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In vitro Activity of WCK 5999, a Carbapenem/β-lactamase Inhibitor Combination Tested against Contemporary KPC-producing Enterobacteriaceae

M CASTANHEIRA, PR RHOMBERG, BA SCHAEFER, RN JONES, HS SADER
JMI Laboratories, North Liberty, Iowa, USA

Mariana Castanheira, PhD
JMI Laboratories
345 Beaver Kreek Centre, Suite A
North Liberty, Iowa 52317
Phone: (319) 665-3370
mariana-castanheira@jmilabs.com

Amended Abstract

Background: KPC serine-carbapenemases are prevalent worldwide and are usually resistant to all β-lactams and often resistant to other antimicrobial classes. WCK 5999 is a combination of meropenem (MEM) and WCK 4234, a β-lactamase inhibitor with enhanced inhibitory activity against class D oxacillinases (including carbapenem hydrolyzing) as well as activity towards class A and C. We tested MEM-WCK 4234 against 156 KPC-producing Enterobacteriaceae isolates from nine bacterial species.

Methods: 156 clinical KPC-producing isolates collected during 2014-2015 from USA hospitals were susceptibility tested against MEM-WCK 4234 (WCK 4234 at fixed 4 and 8 µg/mL), MEM and WCK 4234 alone according to CLSI guidelines. The presence of bla_{KPC} was determined by PCR/sequencing.

Results: MEM alone ($MIC_{50/90}$, 8/>64 µg/mL) had a very limited activity based on CLSI interpretative criteria (≤1 µg/mL). MIC_{50} values for MEM-WCK 4234 were ≤0.03 µg/mL for both inhibitor concentrations and MIC_{90} was 0.5 and 0.12 µg/mL for fixed 4 and 8 µg/mL, respectively. MEM-WCK 4234 inhibited 98.1 and 99.4% of the isolates at ≤1 µg/mL using fixed 4 and 8 µg/mL of inhibitor, respectively. Highest MEM-WCK 4234 MIC value among KPC-producing *K. pneumoniae* (n=124, $MIC_{50/90}$, ≤0.03/0.25 µg/mL) was 2 µg/mL (two isolates at this MIC) when tested at fixed 4 µg/mL of inhibitor and 1 µg/mL using fixed 8 µg/mL of WCK 4234. All six KPC-producing *E. coli* and one *C. freundii* isolates tested were inhibited by MEM-WCK 4234 at ≤0.03 µg/mL; five *K. oxytoca* and 12 *E. cloacae* tested were inhibited at ≤0.5 and ≤1 µg/mL, respectively. MEM-WCK 4234 MIC results for five *S. marcescens* tested were ≤4 µg/mL and ≤2 µg/mL when using WCK4234 at fixed 4 and 8 µg/mL, respectively. WCK 4234 had no activity against the isolates tested (MIC_{50}, >16 µg/mL).

Conclusions: MEM-WCK 4234 displayed promising activity against a collection of very recent (2014-2015) KPC-producing isolates collected from USA hospitals, regardless of the inhibitor concentration used. This combination (WCK 5999) was eight- to >256-fold (mode, 128-fold) more active against KPC-producing isolates when compared to MEM alone. The emergence of pan-drug resistant KPC-producing isolates highlight the need for new therapeutic options for these isolates and the further development of WCK 5999 is warranted.

Introduction

KPC enzymes are the most widespread carbapenemases among Enterobacteriaceae species. Isolates producing these enzymes were initially described in New York and are currently reported from most USA states and several countries worldwide, with a high prevalence in the northeastern USA, Greece, China, Italy, Israel and Brazil among other countries.

KPC-encoding genes are carried by transposons and plasmids that are mobile genetic structures usually harboring resistance genes to additional antimicrobial classes and conferring a multidrug resistance profile to the isolates carrying them. Furthermore, the genes encoding KPC are usually associated with internationally successful *Klebsiella pneumoniae* clones that facilitate its dissemination.

KPC enzymes encode resistance to all β-lactam agents and are poorly inhibited by old β-lactamase inhibitors such as clavulanic acid and tazobactam. WCK 4234 is a novel broader spectrum β-lactamase inhibitor displaying activity against carbapenem-hydrolyzing oxacillinases detected among *Acinetobacter* species. It has been recently demonstrated that WCK 4234 displays a distinct mechanism of KPC inhibition, and unlike avibactam, WCK 4234 does not undergo de-sulfation. In this study, we evaluated the activity of WCK 5999, a combination of meropenem (MEM) and the β-lactamase inhibitor WCK 4234 tested against 156 KPC-producing isolates collected during 2014-2015 from USA medical centers.

Methods

Bacterial isolates. A total of 156 KPC-producing Enterobacteriaceae clinical isolates collected during 2014-2015 were evaluated. These isolates belonged to the following bacterial species: *Citrobacter freundii* species complex, 1 isolate; *Enterobacter aerogenes*, 1; *Enterobacter asburiae*, 1; *Enterobacter cloacae* species complex, 12; *Escherichia coli*, 6; *Klebsiella oxytoca*, 5; *Klebsiella pneumoniae*, 124; *Proteus penneri*, 1 and *Serratia marcescens*, 5. Species identification was confirmed by Matrix-Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS) using the Bruker Daltonics MALDI Biotyper (Billerica, Massachusetts, USA) by following manufacturer instructions.

KPC encoding genes were screened using PCR methods or a microarray based assay (Check-MDR CT101 kit; Check-points, Wageningen, Netherlands). Sequencing of selected amplicons was performed and protein alignments were compared with available sequences.

Susceptibility testing. MIC values for the combination of meropenem + WCK 4234 (WCK 5999) at two fixed concentrations of WCK 4234 (4 and 8 µg/mL) and both compounds alone were determined using Clinical and Laboratory Standards Institute (CLSI) broth microdilution methodology as described in CLSI document M07-A10 (2015). Quality Control (QC) ranges and interpretive criteria for meropenem were as published in CLSI M100-S26 (2016). The tested QC strains included the following: *Escherichia coli* ATCC 25922 and NCTC 13353, *Klebsiella pneumoniae* ATCC 700603 and ATCC BAA-1705, *P. aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212.

Results

- Meropenem ($MIC_{50/90}$, 8/>64 µg/mL) displayed limited activity against KPC-producing Enterobacteriaceae isolates (n=156) and only fourteen (9.0%) isolates were inhibited by this carbapenem at the CLSI susceptibility breakpoint criteria (**Table 1** and **Figure 1**).
- The combination meropenem-WCK 4234 was very active against KPC-producing Enterobacteriaceae and all 156 isolates were inhibited by this combination at ≤4 µg/mL when a fixed 4 µg/mL of inhibitor was tested and at ≤2 µg/mL when testing at a fixed 8 µg/mL of inhibitor (**Table 1**).
- One ceftazidime-avibactam resistant (MIC, 32 µg/mL; data not shown) KPC-producing *K. pneumoniae* isolate was tested and MEM-WCK 4234 MIC results for this isolate were 2 and 0.5 µg/mL using fixed 4 and 8 µg/mL of inhibitor, respectively. Another KPC-producing *K. pneumoniae* isolate displaying an elevated ceftazidime-avibactam MIC value (8 µg/mL; at the susceptible breakpoint) displayed MEM-WCK 4234 MIC results at 0.5 µg/mL using fixed 4 or 8 µg/mL of WCK 4234.
- Against 124 KPC-producing *K. pneumoniae* isolates, meropenem-WCK 4234 ($MIC_{50/90}$, ≤0.03/0.25 and ≤0.03/0.12 µg/mL for fixed 4 and 8 µg/mL of WCK 4234, respectively) inhibited 98.4% and 100.0% of the isolates at ≤1 µg/mL when a fixed 4 and 8 µg/mL of inhibitor was tested, respectively (**Table 1**).
- Meropenem-WCK 4234 ($MIC_{50/90}$, ≤0.03/0.5 and ≤0.03/0.25 µg/mL for fixed 4 and 8 µg/mL of WCK 4234, respectively) inhibited 96.9% of the 32 isolates from the eight other Enterobacteriaceae species using both inhibitor concentrations (**Table 1**).
- Only three isolates displayed MIC values >1 µg/mL when tested against meropenem-WCK 4234 using fixed 4 µg/mL of inhibitor. These isolates were two *K. pneumoniae* displaying MIC values of 2 µg/mL and one *Serratia marscecens* isolate displaying an MIC value of 4 µg/mL for this combination. This isolate was also the only isolate displaying an MIC value of >1 µg/mL (2 µg/mL) for meropenem-WCK 4234 using fixed 8 µg/mL of inhibitor.
- WCK 4234 alone displayed limited activity against KPC-producing Enterobacteriaceae isolates and all isolates had MICs of >16 µg/mL.

Figure 1. Comparative activity of meropenem-WCK 4234 (fixed 4 and 8 µg/mL) and meropenem against 156 KPC-producing isolates.

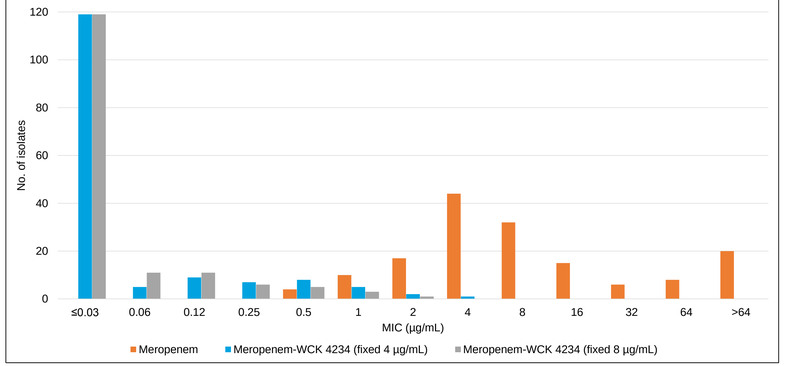

Table 1. Cumulative frequency distribution of MIC results for meropenem ± WCK 4234 when tested against KPC-producing Enterobacteriaceae isolates.

Organism group (no. tested) / Antimicrobial agent	No. of isolates (cumulative percentage) inhibited at MIC (µg/mL) of: ≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64	MIC_{50}	MIC_{90}
Enterobacteriaceae (156)															
Meropenem					4 (2.6)	10 (9.0)	17 (19.9)	44 (48.1)	32 (68.6)	15 (78.2)	6 (82.1)	8 (87.2)	20 (100.0)	8	>64
Meropenem-WCK 4234 (fixed 4 µg/mL)	119 (76.3)	5 (79.5)	9 (85.3)	7 (89.7)	8 (94.9)	5 (98.1)	2 (99.4)	1 (100.0)						≤0.03	0.5
Meropenem-WCK 4234 (fixed 8 µg/mL)	119 (76.3)	11 (83.3)	11 (90.4)	6 (94.2)	5 (97.4)	3 (99.4)	1 (100.0)							≤0.03	0.12
WCK 4234													156 (100.0)	>16	>16
***Klebsiella pneumoniae* (124)**															
Meropenem					1 (0.8)	9 (8.1)	11 (16.9)	37 (46.8)	25 (66.9)	11 (75.8)	6 (80.6)	8 (87.1)	16 (100.0)	8	>64
Meropenem-WCK 4234 (fixed 4 µg/mL)	95 (76.6)	2 (78.2)	8 (84.7)	7 (90.3)	6 (95.2)	4 (98.4)	2 (100.0)							≤0.03	0.25
Meropenem-WCK 4234 (fixed 8 µg/mL)	96 (77.4)	7 (83.1)	10 (91.1)	5 (95.2)	4 (98.4)	2 (100.0)								≤0.03	0.12
WCK 4234													124 (100.0)	>16	>16
Other Enterobacteriaceae[a] (32)															
Meropenem					3 (9.4)	1 (12.5)	6 (31.2)	7 (53.1)	7 (75.0)	4 (87.5)	0 (87.5)	0 (87.5)	4 (100.0)	4	>64
Meropenem-WCK 4234 (fixed 4 µg/mL)	24 (75.0)	3 (84.4)	1 (87.5)	0 (87.5)	2 (93.8)	1 (96.9)	0 (96.9)	1 (100.0)						≤0.03	0.5
Meropenem-WCK 4234 (fixed 8 µg/mL)	23 (71.9)	4 (84.4)	1 (87.5)	1 (90.6)	1 (93.8)	1 (96.9)	1 (100.0)							≤0.03	0.25
WCK 4234													32 (100.0)	>16	>16

a. Other Enterobacteriaceae included *Citrobacter freundii* species complex, 1 isolate; *Enterobacter aerogenes*, 1; *Enterobacter asburiae*, 1; *Enterobacter cloacae* species complex, 12; *Escherichia coli*, 6; *Klebsiella oxytoca*, 5; *Proteus penneri*, 1 and *Serratia marcescens*, 5.

Conclusions

- **MEM-WCK 4234 was very active against contemporary KPC-producing isolates and all isolates were inhibited at ≤4 or ≤2 µg/mL when tested using fixed concentrations of inhibitor at 4 and 8 µg/mL, respectively.**
- **MEM-WCK 4234 was active against one ceftazidime-avibactam resistant KPC-producing *K. pneumoniae* displaying MIC values at 2 and 0.5 µg/mL using different inhibitor concentrations.**
- **The limited options to treat multidrug resistant KPC-producing Enterobacteriaceae and the dissemination of these isolates worldwide warrant the further development of this antibacterial combination.**

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