#257 *In vitro* activity of meropenem/RPX7009, a carbapenem/β-lactamase inhibitor combination tested against contemporary populations of *Enterobacteriaceae* and KPC-producing strains

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Abstract

Background: We evaluated the activity of meropenem (MER) ± RPX7009 (RPX), a serine-β-lactamase inhibitor (BLI) tested against contemporary isolates of Enterobacteriaceae (ENT), including KPC-producing isolates.

Methods: 100 ENT clinical isolates collected during 2012-2013 were tested against MER ± RPX at fixed 4 and 8 µg/ml using CLSI reference broth microdilution methods. Additionally, 100 KPC-producing ENT were tested.

Results: Against all 200 ENT, MER/RPX displayed $MIC_{50/90}$ of ≤0.25/1 and ≤0.25/0.5 µg/ml at fixed 4 and 8 µg/ml, respectively when compared to $MIC_{50/90}$ ≤0.25/>8 µg/ml for MER alone (Table).

Overall, 91.5/95.5% and 96.0/99.0% of ENT were inhibited at ≤1/≤4 µg/ml of MER in the presence of 4 or 8 µg/ml of RPX, respectively. MER/RPX at fixed 4 and 8 µg/ml, inhibited 80.3 and 90.8%, respectively of the *K. pneumoniae* (n=76) isolates at ≤1 µg/ml (MER CLSI susceptible breakpoint), whereas MER inhibited only 21.1% at the same MIC.

Only 32.0% of *E. cloacae* isolates (n=25) were inhibited at 1 µg/ml of MER, but 96.0% of these isolates were inhibited by MER/RPX at both 4 and 8 µg/ml at the same MIC value.

All *E. coli* (n=38), *Serratia* spp. (n=12) and indole-positive Proteae (n=11) isolates were inhibited by ≤0.25 µg/mL of MER/RPX at fixed 4 or 8 µg/ml. MER/RPX inhibited 83.0 and 91.0% and 92 and 98 % of the KPC-producers at ≤1 and ≤4 µg/ml of MER for both BLI concentrations, compared to only 3.0% or 24.0% for MER alone, respectively.

Conclusions: These results demonstrate that MER/RPX is a good candidate for further development that could increase the treatment options against serious infections, including those caused by KPC-producers that are often resistant to most antimicrobial agents.

Organism (no. tested)	Antimicrobial agent[a]	Cumulative % inhibited at MIC (µg/ml)[b]: ≤0.25	0.5	1	2	4	8
ENT (200)	MER	50.0	50.5	51.5	54.5	62.0	71.0
	MER/RPX 4	87.0	89.0	**91.5**	94.0	96.0	98.0
	MER/RPX 8	89.5	**92.5**	95.5	98.5	99.0	99.0
***K. pneumoniae* (76)**	MER	19.7	21.1	21.1	22.4	25.0	36.8
	MER/RPX 4	69.7	72.4	73.7	75.0	77.6	85.5
	MER/RPX 8	72.4	75.0	80.3	85.5	89.5	**94.7**
KPC-producers (100)	MER	0.0	1.0	3.0	9.0	24.0	42.0
	MER/RPX 4	74.0	78.0	83.0	88.0	**92.0**	96.0
	MER/RPX 8	79.0	85.0	**91.0**	97.0	98.0	98.0

a. MER/RPX=meropenem/RPX7009; 4=at fixed 4 µg/ml; 8=at fixed 8 µg/ml
b. MIC_{50} values are underlined and MIC_{90} are bolded

Introduction

Carbapenem resistant Enterobacteriaceae isolates have been detected worldwide mainly due to the spread of carbapenemase-encoding genes.

A large number of acquired carbapenemases have been identified and characterized and among these diverse enzymes, *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria have been detected worldwide.

This carbapenem hydrolyzing β-lactamase is commonly identified in *Klebsiella* spp. clinical isolates, but it can also been found among other Enterobacteriaceae species and these isolates are usually highly resistant to all β-lactam agents.

KPC-encoding genes are carried in plasmids that usually carry resistance determinants to other antimicrobial classes.

Recently, increasing reports of KPC-producing isolates resistant to all antimicrobial agents clinically available, including colistin and tigecycline has increased the awareness to the need of new therapeutic options to treat infections cause by KPC-producing isolates.

In this study, we evaluate the activity of meropenem alone and in combination with RPX7009, a boronic acid-based β-lactamase inhibitor against 200 Enterobacteriaceae isolates, including 100 producing KPC enzymes.

Materials and Methods

Bacterial isolates. One hundred wildtype isolates collected in USA hospitals during 2012 were randomly selected and included 30 *Escherichia coli*, 19 *Klebsiella* spp., 12 *Enterobacter* spp., 12 Indole positive Proteae, 10 *Serratia* spp., 9 *Proteus mirabilis*, 6 *Citrobacter* spp. and 3 isolates belonging to other species.

Additionally, 100 isolates producing KPC-encoding genes collected from 2001 to 2010 were selected to be geographically and temporally diverse and included: 61 *K. pneumoniae*, 17 *E. cloacae*, 8 *E. coli*, 8 *K. oxytoca* and three each of *C. freundii* and *S. marcescens*.

Genes encoding serine-carbapenemases and other β-lactamases were previously identified using various PCR strategies; majority of amplicons were sequenced on both strands.

Susceptibility testing. MIC values were determined using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methodology as described in CLSI document M07-A9 (2012). Meropenem with and without the β-lactamase inhibitor RPX7009 at fixed concentrations of 2, 4 and 8 µg/ml was tested in frozen-form panels using cation-adjusted Mueller-Hinton broth.

Quality control (QC) ranges and interpretive criteria were those published in CLSI M100-S22 (2012); tested QC strains included *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and KPC-producing *K. pneumoniae* BAA-1705.

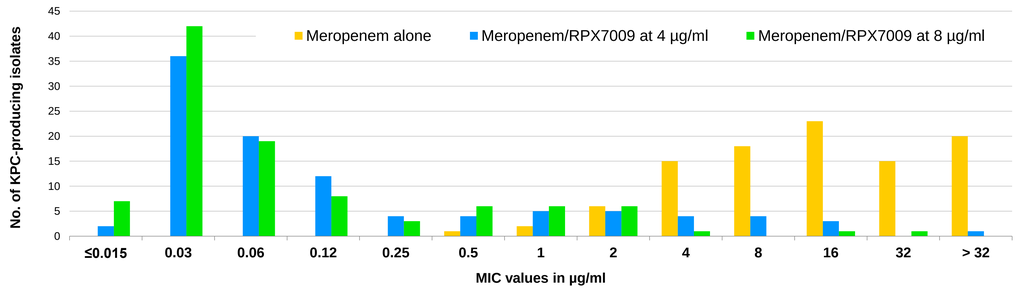
Results

- Against 200 *Enterobacteriaceae* isolates including a population of 100 KPC-producing strains, the MIC_{50} and MIC_{90} values for meropenem alone were 0.12 and 32 µg/ml, respectively and these values were 0.06 to 0.03 and 4 to 0.5 µg/ml, respectively, for meropenem/RPX7009 with inhibitor tested at concentrations of fixed 2, 4 and 8 µg/mL (Table 1). Overall, the coverage of meropenem/RPX7009 was greater using inhibitor concentrations of 4 and 8 µg/mL.
- A total of 94.0 and 98.5% of the isolates were inhibited by ≤2 µg/ml of meropenem/RPX7009 at fixed 4 and 8 µg/mL, respectively, whereas only 54.4% of the isolates were inhibited by meropenem alone (Table 1). At ≤4 and ≤8 µg/ml, 96.0-99.0 and 98.0-99.0% of the isolates were inhibited by meropenem/RPX7009 at fixed 4 and 8 µg/mL, respectively, compared to 62.0 and 71.0% for meropenem at the same concentration (Table 1).
- *K. pneumoniae* strains (n=76) were highly resistant to meropenem alone mainly due to an elevated number of KPC-producers (n=61). $MIC_{50/90}$ values were 16/>32 µg/ml for this carbapenem. When tested with RPX7009 at 4 and 8 µg/ml, $MIC_{50/90}$ values were 0.06/8 and 0.03/1 µg/ml, respectively (Table 1).
- Approximately 79.0% of the *E. coli* strains (n=38) were susceptible to meropenem alone and all isolates were inhibited by ≤0.06 µg/ml of meropenem/RPX7009 at fixed 4 and 8 µg/ml (Table 1).
- MIC_{50} and MIC_{90} results for *E. cloacae* isolates and meropenem were 4 and 16 µg/ml and these results were 0.12 and 0.5 µg/ml and 0.06 and 0.25 µg/ml for meropenem/RPX7009 at fixed 4 and 8 µg/ml, respectively (Table 1).
- $MIC_{50/90}$ values for *S. marcescens* were 0.06/2, 0.06/0.12 and 0.06/0.12 µg/ml for meropenem, meropenem/RPX7009 at fixed 4 and 8 µg/ml, respectively. All Indole-positive Proteae isolates were inhibited by meropenem/RPX7009 at fixed 4 and 8 µg/mL at ≤0.12 µg/ml (data not shown).
- As expected KPC-producing *Enterobacteriaceae* isolates were highly resistant to meropenem and in the presence of RPX7009 MIC values were much lower (Figure 1). Against 100 KPC-producing strains, meropenem/RPX7009 at fixed 4 and 8 µg/ml inhibited over 50% of the isolates at 0.06 µg/ml (Figure 1).
- RPX7009 displayed no activity when tested alone (all MIC values, >32 µg/ml; data not shown).

Table 1. MIC distribution for meropenem alone and in combination with RPX7009 in various concentrations when tested against 200 *Enterobacteriaceae* isolates, including 100 KPC-producers.

Organism (no. tested) / Antimicrobial agent	No. of isolates at MIC in µg/ml (cumulative %): ≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32	MIC 50%	MIC 90%
Enterobacteriaceae (200)															
Meropenem		66 (33.0)	26 (46.0)	8 (50.0)	0 (50.0)	1 (50.5)	2 (51.5)	6 (54.5)	15 (62.0)	18 (71.0)	23 (82.5)	15 (90.0)	20 (100.0)	0.12	32
Meropenem/RPX7009 at 2 µg/ml	19 (9.5)	68 (43.5)	52 (69.5)	19 (79.0)	11 (84.5)	4 (86.5)	2 (87.5)	4 (89.5)	3 (91.0)	6 (94.0)	2 (95.0)	4 (97.0)	6 (100.0)	0.06	4
Meropenem/RPX7009 at 4 µg/ml	20 (10.0)	85 (52.5)	49 (77.0)	16 (85.0)	4 (87.0)	4 (89.0)	5 (91.5)	5 (94.0)	4 (96.0)	4 (98.0)	3 (99.5)	0 (99.5)	1 (100.0)	0.03	1
Meropenem/RPX7009 at 8 µg/ml	25 (12.5)	93 (59.0)	47 (82.5)	11 (88.0)	3 (89.5)	6 (92.5)	6 (95.5)	6 (98.5)	1 (99.0)	0 (99.0)	1 (99.5)	1 (100.0)	--	0.03	0.5
Klebsiella pneumoniae (76)															
Meropenem		12 (15.8)	2 (18.4)	1 (19.7)	0 (19.7)	1 (21.1)	0 (21.1)	1 (22.4)	2 (25.0)	9 (36.8)	17 (59.2)	12 (75.0)	19 (100.0)	16	>32
Meropenem/RPX7009 at 2 µg/ml	1 (1.3)	21 (28.9)	21 (56.6)	5 (63.2)	5 (69.7)	2 (72.4)	1 (73.7)	1 (75.0)	2 (77.6)	6 (85.5)	1 (86.8)	4 (92.1)	6 (100.0)	0.06	32
Meropenem/RPX7009 at 4 µg/ml	2 (2.6)	34 (47.4)	16 (68.4)	2 (71.1)	1 (72.4)	2 (75.0)	4 (80.3)	4 (85.5)	3 (89.5)	4 (94.7)	3 (98.7)	0 (98.7)	1 (100.0)	0.06	8
Meropenem/RPX7009 at 8 µg/ml	6 (7.9)	38 (57.9)	10 (71.1)	2 (73.7)	1 (75.0)	6 (82.9)	6 (90.8)	4 (96.1)	1 (97.4)	0 (97.4)	1 (98.7)	1 (100.0)	--	0.03	1
Escherichia coli (38)															
Meropenem		28 (73.7)	2 (78.9)	0 (78.9)	0 (78.9)	0 (78.9)	0 (78.9)	1 (81.6)	5 (94.7)	1 (97.4)	1 (100.0)	--	--	≤0.03	4
Meropenem/RPX7009 at 2 µg/ml	15 (39.5)	18 (86.8)	4 (97.4)	1 (100.0)	--	--	--	--	--	--	--	--	--	0.03	0.06
Meropenem/RPX7009 at 4 µg/ml	14 (36.8)	19 (86.8)	5 (100.0)	--	--	--	--	--	--	--	--	--	--	0.03	0.06
Meropenem/RPX7009 at 8 µg/ml	15 (39.5)	20 (92.1)	3 (100.0)	--	--	--	--	--	--	--	--	--	--	0.03	0.03
Enterobacter cloacae (25)															
Meropenem		3 (12.0)	3 (24.0)	2 (32.0)	0 (32.0)	0 (32.0)	0 (32.0)	2 (40.0)	4 (56.0)	5 (76.0)	4 (92.0)	2 (100.0)	--	4	16
Meropenem/RPX7009 at 2 µg/ml	0 (0.0)	4 (16.0)	4 (32.0)	7 (60.0)	4 (76.0)	2 (84.0)	0 (84.0)	3 (96.0)	0 (96.0)	0 (96.0)	1 (100.0)	--	--	0.12	2
Meropenem/RPX7009 at 4 µg/ml	0 (0.0)	6 (24.0)	6 (48.0)	7 (76.0)	2 (84.0)	2 (92.0)	1 (96.0)	0 (96.0)	1 (100.0)	--	--	--	--	0.12	0.5
Meropenem/RPX7009 at 8 µg/ml	0 (0.0)	9 (36.0)	10 (76.0)	3 (88.0)	2 (96.0)	0 (96.0)	0 (96.0)	1 (100.0)	--	--	--	--	--	0.06	0.25

Figure 1. Distribution of 100 KPC-producing *Enterobacteriaceae* isolates when tested against meropenem alone and meropenem/RPX7009 with inhibitor at fixed 4 and 8 µg/mL.



Conclusions

- Meropenem/RPX7009 with the β-lactamase inhibitor at fixed concentrations of 4 and 8 µg/ml was very active against contemporary wildtype Enterobacteriaceae isolates and KPC-producing strains from various species.
- The worldwide dissemination of KPC-producing strains, including those that are resistant to all antimicrobial agents clinically available, highlights the need for compounds that are active against these isolates, such as meropenem/RPX7009 combination.

Disclaimers/Acknowledgments

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