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In Vitro Evaluation of the Activity of Imipenem-Relebactam against 451 Recent Clinical Isolates of *Bacteroides* Group and Related Species

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We evaluated the *in vitro* activity of imipenem-relebactam (imipenem-MK7655) against 451 recent clinical isolates within the *Bacteroides* group and related species. Relebactam did not enhance or inhibit the activity of imipenem against *Bacteroides fragilis* or other *Bacteroides* species. No synergistic or antagonistic effect was observed. The MICs of imipenem-relebactam were equal to or within one dilution of the MICs of these isolates to imipenem.

Imipenem-relebactam (imipenem-MK7655) is the combination of imipenem with a novel β-lactamase inhibitor (relebactam) undergoing clinical development (1, 2). The new agent has demonstrated potent inhibitory activity against class A and class C β-lactamases. In addition, relebactam inhibits carbapenemases, such as *Klebsiella pneumoniae* carbapenemase (KPC), a plasmid-encoded carbapenemase produced by some *Enterobacteriaceae*, most commonly by *Klebsiella pneumoniae* (1, 2). Imipenem with relebactam has recently completed a successful phase 2 trial in intra-abdominal infections and is currently in phase 3 development for the treatment of drug-resistant Gram-negative infections (3).

Imipenem has been among the most active agents against the *Bacteroides* species (4–6). Generally, the resistance rates of these species to imipenem have been very low (~0.5 to 1%), However, recently, we have seen some strains with elevated MICs against this agent and other carbapenems (4–6).

The purpose of this study was to evaluate the *in vitro* activity of relebactam combined with imipenem against a large number of *Bacteroides fragilis* group isolates (including strains resistant to imipenem) and to determine whether the addition of the inhibitor will enhance imipenem's spectrum to include resistant strains.

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The evaluation consisted of 451 recent clinical isolates of the *B. fragilis* group and related species from 2011 to 2012. Some of these isolates had been referred from 6 geographically distinct medical centers throughout the United States (Tufts Medical Center, R. M. Alden Labs, Loyola, New York Presbyterian/Weill Cornell Medical Center, Mayo Clinic, and Duke University Health Center). The identification of the isolates was confirmed according to methodology described in the *Wadsworth-KTL Anaerobic Bacteriology Manual* (7). *B. fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741 were used as reference controls and included with each test. The susceptibilities of the isolates were determined by agar dilution, according to the recommendations of Clinical and Laboratory Standards Institute (CLSI) document M11-A8 (8). Imipenem-relebactam (at 4 μg/ml) and imipenem alone were evaluated, along with the following agents: doripenem, ertapenem, meropenem, ampicillin-sulbactam, piperacillin-tazobactam, cefoxitin, moxifloxacin, tigecycline, clindamycin, linezolid, metronidazole, and chloramphenicol. Table 1 lists the antimicrobial agents, the range of concentrations tested, and their breakpoints for resistance. CLSI recommendations for resistance breakpoints were used for the analysis (8, 9). For tigecycline, the FDA-recommended resistance breakpoint for anaerobes was used (10).

TABLE 1 Test range and recommended breakpoints for resistance

Antimicrobial agent	Testing range (μg/ml)	CLSI resistance breakpoint[a,b]
Imipenem	0.06–16	≥16
Imipenem-relebactam	0.06–16	≥16
Ampicillin-sulbactam	0.5–128	≥32
Piperacillin-tazobactam	0.5–256	≥128
Doripenem	0.06–16	≥8
Ertapenem	0.06–16	≥16
Meropenem	0.06–16	≥16
Cefoxitin	2–128	≥64
Clindamycin	0.5–16	≥8
Linezolid[a]	0.5–16	≥8
Moxifloxacin	0.5–16	≥8
Tigecycline[b]	0.06–64	≥16
Chloramphenicol[c]	2, 8	≥32
Metronidazole	1–16	≥32

[a] There are no breakpoint recommendations for linezolid against anaerobes.
[b] For tigecycline, the FDA-recommended breakpoint for anaerobes of ≥16 μg/ml was used.
[c] Chloramphenicol was tested only at concentrations of 2 and 8 μg/ml.

A summary of the comparative activities of the antimicrobial agents against all the species within the group is shown in Table 2. The addition of relebactam (MK-7655) to imipenem did not enhance or detract from the activity of imipenem alone against any of the species within the group. The MICs of the combination against all isolates were equal to or within one dilution of the MICs of imipenem alone.

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TABLE 2 Activities of antibiotics versus *Bacteroides fragilis* group and related species

Species tested	Antimicrobial agent	MIC range	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% resistant[a]
All isolates ($n = 451$)	Imipenem	≤0.06 to 16	0.25	1	0.7
	Imipenem-relebactam	≤0.06 to 16	0.25	0.5	0.7
	Piperacillin-tazobactam	≤0.5 to >256	4	16	1.1
	Ampicillin-sulbactam	≤0.5 to >128	2	16	3.3
	Ertapenem	0.12 to >16	0.5	2	1.6
	Meropenem	0.12 to >16	0.25	1	1.3
	Doripenem	0.12 to >16	0.5	1	2.2
	Cefoxitin	≤2 to >128	16	32	7.3
	Tigecycline	≤0.06 to 32	1	8	3.5
	Moxifloxacin	≤0.5 to >16	2	>16	30.6
	Clindamycin	≤0.5 to >16	2	>16	39.5
	Linezolid	≤0.5 to 16	2	4	1.6
	Chloramphenicol[b]	>2 to ≤8	≤8	≤8	0.0
	Metronidazole	≤1 to 2	≤ 1	2	0.0
B. fragilis ($n = 220$)	Imipenem	≤0.06 to 16	0.25	0.5	0.9
	Imipenem-relebactam	≤0.06 to 16	0.25	0.5	0.9
	Piperacillin-tazobactam	≤0.5 to >256	2	8	0.9
	Ampicillin-sulbactam	≤0.5 to >128	2	16	2.3
	Ertapenem	0.12 to >16	0.5	2	1.8
	Meropenem	0.12 to >16	0.25	1	1.8
	Doripenem	0.12 to >16	0.5	1	2.7
	Cefoxitin	≤2 to >128	16	32	4.1
	Tigecycline	≤0.06 to 32	1	8	4.5
	Moxifloxacin	≤0.5 to >16	1	8	22.3
	Clindamycin	≤0.5 to >16	1	>16	28.2
	Linezolid	≤0.5 to 8	2	4	1.4
	Chloramphenicol	>2 to ≤8	≤8	≤8	0.0
	Metronidazole	≤1 to 2	≤1	≤1	0.0
Bacteroides non-*fragilis* group species ($n = 231$)	Imipenem	≤0.06 to 16	0.5	1	0.4
	Imipenem-relebactam	≤0.06 to 16	0.25	1	0.4
	Piperacillin-tazobactam	≤0.5 to 128	4	16	1.3
	Ampicillin-sulbactam	≤0.5 to 128	4	16	4.3
	Ertapenem	0.12 to 16	1	2	1.3
	Meropenem	0.12 to 16	0.25	1	0.9
	Doripenem	0.12 to 16	0.5	1	1.7
	Cefoxitin	≤2 to >128	16	64	10.4
	Tigecycline	≤0.06 to 32	1	8	2.6
	Moxifloxacin	≤0.5 to >16	2	>16	38.5
	Clindamycin	≤0.5 to >16	8	>16	50.2
	Linezolid	≤0.5 to 16	4	4	1.7
	Chloramphenicol	>2 to ≤8	≤8	≤8	0.0
	Metronidazole	≤1 to 2	≤1	2	0.0
Bacteroides ovatus ($n = 43$)	Imipenem	≤0.06 to 4	0.25	1	0.0
	Imipenem-relebactam	0.12 to 4	0.25	0.5	0.0
	Piperacillin-tazobactam	≤0.5 to 32	4	16	0.0
	Ampicillin-sulbactam	≤0.5 to 16	2	16	0.0
	Ertapenem	0.25 to 16	1	4	2.3
	Meropenem	0.12 to 8	0.25	2	0.0
	Doripenem	0.25 to 4	0.5	1	0.0
	Cefoxitin	8 to >128	32	64	18.6
	Tigecycline	≤0.06 to 16	1	8	2.3
	Moxifloxacin	≤0.5 to >16	4	>16	37.2
	Clindamycin	≤0.5 to >16	>16	>16	60.5
	Linezolid	1 to 4	4	4	0.0
	Chloramphenicol	>2 to ≤8	≤8	≤8	0.0
	Metronidazole	≤1 to 2	≤1	2	0.0

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TABLE 2 (Continued)

Species tested	Antimicrobial agent	MIC range	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% resistant[a]
B. thetaiotaomicron (*n* = 103)	Imipenem	≤0.06 to 16	0.25	1	1.0
	Imipenem-relebactam	≤0.06 to 16	0.25	0.5	1.0
	Piperacillin-tazobactam	≤0.5 to 128	8	32	1.0
	Ampicillin-sulbactam	≤0.5 to 128	2	16	1.0
	Ertapenem	0.12 to 16	1	2	1.0
	Meropenem	0.12 to 16	0.25	1	1.0
	Doripenem	0.12 to >16	0.5	1	1.0
	Cefoxitin	≤2 to 128	16	64	10.7
	Tigecycline	≤0.06 to 32	1	8	4.9
	Moxifloxacin	≤0.5 to >16	2	>16	25.2
	Clindamycin	≤0.5 to >16	8	>16	52.4
	Linezolid	≤0.5 to 16	4	4	2.9
	Chloramphenicol	>2 to ≤8	≤8	≤8	0.0
	Metronidazole	≤1 to 2	≤1	2	0.0
Parabacteroides distasonis (*n* = 24)	Imipenem	0.12 to 4	0.5	1	0.0
	Imipenem-relebactam	0.12 to 4	0.5	1	0.0
	Piperacillin-tazobactam	≤0.5 to 128	8	64	4.2
	Ampicillin-sulbactam	≤0.5 to 64	16	32	29.2
	Ertapenem	0.12 to 8	1	4	0.0
	Meropenem	0.12 to 8	0.5	4	0.0
	Doripenem	0.25 to 8	1	4	8.3
	Cefoxitin	4 to 64	32	32	4.2
	Tigecycline	0.12 to 8	1	4	0.0
	Moxifloxacin	≤0.5 to >16	2	16	45.8
	Clindamycin	≤0.5 to >16	4	>16	37.5
	Linezolid	2 to 4	2	4	0.0
	Chloramphenicol	>2 to ≤8	≤8	≤8	0.0
	Metronidazole	≤1 to 2	≤1	2	0.0
Bacteroides uniformis (*n* = 22)	Imipenem	≤0.06 to 1	0.25	0.5	0.0
	Imipenem-relebactam	0.12 to 1	0.25	0.5	0.0
	Piperacillin-tazobactam	≤0.5 to 16	2	8	0.0
	Ampicillin-sulbactam	≤0.5 to 16	2	8	0.0
	Ertapenem	0.12 to 2	0.5	1	0.0
	Meropenem	0.12 to 2	0.25	0.5	0.0
	Doripenem	0.25 to 4	0.5	1	0.0
	Cefoxitin	≤2 to 128	16	32	9.1
	Tigecycline	0.12 to 8	1	8	0.0
	Moxifloxacin	≤0.5 to >16	8	>16	54.5
	Clindamycin	≤0.5 to >16	2	>16	27.3
	Linezolid	1 to 4	4	4	0.0
	Chloramphenicol	>2 to ≤8	≤8	≤8	0.0
	Metronidazole	≤1 to 2	≤1	≤1	0.0
Bacteroides vulgatus (*n* = 22)	Imipenem	0.25 to 4	0.5	1	0.0
	Imipenem-relebactam	0.12 to 2	0.5	1	0.0
	Piperacillin-tazobactam	1 to 128	4	16	4.5
	Ampicillin-sulbactam	2 to 64	4	16	4.5
	Ertapenem	0.12 to 16	0.5	2	4.5
	Meropenem	0.12 to 16	0.5	2	4.5
	Doripenem	0.25 to 16	0.5	1	4.5
	Cefoxitin	≤2 to 128	8	16	4.5
	Tigecycline	0.12 to 8	1	4	0.0
	Moxifloxacin	≤0.5 to >16	>16	>16	77.3
	Clindamycin	≤0.5 to >16	>16	>16	68.2
	Linezolid	1 to 16	2	4	4.5
	Chloramphenicol	>2 to ≤8	≤8	≤8	0.0
	Metronidazole	≤1 to 2	≤1	≤1	0.0

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TABLE 2 (Continued)

Species tested	Antimicrobial agent	MIC range	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% resistant[a]
Bacteroides cacae ($n = 10$)	Imipenem	≤0.06 to 1	0.25	0.25	0.0
	Imipenem-relebactam	≤0.06 to 1	0.25	0.25	0.0
	Piperacillin-tazobactam	1 to 16	4	8	0.0
	Ampicillin-sulbactam	1 to 32	2	4	10.0
	Ertapenem	0.25 to 1	0.5	1	0.0
	Meropenem	0.12 to 0.5	0.25	0.25	0.0
	Doripenem	0.25 to 2	0.25	0.5	0.0
	Cefoxitin	≤2 to 32	16	32	0.0
	Tigecycline	0.25 to 8	0.5	4	0.0
	Moxifloxacin	1 to >16	4	>16	50.0
	Clindamycin	≤0.5 to >16	2	>16	30.0
	Linezolid	2 to 4	2	4	0.0
	Chloramphenicol	>2 to ≤8	≤8	≤8	0.0
	Metronidazole	≤1 to 2	≤1	≤1	0.0
Other species within group ($n = 7$)[c]	Imipenem	0.12 to 4			0.0
	Imipenem-relebactam	0.25 to 4			0.0
	Piperacillin-tazobactam	2 to 16			0.0
	Ampicillin-sulbactam	2 to 16			0.0
	Ertapenem	0.5 to 2			0.0
	Meropenem	0.25 to 1			0.0
	Doripenem	0.5 to 2			0.0
	Cefoxitin	8 to 64			14.3
	Tigecycline	0.5 to 8			0.0
	Moxifloxacin	1 to 16			28.6
	Clindamycin	1 to >16			42.9
	Linezolid	2 to 4			0.0
	Chloramphenicol	>2 to ≤8			0.0
	Metronidazole	All ≤1			0.0

[a] Percent resistance is calculated using CLSI and FDA-recommended breakpoints (see Table 1).
[b] Chloramphenicol tested only at concentrations of 2 and 8 μg ml.
[c] Three *Parabacteroides goldsteinii*, 2 *Bacteroides eggerthii*, 1 *Bacteroides stercoris*, and 1 *Bacteroides dorei*.

For all *in vitro* testing, the MICs of the control organisms, *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741, were within the range specified by the CLSI and FDA for each agent (8–10).

A comparison of the activities of the antimicrobial agents against all the isolates showed equal MIC_{90}s of 1 μg/ml for imipenem-relebactam and imipenem alone. The resistance rate for both imipenem and imipenem-relebactam was 0.7%.

The lack of enhanced activity of the combination suggests that relebactam does not inhibit the metalloenzyme (*cfiA* gene) produced by *Bacteroides fragilis* group or that other resistance mechanisms, such as a porin mutation, might also be the cause for resistance to imipenem and other carbapenems. We did not specifically examine isolates for resistance mechanisms as part of this study and cannot comment on the proportion of isolates that might have a particular mechanism of resistance. In conclusion, relebactam does not add activity to imipenem against *Bacteroides* species.

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