

# Activity of Meropenem Combined with RPX7009, a Novel $\beta$ -Lactamase Inhibitor, against Gram-Negative Clinical Isolates in New York City

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Multidrug-resistant *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacteriaceae* are endemic to hospitals in New York City and other regions. RPX7009 is a novel  $\beta$ -lactamase inhibitor with activity against serine carbapenemases. We tested the activity of meropenem plus RPX7009 against 4,500 recent Gram-negative clinical isolates from 11 New York City hospitals. The meropenem-RPX7009 combination was found to have excellent *in vitro* activity against *Escherichia coli*, *K. pneumoniae*, and *Enterobacter* spp., including multidrug-resistant (MDR) KPC-producing strains. Overall, 131/133 (98.5%) KPC-producing *Enterobacteriaceae* strains were inhibited by meropenem ( $\leq 1$   $\mu$ g/ml) plus RPX7009 (8  $\mu$ g/ml). In a limited number of strains, the combination appeared to have reduced activity against KPC-producing *K. pneumoniae* isolates with diminished *ompK35* and *ompK36* expression. The addition of RPX7009 did not affect the activity of meropenem against *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. The meropenem-RPX7009 combination shows promise as a novel agent against KPC-producing *Enterobacteriaceae* and deserves further study. Other approaches will be needed to address multidrug-resistant *A. baumannii* and *P. aeruginosa*, which typically possess different mechanisms of carbapenem resistance.

Carbapenem resistance has been reported worldwide among the *Enterobacteriaceae*, in *Acinetobacter baumannii*, and in *Pseudomonas aeruginosa* (1–3). Mechanisms of carbapenem resistance include production of carbapenem-hydrolyzing enzymes, loss or alteration of porins, and increased efflux system activity (4–6). Historically, the old  $\beta$ -lactamase inhibitors (clavulanate, sulbactam, and tazobactam), in combination with  $\beta$ -lactams, have been useful against organisms producing various types of  $\beta$ -lactamases. However, these compounds have not been found to be active against many carbapenemases, including *Klebsiella pneumoniae* carbapenemase (KPC) and metalloenzymes (7). Several new  $\beta$ -lactamase inhibitors are being developed in the hope of preserving  $\beta$ -lactam activity (7, 8).

RPX7009 is a boron-containing serine  $\beta$ -lactamase inhibitor. Unlike the old  $\beta$ -lactamase inhibitors, boronic acid  $\beta$ -lactamase inhibitors work via formation of a covalent bond between the boron moiety and the serine side chain of the  $\beta$ -lactamase (8). They are also resistant to hydrolysis by serine  $\beta$ -lactamases (8). Activity of RPX7009, in combination with biapenem (formerly RPX2003) or meropenem, has been demonstrated against a variety of carbapenem-resistant *Enterobacteriaceae*. The biapenem-RPX7009 combination has been shown to overcome resistance in the majority of KPC-producing isolates tested, but no inhibition was shown for class B- and D-producing isolates (9). RPX7009 also has activity against some AmpC  $\beta$ -lactamases and restored the activity of cefepime against some *Enterobacter cloacae* isolates with hyperproduction of AmpC (8).

This study evaluated the activity of the meropenem-RPX7009 combination compared to meropenem alone against clinical isolates of *Enterobacteriaceae*, *A. baumannii*, and *P. aeruginosa* collected from various New York City hospitals during 2013 and 2014. The activity of meropenem with and without RPX7009 was also evaluated against isolates of *K. pneumoniae*, *A. baumannii*,

and *P. aeruginosa* with previously characterized mechanisms of resistance.

## MATERIALS AND METHODS

Single patient clinical isolates of *Escherichia coli*, *Enterobacter* spp., *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* were obtained from hospitals located in Brooklyn and Queens, NY, from November 2013 through January 2014. Susceptibility testing was done by either the broth microdilution method (for meropenem with and without RPX7009) or the agar dilution method (for all other antibiotics), as described previously (10), and the MICs were interpreted according to CLSI guidelines (11). RPX7009 (Rempex, San Diego, CA, USA) was combined with meropenem at fixed concentrations of 4 and 8  $\mu$ g/ml, which were previously shown to optimally enhance the activity of biapenem (9). *E. coli* ATCC 25922, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 27853 were used as control organisms. Cephalosporin-resistant isolates were screened by PCR, using previously described primers and conditions (12–15), for the following carbapenemase genes: *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>OXA48</sub>, *bla*<sub>OXA58</sub>, *bla*<sub>OXA23-like</sub>, and *bla*<sub>OXA24-like</sub>. The MICs of meropenem with and without RPX7009 were also determined for *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* isolates previously characterized for mechanisms of resistance (5, 6, 16).

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**TABLE 1** Susceptibility patterns of meropenem in combination with RPX7009 and other antibiotics against carbapenem-resistant Gram-negative organisms in New York City

Drug(s)	MIC <sub>50</sub> (μg/ml)	MIC <sub>90</sub> (μg/ml)	Range (μg/ml)	% Susceptible <sup>a</sup>
<i>K. pneumoniae</i> (KPC <sup>+</sup> ) (n = 121)				
Piperacillin-tazobactam	>128/4	>128/4	2/4 to >128/4	1
Ceftazidime	>16	>16	8 to >16	0
Gentamicin	>8	>8	0.5 to >8	37
Amikacin	32	64	≤0.5 to >64	46
Ciprofloxacin	>4	>4	≤0.125 to >4	9
Trimethoprim-sulfamethoxazole	>4	>4	≤0.5 to >4	13
Meropenem	8	64	0.25 to >64	7
Meropenem-RPX7009 (4 μg/ml)	0.06/4	2/4	0.008/4 to >64/4	NA
Meropenem-RPX7009 (8 μg/ml)	0.03/8	0.5/8	≤0.004/8 to >64/8	NA
<i>A. baumannii</i> (n = 84)				
Ampicillin-sulbactam	32/16	>32/16	4/2 to >32/16	17
Piperacillin-tazobactam	>128/4	>128/4	>128/4 to >128/4	4
Ceftazidime	>16	>16	4 to >16	2
Gentamicin	>8	>8	≤0.25 to >8	20
Amikacin	4	>64	1 to >64	59
Ciprofloxacin	>4	>4	≤0.125 to >4	4
Trimethoprim-sulfamethoxazole	>4	>4	≤0.5 to >4	10
Meropenem	32	64	4 to >64	0
Meropenem-RPX7009 (4 μg/ml)	32/4	64/4	0.25/4 to >64/4	NA
Meropenem-RPX7009 (8 μg/ml)	32/8	64/8	1/8 to >64/8	NA
<i>P. aeruginosa</i> (n = 98)				
Piperacillin-tazobactam	16/4	>128/4	16/4 to >128/4	52
Ceftazidime	8	>16	1 to >16	37
Amikacin	4	16	≤0.5 to >64	94
Ciprofloxacin	>4	>4	≤0.125 to >4	35
Meropenem	8	32	4 to >64	0
Meropenem-RPX7009 (4 μg/ml)	8/4	32/4	0.125/4 to >64/4	NA
Meropenem-RPX7009 (8 μg/ml)	8/8	32/8	0.25/8 to 64/8	NA

<sup>a</sup> NA, not available (breakpoint not established).

## RESULTS

**Escherichia coli.** A total of 2,770 isolates of *E. coli* were tested. Almost 100% of the isolates were susceptible to meropenem (99.9%), with all the isolates inhibited by meropenem (≤1 μg/ml) in combination with RPX7009. Five isolates were found to possess *bla*<sub>KPC</sub>. The meropenem MICs were 0.125, 0.25, 0.5, 2, and 4 μg/ml. These MICs decreased to 0.03, 0.015, 0.03, 0.03, and 0.03 μg/ml, respectively, with RPX7009 at 4 μg/ml and to 0.015, 0.015, 0.03, 0.015, and 0.015 μg/ml, respectively, with RPX7009 at 8 μg/ml. Except for *bla*<sub>KPC</sub>, no carbapenemase genes were detected.

**Klebsiella pneumoniae.** Antibiotic susceptibilities were determined for 894 isolates of *K. pneumoniae*. Eighty-eight percent of the isolates were susceptible to meropenem alone; this increased to 98.8% inhibited by meropenem at 1 μg/ml combined with RPX7009 at 4 μg/ml and to 99.8% combined with RPX7009 at 8 μg/ml. There were 121 isolates of *K. pneumoniae* found to possess *bla*<sub>KPC</sub> (Table 1). The addition of RPX7009 resulted in a 64- to 512-fold decrease in the meropenem MIC in the majority of KPC-positive isolates (range, 0- to 2,048-fold decrease). All but 2 of these isolates (98.3%) were inhibited by 1 μg/ml meropenem combined with RPX7009 at 8 μg/ml. No carbapenemase genes other than *bla*<sub>KPC</sub> were detected.

Isolates previously characterized for mechanisms of resistance were also tested (Table 2). The isolates were previously screened for the presence of *bla*<sub>KPC</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>TEM</sub> and for expression

of *bla*<sub>KPC</sub>, the porin genes *ompK35* and *ompK36*, and the efflux system gene *acrB*. A total of 26 isolates representing 7 clonal groups were available for testing. The KPC-negative isolates were all susceptible to meropenem. Among the isolates that possessed *bla*<sub>KPC</sub> (n = 12), meropenem MICs ranged from 0.015 to 8 μg/ml (with 4 μg/ml of RPX7009) and 0.008 to 2 μg/ml (with 8 μg/ml of RPX7009). *OmpK35* was not produced by nearly all of the KPC-producing isolates and was not included in further analysis. No correlation was found between MICs of meropenem combined with RPX7009 at 8 μg/ml and relative expression of *bla*<sub>KPC</sub>, *ompK36*, or *acrB*. However, two isolates (KC850 and CI855) (Table 2) had relatively high meropenem MICs despite the addition of RPX7009 (MIC = 8 μg/ml with 4 μg/ml of RPX7009 and MIC = 1 and 2 μg/ml with 8 μg/ml of RPX7009). Both of these isolates had decreased expression of *ompK36* compared to only 1 of 10 isolates with meropenem-RPX7009 (8 μg/ml) MICs ≤ 0.5 μg/ml (P = 0.046). The significance of this finding requires further investigation given the small number of isolates studied.

**Enterobacter species.** A total of 211 *Enterobacter* species isolates were collected, including 121 *E. cloacae* and 88 *Enterobacter aerogenes*. Seven isolates were found to have *bla*<sub>KPC</sub> on screening: three were *E. aerogenes*, while four were *E. cloacae*. For meropenem alone, the MICs were 0.06, 2, 4, >8, >8, >8, and >8 μg/ml; the MICs decreased to 0.03, 0.06, 0.06, 0.03, 0.03, 0.06, and 2 μg/ml, respectively, with RPX7009 at 4 μg/ml and to 0.015, 0.03,

TABLE 2 Susceptibilities to meropenem in combination with RPX7009 of characterized isolates of *K. pneumoniae*

Isolate	Clonal group	Other $\beta$ -lactamase(s)	Relative expression <sup>b</sup>			MIC ( $\mu$ g/ml) <sup>c</sup>		
			<i>bla</i> <sub>KPC</sub>	<i>acrB</i>	<i>ompK36</i>	MEM	MEM + R4	MEM + R8
CI512	B	SHV12; TEM1		1.07	8.22	0.015	0.015	0.008
VA302	C	SHV5; TEM1		0.21	1.32	0.015	0.015	0.015
DM152	I	None		1.71	1.92	0.015	0.015	0.008
KB351	U	SHV12		0.34	1.97	0.015	0.015	0.008
MA340	H	SHV11; TEM1		0.24	1.04	0.015	0.015	0.015
CI504	A	SHV11; SHV12		0.45	0.25	0.015	0.015	0.008
KB417	J	SHV12; TEM1		1.24	1.36	0.03	0.03	0.03
CI505	A	SHV11		0.02	1.31	0.03	0.015	0.008
CI518	A	SHV12		0.02	1.01	0.03	0.015	0.015
VM522	A	SHV12		0.003	0.88	0.03	0.008	0.008
CI511	B	SHV12; TEM1		0.5	3.11	0.06	0.06	0.06
WH307	C	TEM1		0.38	1.41	0.06	0.015	0.008
KB370	C	TEM1		0.07	0.86	0.06	0.015	0.015
CI806	B	SHV12; TEM1		0.11	1.81	0.12	0.06	0.03
VM9 <sup>a</sup>	A	SHV11; TEM1	1.1	0.05	1.7	2	0.015	0.015
CI302 <sup>a</sup>	D	SHV5; TEM1	40	1.6	8.19	2	0.015	0.008
CI516 <sup>a</sup>	A	SHV11; TEM1	1	0.2	4.19	4	0.015	0.015
DM834 <sup>a</sup>	A	SHV12; TEM1	1.25	0.32	2.94	4	0.015	0.015
CI513 <sup>a</sup>	B	SHV12; TEM1	18.75	1.64	7.71	4	0.12	0.06
CI839 <sup>a</sup>	B	SHV12; TEM1	5	0.74	7.59	8	0.25	0.12
MA31 <sup>a</sup>	A	SHV12	1	0.49	0.25	16	0.12	0.06
KB528 <sup>a</sup>	B	SHV11; TEM1	22.5	0.56	3.28	32	2	0.5
BD503 <sup>a</sup>	D	SHV5; TEM1	198.75	6.01	4.51	32	0.06	0.06
WO822 <sup>a</sup>	B	SHV12; TEM1	1.1	0.52	5.7	64	0.5	0.12
KC850 <sup>a</sup>	A	SHV12; TEM1	2.5	0.04	0.004	>64	8	1
CI8551 <sup>a</sup>	C	SHV12; TEM1	15	0.17	0.18	>64	8	2

<sup>a</sup> KPC-positive.<sup>b</sup> Relative expression compared with control (set to 1).<sup>c</sup> MEM meropenem; MEM + R4 meropenem, + RPX7009 (4  $\mu$ g/ml); MEM + R8, meropenem + RPX7009 (8  $\mu$ g/ml).

0.03, 0.03, 0.03, 0.03, and 0.5  $\mu$ g/ml, respectively, with RPX7009 at 8  $\mu$ g/ml. Genes for carbapenemases other than *bla*<sub>KPC</sub> were not detected. One isolate of *E. aerogenes* lacking *bla*<sub>KPC</sub> had a meropenem MIC of 2  $\mu$ g/ml; the MICs for this isolate were 1 and 0.5  $\mu$ g/ml with the addition of RPX7009 at 4 and 8  $\mu$ g/ml, respectively. All the remaining KPC-negative isolates were susceptible to meropenem alone (MIC<sub>50</sub>/MIC<sub>90</sub> = 0.06/0.125  $\mu$ g/ml; range, 0.03 to 0.5  $\mu$ g/ml).

***Acinetobacter baumannii*.** The susceptibilities of 158 isolates of *A. baumannii* were determined. Overall, 47% of the isolates were susceptible to meropenem. Among the meropenem-nonsusceptible isolates, MICs were largely unchanged by the addition of RPX7009 (Table 1). A 4-fold or greater decrease in the meropenem MIC was seen in only 4/84 and 2/84 meropenem-nonsusceptible isolates with RPX7009 at 4 and 8  $\mu$ g/ml, respectively. One isolate was found to possess *bla*<sub>KPC</sub>. The MIC for meropenem alone was >64  $\mu$ g/ml; this decreased to 16  $\mu$ g/ml and 8  $\mu$ g/ml with RPX7009 at 4  $\mu$ g/ml and 8  $\mu$ g/ml, respectively. Of the isolates screened, 58 were found to possess *bla*<sub>OXA23-like</sub>. The MIC<sub>50</sub> and MIC<sub>90</sub> for meropenem were 32 and 64  $\mu$ g/ml, respectively, with or without RPX7009. Two isolates were found to have *bla*<sub>OXA24-like</sub>. For one isolate, the MICs for meropenem were >64  $\mu$ g/ml with or without RPX7009; for the other isolate, the MICs were 32, 64, and 32  $\mu$ g/ml, respectively, for meropenem alone, with RPX7009 at 4  $\mu$ g/ml, and with RPX7009 at 8  $\mu$ g/ml. Genes for the carbapenemases NDM, IMP, VIM, and OXA58 were not detected.

Thirty isolates previously characterized for mechanisms of

resistance were also tested (data not shown). Expression of the  $\beta$ -lactamase genes *ampC* and *bla*<sub>OXA51</sub>, the porin gene *ompA*, and the efflux system genes *adeB* and *abeM* were previously determined. Twenty-five isolates were found to be nonsusceptible to meropenem; for these isolates, there was no change in meropenem MICs with the addition of RPX7009 at 8  $\mu$ g/ml, regardless of the expression of *ampC*, *bla*<sub>OXA51</sub>, *ompA*, *adeB*, and *abeM*.

***Pseudomonas aeruginosa*.** A total of 467 isolates of *P. aeruginosa* were collected, and their susceptibilities were determined. Overall, 79% of the isolates were susceptible to meropenem. Among the meropenem-nonsusceptible isolates, the MIC<sub>50</sub> and MIC<sub>90</sub> were largely unchanged by the addition of RPX7009 (Table 1). A 4-fold or greater decrease in the meropenem MIC was seen in 9/98 and 6/98 meropenem-nonsusceptible isolates with RPX7009 at 4 and 8  $\mu$ g/ml, respectively. None of the isolates possessed any of the carbapenemase genes screened.

Thirty isolates previously characterized for mechanisms of resistance were also tested (data not shown). Expression of the  $\beta$ -lactamase gene *ampC*; the porin gene *oprD*; and the efflux system genes *mexA*, *mexC*, *mexE*, and *mexX* were previously determined. Twenty-three isolates were found to be nonsusceptible to meropenem; for these isolates, there was no change in meropenem MICs with the addition of RPX7009 at 8  $\mu$ g/ml, regardless of the expression of *ampC*, *oprD*, *mexA*, *mexC*, *mexE*, and *mexX*.



## DISCUSSION

RPX7009, a new boronic acid  $\beta$ -lactamase inhibitor, is one of several  $\beta$ -lactamase inhibitors currently being developed to address the growing carbapenem resistance in *Enterobacteriaceae*, *A. baumannii*, and *P. aeruginosa*. It has been previously shown to preserve meropenem activity against KPC-producing isolates (17, 18). The meropenem-RPX7009 combination was also tested using an *in vitro* hollow-fiber model to simulate human exposure (19). Simulating human exposures of 2 g meropenem plus 2 g RPX7009 dosed every 8 h and infused over 3 h, the combination demonstrated bactericidal activity against KPC-producing isolates of *Enterobacteriaceae*. The meropenem-RPX7009 combination also showed efficacy *in vivo* against KPC-producing isolates of *K. pneumoniae*, *E. coli*, and *E. cloacae* with meropenem-RPX7009 (8  $\mu$ g/ml) MICs ranging from  $\leq 0.06$  to 8  $\mu$ g/ml using a murine thigh infection model (20). A study evaluating the efficacy, safety, and tolerability of the combination in adults with serious infections due to carbapenem-resistant *Enterobacteriaceae* is under way (<http://clinicaltrials.gov/ct2/show/NCT02168946>).

Our study confirmed the *in vitro* activity of meropenem plus RPX7009 against a large recent collection of Gram-negative clinical isolates from New York City, a region particularly affected by KPC-producing *Enterobacteriaceae*. The addition of RPX7009 resulted in a significant decrease in meropenem MICs in all but one of the *bla*<sub>KPC</sub>-possessing *Enterobacteriaceae*, regardless of species. Although the number of characterized isolates was small, the activity of meropenem-RPX7009 against KPC-producing *K. pneumoniae* isolates did not appear to be affected by expression of *bla*<sub>KPC</sub> or *acrB* or by diminished *ompK35* alone. However, meropenem-RPX7009 MICs were relatively high in isolates with concomitantly diminished *ompK35* and *ompK36* expression, particularly when the lower RPX7009 concentration of 4  $\mu$ g/ml was used. Previous studies have shown an association between carbapenem resistance and reduced *ompK36* expression in *K. pneumoniae* (16), although the concomitant presence of both reduced *ompK35* and *ompK36* may be necessary (21). The combined presence of outer membrane porin deficiency along with  $\beta$ -lactamases has been previously reported to diminish the effect of novel  $\beta$ -lactamase inhibitors (9, 22) and might limit the utility of these agents against some strains. Additional studies of the effect of porin loss on the activity of meropenem-RPX7009 are needed.

The enhanced activity of meropenem in the presence of RPX7009 was limited to isolates where KPC production was the main mechanism of carbapenem resistance. Despite having activity against some class C  $\beta$ -lactamases, RPX7009 did not enhance the effect of meropenem against most *A. baumannii* and *P. aeruginosa* isolates in this study. Differing mechanisms of carbapenem resistance, including production of class D  $\beta$ -lactamases, loss or alteration of porins, and increased activity of efflux systems, may explain the lack of effect of RPX7009 against *A. baumannii* strains from this region. Decreased permeability and increased efflux, the primary mechanisms of carbapenem resistance among *P. aeruginosa* isolates from this region (5), would also not be affected by the addition of RPX7009. Other approaches will be necessary to address the problem of multidrug-resistant (MDR) *A. baumannii* and *P. aeruginosa*.

The meropenem-RPX7009 combination shows promise as a potential therapeutic agent against Gram-negative organisms, including KPC-producing strains, given the limited treatment op-

tions currently available. Additional studies are warranted to determine the clinical efficacy of the combination.

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## REFERENCES

- Castanheira M, Deshpande LM, Costello A, Davies TA, Jones RN. 2014. Epidemiology and carbapenem resistance mechanisms of carbapenem-non-susceptible *Pseudomonas aeruginosa* collected during 2009–11 in 14 European and Mediterranean countries. *J Antimicrob Chemother* 69:1804–1814. <http://dx.doi.org/10.1093/jac/dku048>.
- Nordmann P, Naas T, Poirel L. 2011. Global spread of carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis* 17:1791–1798. <http://dx.doi.org/10.3201/eid1710.110655>.
- Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. 2007. Global challenge of multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 51:3471–3484. <http://dx.doi.org/10.1128/AAC.01464-06>.
- Logan LK. 2012. Carbapenem-resistant *Enterobacteriaceae*: an emerging problem in children. *Clin Infect Dis* 55:852–859. <http://dx.doi.org/10.1093/cid/cis543>.
- Quale J, Bratu S, Gupta J, Landman D. 2006. Interplay of efflux system, *ampC*, and *oprD* expression in carbapenem resistance of *Pseudomonas aeruginosa* clinical isolates. *Antimicrob Agents Chemother* 50:1633–1641. <http://dx.doi.org/10.1128/AAC.50.5.1633-1641.2006>.
- Bratu S, Landman D, Martin DA, Georgescu C, Quale J. 2008. Correlation of antimicrobial resistance with beta-lactamases, the *OmpA*-like porin, and efflux pumps in clinical isolates of *Acinetobacter baumannii* endemic to New York City. *Antimicrob Agents Chemother* 52:2999–3005. <http://dx.doi.org/10.1128/AAC.01684-07>.
- Toussaint KA, Gallagher JC. 2015.  $\beta$ -Lactam/ $\beta$ -lactamase inhibitor combinations: from then to now. *Ann Pharmacother* 49:86–98. <http://dx.doi.org/10.1177/1060028014556652>.
- Hecker SJ, Reddy KR, Totrov M, Hirst GC, Lomovskaya O, Griffith DC, King P, Tsivkovski R, Sun D, Sabet M, Tarazi Z, Clifton MC, Atkins K, Raymond A, Potts KT, Abendroth J, Boyer SH, Loutit JS, Morgan EE, Durso S, Dudley MN. 17 March 2015. Discovery of a cyclic boronic acid  $\beta$ -lactamase inhibitor (RPX7009) with utility vs. class A serine carbapenemases. *J Med Chem* <http://dx.doi.org/10.1021/acs.jmedchem.5b00127>.
- Livermore DM, Mushtaq S. 2013. Activity of biapenem (RPX2003) combined with the boronate  $\beta$ -lactamase inhibitor RPX7009 against carbapenem-resistant *Enterobacteriaceae*. *J Antimicrob Chemother* 68:1825–1831. <http://dx.doi.org/10.1093/jac/dkt118>.
- Clinical and Laboratory Standards Institute. 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—9th ed. CLSI document M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2014. Performance standards for antimicrobial susceptibility testing; 24th informational supplement. CLSI document M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.
- Bradford PA, Bratu S, Urban C, Visalli M, Mariano N, Landman D, Rahal JJ, Brooks S, Cebular S, Quale J. 2004. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. *Clin Infect Dis* 39:55–60. <http://dx.doi.org/10.1086/421495>.
- Nordmann P, Poirel L. 2002. Emerging carbapenemases in Gram-negative aerobes. *Clin Microbiol Infect* 8:321–331. <http://dx.doi.org/10.1046/j.1469-0691.2002.00401.x>.
- Poirel L, Heritier C, Tolun V, Nordmann P. 2004. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 48:15–22. <http://dx.doi.org/10.1128/AAC.48.1.15-22.2004>.
- Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, Amyes SG, Livermore DM. 2006. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents* 27:351–353. <http://dx.doi.org/10.1016/j.ijantimicag.2006.01.004>.
- Landman D, Bratu S, Quale J. 2009. Contribution of *OmpK36* to car-

- bapenem susceptibility in KPC-producing *Klebsiella pneumoniae*. J Med Microbiol 58:1303–1308. <http://dx.doi.org/10.1099/jmm.0.012575-0>.
17. Castanheira M, Becker HK, Rhomberg PR, Jones RN. 2014. Effect of the  $\beta$ -lactamase inhibitor RPX7009 combined with meropenem tested against a large collection of KPC-producing *Enterobacteriaceae*, abstr C-777. Intersci Conf Antimicrob Agents Chemother, Washington, DC, 5 to 9 September 2014.
  18. Castanheira M, Rhomberg PR, Watters A, Jones RN. 2014. *In vitro* activity of meropenem/RPX7009, a carbapenem/ $\beta$ -lactamase inhibitor combination tested against contemporary populations of *Enterobacteriaceae* and KPC-producing strains. Open Forum Infect Dis 1(Suppl 1):S110.
  19. Tarazi Z, Sabet M, Rubio-Aparicio D, Nolan T, Parkinson J, Lomovskaya O, Dudley MN, Griffith DC. 2014. Efficacy of simulated human exposures of Carbavance (meropenem-RPX7009) against carbapenem-resistant *Enterobacteriaceae* in an *in vitro* hollow fiber model, abstr F-959. Intersci Conf Antimicrob Agents Chemother, Washington, DC, 5 to 9 September 2014.
  20. Sabet M, Tarazi Z, Nolan T, Parkinson J, Rubio-Aparicio D, Lomovskaya O, Dudley MN, Griffith DC. 2014. *In vivo* efficacy of carbavance (meropenem/RPX7009) against KPC-producing *Enterobacteriaceae*, abstr F-958. Intersci Conf Antimicrob Agents Chemother, Washington, DC, 5 to 9 September 2014.
  21. Doumith M, Ellington MJ, Livermore DM, Woodford N. 2009. Molecular mechanisms disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter* spp. clinical isolates from the UK. J Antimicrob Chemother 63:659–667. <http://dx.doi.org/10.1093/jac/dkp029>.
  22. Livermore DM, Mushtaq S, Barker K, Hope R, Warner M, Woodford N. 2012. Characterization of  $\beta$ -lactamase and porin mutants of *Enterobacteriaceae* selected with ceftaroline + avibactam (NXL104). J Antimicrob Chemother 67:1354–1358. <http://dx.doi.org/10.1093/jac/dks079>.