In Vitro Antibacterial Activity of Imipenem/Relebactam against Clinical Isolates in Japan

Dai Kurihara,[a] Satoru Matsumoto,[b] Naoko Kishi,[b] Yoshikazu Ishii,[c] Masahiko Mori[a]

[a]Japan Development, MSD K.K., Tokyo, Japan
[b]Microbiological Testing Group, Infectious Diseases Testing Department, Clinical Laboratory Center, Medical Solution Segment, LSI Medience Corporation, Tokyo, Japan
[c]Department of Microbiology and Infectious Diseases, Toho University School of Medicine, Tokyo, Japan

ABSTRACT Relebactam is a novel β-lactamase inhibitor of Ambler class A and C β-lactamases that has been developed in combination with imipenem/cilastatin for the treatment of carbapenem-resistant bacterial infections. In this study, we evaluated the *in vitro* antibacterial activity of imipenem/relebactam (IMR) against imipenem-nonsusceptible Enterobacterales and *Pseudomonas aeruginosa* isolates from Japan. Two sets of antibacterial susceptibility tests were conducted according to the susceptibility testing standard of the Clinical and Laboratory Standards Institute. In the first set, antibacterial susceptibility as measured by the $MIC_{50/90}$ (MIC range) of IMR was assessed for the following 61 imipenem-nonsusceptible strains: 2 *Enterobacter cloacae* complex (not determined [0.25 μg/mL]), 33 *Klebsiella aerogenes* (0.5/1 μg/mL [0.5 to 1 μg/mL]), 2 *Serratia marcescens* (not determined [1 to 2 μg/mL]), and 24 *P. aeruginosa* (2/128 μg/mL [0.25 to >128 μg/mL]). In the second set, antibacterial susceptibility was assessed for the following 8 imipenem-nonsusceptible strains: 4 *Escherichia coli*, 1 *E. cloacae* complex and 3 *Klebsiella pneumoniae*. The MIC ranges of IMR for these strains were 0.25 to 0.5 μg/mL, 0.5 μg/mL, and 0.5 to 16 μg/mL, respectively. The antibacterial activity of IMR was similar to or lower than that of amikacin and comparable to or greater than those of other reference drugs. In conclusion, IMR has shown antibacterial activity against clinical isolates from Japan and, therefore, is expected to become a new therapeutic option for carbapenem-resistant infections in Japan.

IMPORTANCE Carbapenem-resistant Enterobacterales and carbapenem-resistant *Pseudomonas aeruginosa* strains pose a global threat. Antibacterial activity of imipenem/relebactam (IMR) against clinical isolates of these bacteria from several global regions has been shown; however, as yet there are no reports on Japanese isolates. In this study, we evaluated the *in vitro* antibacterial activity of IMR against imipenem-nonsusceptible Enterobacterales and *Pseudomonas aeruginosa* isolates from Japan. The antibacterial activity of IMR against imipenem-nonsusceptible Enterobacterales was generally comparable to that of amikacin (AMK) and comparable to or higher than those of other reference drugs tested. The antibacterial activity of IMR against imipenem-nonsusceptible *P. aeruginosa* isolates was lower than that of AMK but comparable to or higher than those of other drugs. These results support the use of IMR as a new treatment option for infections due to Enterobacterales and *P. aeruginosa* strains that are resistant to existing β-lactams and other antibacterial agents.

KEYWORDS carbapenem-resistant, imipenem, relebactam, susceptibility

R elebactam (REL) is a novel, non–β-lactam bicyclic diazabicyclooctane β-lactamase inhibitor that blocks Ambler class A and C β-lactamases like *Klebsiella pneumoniae* carbapenemase (KPC) and AmpC. The combination drug of REL and imipenem/cilastatin (IMI) has been approved as IMI/REL in the United States, Europe, and Japan. IMI is a combination product of imipenem, a carbapenem antibacterial agent, and cilastatin, a

Address correspondence to Dai Kurihara, dai.kurihara@merck.com.

dehydropeptidase-I inhibitor. Imipenem has strong antibacterial activity against Gram-positive and Gram-negative aerobes and anaerobes (1). Since imipenem is inactivated by dehydropeptidase-I in the kidneys, cilastatin is combined with imipenem (1). Cilastatin has no independent antibacterial activity (2). Because of this characteristic, susceptibility tests for IMI/REL are generally conducted using imipenem/relebactam (IMR) without the addition of cilastatin (3).

Gram-negative bacteria cause infections throughout the body, including the bloodstream, respiratory organs, intra-abdominal organs, etc. The emergence of drug-resistant bacteria has become a problem, and in particular, carbapenem-resistant Enterobacterales and carbapenem-resistant *Pseudomonas aeruginosa* pose a global threat (4). The mechanisms of carbapenem resistance include upregulation of multidrug efflux systems to promote antibacterial expulsion (though imipenem is not subject to efflux [5]), increased production of AmpC or extended-spectrum β-lactamase (ESBL) with concomitant loss of porins that then limits entry of carbapenem into cells (e.g., OprD in the case of imipenem), and production of carbapenemases (6, 7). REL has been shown to restore the antibacterial activity of imipenem against these resistant strains by inhibiting Ambler class A and C β-lactamases, including ESBLs, KPCs, and AmpCs (8). In a global phase 3 study in patients with imipenem-resistant bacterial infections, the efficacy rate for the overall response to IMI/REL was comparable to that of IMI + colistimethate sodium, a comparator drug, and the total mortality was low (9). In Japan, IMI/REL is indicated for treatment of various infections caused by *E. coli*, *Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp., *P. aeruginosa*, and *Acinetobacter* spp. that are resistant to carbapenems and susceptible to IMR.

Antibacterial activity of IMR against clinical isolates collected from several global regions has been shown (10, 11); however, as yet there are no reports on Japanese isolates. In this study, the *in vitro* antibacterial activity of IMR against clinical isolates collected from Japan was investigated in 2 analyses. The first was a set of susceptibility tests using clinical isolates collected prospectively from medical institutions across Japan in 2016. A part of the results was previously reported by Hidaka et al. (12), but the current study reports the antibacterial activity of IMR and antibacterial susceptibility of imipenem-nonsusceptible strains. The second analysis involves antibacterial susceptibility testing of imipenem-nonsusceptible bacterial stocks. In both tests, the antibacterial activity of IMR was compared with that of control drugs to identify the *in vitro* antibacterial profiles against clinical isolates from Japan.

RESULTS

In this prospective antibacterial susceptibility study, 850 isolates of species listed in the indication for IMI/REL (approved in 2021 in Japan) were collected, of which 2 *Enterobacter cloacae* complex isolates, 33 *Klebsiella aerogenes* isolates, 2 *Serratia marcescens* isolates, and 24 *P. aeruginosa* isolates were imipenem-nonsusceptible. All of the *E. cloacae* complex isolates and *K. aerogenes* isolates were imipenem-intermediate, and there were no imipenem-resistant isolates. Minimum inhibitory concentrations (MIC) were interpreted as susceptible, intermediate, and resistant using Clinical and Laboratory Standards Institute (CLSI) breakpoints (3). Of the 2 *S. marcescens* isolates, 1 was imipenem intermediate and the other was imipenem resistant. Three of the 24 *P. aeruginosa* isolates were imipenem intermediate, and 21 of 24 were imipenem resistant. The antibacterial activities of IMR, imipenem, and reference drugs (meropenem [MEM], tazobactam/piperacillin [TZP], ceftazidime [CAZ], cefepime [FEP], levofloxacin [LVX], amikacin [AMK], colistin [CST], and tigecycline [TGC]) against these strains are shown in Table 1.

The isolates of *E. cloacae* complex and *K. aerogenes* were all susceptible to IMR. The MIC of IMR against *E. cloacae* complex isolates was 0.25 μg/mL, 8-fold lower than that of imipenem. The MIC$_{50/90}$ of IMR against *K. aerogenes* was 0.5/1 μg/mL, 2- to 4-fold lower than that of imipenem. The antibacterial activities of most reference drugs against these strains were comparable to those of IMR; the MIC$_{50/90}$ values were low and the percentages of susceptibility were high. As an exception, the MIC of CST against imipenem-nonsusceptible

TABLE 1 Antibacterial activity of IMR and other agents against imipenem-nonsusceptible isolates collected in the prospective study

Species	No. of isolates	Antibacterial agent[a]	MIC (μg/mL)			No. (%) with MIC interpretation[b]:		
			Range	MIC$_{50}$	MIC$_{90}$	Susceptible	Intermediate	Resistant
Enterobacter cloacae complex	2	IMR	0.25	—[c]	—	2 (100.0)	0	0
		Imipenem	2	—	—	0	2 (100.0)	0
		MEM	≤0.06	—	—	2 (100.0)	0	0
		TZP	0.5 to 4	—	—	2 (100.0)	0	0
		CAZ	0.25 to 0.5	—	—	2 (100.0)	0	0
		FEP	≤0.06 to 0.12	—	—	2 (100.0)	0[d]	0
		LVX	≤0.06	—	—	2 (100.0)	0	0
		AMK	1 to 2	—	—	2 (100.0)	0	0
		CST	>32	—	—	—	0	2 (100.0)
		TGC	0.5 to 2	—	—	1 (50.0)	—	1 (50.0)
Klebsiella aerogenes	33	IMR	0.5 to 1	0.5	1	33 (100.0)	0	0
		Imipenem	2	2	2	0	33 (100.0)	0
		MEM	≤0.06 to 0.12	≤0.06	≤0.06	33 (100.0)	0	0
		TZP	2 to 64	2	16	30 (90.9)	3 (9.1)	0
		CAZ	0.12 to 64	0.5	2	30 (90.9)	0	3 (9.1)
		FEP	≤0.06 to 0.25	≤0.06	0.12	33 (100.0)	0[d]	0
		LVX	≤0.06 to 1	≤0.06	0.12	32 (97.0)	1 (3.0)	0
		AMK	1 to 4	2	2	33 (100.0)	0	0
		CST	0.5 to 2	1	1	—	33 (100.0)	0
		TGC	0.5 to 8	0.5	1	21 (63.6)	—	12 (36.4)
Serratia marcescens	2	IMR	1 to 2	—	—	1 (50.0)	1 (50.0)	0
		Imipenem	2 to 16	—	—	0	1 (50.0)	1 (50.0)
		MEM	≤0.06 to 2	—	—	1 (50.0)	1 (50.0)	0
		TZP	8 to 16	—	—	2 (100.0)	0	0
		CAZ	0.5 to 8	—	—	1 (50.0)	1 (50.0)	0
		FEP	0.12 to 8	—	—	1 (50.0)	1 (50.0)[d]	0
		LVX	≤0.06 to 16	—	—	1 (50.0)	0	1 (50.0)
		AMK	2 to 4	—	—	2 (100.0)	0	0
		TGC	1 to 4	—	—	0	—	2 (100.0)
Pseudomonas aeruginosa	24	IMR	0.25 to >128	2	128	13 (54.2)	3 (12.5)	8 (33.3)
		Imipenem	4 to >128	16	128	0	3 (12.5)	21 (87.5)
		MEM	1 to >128	32	>128	4 (16.7)	3 (12.5)	17 (70.8)
		TZP	0.5 to >128	32	128	10 (41.7)	9 (37.5)	5 (20.8)
		CAZ	1 to >128	8	>128	12 (50.0)	1 (4.2)	11 (45.8)
		FEP	0.5 to >128	8	>128	13 (54.2)	3 (12.5)	8 (33.3)
		LVX	0.5 to >128	16	>128	3 (12.5)	2 (8.3)	19 (79.2)
		AMK	1 to >128	4	128	19 (79.2)	1 (4.2)	4 (16.7)
		CST	1 to 2	2	2	—	24 (100.0)	0
		TGC	4 to 64	16	64	—	—	—

[a]AMK, amikacin; FEP, cefepime; CAZ, ceftazidime; CST, colistin; IMR, imipenem/relebactam; LVX, levofloxacin; MEM, meropenem; TZP, tazobactam/piperacillin; TGC, tigecycline.

[b]For TGC against Enterobacterales, European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2021 breakpoints were applied because Clinical and Laboratory Standards Institute (CLSI) breakpoints were not defined.

[c]—, Not applicable.

[d]Susceptible, dose-dependent.

E. cloacae complex isolates was high (>32 μg/mL) and 2 of 2 isolates were resistant to CST. In addition, 36.4% of imipenem-nonsusceptible *K. aerogenes* isolates were resistant to TGC.

The MICs of imipenem against 2 imipenem-nonsusceptible strains of *S. marcescens* were 2 μg/mL and 16 μg/mL, while the MICs of IMR were 1 μg/mL and 2 μg/mL, respectively, 2- to 8-fold lower than those of imipenem alone. One isolate was imipenem-resistant and another was imipenem-intermediate. As for the reference drugs, both isolates were susceptible to TZP and AMK and resistant to TGC. For the other drugs, the antibacterial activities were generally similar to those of IMR.

The MIC$_{50}$ of IMR against imipenem-nonsusceptible *P. aeruginosa* isolates was 2 μg/mL, 8-fold lower than that of imipenem. The MIC$_{90}$ of IMR was 128 μg/mL, equivalent to that of imipenem. The percentage of susceptibility to IMR was 54.2%, showing a

TABLE 2 Antibacterial activity of IMR and other agents against preserved imipenem-nonsusceptible isolates

Species	No. of isolates	Antibacterial agent[a]	MIC range (μg/mL)	No. (%) with MIC interpretation[b]:		
				Susceptible	Intermediate	Resistant
Escherichia coli	4	IMR	0.2 to 0.5	4 (100.0)	0	0
		Imipenem	2 to 8	0	2 (50.0)	2 (50.0)
		MEM	4 to 8	0	0	4 (100.0)
		TZP	128 to >128	0	0	4 (100.0)
		CAZ	128 to >128	0	0	4 (100.0)
		FEP	8 to >128	0	1 (25.0)[c]	3 (75.0)
		LVX	16 to 32	0	0	4 (100.0)
		AMK	2 to 4	4 (100.0)	0	0
		CST	1	—[d]	4 (100.0)	0
		TGC	0.25 to 0.5	4 (100.0)	—	0
Enterobacter cloacae complex	1	IMR	0.5	1 (100.0)	0	0
		Imipenem	8	0	0	1 (100.0)
		MEM	8	0	0	1 (100.0)
		TZP	>128	0	0	1 (100.0)
		CAZ	>128	0	0	1 (100.0)
		FEP	>128	0	0[c]	1 (100.0)
		LVX	2	0	0	1 (100.0)
		AMK	1	1 (100.0)	0	0
		CST	1	—	1 (100.0)	0
		TGC	1	0	—	1 (100.0)
Klebsiella pneumoniae	3	IMR	0.5 to 16	1 (33.3)	1 (33.3)	1 (33.3)
		Imipenem	2 to 32	0	1 (33.3)	2 (66.7)
		MEM	16	0	0	3 (100.0)
		TZP	16 to >128	1 (33.3)	0	2 (66.7)
		CAZ	4 to >128	1 (33.3)	0	2 (66.7)
		FEP	1 to >128	1 (33.3)	0[c]	2 (66.7)
		LVX	0.12 to >128	1 (33.3)	0	2 (66.7)
		AMK	2 to >128	2 (66.7)	0	1 (33.3)
		CST	0.5 to 1	—	3 (100.0)	0
		TGC	0.25 to 1	2 (66.6)	—	1 (33.3)

[a]AMK, amikacin; FEP, cefepime; CAZ, ceftazidime; CST, colistin; IMR, imipenem/relebactam; LVX, levofloxacin; MEM, meropenem; TZP, tazobactam/piperacillin; TGC, tigecycline.
[b]For TGC against Enterobacterales, EUCAST breakpoints in 2021 were applied because CLSI breakpoints were not defined.
[c]Susceptible, dose-dependent.
[d]—, Not applicable.

large decrease in the resistance rate and an increase in the susceptibility rate compared with those of imipenem. The MIC_{90} values of the reference drugs were also high, except for CST. The percentage of susceptibility was the highest for AMK at 79.2%, while the percentages of susceptibility to MEM, TZP, and LVX were relatively low, and the percentages of susceptibility to CAZ and FEP were comparable to that of IMR.

Since the imipenem-nonsusceptible strains collected in the prospective study only covered some of the bacterial species listed in the indication for IMI/REL, a second set of antibacterial susceptibility tests was performed using the following existing stocks of imipenem-nonsusceptible clinical isolates: 4 *E. coli*, 1 *E. cloacae* complex and 3 *K. pneumoniae*. The antibacterial activities of IMR, imipenem, and the reference drugs against these strains are shown in Table 2.

The MICs of IMR against imipenem-nonsusceptible *E. coli* isolates were 0.25 to 0.5 μg/mL, 4- to 32-fold lower than those of imipenem. All 4 strains were susceptible to IMR, AMK, and TGC, intermediate to CST, and resistant to almost all other reference drugs. In particular, the MICs of TZP and CAZ were as high as 128 to >128 μg/mL.

The MIC of IMR against the imipenem-nonsusceptible *E. cloacae* complex isolate was 0.5 μg/mL, 16-fold lower than that of imipenem. This strain was susceptible to IMR and AMK and intermediate to CST but resistant to other drugs. In particular, the MICs of TZP, CAZ, and FEP were as high as >128 μg/mL.

The MIC range of IMR against imipenem-nonsusceptible *K. pneumoniae* isolates was 0.5 to 16 μg/mL, 2- to 8-fold lower than the MIC range of imipenem. The number of isolates susceptible, intermediate, and resistant to IMR was 1 each. For the reference drugs AMK and TGC, 2 isolates and 1 isolate were susceptible and resistant, respectively, and all were intermediate to CST. All isolates were resistant to MEM, and 2 of 3 isolates were resistant to other drugs. One imipenem-nonsusceptible *K. pneumoniae* strain was resistant to all drugs except CST, and the MICs were very high (multidrug-resistant strain).

Table 3 shows the antibacterial activities of IMR, imipenem, and the reference drugs against imipenem-nonsusceptible strains of species listed in the indication for IMI/REL for which β-lactamases were identified in the 2 studies. The numbers of AmpC-positive imipenem-nonsusceptible strains obtained in the 2 studies were as follows: 1 for *E. coli*, 3 for *K. aerogenes*, 1 for *K. pneumoniae*, and 1 for *S. marcescens*. In addition, 5 strains of *P. aeruginosa* were found to be constitutive producers of AmpC. The *K. pneumoniae* isolate was the multidrug-resistant strain described above. Among these 11 isolates, 1 *E. coli* isolate, 3 *K. aerogenes* isolates, and 4 *P. aeruginosa* isolates were susceptible to IMR. The MICs of IMR were lower than those of imipenem even in isolates that were not susceptible to IMR (data not shown), which is consistent with previous reports (5, 13, 14). Among the reference drugs, AMK showed a higher antibacterial activity than IMR. All but 1 *K. pneumoniae* isolate were susceptible to AMK. All isolates were intermediate to CST. The other reference drugs had lower antibacterial activities against *P. aeruginosa*, and only 1 isolate was susceptible to each drug. The antibacterial activities of TZP and CAZ against constitutive AmpC-producing isolates were generally low regardless of species, with 18.2% of AmpC-producing isolates susceptible to TZP (*S. marcescens*, N = 1; *P. aeruginosa*, N = 1) and 9.1% susceptible to CAZ (*P. aeruginosa*, N = 1).

ESBL-positive imipenem-nonsusceptible strains comprised 3 *E. coli* isolates and 1 *K. pneumoniae* isolate. All 4 of these isolates possessed CTX-M-type ESBLs and were susceptible to IMR. As for the reference drugs, the isolates were all susceptible to AMK and TGC, intermediate to CST, and resistant to the others. One imipenem-nonsusceptible *K. pneumoniae* isolate was positive for the Guiana extended-spectrum (GES)–type carbapenemase and was intermediate to IMR. This isolate was resistant to MEM, intermediate to CST, and susceptible to other drugs. Six imipenem-nonsusceptible *P. aeruginosa* isolates possessed metallo-β-lactamases (MBLs). These 6 isolates were resistant to all drugs, including IMR, except for TZP, AMK, and CST. The numbers of isolates susceptible to TZP and AMK were 2 and 1, respectively, and all were intermediate to CST.

The antibacterial activities of IMR and imipenem against isolates of species listed in the Japanese indication for IMI collected in the prospective study are shown in Table 4. Overall, the rate of susceptibility of aerobic Gram-negative bacteria to IMR was high. The MIC_{90} range of IMR in Enterobacterales other than *Morganellaceae* was 0.12 to 1 μg/mL, indicating that almost all strains were susceptible to IMR. Among them, no imipenem-nonsusceptible isolates were collected for *Citrobacter* spp. and *E. coli*; however, the MIC_{50} and MIC_{90} values of IMR for these isolates were lower than those of imipenem (13, 15). The $MIC_{50/90}$ values of IMR for isolates of *P. aeruginosa* and *Acinetobacter* spp., the other species listed in the indication for IMI/REL, were 0.25/4 μg/mL and 0.12/0.25 μg/mL, respectively, and the percentages of susceptibility to IMR were 89.0% and 100%, respectively. In the *Morganellaceae* (*Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, and *Providencia* spp.), there were imipenem-nonsusceptible isolates, but enhancement of the antibacterial activity of imipenem by REL was nearly absent except for *M. morganii*. The *Haemophilus influenzae* isolates were 100% susceptible to IMR and imipenem.

The antibacterial activities of IMR against aerobic Gram-positive bacteria were similar to those of imipenem. While the MIC_{90} values of IMR and imipenem were high for methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), methicillin-resistant coagulase-negative *Staphylococcus* (MRCNS;

TABLE 3 β-Lactamase profiles and antibiograms of imipenem-nonsusceptible isolates[a]

β-Lactamase	Species	No. of isolates: Tested	Positive for β-lactamase tested	MIC interpretation (susceptible/intermediate/resistant)[b]: IMR	Imipenem	MEM	TZP	CAZ	FEP	LVX	AMK	CST	TGC	Data source
AmpC	Escherichia coli	4	1	1/0/0	0/0/1	0/0/1	0/0/1	0/0/1	0/1[c]/0	0/0/1	1/0/0	—[d]/1/0	1/—/0	Bacterial stocks
	Klebsiella aerogenes	33	3[e]	3/0/0	0/3/0	3/0/0	0/3/0	0/0/3	3/0[c]/0	2/1/0	3/0/0	—/3/0	2/—/1	Prospective study
	Klebsiella pneumoniae	3	1	0/0/1	0/0/1	0/0/1	0/0/1	0/0/1	0/0[c]/1	0/0/1	0/0/1	—/1/0	0/—/1	Bacterial stocks
	Serratia marcescens	2	1[e]	0/1/0	0/0/1	0/1/0	1/0/0	0/1/0	0/1[c]/0	0/0/1	1/0/0	—	0/—/1	Prospective study
	Pseudomonas aeruginosa	24	5[e]	4/1/0	0/0/5	1/0/4	1/1/3	1/0/4	1/2/2	1/0/4	5/0/0	—/5/0	—	Prospective study
ESBL	E. coli	4	3	3/0/0	0/2/1	0/0/3	0/0/3	0/0/3	0/0[c]/3	0/0/3	3/0/0	—/3/0	3/—/0	Bacterial stocks
	K. pneumoniae	3	1	1/0/0	0/1/0	0/0/1	0/0/1	0/0/1	0/0[c]/1	0/0/1	1/0/0	—/1/0	1/—/0	Bacterial stocks
GES-type carbapenemase	K. pneumoniae	3	1	0/1/0	0/0/1	0/0/1	1/0/0	1/0/0	1/0[c]/0	1/0/0	1/0/0	—/1/0	1/—/0	Bacterial stocks
MBL	P. aeruginosa	24	6	0/0/6	0/0/6	0/0/6	2/3/1	0/0/6	0/0/6	0/0/6	1/1/4	—/6/0	—	Prospective study

[a]AMK, amikacin; FEP, cefepime; CAZ, ceftazidime; CST, colistin; ESBL, extended-spectrum β-lactamase; GES, Guiana extended spectrum; IMR, imipenem/relebactam; LVX, levofloxacin; MEM, meropenem; MBL, metallo-β-lactamase; MIC, MIC; TZP, tazobactam/piperacillin; TGC, tigecycline.

[b]For TGC against Enterobacterales, EUCAST breakpoints in 2021 were applied because CLSI breakpoints were not defined.

[c]Susceptible, dose-dependent.

[d]—, Not applicable.

[e]Isolate(s) constitutively express AmpC.

TABLE 4 Antibacterial activity of IMR and other agents against isolates collected in this prospective study

Species[a]	No. of isolates	Antibacterial agent[b]	MIC (μg/mL)			No. (%) with MIC interpretation		
			Range	MIC$_{50}$	MIC$_{90}$	Susceptible	Intermediate	Resistant
Gram-positive aerobes								
MSSA	50	IMR	≤0.06	≤0.06	≤0.06	—[c]	—	—
		Imipenem	≤0.06	≤0.06	≤0.06	—	—	—
MRSA	50	IMR	≤0.06 to 64	≤0.06	32	—	—	—
		Imipenem	≤0.06 to 64	≤0.06	32	—	—	—
MSSE	20	IMR	≤0.06	≤0.06	≤0.06	—	—	—
		Imipenem	≤0.06	≤0.06	≤0.06	—	—	—
MRSE	50	IMR	≤0.06 to 32	0.12	4	—	—	—
		Imipenem	≤0.06 to 32	0.12	4	—	—	—
MSCNS	20	IMR	≤0.06	≤0.06	≤0.06	—	—	—
		Imipenem	≤0.06	≤0.06	≤0.06	—	—	—
MRCNS	50	IMR	≤0.06 to 128	0.12	64	—	—	—
		Imipenem	≤0.06 to 128	0.12	64	—	—	—
Enterococcus faecalis	50	IMR	0.5 to 4	1	1	—	—	—
		Imipenem	0.5 to 4	1	1	—	—	—
Enterococcus faecium	50	IMR	128 to >128	>128	>128	—	—	—
		Imipenem	128 to >128	>128	>128	—	—	—
Enterococcus avium	25	IMR	0.5 to 32	2	8	—	—	—
		Imipenem	0.5 to 32	2	8	—	—	—
PSSP	25	IMR	≤0.06	≤0.06	≤0.06	25 (100.0)[d]	0[d]	0[d]
		Imipenem	≤0.06	≤0.06	≤0.06	25 (100.0)	0	0
PRSP	25	IMR	0.25 to 0.5	0.25	0.5	0[d]	25 (100.0)[d]	0[d]
		Imipenem	0.25 to 0.5	0.25	0.5	0	25 (100.0)	0
Streptococcus pyogenes	50	IMR	≤0.06	≤0.06	≤0.06	—	—	—
		Imipenem	≤0.06	≤0.06	≤0.06	—	—	—
Streptococcus agalactiae	50	IMR	≤0.06	≤0.06	≤0.06	—	—	—
		Imipenem	≤0.06	≤0.06	≤0.06	—	—	—
Streptococcus mitis group	20	IMR	≤0.06	≤0.06	≤0.06	—	—	—
		Imipenem	≤0.06	≤0.06	≤0.06	—	—	—
Streptococcus anginosus	20	IMR	≤0.06	≤0.06	≤0.06	—	—	—
		Imipenem	≤0.06	≤0.06	≤0.06	—	—	—
Streptococcus constellatus	20	IMR	≤0.06	≤0.06	≤0.06	—	—	—
		Imipenem	≤0.06	≤0.06	≤0.06	—	—	—
Streptococcus salivarius	10	IMR	≤0.06	≤0.06	≤0.06	—	—	—
		Imipenem	≤0.06	≤0.06	≤0.06	—	—	—
Staphylococcus intermedius	10	IMR	≤0.06	≤0.06	≤0.06	—	—	—
		Imipenem	≤0.06	≤0.06	≤0.06	—	—	—
Gram-negative aerobes								
Citrobacter spp.	100	IMR	0.12 to 1	0.12	0.25	100 (100.0)	0	0
		Imipenem	0.12 to 1	0.25	1	100 (100.0)	0	0
Escherichia coli	100	IMR	≤0.06 to 1	0.12	0.12	100 (100.0)	0	0
		Imipenem	≤0.06 to 1	0.12	0.25	100 (100.0)	0	0
Enterobacter cloacae complex	100	IMR	0.12 to 0.5	0.25	0.25	100 (100.0)	0	0
		Imipenem	0.12 to 2	0.5	1	98 (98.0)	2 (2.0)	0
Klebsiella pneumoniae	100	IMR	≤0.06 to 1	0.12	0.5	100 (100.0)	0	0
		Imipenem	≤0.06 to 1	0.12	0.5	100 (100.0)	0	0
Klebsiella oxytoca	100	IMR	0.12 to 0.25	0.12	0.25	100 (100.0)	0	0
		Imipenem	0.12 to 0.25	0.12	0.25	100 (100.0)	0	0
Klebsiella aerogenes	100	IMR	0.12 to 1	0.5	1	100 (100.0)	0	0
		Imipenem	0.25 to 2	1	2	67 (67.0)	33 (33.0)	0
Serratia marcescens	100	IMR	0.12 to 2	0.5	1	99 (99.0)	1 (1.0)	0
		Imipenem	0.25 to 16	0.5	1	98 (98.0)	1 (1.0)	1 (1.0)
Morganella morganii	100	IMR	0.5 to 2	1	2	60 (60.0)[d]	40 (40.0)[d]	0[d]
		Imipenem	0.5 to 4	2	2	35 (35.0)	60 (60.0)	5 (5.0)
Proteus mirabilis	100	IMR	≤0.06 to 4	0.5	2	85 (85.0)[d]	13 (13.0)[d]	2 (2.0)[d]
		Imipenem	≤0.06 to 4	0.5	2	85 (85.0)	12 (12.0)	3 (3.0)
Proteus vulgaris	100	IMR	≤0.06 to 2	1	2	71 (71.0)[d]	29 (29.0)[d]	0[d]
		Imipenem	0.12 to 4	1	2	69 (69.0)	30 (30.0)	1 (1.0)
Providencia spp.	100	IMR	0.5 to 4	1	2	81 (81.0)[d]	17 (17.0)[d]	2 (2.0)[d]
		Imipenem	0.5 to 4	1	2	81 (81.0)	17 (17.0)	2 (2.0)

(Continued on next page)

TABLE 4 (Continued)

Species[a]	No. of isolates	Antibacterial agent[b]	MIC (μg/mL)			No. (%) with MIC interpretation		
			Range	MIC$_{50}$	MIC$_{90}$	Susceptible	Intermediate	Resistant
Pseudomonas aeruginosa	100	IMR	0.12 to >128	0.25	4	89 (89.0)	3 (3.0)	8 (8.0)
		Imipenem	0.5 to >128	2	32	76 (76.0)	3 (3.0)	21 (21.0)
Acinetobacter spp.	50	IMR	≤0.06 to 0.5	0.12	0.25	50 (100.0)[d]	0[d]	0[d]
		Imipenem	≤0.06 to 1	0.12	0.25	50 (100.0)	0	0
Burkholderia cepacia complex	25	IMR	≤0.06 to 4	0.25	1	—	—	—
		Imipenem	1 to 16	4	8	—	—	—
BLNAR	25	IMR	0.25 to 4	1	2	25 (100.0)[d]	—	—
		Imipenem	0.25 to 4	1	2	25 (100.0)	—	—
Haemophilus influenzae (other than BLNAR)	25	IMR	≤0.06 to 2	0.5	1	25 (100.0)[d]	—	—
		Imipenem	≤0.06 to 2	0.5	1	25 (100.0)	—	—
Anaerobes								
Peptostreptococcus spp.	25	IMR	≤0.06	≤0.06	≤0.06	25 (100.0)	0	0
		Imipenem	≤0.06	≤0.06	≤0.06	25 (100.0)	0	0
Bacteroides spp.	25	IMR	≤0.06 to 8	0.25	1	24 (96.0)	1 (4.0)	0
		Imipenem	≤0.06 to 16	0.25	2	24 (96.0)	0	1 (4.0)
Prevotella spp.	25	IMR	≤0.06 to 0.12	≤0.06	≤0.06	25 (100.0)	0	0
		Imipenem	≤0.06 to 0.12	≤0.06	≤0.06	25 (100.0)	0	0

[a]BLNAR, β-lactamase–negative ampicillin-resistant *H. influenzae*; MSCNS, methicillin-susceptible coagulase-negative *Staphylococcus* (other than *Staphylococcus epidermidis*); MSSA, methicillin-susceptible *Staphylococcus aureus*; MSSE, methicillin-susceptible *S. epidermidis*; MRCNS, methicillin-resistant coagulase-negative *Staphylococcus* (other than *S. epidermidis*); MRSE, methicillin-resistant *S. epidermidis*; MRSA, methicillin-resistant *S. aureus*; PSSP, penicillin-susceptible *Streptococcus pneumoniae*; PRSP, penicillin-resistant *S. pneumoniae*.
[b]IMR, imipenem/relebactam.
[c]—, Not applicable.
[d]Calculated according to imipenem breakpoints because breakpoints for IMR were not defined.

other than *S. epidermidis*), *Enterococcus faecium*, and *Enterococcus avium* isolates, those for the other species were low (≤0.06 to 1 μg/mL).

Anaerobic bacteria were highly susceptible to IMR. The MIC$_{50/90}$ values for *Peptostreptococcus* spp. and *Prevotella* spp. were ≤0.06/≤0.06 μg/mL, and the percentage of susceptibility was 100%. The MIC$_{50/90}$ for *Bacteroides* spp. was 0.25/1 μg/mL, and the percentage of susceptibility was 96.0%. Enhancement of imipenem antibacterial activity by REL was observed in *Bacteroides* spp.

DISCUSSION

In this study, the antibacterial activities of IMR and reference drugs against clinical isolates from Japan were evaluated in 2 analyses. Among the species listed in the indication for IMI/REL, imipenem-nonsusceptible isolates of *E. coli*, *E. cloacae* complex, *K. pneumoniae*, *K. aerogenes*, *S. marcescens*, and *P. aeruginosa* were collected. Overall, the susceptibility of these isolates to IMR was high. All isolates of *E. coli*, *E. cloacae* complex, and *K. aerogenes* were susceptible to IMR. Based on the CLSI breakpoint for IMR (MIC of ≤1 μg/mL) (3), there were IMR-nonsusceptible isolates of *K. pneumoniae*, *S. marcescens*, and *P. aeruginosa*. Of these isolates, the MIC of 1 *K. pneumoniae* isolate and 1 *S. marcescens* isolate was 2 μg/mL, which is considered intermediate susceptibility according to the CLSI breakpoint for IMR (MIC of 2 μg/mL). However, it has been reported that at the recommended IMI/REL dosing regimens, >90% of patients were predicted to achieve joint pharmacokinetic/pharmacodynamic targets at an MIC breakpoint of ≤2 μg/mL, which aligns with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint for IMR (16, 17). Taking this into consideration, IMR may be effective for 2/3 and 2/2 of imipenem-nonsusceptible isolates of *K. pneumoniae* and *S. marcescens* collected in this study, respectively, and thus, these isolates would be considered susceptible by EUCAST breakpoints but of intermediate susceptibility by CLSI breakpoints.

Comparison of the antibacterial activities of IMR with those of the reference drugs showed that the antibacterial activities of IMR against imipenem-nonsusceptible Enterobacterales were generally comparable to those of AMK and comparable to or

higher than those of other drugs. In particular, the differences between IMR and MEM, TZP, CAZ, and FEP were notable for isolates with low susceptibility to imipenem. The antibacterial activities of IMR against imipenem-nonsusceptible *P. aeruginosa* isolates were lower than that of AMK and comparable to or higher than those of other drugs. The percentages of susceptibility to CST were intermediate for both imipenem-nonsusceptible Enterobacterales and *P. aeruginosa* isolates. CLSI does not define a susceptibility breakpoint for CST; however, pharmacokinetic/pharmacodynamic analysis has shown that less than 50% of patients can achieve the blood concentrations of CST required for killing bacteria due to the high risk of nephrotoxicity (18). These results indicate that IMR can be a new treatment option for Enterobacterales and *P. aeruginosa* isolates that are resistant to existing β-lactams and other antibacterial agents.

The antibacterial activities against imipenem-nonsusceptible isolates possessing β-lactamases were also assessed. The antibacterial activities of IMR were lower than those of AMK and higher than those of the other reference drugs against isolates with AmpC, and were similar to those of AMK and TGC, and higher than those of the other reference drugs against isolates with ESBL. As the production of AmpC or ESBL concomitant with entry porin deficiency is a known mechanism of carbapenem resistance (6, 7), these results suggest the potential utility of IMR against bacteria that are carbapenem resistant due to these mechanisms.

In 2 sets of antibacterial susceptibility tests, 8 *P. aeruginosa* isolates and 1 *K. pneumoniae* isolate were resistant to IMR. Six of 8 IMR-resistant *P. aeruginosa* strains possessed an MBL. Since REL does not inhibit MBL (19), this result was expected. The IMR-resistant *K. pneumoniae* strain was multidrug-resistant and may have possessed multiple resistance factors like penicillin-binding protein mutations and increased drug efflux pumps in addition to AmpC production; although these are possible factors for resistance in *K. pneumoniae*, it is important to note that imipenem is not subject to efflux, and therefore, efflux pumps would not be a mechanism of resistance for IMR. Overexpression or modification of drug efflux pumps and mutation of penicillin-binding protein have been reported as mechanisms of resistance to IMR (20); however, this mechanism is not definitive, as another study did not find evidence to support that imipenem or REL was subject to efflux (5). These mechanisms of resistance were not analyzed in the present study, and therefore, mechanisms of IMR resistance other than MBL in the studied isolates are unknown.

In the prospective study, antibacterial susceptibility was also measured for bacterial species listed in the IMI indication in Japan. The antibacterial activities of IMR were equal to or higher than those of imipenem against all bacterial species collected, and it was confirmed that the antibacterial activity of imipenem was not weakened by the combination with REL. There were imipenem-nonsusceptible isolates of *P. mirabilis*, *P. vulgaris*, and *Providencia* spp., but the enhancement of antibacterial activity of imipenem by REL was minimal. Since these species are known to be carbapenem-resistant by mechanisms other than β-lactamases (3, 21), REL was not effective in restoring imipenem susceptibility in these species. In *Morganellaceae*, enhancement of the antibacterial activity of imipenem by REL was observed only in *M. morganii*. With the exception of 1 strain, IMR-susceptible *M. morganii* strains did not show phenotypes of ESBL expression or constitutive expression of AmpC (data not shown). Since AmpC production is known to be one of the mechanisms of carbapenem resistance in *M. morganii* (22), REL may have restored the activity of imipenem by inhibiting AmpC induced by imipenem. Although IMI is indicated for infections due to *Staphylococcus* spp. and *Enterococcus* spp., the MIC_{90} values of IMR and imipenem against MRSA, MRSE, methicillin-resistant coagulase-negative *Staphylococcus* (MRCNS; other than *S. epidermidis*), *E. faecium*, and *E. avium* isolates were high. It is known that there are some strains of these species that are less susceptible to imipenem (23, 24), and the results of this study were consistent with those reports.

Comparison of the results of prospective studies with overseas surveillance revealed differences in the IMR susceptibilities of *P. aeruginosa* and *Acinetobacter* species isolates (10, 11). In the United States and Europe, about 10% of imipenem-nonsusceptible *P. aeruginosa*

strains were resistant to IMR, while about 30% were resistant in this study. This difference is considered to be due to the higher proportion of MBL-possessing isolates in this study than in the United States (10) or Europe (11). The percentages of susceptibility of *Acinetobacter* spp. isolates to IMR were approximately 50% and 10% in the United States and Europe, respectively, while that observed in this study was 100%. The main mechanism of carbapenem resistance in *Acinetobacter* spp. is OXA-type carbapenemases, which are not inhibited by REL, and distribution of them is common overseas yet rare in Japan (25). This may account for the difference in the susceptibilities of *Acinetobacter* species isolates to IMR.

Several limitations should be considered when interpreting the results of this study. Primarily, there was a small number of imipenem-nonsusceptible isolates and the number of bacterial species was limited. Among the species listed in the indication of IMI/REL, imipenem-nonsusceptible isolates of *Citrobacter* spp. and *Acinetobacter* spp. were not collected in this study. However, a lower MIC$_{50/90}$ of IMR than of imipenem was observed for *Citrobacter* spp., suggesting that IMR may be effective against imipenem-nonsusceptible *Citrobacter* spp. In addition, this study lacked the genetic analysis needed to confirm the presence of β-lactamases in phenotypically β-lactamase-positive isolates. Furthermore, resistance mechanisms other than β-lactamases were not analyzed.

In summary, IMR generally showed antibacterial activity against imipenem-nonsusceptible Enterobacterales other than *Morganellaceae* and *P. aeruginosa* isolates tested in this study, which often correspond to the indicated pathogens of IMI/REL in Japan. The antibacterial activities of IMR against imipenem-nonsusceptible isolates were lower than those of AMK and comparable to or greater than those of other drugs. The activity of IMR was superior to those of the reference drugs other than AMK and TGC for isolates with low susceptibility to imipenem, including isolates with AmpC or ESBL, which are 2 resistance mechanisms that are affected by REL. Although the efficacy was comparable to or less than those of existing drugs like AMK and TGC, issues with these drugs related to safety (26–28) and resistance (29–31) suggest that IMR is expected to be a new option for treatment of infections caused by carbapenem-resistant pathogens.

MATERIALS AND METHODS

Bacterial isolates. In the prospective study, aerobic bacteria (*N* = 1,920 isolates, including *Staphylococcus* spp., *Enterococcus* spp., *Streptococcus* spp., *Citrobacter* spp., *E. cloacae* complex, *E. coli*, *K. pneumoniae*, *Klebsiella oxytoca*, *K. aerogenes*, *S. marcescens*, *M. morganii*, *P. mirabilis*, *P. vulgaris*, *Providencia* spp., *P. aeruginosa*, *Acinetobacter* spp., *Burkholderia cepacia* complex, and *H. influenzae*) and anaerobic bacteria (*N* = 75 isolates, including *Peptostreptococcus* spp., *Bacteroides* spp., and *Prevotella* spp.) were isolated from various clinical specimens of patients in 605 medical institutions throughout Japan from January 2016 to September 2016 by LSI Medience Corporation. Identification of the isolates was carried out in accordance with the *Manual of Clinical Microbiology* (32), and strains were frozen at −70°C in skim milk. Collection of these strains was conducted in compliance with the "Ethical Guidelines for Epidemiologic Research" issued by the Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labor and Welfare (33) and the notification of the Japanese Society for Clinical Microbiology.

In the antibacterial susceptibility test of bacterial stocks, imipenem-nonsusceptible strains of *E. coli*, *E. cloacae* complex, and *K. pneumoniae* (*N* = 8) were collected by Toho University from 6 hospitals in Japan between 2013 and 2016 and frozen at −80°C in 15% glycerol. Identification of bacterial species was performed using draft whole-genome sequencing as described previously (34).

Antibacterial susceptibility testing. Following the CLSI guidelines (3, 35, 36), the broth microdilution method and agar dilution method were used for aerobic bacteria and anaerobic bacteria, respectively. Ninety-six-well frozen plates (Eiken Chemical Co., Ltd., Tokyo, Japan) containing drugs were used for testing. All isolates were tested against IMR (REL was fixed at 4 μg/mL), imipenem, MEM, TZP (tazobactam was fixed at 4 μg/mL), CAZ, FEP, LVX, AMK, CST, and TGC. The concentration range of drugs other than CST was 0.06 to 128 μg/mL, and that of CST was 0.06 to 32 μg/mL. MICs were interpreted as susceptible, intermediate, and resistant using CLSI breakpoints (3). MIC values for IMR and TZP are presented for imipenem and piperacillin, respectively.

Detection of β-lactamases. In the prospective study, *E. coli*, *Klebsiella* species, and *P. mirabilis* isolates were tested for ESBLs, and Enterobacterales, *P. aeruginosa*, and *Acinetobacter* species isolates were tested for MBL and AmpC. An MIC of ≥2 μg/mL for CAZ or cefotaxime (CTX) and an ≥8-fold decrease in the MIC of CAZ or CTX in combination with clavulanic acid versus that of CAZ or CTX alone was considered positive for ESBLs. An MIC of ≥2 μg/mL for CAZ or CTX, an ≥8-fold decrease in the MIC of CAZ or CTX in combination with 3-aminophenylboronic acid versus that of CAZ or CTX alone, and a negative

result in the modified carbapenem inactivation method (3) was considered positive for constitutive expression of AmpC (37). Unlike imipenem (38), both CAZ and CTX are not strong inducers of AmpC (37); therefore, this test only detected constitutive production of AmpC. Since many species of bacteria encode a chromosomal AmpC (e.g., *E. cloacae* and *P. aeruginosa*) and since imipenem is a strong inducer of AmpC, all members of these species should be considered to hyperproduce AmpC when imipenem is present, either in an *in vitro* susceptibility test or in a patient. A decrease of ≥8-fold in the MIC of CAZ or imipenem in combination with dipicolinic acid versus that of CAZ or imipenem alone was considered positive for MBL. The final concentrations of clavulanic acid, 3-aminophenylboronic acid, and dipicolinic acid were fixed at 4 μg/mL, 200 μg/mL, and 175 μg/mL, respectively.

In the antibacterial susceptibility test using bacterial stocks, the β-lactamase gene profile was identified using draft whole-genome sequencing as described in a previous study (34). Briefly, bacterial DNA libraries were sequenced on a MiSeq system. Acquired antibacterial resistance genes were identified using the ResFinder database, version 2.1.

Determination of antibacterial-resistant strains. *S. aureus* was defined as methicillin-susceptible *S. aureus* when the MIC of OXA was ≤2 μg/mL and as MRSA when the MIC of OXA was ≥4 μg/mL. *S. epidermidis* was defined as methicillin-susceptible *S. epidermidis* when the MIC of OXA was ≤0.25 μg/mL and as MRSE when the MIC of OXA was ≥0.5 μg/mL. Coagulase-negative *Staphylococcus* was defined as methicillin-susceptible coagulase-negative *Staphylococcus* (MSCNS) when the MIC of OXA was ≤0.25 μg/mL and as methicillin-resistant coagulase-negative *Staphylococcus* (MRCNS) when the MIC of OXA was ≥0.5 μg/mL. *Streptococcus pneumoniae* was defined as penicillin-susceptible *S. pneumoniae* when the MIC of penicillin G was ≤0.06 μg/mL and as penicillin-resistant *S. pneumoniae* when the MIC of penicillin G was ≥2 μg/mL. β-Lactamase–negative *H. influenzae* with an ampicillin MIC of ≥2 μg/mL was classified as β-lactamase–negative ampicillin-resistant *H. influenzae* (BLNAR). β-Lactamase activity was detected by the nitrocefin spot plate method.

REFERENCES

1. Rodloff AC, Goldstein EJ, Torres A. 2006. Two decades of imipenem therapy. J Antimicrob Chemother 58:916–929. https://doi.org/10.1093/jac/dkl354.
2. Clissold SP, Todd PA, Campoli-Richards DM. 1987. Imipenem/cilastatin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy. Drugs 33:183–241. https://doi.org/10.2165/00003495-198733030-00001.
3. Clinical and Laboratory Standards Institute. 2021. Performance standards for antimicrobial susceptibility testing, 31st ed. CLSI supplement M100. CLSI, Wayne, PA, USA.
4. World Health Organization. 2017. Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis. WHO, Geneva, Switzerland.
5. Young K, Painter RE, Raghoobar SL, Hairston NN, Racine F, Wisniewski D, Balibar CJ, Villafania A, Zhang R, Sahm DF, Blizzard T, Murgolo N, Hammond ML, Motyl MR. 2019. In vitro studies evaluating the activity of imipenem in combination with relebactam against *Pseudomonas aeruginosa*. BMC Microbiol 19:150. https://doi.org/10.1186/s12866-019-1522-7.
6. Martinez-Martinez L. 2008. Extended-spectrum beta-lactamases and the permeability barrier. Clin Microbiol Infect 14:82–89. https://doi.org/10.1111/j.1469-0691.2007.01860.x.
7. Poole K. 2011. *Pseudomonas aeruginosa*: resistance to the max. Front Microbiol 2:65. https://doi.org/10.3389/fmicb.2011.00065.
8. Livermore DM, Warner M, Mushtaq S. 2013. Activity of MK-7655 combined with imipenem against Enterobacteriaceae and *Pseudomonas aeruginosa*. J Antimicrob Chemother 68:2286–2290. https://doi.org/10.1093/jac/dkt178.
9. Motsch J, Murta de Oliveira C, Stus V, Koksal I, Lyulko O, Boucher HW, Kaye KS, File TM, Brown ML, Khan I, Du J, Joeng HK, Tipping RW, Aggrey A, Young K, Kartsonis NA, Butterton JR, Paschke A. 2020. RESTORE-IMI 1: a multicenter, randomized, double-blind trial comparing efficacy and safety

of imipenem/relebactam vs colistin plus imipenem in patients with imipenem-nonsusceptible bacterial infections. Clin Infect Dis 70:1799–1808. https://doi.org/10.1093/cid/ciz530.

10. Lob SH, Hackel MA, Kazmierczak KM, Young K, Motyl MR, Karlowsky JA, Sahm DF. 2017. *In vitro* activity of imipenem-relebactam against gram-negative ESKAPE pathogens isolated by clinical laboratories in the United States in 2015 (results from the SMART Global Surveillance Program). Antimicrob Agents Chemother 61:e02209-16. https://doi.org/10.1128/AAC.02209-16.

11. Karlowsky JA, Lob SH, Kazmierczak KM, Hawser SP, Magnet S, Young K, Motyl MR, Sahm DF. 2018. *In vitro* activity of imipenem/relebactam against Gram-negative ESKAPE pathogens isolated in 17 European countries: 2015 SMART surveillance programme. J Antimicrob Chemother 73:1872–1879. https://doi.org/10.1093/jac/dky107.

12. Hidaka Y, Kishi N, Matsumoto S, Yoshinari T, Mori M. 2018. *In vitro* antimicrobial activity of ceftolozane/tazobactam against clinical isolates from Japan. Jpn J Chemother 66:567–577.

13. Hilbert DW, DeRyke CA, Moise P, Motyl MR, Hackel MA, Young K. 2021. Relebactam increases susceptibility of Enterobacterales and Pseudomonas aeruginosa to imipenem in both imipenem-susceptible and imipenem-nonsusceptible gram-negative bacteria–Global SMART 2017–2019. Poster 01590 31st Eur Congr Clin Microbiol Infect Dis (ECCMID), Virtual Meeting, 9 to 12 July 2021.

14. Young K, Hilbert DW, DeRyke CA, Sahm DF, Motyl MR. 2021. Relebactam enhances the activity of imipenem against Burkholderia cepacia complex (BCC) isolates SMART 2016–2019. World Microbe Forum, Virtual Meeting, 20 to 24 June 2021.

15. Hilbert DW, DeRyke CA, Losada MC, Chen LF, Moise PA, Young K. 2021. Relebactam increases imipenem activity against imipenem-nonsusceptible and -susceptible *Pseudomonas aeruginosa* and *Enterobacterales*: assessment of isolates from RESTORE-IMI 2. Poster 1071331 IDWeek 2021, Virtual Meeting, 29 September to 3 October 2021.

16. Patel M, Bellanti F, Daryani NM, Noormohamed N, Hilbert DW, Young K, Kulkarni P, Copalu W, Gheyas F, Rizk ML. 2022. Population pharmacokinetic/pharmacodynamic assessment of imipenem/cilastatin/relebactam in patients with hospital-acquired/ventilator-associated bacterial pneumonia. Clin Transl Sci 15:396–408. https://doi.org/10.1111/cts.13158.

17. European Committee on Antimicrobial Susceptibility Testing. 2021. Breakpoint tables for interpretation of MICs and zone diameters, version 11.0, 2021. EUCAST, Växjö, Sweden.

18. Satlin MJ, Lewis JS, Weinstein MP, Patel J, Humphries RM, Kahlmeter G, Giske CG, Turnidge J. 2020. Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing position statements on polymyxin B and colistin clinical breakpoints. Clin Infect Dis 71:e523–e529. https://doi.org/10.1093/cid/ciaa121.

19. Zhanel GG, Lawrence CK, Adam H, Schweizer F, Zelenitsky S, Zhanel M, Lagace-Wiens PRS, Walkty A, Denisuik A, Golden A, Gin AS, Hoban DJ, Lynch JP, III, Karlowsky JA. 2018. Imipenem-relebactam and meropenem-vaborbactam: two novel carbapenem-β-lactamase inhibitor combinations. Drugs 78:65–98. https://doi.org/10.1007/s40265-017-0851-9.

20. Gomis-Font MA, Cabot G, Sánchez-Diener I, Fraile-Ribot PA, Juan C, Moya B, Zamorano L, Oliver A. 2020. *In vitro* dynamics and mechanisms of resistance development to imipenem and imipenem/relebactam in *Pseudomonas aeruginosa*. J Antimicrob Chemother 75:2508–2515. https://doi.org/10.1093/jac/dkaa206.

21. Neuwirth C, Siébor E, Duez J-M, Péchinot A, Kazmierczak A. 1995. Imipenem resistance in clinical isolates of *Proteus mirabilis* associated with alterations in penicillin-binding proteins. J Antimicrob Chemother 36:335–342. https://doi.org/10.1093/jac/36.2.335.

22. Wozniak A, Villagra NA, Undabarrena A, Gallardo N, Keller N, Moraga M, Román JC, Mora GC, García P. 2012. Porin alterations present in non-carbapenemase-producing *Enterobacteriaceae* with high and intermediate

levels of carbapenem resistance in Chile. J Med Microbiol 61:1270–1279. https://doi.org/10.1099/jmm.0.045799-0.

23. Yamaguchi K, Tateda K, Ohno A, Ishii Y, Murakami H. 2016. Surveillance of in vitro susceptibilities to levofloxacin and various antibacterial agents for 11,762 clinical isolates obtained from 69 centers in 2013. Jpn J Antibiot 69:1–25.

24. Ishihara S, Yamada T, Yokoi S, Ito M, Yasuda M, Nakano M, Kawada Y, Deguchi T. 2002. Antimicrobial activity of imipenem against isolates from complicated urinary tract infections. Int J Antimicrob Agents 19:565–569. https://doi.org/10.1016/s0924-8579(02)00099-7.

25. Matsui M, Suzuki M, Suzuki M, Yatsuyanagi J, Watahiki M, Hiraki Y, Kawano F, Tsutsui A, Shibayama K, Suzuki S. 2018. Distribution and molecular characterization of *Acinetobacter baumannii* international clone II lineage in Japan. Antimicrob Agents Chemother 62:e02190-17. https://doi.org/10.1128/AAC.02190-17.

26. Ortwine JK, Sutton JD, Kaye KS, Pogue JM. 2015. Strategies for the safe use of colistin. Expert Rev Anti Infect Ther 13:1237–1247. https://doi.org/10.1586/14787210.2015.1070097.

27. Kaewpoowat Q, Ostrosky-Zeichner L. 2015. Tigecycline: a critical safety review. Expert Opin Drug Saf 14:335–342. https://doi.org/10.1517/14740338.2015.997206.

28. Selimoglu E. 2007. Aminoglycoside-induced ototoxicity. Curr Pharm Des 13:119–126. https://doi.org/10.2174/138161207779313731.

29. Garneau-Tsodikova S, Labby KJ. 2016. Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives. Medchemcomm 7:11–27. https://doi.org/10.1039/C5MD00344J.

30. Mushtaq S, Reynolds R, Gilmore MC, Esho O, Adkin R, García-Romero I, Chaudhry A, Horner C, Bartholomew TL, Valvano MA, Dry M, Murray J, Pichon B, Livermore DM. 2020. Inherent colistin resistance in genogroups of the *Enterobacter cloacae* complex: epidemiological, genetic and biochemical analysis from the BSAC Resistance Surveillance Programme. J Antimicrob Chemother 75:2452–2461. https://doi.org/10.1093/jac/dkaa201.

31. Pan Y, Awan F, Zhenbao M, Zhang X, Zeng J, Zeng Z, Xiong W. 2020. Preliminary view of the global distribution and spread of the tet(X) family of tigecycline resistance genes. J Antimicrob Chemother 75:2797–2803. https://doi.org/10.1093/jac/dkaa284.

32. Carroll KC, Pfaller MA, Landry ML, McAdam AJ, Patel R, Richter SS, Warnock DW. 2019. Manual of clinical microbiology, 12th ed. ASM Press, Washington, DC, USA.

33. Ministry of Health, Labour and Welfare, Ministry of Education, Culture, Sports, Science and Technology. 2002. Ethical guidelines for epidemiologic research. Ministry of Health, Labour and Welfare and Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan. https://www.mhlw.go.jp/general/seido/kousei/i-kenkyu/ekigaku/0504sisin.html.

34. Aoki K, Harada S, Yahara K, Ishii Y, Motooka D, Nakamura S, Akeda Y, Iida T, Tomono K, Iwata S, Moriya K, Tateda K. 2018. Molecular characterization of IMP-1-producing *Enterobacter cloacae* complex isolates in Tokyo. Antimicrob Agents Chemother 62:e02091-17. https://doi.org/10.1128/AAC.02091-17.

35. Clinical and Laboratory Standards Institute. 2012. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard, 8th ed. CLSI document M11-A8. CLSI, Wayne, PA, USA.

36. Clinical and Laboratory Standards Institute. 2018. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 11th ed. CLSI standard M07. CLSI, Wayne, PA, USA.

37. Jacoby GA. 2009. AmpC β-lactamases. Clin Microbiol Rev 22:161–182. https://doi.org/10.1128/CMR.00036-08.

38. Dunne WM, Jr, Hardin DJ. 2005. Use of several inducer and substrate antibiotic combinations in a disk approximation assay format to screen for AmpC induction in patient isolates of *Pseudomonas aeruginosa*, *Enterobacter* spp., *Citrobacter* spp., and *Serratia* spp. J Clin Microbiol 43:5945–5949. https://doi.org/10.1128/JCM.43.12.5945-5949.2005.