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In Vitro Activity of Biapenem plus RPX7009, a Carbapenem Combined with a Serine β-Lactamase Inhibitor, against Anaerobic Bacteria

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Biapenem is a carbapenem being developed in combination with RPX7009, a new inhibitor of serine β-lactamases. Biapenem was tested alone and in combination with fixed concentrations of RPX7009 by agar dilution against 377 recent isolates of anaerobes. A separate panel of 27 isolates of *Bacteroides* spp. with decreased susceptibility or resistance to imipenem was also tested. Comparator drugs included meropenem, piperacillin-tazobactam, ampicillin-sulbactam, cefoxitin, ceftazidime, metronidazole, clindamycin, and tigecycline plus imipenem, doripenem, and ertapenem for the 27 selected strains. For recent consecutive strains of *Bacteroides* species, the MIC_{90} for biapenem-RPX7009 was 1 μg/ml, with a MIC_{90} of 4 μg/ml for meropenem. Other *Bacteroides fragilis* group species showed a MIC_{90} of 0.5 μg/ml for both agents. The MIC_{90}s for biapenem-RPX7009 were 0.25 μg/ml for *Prevotella* spp., 0.125 μg/ml for *Fusobacterium nucleatum* and *Fusobacterium necrophorum*, 2 μg/ml for *Fusobacterium mortiferum*, 0.5 μg/ml for *Fusobacterium varium*, ≤0.5 μg/ml for Gram-positive cocci and rods, and 0.03 to 8 μg/ml for clostridia. Against 5 *B. fragilis* strains harboring a known metallo-beta-lactamase, biapenem-RPX7009 MICs were comparable to those of other carbapenems (≥32 μg/ml). Against *Bacteroides* strains with an imipenem MIC of 2 μg/ml, biapenem-RPX7009 had MICs of 0.5 to 2 μg/ml, with MICs of 0.5 to 32 μg/ml for meropenem, doripenem, and ertapenem. For strains with an imipenem MIC of 4 μg/ml, the MICs for biapenem-RPX7009 were 4 to 16 μg/ml, with MICs of 8 to >32 μg/ml for meropenem, doripenem, and ertapenem. The inhibitor RPX7009 had no antimicrobial activity when tested alone, and it showed little or no potentiation of biapenem versus anaerobes. Biapenem-RPX7009 showed activity comparable to that of imipenem and was superior to meropenem, doripenem, and ertapenem against imipenem-nonsusceptible *Bacteroides* spp.

Biapenem is a carbapenem with a broad spectrum of activity against Gram-positive and Gram-negative aerobes and anaerobes and with a methyl group on the 1-beta position which confers stability against dehydropeptidase I (1, 2), and it appears to be more stable to hydrolysis by the CcrA enzyme than imipenem or meropenem (3). Its *in vitro* activity against anaerobes was the subject of a number of studies in the 1990s, around the time of its development (1, 4–10). Biapenem was approved for clinical use in Japan in 2001 and was launched in 2002. It was studied clinically against intra-abdominal infections (11) and was found to be equivalent to imipenem. Scant data have been published regarding biapenem's activity against contemporary anaerobic isolates.

Carbapenem resistance in anaerobic bacteria is rare, although strains of *Bacteroides* spp. with class B zinc metallo-beta-lactamases (*cfiA* or *ccrA*) have been reported (12). Although not all *cfiA*-positive *Bacteroides fragilis* strains are resistant to carbapenems, they all have the possibility of becoming resistant to this group of antibiotics by acquisition of an appropriate insertion (IS) element for full expression of the *cfiA* gene, leading to possible treatment failure (12). RPX7009 is a new serine beta-lactamase inhibitor that has been combined with biapenem to enhance its spectrum against carbapenemase-producing bacteria, including KPC-producing isolates. No prior studies have reported RPX7009's activity against anaerobes or the effect of its potential combination with biapenem. Consequently, we studied the effects of three different fixed concentrations of RPX7009 (2, 4, and 8 μg/ml) on the activity of biapenem against 377 recent isolates of anaerobes. In addition, we studied the activity of biapenem alone and in combination with RPX7009 against 27 isolates of *Bacteroides* spp. with various levels of known resistance to imipenem.

MATERIALS AND METHODS

Bacterial strains. The organisms were recovered from recent clinical samples (2009–2011) from humans, mostly from intra-abdominal sources. Some *Prevotella* species came from respiratory sources, and some Gram-positive cocci came from skin and soft tissue infections. Isolates were identified by standard methods (13, 14) and stored in 20% skim milk at −70°C. They were taken from the freezer and transferred at least twice on blood agar to ensure purity and good growth.

In addition to the recent clinical samples, a separate panel of strains from our collection that exhibited diminished imipenem susceptibility was tested. These strains were as follows: for *B. fragilis*, 10 strains with imipenem MICs of 1 to 4 μg/ml and 5 strains with MICs of >32 μg/ml; for *Parabacteroides*, 7 strains (4 *Parabacteroides distasonis* strains and 3 *Parabacteroides goldsteinii* strains) with imipenem MICs of 1 to 8 μg/ml; 1 strain each of *Bacteroides caccae* (imipenem MIC, 16 μg/ml), *Bacteroides ovatus* (imipenem MIC, 2 μg/ml), and *Bacteroides thetaiotaomicron* (imipenem MIC, 4 μg/ml); and 2 strains of *Bacteroides uniformis* (imipenem MICs, 4 and 8 μg/ml).

Drugs tested. Biapenem and RPX7009 (Rempex Pharmaceuticals, San Diego, CA) were tested alone and in combination. Comparator drugs were meropenem, piperacillin-tazobactam, ampicillin-sulbactam, cefoxitin, ceftazidime, metronidazole, clindamycin, and tigecycline (Sigma, St. Louis, MO, or USP, Rockville, MD) and were reconstituted according to

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the manufacturers' instructions. Additionally, 27 selected strains which showed either diminished imipenem susceptibility or imipenem resistance were also tested against imipenem, doripenem, and ertapenem.

MICs were determined using the agar dilution method according to CLSI guidelines (15). Biapenem was tested at concentrations of 0.03 to 32 μg/ml, RPX7009 at 0.125 to 128 μg/ml, and comparator agents at various concentrations from 0.03 to 128 μg/ml. Biapenem was tested alone and in combination with a fixed concentration of RPX7009 (2, 4, or 8 μg/ml). Quality control strains included *B. fragilis* ATCC 25285, *Clostridium difficile* ATCC 700057, *Klebsiella pneumonia* BAA-1705 (a KPC-positive strain), and *Escherichia coli* ATCC 25922. The MIC was defined as the lowest concentration that yielded no visible growth or a major reduction in growth compared to the growth control.

RESULTS

Biapenem alone and in combination with RPX7009 showed excellent activity that was comparable to that of meropenem (Table 1). RPX7009 had no inhibitory activity against any strain tested, with all MICs being >128 μg/ml. The MIC_{90}s for Gram-positive cocci and rods were ≤0.5 μg/ml. *Finegoldia magna*, *Parvimonas micra*, and *Peptoniphilus harei* isolates had a maximum biapenem and biapenem-RPX7009 MIC of 0.5 μg/ml, while 10 strains of *Peptostreptococcus anaerobius* had MIC_{50}s of 0.5 μg/ml but MIC_{90}s of 8 and 16 μg/ml for biapenem alone and biapenem-RPX 7009 (at 2 μg/ml), respectively. The MIC_{90} for *Clostridium perfringens* isolates was 0.06 μg/ml, and that for *C. difficile* was 8 μg/ml. For other *Clostridium* species, the MIC range was 0.125 to 4 μg/ml, depending on the species.

For 36 recent isolates of *B. fragilis*, the MIC_{90} was 1 μg/ml for biapenem and 0.5 μg/ml for biapenem-RPX7009, both of which were more active than meropenem (MIC_{90}, 4 μg/ml). For *B. caccae* and *B. thetaiotaomicron*, the MIC_{90} for biapenem and biapenem-RPX7009 was 0.5 μg/ml. Other *B. fragilis* group species, including *B. ovatus* and *Bacteroides vulgatus*, showed a MIC_{90} of 1 μg/ml for both biapenem-RPX7009 and meropenem.

The MIC_{90} for biapenem-RPX7009 for *Prevotella* spp. was 0.25 μg/ml, and that for *Fusobacterium nucleatum* and *Fusobacterium necrophorum* was 0.125 μg/ml. Biapenem-RPX7009 activity was lower against *Fusobacterium mortiferum* and *Fusobacterium varium*, with MIC_{90}s of 2 and 0.5 μg/ml, respectively. Most *Bilophila* strains were resistant to all beta-lactams, including biapenem-RPX7009 and control carbapenems.

Regarding the comparator agents, clindamycin resistance was found in 17% of *B. fragilis* isolates and 72% of *B. thetaiotaomicron* isolates. Resistance in other *Bacteroides* and *Parabacteroides* species ranged from 26% to 42%. Tigecycline resistance was found in 8.3% of *B. fragilis* isolates and 33.3% of *B. thetaiotaomicron* isolates. Ampicillin-sulbactam resistance varied from 3% for *B. caccae* to 11% for *B. ovatus* and *B. thetaiotaomicron*, 17% for *B. fragilis*, and 50% for other *B. fragilis* group species. Comparatively, there was very little piperacillin-tazobactam resistance among all the *B. fragilis* group species, with only 2.7% resistance in *B. fragilis* itself.

In a subset of clinical isolates of *B. fragilis* and *Parabacteroides* spp. with meropenem MICs of >2 μg/ml (range, 4 to 32 μg/ml), the MIC_{50}s/MIC_{90}s of biapenem and biapenem-RPX7009 were 2/8 and 2/8 μg/ml, respectively, for *B. fragilis* isolates and 2/2 and 2/2 μg/ml, respectively, for *Parabacteroides* isolates. The MIC results for the 27 selected *B. fragilis* group strains previously demonstrated to have diminished imipenem susceptibility are shown in Table 2. Against the five *B. fragilis* strains harboring a metallo-beta-lactamase, biapenem-RPX7009 MICs were comparable to those of the three comparator carbapenems (MICs of ≥32 μg/ml), and the strains also showed resistance to piperacillin-tazobactam, ampicillin-sulbactam, and cefoxitin. Against *Bacteroides* strains with an intermediate imipenem MIC of 2 μg/ml, biapenem-RPX7009 had MICs ranging from 0.5 to 2 μg/ml, while MICs for meropenem, doripenem, and ertapenem were 0.5 to 32 μg/ml. For strains with an imipenem MIC of 4 μg/ml, biapenem-RPX7009 had MICs of 4 to 16 μg/ml, while meropenem, doripenem, and ertapenem had 2- to 3-fold higher MICs of 8 to >32 μg/ml. One strain of *P. distasonis* was unusual, with a much higher MIC than that of the other *Parabacteroides* isolates.

DISCUSSION

A number of studies from the 1990s established the activity of biapenem against a variety of anaerobic bacteria (1, 4–10). Using an agar dilution method with Wilkins-Chalgren agar supplemented with 5% horse blood, Catchpole et al. (1) studied 23 *B. fragilis* strains and found a MIC_{90} of 2 μg/ml (range, 0.25 to 4 μg/ml). One year later, Malanoski et al. (8) used unsupplemented Wilkins-Chalgren agar and reported a MIC_{90} of 0.5 μg/ml (range, 0.25 to 1 μg/ml) against 20 *B fragilis* strains. In more systematic studies, Aldridge et al. (4) used a broth microdilution method and studied 339 *B. fragilis* group isolates, including 176 strains of *B. fragilis*, and they found a MIC_{90} of 0.25 μg/ml (range, 0.12 to 2 μg/ml) for both groups. Since then, no English-language reports on biapenem's anaerobic activity have been published. Our results, obtained with contemporary isolates, show that biapenem has maintained an excellent activity against anaerobes that is generally equivalent to that of the other carbapenems studied.

Comparatively, clindamycin, cefoxitin, and tigecycline resistance was common in *B. thetaiotaomicron* isolates. For clindamycin, 72% of our *B. thetaiotaomicron* isolates were resistant, compared to 17% of *B. fragilis* strains, while resistance in other *Bacteroides* and *Parabacteroides* species ranged from 26% to 42%. A recent study from Canada (16) reported that 34% of 232 *B. fragilis* isolates and 78% of 49 *B. thetaiotaomicron* isolates, collected between 2010 and 2011, were resistant to clindamycin. Snydman et al. (17) studied 1,021 *B. fragilis* isolates collected between 2006 and 2009 from eight geographically distributed U.S. hospitals and found 30% resistance to clindamycin, with 43% resistance for 418 *B. thetaiotaomicron* isolates. Karlowsky et al. (16) also noted that 14% of *B. fragilis* isolates and 31% of *B. thetaiotaomicron* isolates were resistant to tigecycline, compared to 5% and 2%, respectively, in the work of Snydman et al. (17). In contrast, we found tigecycline resistance in 8.3% of *B. fragilis* isolates and 33.3% of *B. thetaiotaomicron* isolates. Ampicillin-sulbactam resistance varied by species of the *B. fragilis* group, from 3% for *B. caccae* to 11% for *B. ovatus* and *B. thetaiotaomicron*, 17% for *B. fragilis*, and 50% for other *B. fragilis* group species. We found very little piperacillin-tazobactam resistance among all the *B. fragilis* group species, with only 2.7% resistance in *B. fragilis* itself. Similarly, both Karlowsky et al. (16) and Snydman et al. (17) found 1% piperacillin-tazobactam resistance in *B. fragilis* isolates, with <1% resistance for *B. thetaiotaomicron*.

Biapenem and biapenem-RPX7009 showed excellent activity against clinical isolates of Gram-positive and Gram-negative anaerobes that was comparable to that of meropenem. RPX7009 showed no antimicrobial activity when tested alone, and overall,

TABLE 1 Comparative *in vitro* activities of biapenem, alone and in combination with RPX7009, and other antimicrobial agents against anaerobic bacteria

Organism (no. of strains) and antimicrobial agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% I or R[a]
Gram-negative organisms				
Bacteroides fragilis (36)				
Biapenem	0.125–>32	0.25	1	2.7
Biapenem plus RPX7009 (2 μg/ml)	0.125–>32	0.25	0.5	
Biapenem plus RPX7009 (4 μg/ml)	0.125–>32	0.25	0.5	
Biapenem plus RPX7009 (8 μg/ml)	0.125–>32	0.25	0.5	
Meropenem	0.125–>32	0.25	4	5.5
Piperacillin-tazobactam	0.125–>128	0.5	4	2.7
Ampicillin-sulbactam	0.5–>128	1	16	16.7
Cefoxitin	4–128	8	32	13.9
Ceftazidime	16–>128	64	>128	NA
Metronidazole	0.5–2	1	2	0
Clindamycin	≤0.06–>128	0.5	>128	16.7
Tigecycline	0.25–16	0.5	4	8.3
RPX7009	>128–>128	>128	>128	NA
B. caccae (19)				
Biapenem	0.125–1	0.25	0.5	0
Biapenem plus RPX7009 (2 μg/ml)	0.125–1	0.25	0.5	
Biapenem plus RPX7009 (4 μg/ml)	0.125–1	0.25	0.5	
Biapenem plus RPX7009 (8 μg/ml)	0.125–1	0.25	0.5	
Meropenem	0.06–2	0.25	1	0
Piperacillin-tazobactam	≤0.06–16	4	8	0
Ampicillin-sulbactam	0.5–16	2	8	2.7
Cefoxitin	4–128	16	32	26.3
Ceftazidime	32–>128	128	>128	NA
Metronidazole	0.5–4	2	2	0
Clindamycin	0.5–>128	4	>128	52.6
Tigecycline	0.125–32	4	16	42
RPX7009	>128–>128	>128	>128	NA
B. ovatus (19)				
Biapenem	0.25–1	0.5	1	0
Biapenem plus RPX7009 (2 μg/ml)	0.25–1	0.5	1	
Biapenem plus RPX7009 (4 μg/ml)	0.25–1	0.5	0.06	
Biapenem plus RPX7009 (8 μg/ml)	0.25–1	0.5	0.06	
Meropenem	0.25–2	0.25	1	0
Piperacillin-tazobactam	2–16	4	16	0
Ampicillin-sulbactam	0.5–16	2	16	10.5
Cefoxitin	16–128	32	64	52.6
Ceftazidime	128–>128	>128	>128	NA
Metronidazole	0.5–2	1	2	0
Clindamycin	0.5–>128	2	>128	42.1
Tigecycline	≤0.06–16	1	8	10.5
RPX7009	>128–>128	>128	>128	NA
B. thetaiotaomicron (18)				
Biapenem	0.25–0.5	0.25	0.5	0
Biapenem plus RPX7009 (2 μg/ml)	0.25–0.5	0.25	0.5	
Biapenem plus RPX7009 (4 μg/ml)	0.25–0.5	0.25	0.5	
Biapenem plus RPX7009 (8 μg/ml)	0.25–0.5	0.25	0.5	
Meropenem	0.125–1	0.25	0.5	0
Piperacillin-tazobactam	4–32	16	16	0
Ampicillin-sulbactam	1–16	2	16	11.1
Cefoxitin	16–64	32	64	66.7
Ceftazidime	>128–>128	>128	>128	NA
Metronidazole	0.25–2	2	2	0
Clindamycin	2–129	4	>128	72.2
Tigecycline	0.125–32	4	32	33.3
RPX7009	>128–>128	>128	>128	NA
B. vulgatus (10)				
Biapenem	0.125–1	0.5	1	0
Biapenem plus RPX7009 (2 μg/ml)	0.125–1	0.25	1	

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TABLE 1 (Continued)

Organism (no. of strains) and antimicrobial agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% I or R[a]
Biapenem plus RPX7009 (4 μg/ml)	0.125–1	0.5	1	
Biapenem plus RPX7009 (8 μg/ml)	0.125–1	0.25	1	
Meropenem	0.125–1	0.5	1	0
Piperacillin-tazobactam	0.25–8	1	4	0
Ampicillin-sulbactam	1–16	8	8	10
Cefoxitin	4–128	16	16	10
Ceftazidime	32–>128	128	>128	NA
Metronidazole	0.5–1	1	1	0
Clindamycin	≤0.06–>128	0.25	>128	40
Tigecycline	0.125–8	0.5	8	20
RPX7009	>128–>128	>128	>128	NA
Other *Bacteroides* spp. (20)[b]				
Biapenem	0.25–2	0.5	1	0
Biapenem plus RPX7009 (2 μg/ml)	0.25–2	0.25	1	
Biapenem plus RPX7009 (4 μg/ml)	0.25–1	0.05	1	
Biapenem plus RPX7009 (8 μg/ml)	0.25–2	0.5	1	
Meropenem	0.125–32	0.5	1	5
Piperacillin-tazobactam	≤0.06–16	2	8	0
Ampicillin-sulbactam	1–32	4	16	50
Cefoxitin	4–64	16	64	25
Ceftazidime	16–>128	>128	>128	NA
Metronidazole	0.5–2	2	2	0
Clindamycin	≤0.06–>128	2	>128	35
Tigecycline	0.125–32	0.5	8	25
RPX7009	>128–>128	>128	>128	NA
Parabacteroides spp. (23)[c]				
Biapenem	0.25–2	1	2	0
Biapenem plus RPX7009 (2 μg/ml)	0.25–2	1	2	
Biapenem plus RPX7009 (4 μg/ml)	0.25–2	1	2	
Biapenem plus RPX7009 (8 μg/ml)	0.25–2	1	2	
Meropenem	0.25–16	1	8	21.7
Piperacillin-tazobactam	2–>128	4	16	4.3
Ampicillin-sulbactam	1–64	8	32	21.7
Cefoxitin	8–128	32	64	39.1
Ceftazidime	16–>128	>128	>128	NA
Metronidazole	0.5–2	1	2	0
Clindamycin	≤0.06–>128	4	>128	39.1
Tigecycline	0.5–8	1	8	8.7
RPX7009	>128–>128	>128	>128	NA
Prevotella bivia (10)				
Biapenem	0.125–0.25	0.25	0.25	0
Biapenem plus RPX7009 (2 μg/ml)	0.125–0.25	0.25	0.25	
Biapenem plus RPX7009 (4 μg/ml)	0.125–0.25	0.25	0.25	
Biapenem plus RPX7009 (8 μg/ml)	0.06–0.25	0.125	0.25	
Meropenem	0.06–0.25	0.125	0.125	0
Piperacillin-tazobactam	≤0.06	≤0.06	≤0.06	0
Ampicillin-sulbactam	0.125–4	2	4	0
Cefoxitin	2–4	2	4	0
Ceftazidime	2–>128	64	>128	NA
Metronidazole	1–4	2	4	0
Clindamycin	≤0.06–>128	>128	>128	70
Tigecycline	≤0.06–0.125	≤0.06	≤0.06	0
RPX7009	>128–>128	>128	>128	NA
Prevotella buccae (10)				
Biapenem	0.06–0.25	0.125	0.25	0
Biapenem plus RPX7009 (2 μg/ml)	0.06–0.25	0.125	0.25	
Biapenem plus RPX7009 (4 μg/ml)	0.06–0.25	0.06	0.25	
Biapenem plus RPX7009 (8 μg/ml)	0.06–0.25	0.06	0.25	
Meropenem	≤0.03–0.5	0.06	0.25	0
Piperacillin-tazobactam	≤0.06–4	≤0.06	2	0
Ampicillin-sulbactam	0.125–4	0.125	2	0

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TABLE 1 (Continued)

Organism (no. of strains) and antimicrobial agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% I or R[a]
Cefoxitin	1–16	1	4	0
Ceftazidime	2–>128	32	128	NA
Metronidazole	0.25–1	1	1	0
Clindamycin	≤0.06–>128	≤0.06	>128	20
Tigecycline	≤0.06–4	0.125	0.5	0
RPX7009	>128–>128	>128	>128	NA
Prevotella melaninogenica (10)				
Biapenem	≤0.03–0.25	0.06	0.125	0
Biapenem plus RPX7009 (2 μg/ml)	≤0.03–0.25	0.125	0.125	
Biapenem plus RPX7009 (4 μg/ml)	≤0.03–0.25	0.06	0.25	
Biapenem plus RPX7009 (8 μg/ml)	≤0.03–0.25	0.06	0.125	
Meropenem	≤0.03–0.25	0.06	0.25	0
Piperacillin-tazobactam	≤0.06	≤0.06	≤0.06	0
Ampicillin-sulbactam	0.125–4	1	1	0
Cefoxitin	1–8	2	4	0
Ceftazidime	1–64	16	32	NA
Metronidazole	0.5–2	1	2	0
Clindamycin	≤0.06–>128	≤0.06	>128	40
Tigecycline	≤0.06	≤0.06	≤0.06	0
RPX7009	>128–>128	>128	>128	NA
Prevotella oralis (10)				
Biapenem	≤0.03–0.25	≤0.03	0.125	
Biapenem plus RPX7009 (2 μg/ml)	≤0.03–0.25	≤0.03	0.125	
Biapenem plus RPX7009 (4 μg/ml)	≤0.03–0.25	≤0.03	0.125	
Biapenem plus RPX7009 (8 μg/ml)	≤0.03–0.25	≤0.03	0.125	
Meropenem	≤0.03–0.25	≤0.03	0.06	0
Piperacillin-tazobactam	≤0.06	≤0.06	≤0.06	0
Ampicillin-sulbactam	≤0.06–1	≤0.06	0.125	0
Cefoxitin	0.25–2	2	2	0
Ceftazidime	0.5–>128	4	8	NA
Metronidazole	0.06–1	0.125	0.5	0
Clindamycin	≤0.06–>128	≤0.06	>128	30
Tigecycline	≤0.06	≤0.06	≤0.06	0
RPX7009	>128–>128	>128	>128	NA
Fusobacterium necrophorum (10)				
Biapenem	≤0.03–0.125	0.06	0.125	
Biapenem plus RPX7009 (2 μg/ml)	≤0.03–0.125	0.125	0.125	
Biapenem plus RPX7009 (4 μg/ml)	≤0.03–0.125	0.125	0.125	
Biapenem plus RPX7009 (8 μg/ml)	≤0.03–0.125	0.06	0.125	
Meropenem	≤0.03	≤0.03	≤0.03	0
Piperacillin-tazobactam	≤0.06	≤0.06	≤0.06	0
Ampicillin-sulbactam	≤0.06–0.25	0.125	0.125	0
Cefoxitin	025–0.5	0.5	0.5	0
Ceftazidime	0.5–1	1	1	NA
Metronidazole	0.25–0.5	0.25	0.5	0
Clindamycin	≤0.06	≤0.06	≤0.06	0
Tigecycline	≤0.06	≤0.06	≤0.06	0
RPX7009	>128–>128	>128	>128	NA
Fusobacterium nucleatum (11)				
Biapenem	≤0.03–0.125	0.06	0.125	
Biapenem plus RPX7009 (2 μg/ml)	≤0.03–0.125	0.06	0.125	
Biapenem plus RPX7009 (4 μg/ml)	≤0.03–0.125	0.06	0.125	
Biapenem plus RPX7009 (8 μg/ml)	≤0.03–0.125	≤0.03	0.125	
Meropenem	≤0.03–0.06	≤0.03	0.06	
Piperacillin-tazobactam	≤0.06	≤0.06	0.06	0
Ampicillin-sulbactam	≤0.06–0.125	≤0.06	0.125	0
Cefoxitin	0.125–1	0.25	0.5	0
Ceftazidime	1–8	4	4	NA
Metronidazole	≤0.03–0.25	0.06	0.25	0
Clindamycin	≤0.06–0.125	0.06	0.125	0
Tigecycline	≤0.06–0.25	≤0.06	0.25	0
RPX7009	>128–>128	>128	>128	NA

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TABLE 1 (Continued)

Organism (no. of strains) and antimicrobial agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% I or R[a]
Fusobacterium mortiferum (10)				
Biapenem	1–4	2	2	0
Biapenem plus RPX7009 (2 μg/ml)	1–4	1	1	
Biapenem plus RPX7009 (4 μg/ml)	1–2	1	1	
Biapenem plus RPX7009 (8 μg/ml)	1–4	1	1	
Meropenem	0.25–0.5	0.5	0.5	0
Piperacillin-tazobactam	0.25–4	1	1	0
Ampicillin-sulbactam	1–8	1	8	0
Cefoxitin	4–8	8	8	0
Ceftazidime	1,280–>128	>128	>128	NA
Metronidazole	0.5–2	0.5	1	0
Clindamycin	≤0.06–0.125	≤0.06	0.125	0
Tigecycline	0.125–0.5	0.25	0.5	0
RPX7009	>128–>128	>128	>128	NA
Fusobacterium varium (11)				
Biapenem	≤0.03–2	0.25	0.5	0
Biapenem plus RPX7009 (2 μg/ml)	≤0.03–2	0.25	0.25	
Biapenem plus RPX7009 (4 μg/ml)	≤0.03–2	0.25	0.5	
Biapenem plus RPX7009 (8 μg/ml)	≤0.03–2	0.25	0.5	
Meropenem	≤0.03–0.25	0.125	0.125	0
Piperacillin-tazobactam	≤0.06–4	2	4	0
Ampicillin-sulbactam	0.125–4	1	2	0
Cefoxitin	0.25–8	4	8	0
Ceftazidime	1–32	8	16	NA
Metronidazole	0.25–1	0.5	1	0
Clindamycin	≤0.06–32	4	8	63.6
Tigecycline	0.125–0.25	0.25	0.25	0
RPX7009	>128–>128	>128	>128	NA
Porphyromonas species (10)[d]				
Biapenem	≤0.03–0.06	0.06	0.06	0
Biapenem plus RPX7009 (2 μg/ml)	≤0.03–0.125	0.06	0.06	
Biapenem plus RPX7009 (4 μg/ml)	≤0.03–0.25	≤0.03	0.06	
Biapenem plus RPX7009 (8 μg/ml)	≤0.03–0.125	≤0.03	0.06	
Meropenem	≤0.03–0.125	≤0.03	≤0.03	
Piperacillin-tazobactam	≤0.06	≤0.06	≤0.06	0
Ampicillin-sulbactam	≤0.06–2	≤0.06	1	0
Cefoxitin	0.25–2	0.5	1	0
Ceftazidime	0.25–32	0.5	8	NA
Metronidazole	0.125–2	0.25	1	0
Clindamycin	≤0.06–>128	≤0.06	1	10
Tigecycline	≤0.06–0.5	≤0.06	≤0.06	0
RPX7009	>128–>128	>128	>128	NA
Veillonella species (10)				
Biapenem	≤0.03–1	0.5	1	0
Biapenem plus RPX7009 (2 μg/ml)	0.06–4	0.5	4	
Biapenem plus RPX7009 (4 μg/ml)	≤0.03–4	0.125	1	
Biapenem plus RPX7009 (8 μg/ml)	≤0.03–2	0.25	2	
Meropenem	≤0.03–0.25	≤0.03	≤0.03	0
Piperacillin-tazobactam	0.25–4	2	4	0
Ampicillin-sulbactam	≤0.06–2	0.5	1	0
Cefoxitin	0.25–8	4	4	0
Ceftazidime	2–32	8	32	NA
Metronidazole	0.25–8	2	4	0
Clindamycin	≤0.06–0.125	≤0.06	≤0.06	0
Tigecycline	≤0.06–0.5	0.25	0.5	0
RPX7009	>128–>128	>128	>128	NA
Bilophila wadsworthia (10)				
Biapenem	1–>32	32	>32	90
Biapenem plus RPX7009 (2 μg/ml)	0.5–>32	32	>32	
Biapenem plus RPX7009 (4 μg/ml)	2–>32	32	>32	
Biapenem plus RPX7009 (8 μg/ml)	1–>32	32	>32	

(Continued on following page)

TABLE 1 (Continued)

Organism (no. of strains) and antimicrobial agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% I or R[a]
Meropenem	0.06–>32	32	>32	60
Piperacillin-tazobactam	16–>128	32	>128	40
Ampicillin-sulbactam	8–>128	5	>128	50
Cefoxitin	64–>128	128	>128	100
Ceftazidime	32–>128	128	>128	NA
Metronidazole	0.06–0.5	0.125	0.25	0
Clindamycin	0.5–>128	0.5	2	30
Tigecycline	0.5–1	0.5	0.5	0
RPX7009	>128–>128	>128	>128	NA
Gram-positive organisms				
Finegoldia magna (10)				
Biapenem	0.125–0.25	0.125	0.125	0
Biapenem plus RPX7009 (2 μg/ml)	0.25–0.25	0.25	0.25	
Biapenem plus RPX7009 (4 μg/ml)	0.125–0.25	0.125	0.125	
Biapenem plus RPX7009 (8 μg/ml)	0.125–0.25	0.125	0.125	
Meropenem	0.06–0.125	0.06	0.125	0
Piperacillin-tazobactam	0.125–0.5	0.125	0.25	0
Ampicillin-sulbactam	0.25–0.5	0.25	0.25	0
Cefoxitin	0.5–2	1	1	0
Ceftazidime	16–64	32	64	NA
Metronidazole	0.25–1	0.5	1	0
Clindamycin	0.125–>128	0.5	>128	30
Tigecycline	0.125–0.5	0.25	0.25	0
RPX7009	>128–>128	>128	>128	NA
Parvimonas micra (10)				
Biapenem	≤0.03–0.5	0.125	0.5	0
Biapenem plus RPX7009 (2 μg/ml)	0.06–0.5	0.125	0.25	
Biapenem plus RPX7009 (4 μg/ml)	0.06–0.25	0.125	0.25	
Biapenem plus RPX7009 (8 μg/ml)	≤0.03–0.5	0.125	0.5	
Meropenem	≤0.03–0.25	0.06	0.25	0
Piperacillin-tazobactam	≤0.06–0.125	≤0.06	0.125	0
Ampicillin-sulbactam	≤0.06–0.5	0.125	0.5	0
Cefoxitin	0.25–4	1	4	0
Ceftazidime	0.5–32	2	4	NA
Metronidazole	0.06–0.5	0.25	0.5	0
Clindamycin	≤0.06–32	0.25	0.5	10
Tigecycline	≤0.06–0.125	≤0.06	≤0.06	0
RPX7009	>128–>128	>128	>128	NA
Peptoniphilus harei (10)				
Biapenem	<0.03–0.125	≤0.03	0.125	0
Biapenem plus RPX7009 (2 μg/ml)	<0.03–0.125	≤0.03	0.125	
Biapenem plus RPX7009 (4 μg/ml)	<0.03–0.125	≤0.03	0.125	
Biapenem plus RPX7009 (8 μg/ml)	<0.03–0.125	≤0.03	0.125	
Meropenem	≤0.03	≤0.03	≤0.03	0
Piperacillin-tazobactam	≤0.06	≤0.06	≤0.06	0
Ampicillin-sulbactam	≤0.06–0.25	0.125	0.25	0
Cefoxitin	0.125–0.5	0.25	0.5	0
Ceftazidime	0.5–1	1	1	NA
Metronidazole	0.125–2	0.5	2	0
Clindamycin	0.125–>128	1	>128	20
Tigecycline	≤0.06–0.125	≤0.06	0.125	0
RPX7009	>128–>128	>128	>128	NA
Peptostreptococcus anaerobius (10)				
Biapenem	0.25–8	0.5	8	20
Biapenem plus RPX7009 (2 μg/ml)	0.25–16	0.5	16	
Biapenem plus RPX7009 (4 μg/ml)	0.25–8	0.5	8	
Biapenem plus RPX7009 (8 μg/ml)	0.25–8	0.5	8	
Meropenem	0.25–8	0.5	4	10
Piperacillin-tazobactam	0.25–16	0.25	16	
Ampicillin-sulbactam	0.125–16	0.25	16	20
Cefoxitin	0.5–16	1	16	

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TABLE 1 (Continued)

Organism (no. of strains) and antimicrobial agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% I or R[a]
Ceftazidime	2–64	8	64	NA
Metronidazole	0.5–1	0.5	1	0
Clindamycin	≤0.06–0.25	0.125	0.25	0
Tigecycline	≤0.06	≤0.06	≤0.06	0
RPX7009	>128–>128	>128	>128	NA
Clostridium difficile (10)				
Biapenem	4–16	8	8	70
Biapenem plus RPX7009 (2 μg/ml)	4–16	8	8	
Biapenem plus RPX7009 (4 μg/ml)	4–16	8	16	
Biapenem plus RPX7009 (8 μg/ml)	4–16	8	8	
Meropenem	1–4	2	4	0
Piperacillin-tazobactam	8–16	8	16	0
Ampicillin-sulbactam	2–4	2	4	0
Cefoxitin	128–>128	128	>128	100
Ceftazidime	>128–>128	>128	>128	100
Metronidazole	0.5–2	1	2	0
Clindamycin	8–>128	8	>128	100
Tigecycline	≤0.06–0.125	0.125	0.125	0
RPX7009	>128–>128	>128	>128	NA
Clostridium clostridioforme group (10)[e]				
Biapenem	0.25–4	1	4	0
Biapenem plus RPX7009 (2 μg/ml)	0.25–4	1	4	
Biapenem plus RPX7009 (4 μg/ml)	0.25–4	1	4	
Biapenem plus RPX7009 (8 μg/ml)	0.25–4	1	4	
Meropenem	0.25–4	0.5	2	0
Piperacillin-tazobactam	≤0.06–16	4	8	0
Ampicillin-sulbactam	0.5–2	1	2	0
Cefoxitin	4–16	16	16	0
Ceftazidime	16–>128	32	>128	NA
Metronidazole	0.06–0.5	0.06	0.25	0
Clindamycin	≤0.06–>128	0.5	32	20
Tigecycline	≤0.06–0.125	≤0.06	≤0.06	0
RPX7009	>128–>128	>128	>128	NA
Clostridium innocuum (10)				
Biapenem	2–4	2	4	0
Biapenem plus RPX7009 (2 μg/ml)	2–4	2	4	
Biapenem plus RPX7009 (4 μg/ml)	2–4	2	4	
Biapenem plus RPX7009 (8 μg/ml)	2–4	2	4	
Meropenem	1–2	1	2	0
Piperacillin-tazobactam	1–4	2	2	0
Ampicillin-sulbactam	0.25–0.5	0.25	0.5	0
Cefoxitin	32–128	64	128	100
Ceftazidime	64–>128	128	128	NA
Metronidazole	0.5–16	1	4	10
Clindamycin	0.25–>128	1	2	10
Tigecycline	≤0.06	≤0.06	≤0.06	0
RPX7009	>128–>128	>128	>128	NA
Clostridium perfringens (10)				
Biapenem	≤0.03–0.125	0.06	0.06	0
Biapenem plus RPX7009 (2 μg/ml)	≤0.03–0.125	0.06	0.06	
Biapenem plus RPX7009 (4 μg/ml)	≤0.03–0.125	0.06	0.06	
Biapenem plus RPX7009 (8 μg/ml)	≤0.03–0.125	0.06	0.06	
Meropenem	≤0.03	≤0.03	≤0.03	0
Piperacillin-tazobactam	≤0.06–1	0.125	1	0
Ampicillin-sulbactam	≤0.060–0.5	0.125	0.25	0
Cefoxitin	1–2	2	2	0
Ceftazidime	4–8	8	8	NA
Metronidazole	1–2	1	2	0
Clindamycin	≤0.06–16	1	2	10
Tigecycline	0.125–4	4	4	0
RPX7009	>128–>128	>128	>128	NA

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TABLE 1 (Continued)

Organism (no. of strains) and antimicrobial agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% I or R[a]
Clostridium ramosum (10)				
Biapenem	1–2	1	2	0
Biapenem plus RPX7009 (2 μg/ml)	1–2	1	1	
Biapenem plus RPX7009 (4 μg/ml)	1–2	1	2	
Biapenem plus RPX7009 (8 μg/ml)	1–2	1	1	
Meropenem	0.5–1	1	1	0
Piperacillin-tazobactam	≤0.06–0.125	≤0.06	0.125	0
Ampicillin-sulbactam	≤0.06–0.5	0.125	0.25	0
Cefoxitin	4–16	8	8	0
Ceftazidime	4–8	8	8	NA
Metronidazole	0.5–16	1	1	10
Clindamycin	2–4	4	4	90
Tigecycline	0.125–0.25	0.125	0.25	0
RPX7009	>128–>128	>128	>128	NA
Clostridium species (10)[f]				
Biapenem	0.125–1	0.25	1	0
Biapenem plus RPX7009 (2 μg/ml)	0.125–1	0.25	1	
Biapenem plus RPX7009 (4 μg/ml)	0.125–1	0.25	1	
Biapenem plus RPX7009 (8 μg/ml)	0.125–1	0.25	1	
Meropenem	0.06–0.5	0.125	0.5	0
Piperacillin-tazobactam	0.125–2	0.5	2	0
Ampicillin-sulbactam	≤0.06–1	0.25	1	0
Cefoxitin	0.25–8	4	8	0
Ceftazidime	0.5–>128	8	>128	NA
Metronidazole	0.125–2	0.5	1	0
Clindamycin	≤0.06–8	1	8	20
Tigecycline	≤0.06–0.5	≤0.06	0.25	0
RPX7009	>128–>128	>128	>128	NA
Eggerthella lenta (10)				
Biapenem	0.5–0.5	0.5	0.5	0
Biapenem plus RPX7009 (2 μg/ml)	0.5–0.5	0.5	0.5	
Biapenem plus RPX7009 (4 μg/ml)	0.5–0.5	0.5	0.5	
Biapenem plus RPX7009 (8 μg/ml)	0.5–0.5	0.5	0.5	
Meropenem	1–2	1	2	0
Piperacillin-tazobactam	16–64	32	32	10
Ampicillin-sulbactam	2–4	2	2	0
Cefoxitin	8–16	8	8	0
Ceftazidime	>128–>128	>128	>128	NA
Metronidazole	0.5–1	0.5	0.5	0
Clindamycin	0.25–0.5	0.25	0.25	0
Tigecycline	0.5–0.5	0.5	0.5	
RPX7009	>128–>128	>128	>128	NA
Non-spore-forming Gram-positive rods (10)[g]				
Biapenem	0.06–0.5	0.06	0.5	0
Biapenem plus RPX7009 (2 μg/ml)	0.06–0.5	0.06	0.5	
Biapenem plus RPX7009 (4 μg/ml)	0.06–0.5	0.125	0.5	
Biapenem plus RPX7009 (8 μg/ml)	0.06–0.5	0.06	0.5	
Meropenem	≤0.03–0.25	0.06	0.25	0
Piperacillin-tazobactam	≤0.06–1	≤0.06	0.25	0
Ampicillin-sulbactam	≤0.06–0.5	0.25	0.5	0
Cefoxitin	0.125–16	2	8	0
Ceftazidime	0.25–32	8	32	NA
Metronidazole	0.25–>32	1	2	10
Clindamycin	≤0.06–>128	≤0.06	2	10
Tigecycline	0.125–0.5	0.125	0.5	0
RPX7009	>128–>128	>128	>128	

[a] I, intermediate; R, resistant; NA, not available.
[b] *Bacteroides uniformis* (8), *Bacteroides cellulosilyticus* (3), *Bacteroides dorei* (2), *Bacteroides nordii* (2), *Bacteroides salyersae* (1), *Bacteroides stercoris* (3), and *Bacteroides xylanisolvens.*
[c] *Parabacteroides distasonis* (6), *Parabacteroides goldsteinii* (9), and *Parabacteroides merdae* (8).
[d] *Porphyromonas asaccharolytica* (5), *Porphyromonas gingivalis* (2), *Porphyromonas somerae* (2), and *Porphyromonas uenonis* (1).
[e] *Clostridium bolteae* (2), *Clostridium citroniae* (1), *Clostridium hathewayi* (1), and *Clostridium clostridioforme* (6).
[f] *Clostridium butyricum* (3), *Clostridium paraputrificum* (2), *Clostridium scindens* (1), *Clostridium sordellii* (2), and *Clostridium symbiosum* (3).
[g] *Collinsella aerofaciens* (1), *Eubacterium limosum* (2), *Pseudoramibacter alactolyticum* (2), *Slackia exigua* (2), *Solobacterium moorei* (1), and *Varibaculum cambriense* (1).

TABLE 2 Comparison of carbapenem and other beta-lactam activities against *Bacteroides* and *Parabacteroides* species with resistance or reduced susceptibility to imipenem[a]

Mechanism and organism	RMA strain no.	MIC(μg/ml)								
		BPM	BPM-RPX7009	MRP	IMI	ERT	DOR	P-T	A-S	FOX
Metalloenzymes										
B. fragilis	5 strains	>32	>32	>32	>32	>32	>32	>128	>128	128
Reduced susceptibility to imipenem										
B. fragilis	9565	2	2	8	1	8	16	1	16	32
	10155	1	1	8	2	16	16	1	16	32
	13778	0.5	0.5	0.5	2	1	0.5	1	32	8
	15507	0.5	0.25	0.25	2	0.5	0.5	1	32	8
	18818	1	1	4	2	4	8	1	16	32
	20361	2	4	16	2	16	32	16	32	32
	16214	8	8	32	4	32	>32	32	64	32
	18473	8	4	8	4	16	8	32	32	>128
	20772	4	4	32	4	32	32	32	64	64
	21925	16	16	16	4	16	16	0.25	8	128
Other *Bacteroides* species with reduced susceptibility to imipenem										
B. caccae	9815	1	2	1	16	2	1	>128	128	64
B. ovatus	14124	2	2	4	2	8	4	8	16	>128
B. thetaiotaomicron	14510	0.5	0.5	0.5	4	2	0.5	64	32	64
B. uniformis	14942	0.5	0.5	0.5	4	2	1	1	32	16
	9853	1	1	1	8	4	1	>128	128	32
Parabacteroides species with reduced susceptibility to imipenem										
P. distasonis	10265	1	1	1	1	4	2	8	32	64
	11017	1	1	0.5	4	1	1	32	32	16
	19958	16	16	32	8	>32	32	8	16	16
	9803	1	1	1	8	4	1	>128	128	64
P. goldsteinii	14939	1	2	8	2	8	4	8	8	64
P. merdae	16062	0.5	0.5	0.5	2	1	1	8	32	16
	18260	1	1	0.5	4	1	1	4	16	64

[a] Abbreviations: RMA, R. M. Alden Research Lab culture collection; BPM, biapenem; BPM-7009, biapenem-RPX7009; MRP, meropenem; IMI, imipenem; ERT, ertapenem; DOR, doripenem; P-T, piperacillin-tazobactam; A-S, ampicillin-sulbactam; FOX, cefoxitin.

there was no significant effect of RPX7009 on biapenem activity against anaerobic bacteria. In some strains of the *B. fragilis* group and the *Parabacteroides* spp., biapenem alone or combined with RPX7009 was more active than imipenem or meropenem. This was particularly the case in the panel of recent isolates with meropenem MICs of >2 μg/ml, as well as for strains from the collection of isolates with reduced susceptibility to imipenem. These data suggest that biapenem-RPX7009 may be useful in the treatment of infections due to anaerobic bacteria. Further studies investigating the utility of this combination are warranted.

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