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Activity of the New Carbapenem/β-Lactamase Inhibitor Combination WCK 5999 against Gram-Negative Isolates Producing Oxacillinases (OXAs)

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Abstract

Background: OXAs are class D serine β-lactamases that can display hydrolytic activity against cephalosporins and/or carbapenems. OXAs with extended- (ESBL) or narrow-spectrum (NS) are detected among Enterobacteriaceae (ENT) and *P. aeruginosa* (PSA). OXAs with carbapenemase (carb) activity are common among *Acinetobacter* spp. (ASP) and ENT. We tested WCK 5999, a combination of meropenem (MEM) with the β-lactamase inhibitor WCK 4234, against 118 OXA-producing isolates.

Methods: 82 isolates producing OXA-carb (55 ASP and 27 ENT) and 36 isolates producing OXA-ESBL/NS (29 ENT and 7 PSA) were tested. Susceptibility (S) testing was performed according to the CLSI guidelines for MEM-WCK 4234 (WCK 4234 at fixed 4 and 8 µg/mL), MEM and WCK 4234. The presence of OXA-encoding genes was previously determined by PCR/sequencing.

Results: Overall, MEM-WCK 4234 inhibited all ENT producing OXAs at ≤0.5 and ≤0.25 µg/mL using WCK 4234 at fixed 4 and 8 µg/mL, respectively. MEM alone inhibited only 64.3% of these isolates at the CLSI S breakpoint. All OXA-ESBL/NS ENT were inhibited by MEM-WCK 4234 at ≤0.12 µg/mL, regardless of the inhibitor concentration and 89.7% of these strains were MEM-S. MEM inhibited only 37.0% of the ENT isolates producing OXA-carb (OXA-48-like); however, MEM-WCK 4234 inhibited all these isolates at ≤0.5 or ≤0.25 µg/mL (WCK 4234 at fixed 4 and 8 µg/mL, respectively). All OXA-producing ASP were resistant to MEM (MIC_{90}, >64 µg/mL), but MEM-WCK 4234 at fixed 8 µg/mL inhibited 65.5 and 90.9% of these isolates at ≤2 and ≤4 µg/mL, respectively. MEM-WCK 4234 had higher activity against the more prevalent OXA-23-producing ASP when compared to isolates producing OXA-24. MEM-WCK 4234 (fixed 8 µg/mL) inhibited 84.4 and 96.9% of the OXA-23 isolates and 29.4 and 76.5% of the OXA-24 isolates at ≤2 and ≤4 µg/mL, respectively. Based on a small set of isolates (n=7), the activity of MEM-WCK 4234 was limited against PSA isolates producing OXA-ESBL/NS.

Conclusions: MEM-WCK 4234 displayed enhanced activity against ENT and ASP isolates producing OXAs with carbapenemase activity when compared to MEM. The activity of MEM-WCK 4234 was very good against ENT producing OXA-ESBL/NS, but showed limited activity against OXA-ESBL/NS-producing PSA since the MEM resistance mechanism in the latter species is not mediated by β-lactamases.

Introduction

Oxacillinases were among the earliest β-lactamases detected; however, until recently these molecular class D β-lactamases were secondary to class A enzymes that were considered more prevalent. The early oxacillinases reported had a limited substrate profile and only hydrolyzed penicillins, but some enzymes reported later were able to confer resistance to cephalosporins.

Carbapenem-resistance due the production of plasmid-encoded β-lactamases categorized as OXA enzymes (OXA-23, OXA-40 and OXA-58) among *Acinetobacter* species has emerged as a major problem. Additionally, the emergence of OXA-48-like enzymes that might encode some level of carbapenem resistance in Enterobacteriaceae has increased the awareness about class D enzymes and their importance.

Different from class A and C β-lactamases, oxacillinases are poorly inhibited by β-lactamase inhibitors (BLIs) in clinical use, including newer inhibitors that have been recently approved or those in a late stage of development. WCK 4234 is a novel broader spectrum β-lactamase inhibitor displaying inhibitory activity against carbapenem-hydrolyzing oxacillinases detected among *Acinetobacter* species, such as OXA-23 and OXA-24. Unlike older and newer BLIs, WCK 4234 has also been recently reported to be a potent inhibitor of carbapenemases such as OXA-48 and cephalosporinases associated with *Pseudomonas* (PDC) and *Acinetobacter* (ADC) species. In this study, we evaluated the activity of WCK 5999, a combination of meropenem with the β-lactamase inhibitor WCK 4234, against 118 OXA-producing isolates of Enterobacteriaceae, *Acinetobacter* spp. and *Pseudomonas aeruginosa* collected worldwide.

Methods

Bacterial isolates: A total of 118 contemporary clinical strains producing class D OXA β-lactamases, including 56 Enterobacteriaceae, 55 *Acinetobacter* spp. and seven *P. aeruginosa* isolates were evaluated. Oxacillinase encoding genes were previously characterized by PCR and sequencing methods. Isolates carrying genes encoding enzymes with an extended-spectrum (ESBL) or a narrow-spectrum (NS) hydrolytic profile were grouped under OXA ESBL/NS and those isolates producing enzymes with carbapenemase activity were categorized as OXA carbapenemase. A summary of the oxacillinase enzymes produced by the isolates tested is displayed in **Table 1**.

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 + WCK 4234 at fixed 4 µg/mL ($MIC_{50/90}$, 1/8 µg/mL) or fixed 8 µg/mL ($MIC_{50/90}$, 0.5/4 µg/mL) were >32-fold more active than meropenem alone ($MIC_{50/90}$, 32/>64 µg/mL) tested against the overall collection of 118 Gram-negative isolates producing oxacillinases (**Figure 1**).
- Meropenem + WCK 4234 ($MIC_{50/90}$, ≤0.03/0.06 µg/mL for both inhibitor concentrations) was very active against ENT isolates producing OXA ESBL/NS enzymes and all isolates were inhibited by meropenem + WCK 4234 at ≤0.12 µg/mL, regardless of the inhibitor combination tested (**Table 2**). Meropenem alone inhibited 25 of 29 isolates at the lowest concentration tested (≤0.5 µg/mL), but four isolates had meropenem MIC results ranging from 1 to 4 µg/mL.
- Against Enterobacteriaceae isolates producing OXA carbapenemases (OXA-48-like), meropenem + WCK 4234 ($MIC_{50/90}$, ≤0.03/0.12 µg/mL for both inhibitor concentrations) inhibited all 27 isolates (100.0%) at ≤0.5 µg/mL and ≤0.25 µg/mL when the inhibitor was tested at fixed 4 and 8 µg/mL, respectively (**Table 2**). Meropenem alone ($MIC_{50/90}$, 4/64 µg/mL) inhibited only 37.0% of the isolates at the current CLSI susceptible breakpoint for this carbapenem.
- *A. baumannii* isolates producing carbapenem-hydrolyzing oxacillinases (n=55) displayed elevated MIC results for meropenem alone ($MIC_{50/90}$, 64/>64 µg/mL; **Table 2**). Meropenem + WCK 4234 MIC values ranged from 0.25 to 64 µg/mL, and 92.7 and 98.2% of the isolates were inhibited at ≤8 µg/mL of meropenem combined with WCK 4234 at fixed 4 ($MIC_{50/90}$, 4/8 µg/mL) and 8 µg/mL ($MIC_{50/90}$, 2/4 µg/mL), respectively.
- The activity of meropenem + WCK 4234 was four-fold greater for *A. baumannii* isolates producing OXA-23 ($MIC_{50/90}$, 2/4 µg/mL for both inhibitor concentrations; **Table 2**) when compared to isolates producing OXA-24 ($MIC_{50/90}$, 8/16 µg/mL for WCK 4234 at fixed 4 µg/mL and 4/8 µg/mL for inhibitor at 8 µg/mL) enzymes.
- Modal MIC values for WCK 5999 against OXA-23-producers (without other enzymes; 4 and 2 µg/mL for fixed 4 µg/mL and 8 µg/mL of inhibitor, respectively) were two-fold lower when compared to isolates producing OXA-24 (without other enzymes; 8 and 4 µg/mL for fixed 4 µg/mL and 8 µg/mL of inhibitor, respectively).
- Against a limited collection of OXA-producing *P. aeruginosa* isolates (n=7), meropenem + WCK 4234 MIC results ranged from 2 to 32 µg/mL (both inhibitor concentrations; **Table 2**) and these values were similar to those of meropenem alone. One OXA-14-producing strain had a four-fold decrease in the meropenem MIC when tested in the presence of WCK 4234.
- WCK 4234 MIC values were >16 µg/mL for all isolates tested (data not shown).

Table 1. Summary of the oxacillinase-producing isolates tested.

Organism (no. tested) Oxacillinase	Category: OXA carbapenemase	Category: OXA ESBL/NS
Enterobacteriaceae (56)	27	29
OXA-1/30		15
OXA-10-like		5
OXA-18/45-like		1
OXA-2-like		7
OXA-35/-101		1
OXA-48, OXA-1/30	2	
OXA-48	17	
OXA-162	1	
OXA-163	1	
OXA-181	3	
OXA-181, OXA-1/30	2	
OXA-232	1	
***Acinetobacter baumannii* (55)**	55	
OXA-23	32	
OXA-23, OXA-24	1	
OXA-23, OXA-24, OXA-58	1	
OXA-24	17	
OXA-58	4	
***Pseudomonas aeruginosa* (7)**		7
OXA-10		2
OXA-14		2
OXA-17		1
OXA-2		1
OXA-226		1

ESBL, extended-spectrum beta-lactamase; NS, narrow spectrum.

Figure 1. Cumulative distribution of meropenem + WCK 4234 (WCK 4234 at fixed 4 and 8 µg/mL) and meropenem values tested against all 118 OXA-producing Gram-negative isolates.

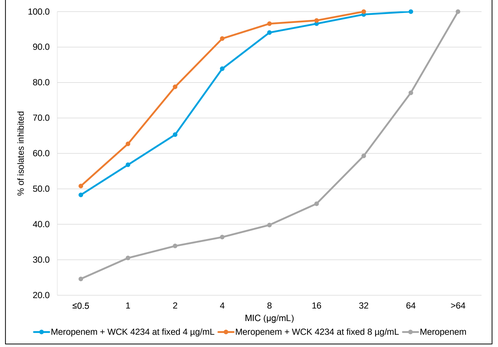

Table 2. Cumulative frequency distribution of MIC results for meropenem + WCK 4234 (WCK 4234 at fixed 4 and 8 µg/mL) and meropenem when tested against OXA-producing Gram-negative organisms.

Organism/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	>a	MIC_{50}	MIC_{90}
Enterobacteriaceae (56)															
Meropenem + WCK 4234 at fixed 4 µg/mL	39 (69.6)	10 (87.5)	5 (96.4)	1 (98.2)	1 (100.0)	--	--	--	--	--	--	--	--	≤0.03	0.12
Meropenem + WCK 4234 at fixed 8 µg/mL	42 (75.0)	8 (89.3)	5 (98.2)	1 (100.0)	--	--	--	--	--	--	--	--	--	≤0.03	0.12
Meropenem	--	--	--	--	29 (51.8)	7 (64.3)	4 (71.4)	3 (76.8)	1 (78.6)	4 (85.7)	5 (94.6)	3 (100.0)	--	≤0.5	32
OXA ESBL/NS Enterobacteriaceae (29)															
Meropenem + WCK 4234 at fixed 4 µg/mL	23 (79.3)	4 (93.1)	2 (100.0)	--	--	--	--	--	--	--	--	--	--	≤0.03	0.06
Meropenem + WCK 4234 at fixed 8 µg/mL	25 (86.2)	2 (93.1)	2 (100.0)	--	--	--	--	--	--	--	--	--	--	≤0.03	0.06
Meropenem	--	--	--	--	25 (86.2)	1 (89.7)	2 (96.6)	1 (100.0)	--	--	--	--	--	≤0.5	2
OXA carbapenemase Enterobacteriaceae (27)															
Meropenem + WCK 4234 at fixed 4 µg/mL	16 (59.3)	6 (81.5)	3 (92.6)	1 (96.3)	1 (100.0)	--	--	--	--	--	--	--	--	≤0.03	0.12
Meropenem + WCK 4234 at fixed 8 µg/mL	17 (63.0)	6 (85.2)	3 (96.3)	1 (100.0)	--	--	--	--	--	--	--	--	--	≤0.03	0.12
Meropenem	--	--	--	--	4 (14.8)	6 (37.0)	2 (44.4)	2 (51.9)	1 (55.6)	4 (70.4)	5 (88.9)	3 (100.0)	--	4	64
***Acinetobacter baumannii* (55)**															
Meropenem + WCK 4234 at fixed 4 µg/mL	--	--	--	--	1 (1.8)	10 (20.0)	10 (38.2)	19 (72.7)	11 (92.7)	2 (96.4)	1 (98.2)	1 (100.0)	--	4	8
Meropenem + WCK 4234 at fixed 8 µg/mL	--	--	--	1 (1.8)	3 (7.3)	14 (32.7)	18 (65.5)	14 (90.9)	4 (98.2)	0 (98.2)	1 (100.0)	--	--	2	4
Meropenem	--	--	--	--	--	--	--	--	--	2 (3.6)	8 (18.2)	18 (50.9)	27 (100.0)	64	>64
OXA-23-producing *A. baumannii* (32)															
Meropenem + WCK 4234 at fixed 4 µg/mL	--	--	--	--	1 (3.1)	8 (28.1)	9 (56.2)	11 (90.6)	2 (96.9)	0 (96.9)	1 (100.0)	--	--	2	4
Meropenem + WCK 4234 at fixed 8 µg/mL	--	--	--	1 (3.1)	3 (12.5)	10 (43.8)	13 (84.4)	4 (96.9)	1 (100.0)	--	--	--	--	2	4
Meropenem	--	--	--	--	--	--	--	--	--	--	6 (18.8)	18 (75.0)	8 (100.0)	64	>64
OXA-24-producing *A. baumannii* (17)															
Meropenem + WCK 4234 at fixed 4 µg/mL	--	--	--	--	--	1 (5.9)	0 (5.9)	4 (29.4)	9 (82.4)	2 (94.1)	0 (94.1)	1 (100.0)	--	8	16
Meropenem + WCK 4234 at fixed 8 µg/mL	--	--	--	--	--	2 (11.8)	3 (29.4)	8 (76.5)	3 (94.1)	0 (94.1)	1 (100.0)	--	--	4	8
Meropenem	--	--	--	--	--	--	--	--	--	--	--	--	17 (100.0)	>64	>64
***Pseudomonas aeruginosa* (7)**															
Meropenem + WCK 4234 at fixed 4 µg/mL	--	--	--	--	--	--	--	3 (42.9)	1 (57.1)	1 (71.4)	2 (100.0)	--	--	8	--
Meropenem + WCK 4234 at fixed 8 µg/mL	--	--	--	--	--	--	1 (14.3)	2 (42.9)	1 (57.1)	1 (71.4)	2 (100.0)	--	--	8	--
Meropenem	--	--	--	--	--	--	--	--	3 (42.9)	1 (57.1)	3 (100.0)	--	--	16	--

a. MIC values are greater than the highest dilution tested.

Conclusions

- **Meropenem + WCK 4234 was very active against Enterobacteriaceae isolates producing oxacillinases with a broad range of hydrolytic profiles that included enzymes with penicillinase, cephalosporinase and carbapenemase activities. Individual MIC results for meropenem + WCK 4234 were at least 16-fold and up to 1024-fold lower than MIC results for meropenem alone against these OXA-producing Enterobacteriaceae isolates.**
- **Meropenem + WCK 4234 was active against *Acinetobacter* spp. isolates carrying *bla*$_{OXA-23}$, that is the most prevalent carbapenem-hydrolyzing oxacillinase detected in these organisms. Meropenem + WCK 4234 activity was relatively modest for OXA-24-producing *Acinetobacter* spp. isolates.**
- **Combinations of meropenem + WCK 4234 were only marginally more active than meropenem alone against a small set of OXA-producing *P. aeruginosa*. Generally, oxacillinases detected among *P. aeruginosa* isolates are usually not carbapenemases and therefore it is likely that meropenem resistance in these pathogens could be associated with non-enzymatic resistance mechanisms such as oprD loss and/or efflux.**
- **OXA-producing isolates are widespread and β-lactamase inhibitors currently available for clinical use display variable activity against these enzymes. Thus, the further development of meropenem + WCK4234 is warranted.**

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References

1. Bethel CR, Papp-Wallace KM, Bonomo RA (2016). Novel Bridged Diazabicyclooctanes (DBOs) are Effective Inhibitors of Representative Class A, C, and D β-Lactamases Expressed by Multidrug Resistant (MDR) Pathogens. Abstr. *ASM MICROBE June 16-20, 2016*, Boston, MA, USA.
2. Castanheira M, Farrell SE, Deshpande LM, Mendes RE, Jones RN (2013). Prevalence of β-lactamase encoding genes among Enterobacteriaceae bacteremia isolates collected in 26 USA hospitals: Report from the SENTRY Antimicrobial Surveillance Program (2010). *Antimicrob Agents Chemother* 57: 3012-3020.
3. Castanheira M, Costello SE, Woosley LN, Deshpande LM, Davies TA, Jones RN (2014). Evaluation of clonality and carbapenem resistance mechanisms among *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex and *Enterobacteriaceae* isolates collected in European and Mediterranean countries and detection of two novel β-lactamases, GES-22 and VIM-35. *Antimicrob Agents Chemother* 58: 7358-7366.
4. Clinical and Laboratory Standards Institute (2015). *M07-A10. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-tenth edition*. Wayne, PA: CLSI.
5. Clinical and Laboratory Standards Institute (2016). *M100-S26. Performance standards for antimicrobial susceptibility testing: 26th informational supplement.* Wayne, PA: CLSI.
6. Evans BA, Amyes SG (2014). OXA beta-lactamases. *Clin Microbiol Rev* 27: 241-263.
7. Nguyen NQ, Papp-Wallace KM, Bonomo RA, Van Den Akker F (2016). Crystallographic Analyses Of Inhibition Of Klebsiella β-lactamase KPC-2 By Novel Bridged Diazabicyclooctane (DBO) β-lactamase Inhibitors. Abstr. *ASM MICROBE June 16-20, 2016*, Boston, MA, USA.
8. Poirel L, Castanheira M, Carrer A, Rodriguez CP, Jones RN, Smayevsky J, Nordmann P (2011). OXA-163, an OXA-48-related class D (beta)-lactamase with extended activity toward expanded-spectrum cephalosporins. *Antimicrob Agents Chemother* 55: 2546-2551.