, France).

Antimicrobial susceptibility testing

Susceptibility testing of imipenem with and without relebactam (4 μg/ml) (Merck & Co., Inc., New Jersey, USA), meropenem (Sigma-Aldrich, St. Louis, MO, USA), doripenem (Sigma-Aldrich), gentamicin (AppliChem, GmbH, Darmstadt, Germany), and colistin (Sigma-Aldrich) was performed by standard broth microdilution, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [8].

Susceptibility testing of ceftazidime-avibactam, fosfomycin, and tigecycline was performed by Liofilchem® MIC Test Strips (MTS) (Liofilchem srl, Roseto degli Abruzzi, Italy), according to manufacturer's instructions. Antimicrobial susceptibility results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing recommendations (EUCAST 2018, version 8.0) [9]. As susceptibility breakpoints for the combination of imipenem-relebactam are not established by EUCAST or CLSI, imipenem breakpoints were applied. Further, in KPC-producing isolates with elevated MICs to imipenem-relebactam (> 2 mg/L), we determined the MIC to meropenem-vaborbactam by MTS. *Escherichia coli* ATCC 25922, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC27853 were used as quality control strains. All isolates were stored at − 80 °C and were sub-cultured twice before testing.

Screening for β-lactamase genes and molecular typing

All *K. pneumoniae* isolates were screened for genes encoding metallo- and serine β-lactamases by PCR as described previously [6]. All isolates with imipenem-relebactam MIC > 2 mg/L were tested further for the presence of genes encoding ESBLs and acquired AmpC β-lactamases [10].

Genetic relatedness of *K. pneumoniae* isolates was evaluated by pulsed field gel electrophoresis (PFGE) analysis as previously described [6].

Whole genome sequencing analysis of selective isolates

Total DNA was extracted and purified using the PureLink® Genomic DNA mini kit (Life Technologies, Invitrogen™, Carlsbad, CA, USA) following manufacturer's Gram Negative Bacterial Cell DNA extraction protocol and whole genome sequencing (WGS) data were generated on Ion Torrent™ platform (Life Technologies). Analyses of WGS were performed by tools provided by the Center for Genomic Epidemiology (CGE) (http://cge.cbs.dtu.dk/services/all.php). Capsule and lipopolysaccharide serotype were predicted by Kaptive Web tool (http://kaptive.holtlab.net/). Selected genes were also aligned to reference genes with the NCBI BLAST algorithm (www.ncbi.nlm.nih.gov/BLAST).

Transferability of *bla*KPC genes

Conjugation and transformation experiments were performed, as previously described [11]. Transconjugant/transformant selection was performed in media containing rifampicin and ceftazidime or ceftazidime alone at 16 mg/L respectively.

Results

A total of 314 *K. pneumoniae* isolates with imipenem and/or meropenem MIC > 2 mg/L were tested. Isolates were obtained from urine ($n = 128$), blood ($n = 62$), specimens of lower respiratory tract ($n = 53$), pus ($n = 40$), CSF ($n = 2$), and other sites ($n = 29$).

All isolates were non-susceptible to imipenem (MICs > 2 mg/L) and doripenem (MICs > 1 mg/L), and 313 (99.7%) were non-susceptible to meropenem (MICs > 2 mg/L). Two hundred ninety-five were KPC-producing, and 19 OXA-48-producing *K. pneumoniae* isolates. MIC_{50} and MIC_{90} against all isolates were 32 and > 64 mg/L, respectively, for imipenem and 0.5 and 2 mg/L for imipenem-relebactam. The addition of relebactam improved imipenem susceptibility rates from 0 to 92.7% (Table 1).

Table 1 MIC and cumulative percent inhibited distributions for imipenem-relebactam and comparators, in relation to the carbapenemase type produced by the 314 *K. pneumoniae* isolates

Organism /Genotype	Agent	No of isolates / (cumulative % of isolates) inhibited at MIC (mg/L)										MIC_{50} (mg/L)	MIC_{90} (mg/L)	S (%)
		≤0.25	0.5	1	2	4	8	16	32	64	>64			
KPC-producers	Imipenem					2 (0.7)	28 (10.2)	74 (35.3)	47 (**51.2**)	109 (88.1)	35 (100)	32	>64	0
(n=295)	Imipenem-relebactam	**154 (52.2)**	**91 (83.1)**	**29 (92.9)**	**15 (98.0)**	6 (100)						0.25	1	98.0
	Meropenem				**1 (0.3)**		22 (7.8)	41 (21.7)	60 (42.0)	44 (56.9)	127 (100)	64	>64	0.3
	Doripenem				3 (1.0)	28 (10.5)	58 (30.2)	32 (41.0)	39 (54.2)	72 (78.6)	63 (100)	32	>64	0
	Colistin		**28 (9.5)**	**133 (54.6)**	**18 (60.7)**	9 (63.7)	11 (67.5)	18 (73.6)	41 (87.5)	21 (94.6)	16 (100)	1	64	63.4
	Fosfomycin					**1 (0.3)**	**13 (4.7)**	**74 (29.8)**	**87 (59.3)**	58 (79.0)	62 (100)	32	1024	59.3
	Tigecycline	**3 (1.0)**	**37 (13.6)**	**103 (48.5)**	114 (87.1)	31 (97.6)	5 (99.3)	2 (100)				2	4	48.5
	Gentamicin		**6 (2.0)**	**51 (19.3)**	**132 (64.1)**	41 (78.0)	5 (79.7)	13 (84.1)	4 (85.4)	5 (87.1)	38 (100)	2	>64	64.1
	Ceftazidime-avibactam	**20 (6.8)**	**83 (34.9)**	**130 (79.0)**	**52 (96.6)**	**9 (99.7)**		1 (100)				1	2	99.7
OXA-producers[a]	Imipenem					6 (31.6)	10 (84.2)	2 (94.7)			1 (100)	8	16	0.0
(n=19)	Imipenem-relebactam				**2 (10.5)**	13 (78.9)	2 (89.5)	1 (94.7)	1 (100)			4	16	10.5
	Meropenem						1 (5.3)	9 (52.6)	7 (89.5)	1 (94.7)	1 (100)	16	64	0.0
	Doripenem						9 (47.4)	9 (94.7)			1 (100)	16	16	0.0
	Colistin		**1 (5.3)**	**7 (42.1)**					8 (84.2)	2 (94.7)	1 (100)	32	64	42.1
	Fosfomycin					**1 (5.3)**		**9 (52.6)**	**3 (68.4)**	1 (73.7)	5 (100)	16	>64	68.4
	Tigecycline		**3 (15.7)**	**9 (63.2)**	4 (84.2)	1 (89.5)	1 (94.7)				1 (100)	1	8	63.2
	Gentamicin			**2 (10.5)**	**2 (21.1)**				1 (26.3)		14 (100)	>64	>64	21.1
	Ceftazidime-avibactam		**7 (36.8)**	**10 (89.5)**	**2 (100)**							1	2	100.0

[a] One isolate harboring also bla_{KPC} is included

Bold indicates susceptible by EUCAST 2018 breakpoint

KPC-producing isolates

For 295 isolates that harbored bla_{KPC}, the imipenem MIC_{50} and MIC_{90} values were 32 and > 64 mg/L, respectively. The addition of relebactam reduced imipenem MICs by a median of 128-fold (range 8- to 512-fold), decreased the MIC_{50} and MIC_{90} values to 0.25 and 1 mg/L, respectively, and improved imipenem susceptibility rates from 0 to 98.0% (Table 1).

PFGE genotyping revealed a multiclonal population of KPC-producing *K. pneumoniae*, with a prevalent PFGE profile (with ten variants) comprising 147 (49.8%) of the isolates, which was detected in 18 centers. Additionally, two PFGE profiles, consisting of three and two variants each, comprised 20 (6.8%) and 18 (6.1%) isolates, respectively. Twenty-four more PFGE profiles were identified that included 2–10 isolates each.

KPC-producing *K. pneumoniae* non-susceptible to imipenem-relebactam

In six isolates (2.0%), all with imipenem MICs ≥ 128 mg/L, the addition of 4 mg/L relebactam reduced imipenem MIC to 4 mg/L, which corresponds to intermediate imipenem resistance, according to the EUCAST breakpoints (Table 2).

WGS analysis determined that the isolates belonged to 3 different sequence types (STs): ST258 ($n = 3$), ST147 ($n = 2$), and ST15 ($n = 1$) (Table 2). The three ST258 strains were isolated in different hospitals, in North Greece, Attica, and South Greece. Only the two ST147 isolates were epidemiologically related as they shared the same PFGE pattern and were isolated in the same hospital. Five isolates harbored bla_{KPC-2} and one bla_{KPC-23} (Table 2).

Table 2 Antimicrobial susceptibility and genotype of the imipenem-relebactam non-susceptible clinical KPC-producing *K. pneumoniae* isolates and their transformant/transconjugant laboratory strains

Isolate	Region/ hospital	KPC-gene	MLST clone	MIC (mg/L)							Other *bla*-genes	Major porin mutations	
				IMP	IMP/REL	CAZ	CAZ/AVI	MER	MER/VAB	DOR		OmpK35	OmpK36
KP-90	NG/A	bla_{KPC-23}	ST258	512	4	> 1024	16	512	4	> 64	bla_{SHV-11}, bla_{TEM-1A}, bla_{OXA-9}[a]	FS_aa89	v1
TOP10/pl90	LS	bla_{KPC-23}	ND	16	0.5	> 1024	8	8	0.06	8	bla_{TEM-1A}, bla_{OXA-9}[a]	NA	NA
KP-128	AT/B	bla_{KPC-2}	ST258	128	4	512	2	512	16	> 64	bla_{SHV-11}, bla_{CMY-4}, bla_{TEM-1A}, bla_{OXA-9}[a], bla_{OXA-1}	FS_aa89	v1-GD
TOP10/pl128	LS	bla_{KPC-2}	ND	16	0.5	128	1	8	0.06	8	bla_{TEM-1A}, bla_{OXA-9}[a]	NA	NA
KP-158	SG/C	bla_{KPC-2}	ST258	512	4	512	0.5	512	1	> 64	bla_{SHV-11}, bla_{CMY-4}, bla_{TEM-1A}, bla_{OXA-9}[a], bla_{OXA-1}	FS_aa89	v1
TOP10/pl158	LS	bla_{KPC-2}	ND	16	0.5	128	1	8	0.06	8	bla_{TEM-1A}, bla_{OXA-9}[a]	NA	NA
KP-74	AT/D	bla_{KPC-2}	ST147	256	4	64	1	256	2	> 64	bla_{SHV-67}, bla_{TEM-1A}, bla_{OXA-9}[a], bla_{OXA-10}	TS_ aa173	v2
TOP10/pl74	LS	bla_{KPC-2}	ND	8	0.25	64	1	8	0.06	8	bla_{TEM-1A}, bla_{OXA-9}[a]	NA	NA
KP-80	AT/D	bla_{KPC-2}	ST147	256	4	64	1	256	4	> 64	bla_{SHV-67}, bla_{TEM-1A}, bla_{OXA-9}[a], bla_{OXA-10}	TS_ aa173	v2
TOP10/pl80	LS	bla_{KPC-2}	ND	8	0.25	64	1	8	0.06	8	bla_{TEM-1A}, bla_{OXA-9}[a]	NA	NA
KP-105	CG/E	bla_{KPC-2}	ST15	256	4	128	2	512	2	> 64	bla_{SHV-28}, $bla_{CTX-M-163}$, bla_{TEM-1B}, bla_{OXA-1}	WT	v1-A323P
RC85/pl105	LS	bla_{KPC-2}	ND	4	0.125	32	1	2	0.03	4	bla_{TEM-1B}	NA	NA
TOP10	LS	–	ND	0.25	0.25	0.5	0.5	0.06	0.03	0.06	–	NA	NA
RC85	LS	–	ND	0.25	0.25	0.25	0.25	0.06	0.03	0.06	–	NA	NA

NG, North Greece; *AT*, Attica; *SG*, South Greece; *CG*, Central Greece; *LS*, laboratory strain

FS_aa89, frameshift due to nt insertion at nt124, resulting in stop codon at amino acid 89; *TS_ aa173*, C to A transition at nt519 resulting in stop codon at amino acid 173; *v1*, OmpK36 variant (GenBank accession number WP_002913005.1); *v1-GD*, OmpK36 v1variant with a glycine-aspartic acid duplication at positions 136 and 137; *v2*, OmpK36 variant (GenBank accession number WP_099130593.1); *v1-A323P*, OmpK36 variant (GenBank accession number WP_002913005.1) with one amino acid substitution (A323P) at B14 β-strand

[a] Non-functional bla_{OXA-9} due to a G to A mutation at nt335 resulting in a stop codon (aa112)

The carbapenemase gene was detected on identical Tn*4401a* transposons, located between IS*Kpn7* and IS*Kpn6*, while no mutations known to contribute to the expression of bla_{KPC} were evident within promoter sequences P1 and P2 in any isolate. All isolates harbored additional β-lactamase genes (Table 2).

Five isolates harbored a non-functional OmpK35. Isolates belonging to ST258 had a truncated protein at codon 89, while the isolates of ST147 had a truncated protein at codon 173. In the ST15 isolate, a wild-type OmpK35 (GenBank accession number WP_004141771.1) was detected.

OmpK36 of the ST258 isolates matched to the OmpK36_v1 (GenBank accession number WP_002913005.1) were full length with only that of KP-128, to harbor a duplication of amino acids G134–D135, located at the conserved loop L3. This insertion contributes to high-level resistance to carbapenems [12]. ST147 isolates harbored OmpK36_v2 (GenBank accession number WP_099130593.1), while the ST15 isolate harbored the OmpK36_v1 variant with one amino acid substitution (A323P) at the B14 β-strand of the protein. No insertion sequences were identified upstream of *ompK*36 in any isolate.

Susceptibility testing of *E. coli* TOP10 transformants and RC85-harboring bla_{KPC-2}- or bla_{KPC-23}-carrying plasmids is shown in Table 2. In all isogenic TOP10 transformants and in RC85/pl105, the addition of 4 mg/L relebactam reduced imipenem MIC from 8 to 16 mg/L to 0.25–0.5 mg/L suggesting that chromosomal genes have contributed to the high imipenem and elevated imipenem-relebactam MICs in parent isolates.

OXA-48-like–producing isolates

No significant difference was observed in imipenem-relebactam vs. imipenem MICs against OXA-48-like carbapenemase producers (median 4 and 8 mg/L, respectively) (Table 1). The addition of relebactam restored the activity of imipenem in only two of the 19 OXA-48-like–producing isolates. All 19 isolates harbored additional β-lactamase genes (Table 3).

The OXA-48-like–producing *K. pneumoniae* isolates were multiclonal (eight PFGE clones). Different clones between hospitals and within hospitals were observed.

WGS analysis was performed in isolate KP-205 co-producing KPC-2 and OXA-48, as it was the isolate exhibiting the highest imipenem-relebactam MIC (32 mg/L). The isolate was assigned to ST716, an infrequently reported clone of capsular type KL110 (*wzc* 941; *wzi* 89) [13]. bla_{KPC-2} was detected on a Tn*4401a*, while bla_{OXA-48} on a Tn*1999* transposon. The isolate harbored additional acquired *bla* genes coding for CTX-M-15, VEB-1, OXA-10, TEM1B, and OXA-1 and had intact OmpK35 and OmpK36 proteins, with OmpK36 matching to the OmpK36_v6 (GenBank accession number WP_004149145.1).

Comparator antimicrobial agents

Of the comparator agents, only ceftazidime-avibactam showed in vitro activity comparable to that of imipenem-relebactam (S, 99.7%). Ceftazidime-avibactam was active against all but one KPC-producing isolate and against all 19 isolates with OXA-48-like carbapenemases. These isolates were ceftazidime-resistant due to CTX-M-type ESBL co-production and the addition of avibactam reduced MICs to 0.5–2 mg/L. The isolate that was resistant to ceftazidime-avibactam (KP-90; MIC, 16 mg/L) produced KPC-23 was highly resistant to ceftazidime (MIC, > 1024 mg/L) and imipenem (MIC, 512 mg/L) and had an MIC of 4 mg/L to imipenem-relebactam (intermediate resistance to imipenem).

Of the other agents tested, gentamicin was the most active in vitro, with 61.5% of the isolates to be inhibited at ≤ 2 mg/L, followed by fosfomycin (S, 59.8%) and colistin (S, 59.6%). Finally, tigecycline demonstrated 49.4% susceptibility rate.

Discussion

Imipenem-relebactam exhibited potent activity, against KPC-producing isolates. Relebactam restored imipenem susceptibility in 98.0% of the KPC isolates. This is in accordance with other studies reporting that relebactam restored imipenem susceptibility in up to 98% of Enterobacteriaceae-producing KPC [2, 14].

In our collection of KPC-producing *K. pneumoniae* isolates, six (2.0%) exhibited high MIC to imipenem (≥ 128 mg/L) and elevated MIC to imipenem-relebactam (4 mg/L). These isolates exhibited various defects in OmpK35 or/and OmpK36. As carbapenems cross the outer membrane using both the OmpK35 and OmpK36 porins, the inactivation of OmpK35 or/and OmpK36 shown in those isolates likely contributed to the high carbapenem MICs observed. The resulting outer membrane porin loss in combination with a carbapenemase has been reported to confer high-level resistance to carbapenems [15]. Downregulation of OmpK36 or major mutations in this porin has been reported to increase imipenem-relebactam MIC up to 8 mg/L [16, 17]. Recently, Balabanian et al. reported that disruption of either *ompK*35 or *ompK*36 alone did not affect the MIC of imipenem-relebactam. However, when both were disrupted, the MIC of imipenem-relebactam increased to 2–512 mg/L depending on the expression of bla_{KPC} [18]. In our study, disrupted OmpK35 was present in five isolates with elevated imipenem-relebactam MIC, and in four of them was the only

Table 3 Antimicrobial susceptibility and genotype of the OXA-48–producing *K. pneumoniae* isolates

Isolate	MIC (mg/L)					Acquired *bla*-genes
	Imipenem	Imipenem-relebactam	Meropenem	Doripenem	Ceftazidime-avibactam	
KP-52	8	4	16	8	1	bla_{OXA-48}, bla_{CTX-M}, $bla_{CMY-2-like}$, bla_{VEB}, bla_{OXA-10}, bla_{TEM}, bla_{OXA-1}
KP-54	8	4	32	16	1	bla_{OXA-48}, bla_{CTX-M}, $bla_{CMY-2-like}$, bla_{VEB}, bla_{OXA-10}, bla_{TEM}, bla_{OXA-1}
KP-56	8	8	32	16	1	bla_{OXA-48}, bla_{CTX-M}, $bla_{CMY-2-like}$, bla_{VEB}, bla_{OXA-10}, bla_{TEM}, bla_{OXA-1}
KP-82	8	4	32	16	1	bla_{OXA-48}, bla_{CTX-M}, $bla_{CMY-2-like}$, $bla_{OXA-7/13}$, bla_{TEM}, bla_{OXA-1}
KP-85	8	4	64	16	1	bla_{OXA-48}, bla_{CTX-M}, $bla_{OXA-7/13}$, bla_{OXA-1}
KP109	4	4	16	8	0.5	bla_{OXA-48}, bla_{CTX-M}, $bla_{OXA-7/13}$, bla_{TEM}, bla_{OXA-1}
KP-115	8	4	32	16	0.5	bla_{OXA-48}, $bla_{OXA-7/13}$
KP-119	8	8	32	16	0.5	bla_{OXA-48}, $bla_{OXA-7/13}$
KP-127	4	4	16	8	1	bla_{OXA-48}, bla_{CTX-M}, $bla_{OXA-7/13}$, bla_{OXA-1}, bla_{TEM}
KP-178	8	4	16	8	0.5	bla_{OXA-48}, bla_{CTX-M}, $bla_{OXA-7/13}$, bla_{OXA-1}, bla_{TEM}
KP-186	4	4	16	8	0.5	bla_{OXA-48}, bla_{CTX-M}, $bla_{OXA-7/13}$, bla_{OXA-1}, bla_{TEM}
KP-188	16	2	16	8	1	bla_{OXA-48}, bla_{CTX-M}, $bla_{OXA-7/13}$, bla_{OXA-1}
KP-190	4	2	16	8	1	bla_{OXA-48}, bla_{CTX-M}, $bla_{OXA-7/13}$, bla_{OXA-1}
KP-205	>64	32	>64	>64	2	bla_{KPC-2}, bla_{OXA-48}, $bla_{CTX-M-15}$, bla_{VEB-1}, bla_{OXA-10}, bla_{TEM-1B}, bla_{OXA-1}
KP-292	4	4	16	8	2	bla_{OXA-48}, bla_{CTX-M}, $bla_{CMY-2-like}$, $bla_{OXA-7/13}$, bla_{TEM}, bla_{OXA-1}
KP-293	8	4	32	16	1	bla_{OXA-48}, bla_{CTX-M}, $bla_{OXA-7/13}$, bla_{OXA-1}, bla_{TEM}
KP-294	16	16	32	16	0.5	bla_{OXA-48}, bla_{CTX-M}, $bla_{OXA-7/13}$, bla_{OXA-1}
KP-295	4	4	8	8	1	bla_{OXA-48}, bla_{CTX-M}, $bla_{OXA-7/13}$, bla_{OXA-1}, bla_{TEM}
KP-413	8	4	16	16	0.5	bla_{OXA-48}, bla_{CTX-M}, $bla_{CMY-2-like}$, $bla_{OXA-7/13}$, bla_{TEM}, bla_{OXA-1}

porin deficiency identified, suggesting that the role of OmpK35 requires further study. Yet, the level of expression of additional β-lactamase genes, which were not studied further, may also have contributed to increased imipenem-relebactam MICs.

Additionally, our results revealed the association of the GD duplication within the L3 internal loop of OmpK36, with reduced susceptibility to meropenem/vaborbactam, [19], but not to imipenem-relebactam. OmpK36, which has a smaller channel than OmpK35, appears to play a more significant role in the passage of vaborbactam across the outer membrane than OmpK35 [19].

Susceptibility studies of isogenic isolates harboring plasmids from the six isolates revealed that chromosomal factors impacted imipenem-relebactam susceptibilities and contributed to elevated MICs.

A limitation of our study is that the in vitro activity of imipenem-relebactam was not evaluated based on approved susceptibility breakpoints as those have not yet been determined. Rather, we evaluated the in vitro activity of the combination based on the reduction of imipenem MICs to the susceptible range when relebactam was added. Additionally, we have not studied OmpK35 and OmpK36 of all KPC isolates to elucidate the impact of mutations in one or both proteins to the level of imipenem-relebactam MICs. Further, functional studies are needed to define the impact of porin gene disruptions on imipenem-relebactam MICs.

In summary, relebactam restored the in vitro activity of imipenem against a large collection of clinical bla_{KPC}-harboring *K. pneumoniae* isolates from a country where carbapenemase producers are endemic, suggesting that it is a promising option for difficult-to-treat infections by these bacteria. Production of KPC carbapenemase represents the main cause of carbapenem resistance among *K. pneumoniae* in Greek hospitals (66.5%) [6] and this carbapenemase appears to be very well inhibited by relebactam. As the global spread of KPC carbapenemases poses a therapeutic challenge for clinicians, the novel combination of imipenem with relebactam is a welcome advancement.

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Compliance with ethical standards

Conflict of interest IG has received research grand and speaker honorarium from ACHAOGEN and speaker honorarium and financial support for attending symposia from MSD; MS has received research grand from ACHAOGEN; HG has received research grand from Pfizer; AA has received research grand by MSD. All other authors: none to declare.

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