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Original Article

Activities of imipenem-relebactam combination against carbapenem-nonsusceptible Enterobacteriaceae in Taiwan

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KEYWORDS
Imipenem;

Abstract *Background:* Imipenem-relebactam is a new β-lactam and β-lactamase inhibitor combination to treat carbapenem-resistant gram-negative bacteria infections. However,

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Relebactam;
Carbapenem-nonsusceptible;
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in vivo;
Caenorhabditis elegans

difference in carbapenem resistant mechanisms existed with geographic variations.
Objective: To evaluate the susceptibility of imipenem-relebactam to 660 carbapenem-nonsusceptible Enterobacteriaceae isolates in Taiwan and to identify the *in vivo* efficacy with a *Caenorhabditis elegans* model.
Methods: 188 carbapenem-nonsusceptible *Escherichia coli* isolates and 472 carbapenem-nonsusceptible *Klebsiella pneumoniae* isolates were collected from a national surveillance study in Taiwan. The antimicrobial susceptibility profiles and carbapenemase distributions were determined. An agar dilution method was performed to evaluate the *in vitro* activities of imipenem monotherapy and imipenem-relebactam combination. Contributions of metallo-carbapenemase to imipenem-relebactam susceptibility was investigated via EDTA treatment. A *C. elegans* model was used to evaluate the *in vivo* efficacy of imipenem-relebactam combination.
Results: 87.8% and 82.2% susceptibility to imipenem-relebactam was observed for 188 carbapenem-nonsusceptible *E. coli* and 472 carbapenem-nonsusceptible *K. pneumoniae*, respectively. However, poor activities of imipenem-relebactam was observed against 23 metallo-carbapenemase producers tested in this study. In the *in vivo C. elegans* model, imipenem-relebactam significantly rescued nematodes from the infection of a bla_{KPC}-producing *K. pneumoniae* isolate.
Conclusion: Our study supports that imipenem-relebactam is a potential therapy against carbapenem-nonsusceptible Enterobacteriaceae, and to our knowledge, this is the first report of evaluation for imipenem-relebactam efficacy against carbapenem-nonsusceptible Enterobacteriaceae in Taiwan.

Introduction

Resistance to carbapenem in gram-negative bacilli is mainly attributed to the transmittable carbapenemases, the loss of porins in combination with bla_{AmpC} β-lactamase over-expression, and active efflux pumps.[1] The rapid dissemination of carbapenemase genes and selection pressure from overconsumption of carbapenems have led to the challenge in treating carbapenem resistant pathogen infections in clinical settings worldwide.[1] Globally epidemiological investigations revealed 0—58.6% of resistant rate in Europe, 0—52% in the Indian subcontinent, 0—25% in Asia—pacific, and 0.4—28.6% in Latin America.[2,3] In Taiwan, 10.5% (71/673) of *Klebsiella pneumoniae* clinical isolates were non-susceptibile to at least one carbapenem in 2017.[4] These studies have indicated an urgent need for the development of novel agents against carbapenem resistance.

Relebactam belongs to a class of bi-cyclic diazabicyclooctan β-lactamase inhibitor, which is structurally similar to avibactam.[5] The combination of imipenem-relebactam, approved by FDA in 2019, possesses anti-carbapenem-resistant Enterobacteriaceae efficacy, except for metallo-carbapenemase producers.[6] For non-metallo-carbapenemase-producing Enterobacteriaceae, 98.5% isolates in USA and 98% isolates in Europe were susceptible to imipenem-relebactam.[7,8] However, an international study involving 194 laboratories from 55 countries revealed different resistant rates to imipenem-relebactam in imipenem-nonsusceptible non-*Proteeae* Enterobacteriaceae isolates, including Africa (64.5—76.1% resistant), Asia (47.2—56.5%), Europe (42.3—50.9%), Latin America (18.7—21.1%), Middle East (66.1—76.4%), USA/Canada (22.9—25.5%), and South Pacific (43.7—48.4%), indicating geographic variations.[9] In the present study, we sought to evaluate the efficacies of imipenem-relebactam combination against 660 isolates of carbapenem-nonsusceptible Enterobacteriaceae collected in Taiwan.

Materials and methods

Bacterial collection

The bacteria collection is from Taiwan national surveillance studies[10,11] in which *Escherichia coli* and *K. pneumoniae* with MICs>1 μg/ml for imipenem or/and meropenem were defined as carbapenem-nonsusceptible Enterobacteriaceae (CnsE) in accordance with the CLSI guideline.[12] All carbapenem-nonsusceptible *E. coli* (CnsEC) from January 2012 to September 2015 were recruited in the study, except for those not viable after culture from storage condition. All available carbapenem-nonsusceptible *K. pneumoniae* (CnsKP) in the year 2014 were recruited in the study. Overall, 660 carbapenem-nonsusceptible Enterobacteriaceae (CnsE) isolates were collected from 16 Taiwanese hospitals. Among the 660 CnsE isolates, 188 isolates (18.5%) were CnsEC, and 472 isolates (71.5%) were CnsKP.[10,11] Urine was the most common isolation source (n = 251; 251/660, 38.0%), followed by sputum/endotracheal aspirates (n = 129; 129/660, 19.6%), blood (n = 56; 56/660, 8.5%), wounds/pus (n = 61; 61/660, 9.2%), stool/rectal swabs (n = 35; 35/660, 5.3%), bile (n = 33; 33/660, 5.0%), ascites (n = 26; 26/660, 3.9%), and abscesses (n = 13; 13/660, 2.0%). 56 isolates (56/660, 8.5%) were isolated from other sources, including PCTD drainage, CVP tips, and blood gas sampling lines.

The antimicrobial susceptibility testing and β-lactamase gene detections were also performed in the previous

national surveillance studies.[10,11] Briefly, the susceptibilities of 18 antimicrobial agents was also determined in the surveillance via broth microdilution method (Sensititre, Trek Diagnostic Systems, Cleveland, OH, USA). Antibiotic susceptibilities were interpreted according to MIC breakpoints established by CLSI.[12] PCR detection was used to investigate the presence of ESBL, AmpC, and carbapenemase genes.[13]

Antimicrobial susceptibility testing

MICs of imipenem and imipenem-relebactam were measured using standard agar dilution test. Based on an average total plasma concentration described in a previous study,[14] relebactam (REL) was assessed at a fixed concentration of 4 μg/ml in combination with a 2-fold dilution of imipenem (IMI). 24 isolates of metallo-carbapenemase producers were subjected to the estimation of MIC values under EDTA treatment at 320 μg/ml.[15]

Caenorhabditis elegans infection model

C. elegans strain N2 was employed to evaluate the treatment effect of imipenem-relebactam against a sequence type (ST) 11 KPC-producer *K. pneumoniae* isolate (CRE-1462). Procedures were executed as described in our earlier study with some modifications.[16] Briefly, CRE-1462 (MICs of imipenem and imipenem-relebactam were 16 and 0.25/4 μg/ml) was cultured in LB broth for 16–18 h at 37 °C, and bacterial suspension was adjusted to OD_{600} = 2 (ca. 7.5 × 10^8 CFU/ml). Thirty μl of the resulting bacterial suspension was subsequently spread onto nematode growth medium (NGM) agar and the plate was cultured at room temperature for overnight to form bacterial lawn. The plate with CRE-1462 was used to infect 300–400 growth synchronized L4 worms for 3 days, and 40 infected worms were subjected to untreated group (0 μg/ml), imipenem (1 μg/ml), and imipenem-relebactam (1/4 μg/ml) treatments on NGM agar, according to the susceptible breakpoint of imipenem suggested in CLSI guideline. Nematodes fed with *E. coli* lab strains OP50 served as an uninfected control. Nematode survival was recorded daily, and worms were transferred onto new plates and treated at the same conditions. Experiments were repeated in triplicate for reproducibility of results.

Statistical analyses

For analysis, the MIC values of imipenem-relebactam were first transformed by the use of log base 2, and the statistical analysis of $\log_2$ MIC values were carried out using GraphPad Prism Version 7.0 software (San Diego, CA) with paired *t*-test. The same software was used to create Kaplan–Meier survival curves and perform analysis using the log-rank (Mantel–Cox) test.

Results

Susceptibility profiles of *enterobacteriaceae* isolates

As shown in Table 1, 19 antimicrobial agents except amikacin and imipenem-relebactam, showed poor activity against 188 isolates of CnsEC. These agents included aztreonam (3.2%), ampicillin (0%), cefazolin (0%), cefoxitin (0.5%), cefotaxime (1.1%), ceftazidime (2.1%), ceftriaxone (0.5%), cefepime

Table 1 Susceptibilities of 19 antimicrobial agents against 660 carbapenem-nonsusceptible Enterobacteriaceae isolates.

Antimicrobial agent	Species and their susceptibility profiles to antimicrobial agent		
	E. coli (n = 188)	*K. pneumoniae* (n = 472)	Total (n = 660)
Aztreonam	3.2% (6/188)	7.4% (35/472)	6.2% (41/660)
Ampicillin	0% (0/188)	0% (0/472)	0% (0/660)
Cefazolin	0% (0/188)	0.2% (1/472)	0.2% (1/660)
Cefoxitin	0.5% (1/188)	0.6% (3/472)	0.6% (4/660)
Cefotaxime	1.1% (2/188)	2.5% (12/472)	2.1% (14/660)
Ceftazidime	2.1% (4/188)	0.4% (2/472)	0.9% (6/660)
Ceftriaxone	0.5% (1/188)	0.2% (1/472)	0.3% (2/660)
Cefepime	24.5% (46/188)	14.2% (67/472)	17.1% (113/660)
Piperacillin-Tazobactam	3.2% (6/188)	4.7% (22/472)	4.2% (28/660)
Doripenem	34.6% (65/188)	21.6% (102/472)	25.3% (167/660)
Ertapenem	0% (0/188)	2.5% (12/472)	1.8% (12/660)
Meropenem	28.7% (54/188)	23.1% (109/472)	24.7% (163/660)
Imipenem	29.3% (55/188)	18.9% (89/472)	21.8% (144/660)
Ciprofloxacin	18.1% (34/188)	8.5% (40/472)	11.2% (74/660)
Levofloxacin	23.9% (45/188)	12.5% (59/472)	15.8% (104/660)
Amikacin	90.4% (170/188)	74.4% (351/472)	78.9% (521/660)
Gentamicin	51.1% (96/188)	39.2% (185/472)	42.6% (281/660)
Trimethoprim/Sulfamethoxazole	31.9% (60/188)	18.9% (89/472)	22.6% (149/660)
Imipenem-Relebactam[a]	87.8% (165/188)	82.2% (388/472)	83.8 (553/660)

[a] CLSI interpretive criteria for single-agent imipenem was used to interpret the susceptibility of imipenem-relebactam combination.

(24.5%), piperacillin-tazobactam (3.2%), doripenem (34.6%), ertapenem (0%), meropenem (28.7%), imipenem (29.3%), ciprofloxacin (18.1%), levofloxacin (23.9%), gentamicin (51.1%) and trimethoprim/sulfamethoxazole (31.9%). An intermediate susceptibility was found in gentamicin (51.1%), whereas amikacin (90.4%) and imipenem-relebactam (87.8%) revealed high susceptible rates against the CnsEC isolates. Among 472 isolates of CnsKP, the same 17 agents and gentamicin revealed poor susceptibilities: aztreonam (7.4%), ampicillin (0%), cefazolin (0.2%), cefoxitin (0.6%), cefotaxime (2.5), ceftazidime (0.4%), ceftriaxone (0.2%), cefepime (14.2%), piperacillin-tazobactam (4.7%), doripenem (21.6%), ertapenem (2.5%), meropenem (23.1%), imipenem (18.9%), ciprofloxacin (8.5%), levofloxacin (12.5%), gentamicin (39.2%), and trimethoprim/sulfamethoxazole (18.9%). An intermediate susceptibility was found in amikacin (74.4%), whereas the imipenem-relebactam showed a high susceptibility (82.2%) against the CnsKP isolates we tested. Overall, our antimicrobial susceptibility test results revealed poor susceptibilities in 19 agents tested against 660 CnsE isolates (188 CnsEC and 472 CnsKP), aztreonam (6.2%), ampicillin (0%), cefazolin (0.2%), cefoxitin (0.6%), cefotaxime (2.1%), ceftazidime (0.9%), ceftriaxone (0.3%), cefepime (17.1%), piperacillin-tazobactam (4.2%), doripenem (25.3%), ertapenem (1.8%), meropenem (24.7%), imipenem (21.8%), ciprofloxacin (11.2%), levofloxacin (15.8%), gentamicin (42.6%), and trimethoprim/sulfamethoxazole (22.6%). Only amikacin possessed an intermediate susceptibility (78.9%), whereas the imipenem-relebactam showed a high susceptibility (83.8%) against the 660 CnsE isolates we tested.

PCR detection results of carbapenemase genes are shown in Table 2. The bla_{KPC} gene was most common and was detected in 123 CnsE isolates, including 3 CnsEC and 120 CnsKP isolates. Among the Amber class B carbapenemase genes we detected, the bla_{NDM} gene was only found in 5 CnsEC isolates, not in CnsKP. The bla_{IMP} gene was found in 1 CnsEC and 9 CnsKP isolates, and bla_{NDM} gene was found in 3 CnsEC and 5 CnsKP isolates. The Amber class D carbapenemase gene, bla_{OXA-48}, was found in 13 CnsE isolates (2 CnsEC and 11 CnsKP).

In vitro imipenem-relebactam activity

The *in vitro* results indicate that imipenem with relebactam were significantly more powerful than the single-agent imipenem (Table 3). Among the 188 CnsEC isolates, MIC values of the imipenem monotherapy ranged from 0.125 to >32 μg/ml, MIC_{50} and MIC_{90} were 4 and 32 μg/ml, respectively, and the susceptibility was 28.7% (54/188). When combined with relebactam, the MIC value range changed to <0.03～>32 μg/ml, MIC_{50} and MIC_{90} significantly decreased to 0.25 and 2 μg/ml, respectively ($p<0.0001$), and the susceptibility increased to 87.8% (165/188). However, no significant improvements were found in class B carbapenemase-producing isolates. The MIC value range, MIC_{50}, MIC_{90}, and susceptibility of the imipenem monotherapy against 9 class B carbapenemase-producing CnsEC isolates were 0.5～>32 μg/ml, 8 μg/ml, >32 μg/ml, and 22.2% (2/9), respectively. Those of imipenem-relebactam combination were 0.125～>32 μg/ml, 4 μg/ml, >32 μg/ml, and 11.1% (1/9), respectively ($p = 0.5588$). The significant differences of the mean $\log_2$ MIC and their 95% confidence intervals were found to be −4.3 (−8.1, −0.5; $p=0.039$) and −3.6 (−3.9, −3.3; $p<0.0001$) for *E. coli* isolates with class A carbapenemase and without carbapenemase, respectively. The MIC difference of all isolates was −3.4 (−3.7, −3.1; $p<0.0001$).

Similar results were noted for 472 CnsKP isolates (Table 4). Compared to the single-agent imipenem, the combination of imipenem and relebactam showed significantly improved antibacterial activity in MIC_{50} (from 16 to 0.5 μg/ml), MIC_{90} (from >32 to 4 μg/ml), and susceptibility (from 18.9% to 82.2%). No statistically significant differences were observed among 14 class B carbapenemase-producing CnsKP isolates ($p=0.1386$), as there were similar MIC ranges, MIC_{50}, MIC_{90} values, and the susceptibility. The significant differences of the mean $\log_2$ MIC and their 95% CIs were found to be −5.9 (−6.2, −5.7; $p<0.0001$), −1.5 (−2.7, −0.3; $p=0.022$), and −3.0 (−3.2, −2.8; $p<0.0001$) for *K. pneumoniae* isolates with class A, D, and no carbapenemase, respectively. The MIC difference of all 472 isolates was −3.6 (−3.8, −3.4; $p<0.0001$).

Table 2 Carbapenemase genes in 660 carbapenem-nonsusceptible Enterobacteriaceae isolates.

Species	Carbapenemase gene				
	bla_{KPC}	bla_{NDM}	bla_{IMP}	bla_{VIM}	bla_{OXA-48}
E. coli (n = 188)	3	5	1	3	2
K. pneumoniae (n = 472)	120[a]	0	9	5	11[a]
Total (n = 660)	123	5	10	8	13

[a] Two CnsKP isolates co-carried a bla_{KPC} and a bla_{OXA-48} gene.

Evaluation of imipenem-relebactam against metallo-carbapenemase producers

Poor activity was observed for both imipenem and imipenem with relebactam against 23 metallo-carbapenemase-producing isolates (9 CnsEC and 14 CnsKP) (Tables 3 and 4). To investigate the contribution of metallo-carbapenemases, EDTA was prepared with imipenem only or combined with relebactam at various concentrations. As shown in Fig. 1, the addition of EDTA significantly improved activity in both cases (Fig. 1, both $p<0.0001$). Significant decreases in MIC values were noted for both imipenem only and imipenem with relebactam, suggesting that metallo-carbapenemases are more relevant to reduce imipenem-relebactam susceptibilities.

In vivo C. elegans study

A *C. elegans* infection model was established with a randomly selected carbapenem-resistant *K. pneumoniae* isolate (CRE-1462), which carried a bla_{KPC} gene. Compared to the uninfected control, the survival of untreated group was significantly reduced than uninfected control ($p<0.0001$), implying the pathogenicity of CRE-1462 in the nematodes (Fig. 2 and Table 5). Statistically, no significant change was found between the

Table 3 MIC for imipenem and imipenem/relebactam combination against 188 carbapenem-nonsusceptible *E. coli*.

Group	MIC and susceptibility[a]	Antimicrobial agents[b]		Mean of difference in log_2 MIC values (95% CI)	p[c]
		IMI	IMI-REL		
Class A carbapenemase (n = 3)	Range	1–8	0.06–0.125	−4.3 (−8.1, −0.5)	0.0390
	MIC_{50}	1	0.125		
	MIC_{90}	8	0.125		
	% susceptible	66.7% (2/3)	100% (3/3)		
	% intermediate	0% (0/3)	0% (0/3)		
	% resistant	33.3% (1/3)	0% (0/3)		
Class B carbapenemase (n = 9)	Range	0.5 ~ >32	0.125 ~ >32	−0.2 (−1.1, 0.6)	0.5588
	MIC_{50}	8	4		
	MIC_{90}	>32	>32		
	% susceptible	22.2% (2/9)	11.1% (1/9)		
	% intermediate	0% (0/9)	0% (0/9)		
	% resistant	77.8% (7/9)	88.9% (8/9)		
Class D carbapenemase (n = 2)	Range	4–8	0.5–2	–	–
	MIC_{50}	4	0.5		
	MIC_{90}	8	2		
	% susceptible	0% (0/2)	50% (1/2)		
	% intermediate	0% (0/2)	50% (1/2)		
	% resistant	100% (2/2)	0% (0/2)		
Non-carbapenemase producer (n = 174)	Range	0.125 ~ >32	<0.06 ~ >32	−3.6 (−3.9, −3.3)	<0.0001
	MIC_{50}	4	0.25		
	MIC_{90}	32	1		
	% susceptible	28.7% (50/174)	92.0% (160/174)		
	% intermediate	12.6% (22/174)	3.4% (6/174)		
	% resistant	58.6% (102/174)	4.6% (8/174)		
Total (n = 188)	Range	0.125 ~ >32	<0.03 ~ >32	−3.4 (−3.7, −3.1)	<0.0001
	MIC_{50}	4	0.25		
	MIC_{90}	32	2		
	% susceptible	28.7% (54/188)	87.8% (165/188)		
	% intermediate	11.7% (22/188)	3.7% (7/188)		
	% resistant	59.6% (112/188)	8.5% (16/188)		

[a] CLSI interpretive criteria for single-agent imipenem was used to interpret the susceptibility of imipenem-relebactam combination.
[b] Abbreviations: IMI, imipenem; IMI-REL, imipenem-relebactam combination.
[c] p value for the MIC data.

untreated group and nematodes with an imipenem single-agent treatment (1 μg/ml) (Fig. 2). This suggests that the administration of 1 μg/ml of imipenem failed to rescue the infected nematodes with a hazard ratio (HR, 1.007; 95% confidence interval [CI] 0.546 to 1.569; $p=0.9664$) (Table 5). Contrary to the imipenem monotherapy, a significantly right-shifted survival curve was noted when the nematodes were administered with 1/4 μg/ml of imipenem-relebactam ($p<0.0001$). Median survival time for the untreated group and the imipenem monotherapy were both 2 days (Table 5). The combination therapy of imipenem-relebactam extended the median survival time from 2 to 4 days, with a significant decrease in the hazard ratio (HR, 0.472; 95% confidence interval [CI] 0.292 to 0.763; $p<0.0001$) (Table 5). Our data indicate that the combination therapy of imipenem-relebactam possessed treatment effect to rescue the *C. elegans* model infected with a carbapenemase-producing *K. pneumoniae* isolate.

Discussion

To date, carbapenem-resistant Enterobacteriaceae (CRE) were reported worldwide in a rapidly increasing rate and usually found with multidrug resistance, thereby limiting clinical treatment choice.[2] The β-lactam-hydrolyzing enzymes, namely the β-lactamases, have been reported as one of the carbapenem-resistant mechanisms,[1] and thus, β-lactamase inhibitors such as avibactam, vaborbactam, and relebactam were developed as an effective strategy to treat infections caused by β-lactamase-producing gram-negative bacilli.[17,18] The bi-cyclic diazabicyclooctan β-lactamase inhibitors were synthesized as a serine β-lactamases inhibitor.[19] The first-in-class inhibitor was avibactam which was combined with ceftazidime and approved by FDA in 2015.[17] Relebactam, also a potent bi-cyclic diazabicyclooctan β-lactamase inhibitor, was approved by the FDA in 2019 as a combination therapy of imipenem-cilastatin-relebactam.[5] In a study of

Table 4 MIC for imipenem and imipenem/relebactam combination against 472 carbapenem-nonsusceptible *K. pneumoniae*.

Group	MIC and susceptibility[a]	Antimicrobial agents[b]		Mean of difference in log_2 MIC values (95% CI)	p[c]
		IMI	IMI-REL		
Class A carbapenemase (n = 121)[c]	Range	0.5 ~ >32	0.125–16	−5.9 (−6.2, −5.7)	<0.0001
	MIC_{50}	32	0.5		
	MIC_{90}	>32	2		
	% susceptible	0.8% (1/121)	88.4% (107/121)		
	% intermediate	1.7% (2/121)	5.0% (6/121)		
	% resistant	97.5% (118/121)	6.6% (8/121)		
Class B carbapenemase (n = 14)	Range	1 ~ >32	0.125 ~ >32	−0.4 (−1.0, 0.2)	0.1386
	MIC_{50}	2	4		
	MIC_{90}	16	16		
	% susceptible	21.4% (3/14)	42.9% (6/14)		
	% intermediate	35.7% (5/14)	0% (0/14)		
	% resistant	42.9% (6/14)	57.1% (8/14)		
Class D carbapenemase (n = 10)[d]	Range	0.5 ~ >32	0.25 ~ >32	−1.5 (−2.7, −0.3)	0.0220
	MIC_{50}	4	2		
	MIC_{90}	>32	2		
	% susceptible	30.0% (3/10)	40.0% (4/10)		
	% intermediate	10.0% (1/10)	50.0% (5/10)		
	% resistant	60.0% (6/10)	10.0% (1/10)		
Non-carbapenemase producer (n = 329)	Range	0.125 ~ >32	<0.03–32	−3.0 (−3.2, −2.8)	<0.0001
	MIC_{50}	8	0.5		
	MIC_{90}	32	4		
	% susceptible	24.9% (82/329)	82.4% (271/329)		
	% intermediate	12.5% (41/329)	6.7% (22/329)		
	% resistant	62.6% (206/329)	10.9% (36/329)		
Total (n = 472)[d]	Range	0.125 ~ >32	<0.03 ~ >32	−3.6 (−3.8, −3.4)	<0.0001
	MIC_{50}	16	0.5		
	MIC_{90}	>32	4		
	% susceptible	18.9% (89/472)	82.2% (388/472)		
	% intermediate	10.4% (49/472)	6.6% (31/472)		
	% resistant	70.6% (334/472)	11.2% (53/472)		

[a] CLSI interpretive criteria for single-agent imipenem was used to interpret the susceptibility of imipenem-relebactam combination.
[b] Abbreviations: IMI, imipenem; IMI-REL, imipenem-relebactam combination.
[c] p value for the MIC data.
[d] Two isolates of *K. pneumoniae* were detected to co-carry a class A and a class D carbapenemase gene.

imipenem-relebactam combined activity against 110 bla_{KPC}-carrying gram-negative bacilli,[20] MIC_{50} and MIC_{90} values from imipenem monotherapy treatment of 110 isolates were 16 and > 128 μg/ml, respectively, with a susceptibility of only 4.5%. When combined with relebactam, imipenem MIC_{50} and MIC_{90} values fell to 0.25 and 1 μg/ml, respectively, with susceptibility restored to 90.9%. In a separate evaluation of imipenem-relebactam antimicrobial activity against 111 bla_{KPC}-possessing *K. pneumoniae* isolates collected in New York, MIC_{50} and MIC_{90} values from imipenem monotherapy were 16 and > 16 μg/ml, respectively, with 9% susceptibility, whereas MIC_{50} and MIC_{90} values for imipenem-relebactam combination therapies were improved to 0.25 and 1 μg/ml, respectively, with 97% susceptibility.[21] In the present study we observed significant restoration of imipenem antibacterial activity when combined with relebactam (p<0.0001) (Tables 3 and 4), and significant improvement in susceptibility against CnsEC (87.8%) and CnsKP (82.2%). Compared to the single-agent imipenem, the overall susceptibility rate of imipenem-relebactam significantly increased from 21.8% to 83.8% (Table 1).

Previous studies have attributed carbapenem resistance to numerous mechanisms, among non-carbapenemase producers, including mutations of porins and AmpCs in combination with ESBL enzymes.[1] The mechanism is also highly recognizable in *Klebsiella* spp., *E. coli* as well as other genera. In our work, among 503 non-carbapenemase producers (174 *E. coli* and 329 *K. pneumoniae* isolates), 132 isolates (26.2%) were susceptible to single-agent imipenem, whereas 431 isolates (85.7%) were susceptible to imipenem-relebactam combination (Tables 3 and 4). According to the detection of bla_{AmpC} and bla_{ESBL} (Table S1), 199 and 155 isolates carried bla_{DHA} and bla_{CMY} (both bla_{AmpC} genes), respectively; 85 and 173 isolates were harbored $bla_{CTX\text{-}M\text{-}G1}$ and $bla_{CTX\text{-}M\text{-}G9}$ (both bla_{ESBL} genes), respectively. Nevertheless, both bla_{AmpC} and bla_{ESBL} could be inhibited by relebactam,[5,22] and thereby, the activity of imipenem would be restored. Our findings were also consistent with previous studies.[21,22]

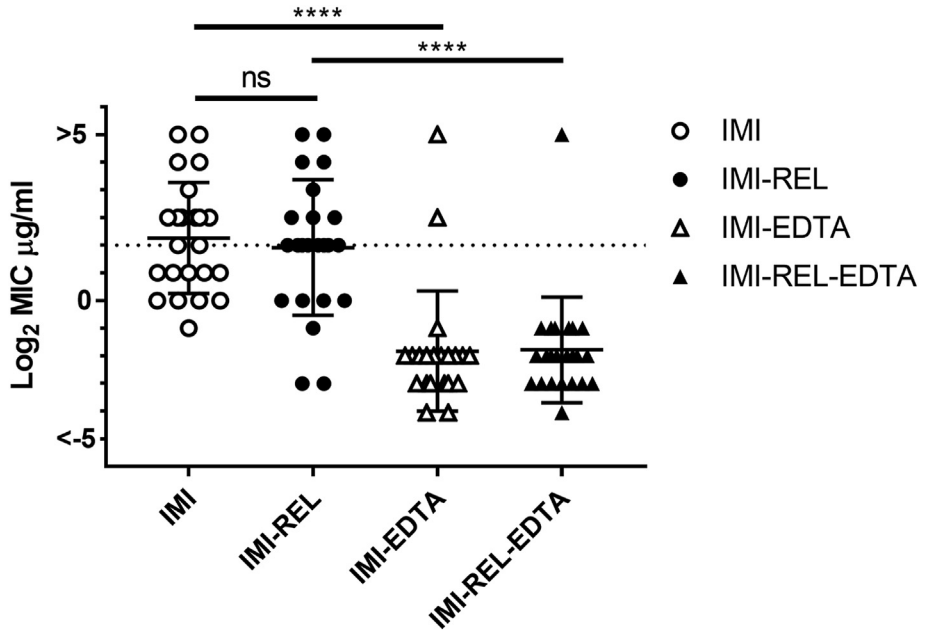

Figure 1. MIC distributions of imipenem and imipenem-relebactam combination following the addition of EDTA. Abbreviations: IMI, imipenem; IMI-REL, imipenem with relebactam. ns, no statistical significance; ****, $p<0.0001$.

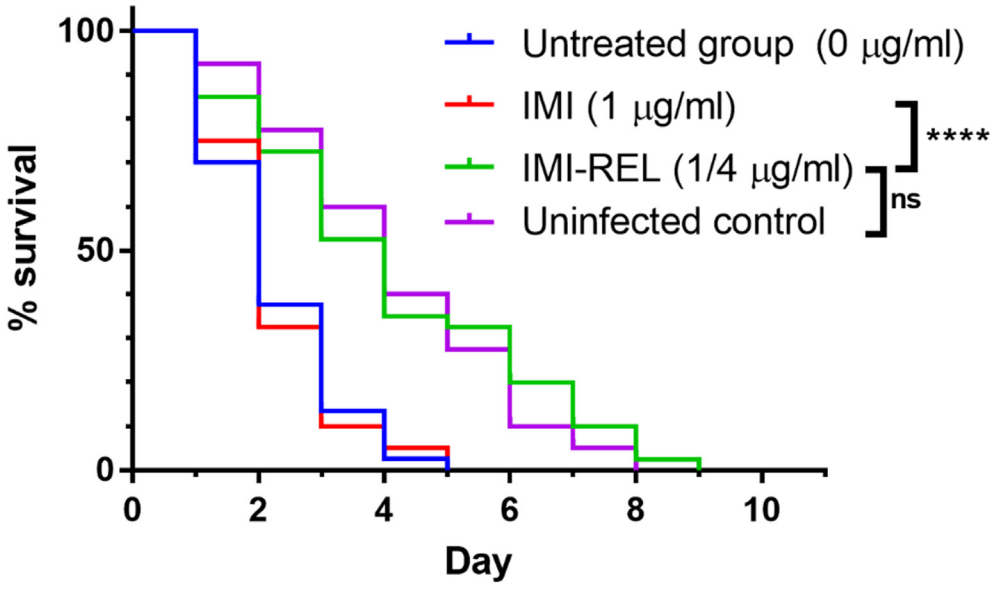

Figure 2. In vivo *C. elegans* study. Infected nematodes (n = 40) were treated with imipenem or imipenem-relebactam combination therapy. Nematodes were consistently treated with the same conditions. Nematodes serving as an uninfected control was fed with a non-toxic *E. coli* lab strain OP50. Nematodes infected by CRE-1462 was treated with 1 μg/ml of imipenem (IMI), 1/4 μg/ml of imipenem-relebactam, and vehicle (0 μg/ml, namely untreated group). Abbreviations: IMI, imipenem; IMI-REL, imipenem with relebactam. ****, $p<0.0001$; ns, no significance.

Relebactam belongs to a class of serine β-lactamase inhibitor,[5,19] and specifically inhibits class A and C β-lactamases and some class D β-lactamases.[23] In a previous study, the MIC values of imipenem ranging from 2 to >16 μg/ml were found for 200 isolates of carbapenemase-producing Enterobacteriaceae collected in the US during 2013–2017.[7] Compared to imipenem monotherapy, relebactam decreased the range of MIC values to <0.125–4 μg/ml, with reduced MIC_{50} (from 8 to <0.125 μg/ml) and MIC_{90} (from >16 to 0.5 μg/ml) values. Another study conducted in Greece revealed that the susceptibilities of imipenem to 314 carbapenemase-producing *K. pneumoniae* isolates (295 KPC-producers and 19 OXA-producers) were 0%.[8] Combined with relebactam, susceptibilities of 295 KPC-producers and 19 OXA-producers to imipenem improved to 98% and 10.5%, respectively, suggesting that relebactam could inhibited

Table 5 *In vivo C. elegans* statistical data and analysis.

Treatment	Median survival time (days)	*p* value	Hazard Ratio		
			Ratio	lower 95%	upper 95%
Untreated group	2	-	1	—	—
Imipenem	2	0.9664	1.007	0.546	1.569
Imipenem-relebactam	4	<0.0001	0.472	0.292	0.763
Uninfected control	4	<0.0001	0.422	0.258	0.690

Note: All experiments were performed in triplicate.

most of class A carbapenemase but partially inhibited the class D carbapenemase. In the present study, we found that the imipenem-relebactam combination was effective against class A and D carbapenemase-producers but ineffective against class B carbapenemase-producing isolates (Table 3, Table 4 and Fig. 1). The addition of EDTA inhibited the activities of metallo-carbapenemases and further improved both susceptibilities of imipenem and imipenem-relebactam (Fig. 1). In summary, relebactam significantly increased imipenem susceptibility in both carbapenemase and non-carbapenemase producing cases, suggesting that it may be a potential alternative therapeutic regimen for carbapenem-resistant Enterobacteriaceae infections. However, our results also suggested that the detection of metallo-carbapenemase should be considered before the usage of imipenem-relebactam in clinical settings.

For the *in vivo* efficacy, the *in vivo C. elegans* model was infected by a randomly selected CRE-1462 bla_{KPC}-containing *K. pneumoniae* clinical isolate. A significant right shift in the survival curve was observed in the imipenem-relebactam combination therapy group ($p<0.0001$) (Fig. 2), with extended median survival times (from 2 days to 4 days) (Table 4). This data is consistent with previous studies which investigated the imipenem-relebactam combination using a mouse model with a disseminated bla_{KPC}-producing *K. pneumoniae* infection.[24] Compared to imipenem monotherapy, imipenem combined with different levels of relebactam resulted in significant decreases in spleen CFUs (2.29—3.06 log).

Conclusion

Our data indicate therapeutic effectiveness for imipenem-relebactam combination against carbapenem-nonsusceptible Enterobacteriaceae, with an improved susceptibility of 83.8% (553/660). The results of the *C. elegans* model also provide evidence of *in vivo* efficacy for imipenem-relebactam combination against KPC-producing clinical isolates, but further clinical investigation in Taiwan is still urgently needed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2021.02.001.