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In Vitro Activity of WCK 5999 against *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates from a Worldwide Surveillance Program (2015)

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Amended Abstract*

Background: WCK 5999 is a novel carbapenem/β-lactamase inhibitor combination comprising meropenem (MEM) and WCK 4234, a broader-spectrum β-lactamase inhibitor with enhanced activity against Class D carbapenemases. The *in vitro* activity of MEM-WCK 4234 using both fixed 4 (F4) and fixed 8 (F8) μg/mL of WCK 4234 was evaluated against *A. baumannii* (ACB) and *P. aeruginosa* (PSA) isolates collected during 2015 from the SENTRY worldwide surveillance program and also included PSA resistant isolate subgroups.

Methods: MIC values for MEM-WCK 4234 (F4 and F8) and comparator compounds were determined using reference broth microdilution methodology. Breakpoint interpretative criteria followed CLSI guidelines.

Results: MEM-WCK 4234 (F4 and F8) was active against ACB isolates (MIC_{50}/MIC_{90} values of 2/16 and 2/8 μg/mL, respectively; **Table**). The addition of WCK 4234 to MEM significantly increased the % of ACB isolates with MEM-WCK 4234 MICs ≤4 μg/mL (based on 2g TID MEM dose) from 37.2% (MEM alone) to 75.3% (F4) and 83.9% (F8). Against PSA, the % of isolates with MEM-WCK 4234 MICs ≤4 μg/mL increased from 76.0% (MEM alone) to 85.2% (F4) and 85.5% (F8). MEM-WCK 4234 MIC_{50} values (F4 and F8) against PSA were also two-fold lower than MEM. MEM-WCK 4234 (F4 and F8) combinations demonstrated enhanced activity against resistant PSA subgroups including ceftazidime (CAZ) non-S (48.9-50.2% S), MDR (41.8-43.4% S), and XDR (29.4-31.1% S) isolates whereas comparator agent S ranged 0.0-30.6%.

Conclusions: WCK 5999 was very active against ACB isolates and demonstrated enhanced activity over MEM alone against PSA, most notably against CAZ NS, MDR, and XDR PSA isolates. These data support the continued development of this promising antibacterial combination.

Organism / Phenotype (n)	MIC_{50}/MIC_{90} μg/mL (%Susceptible[a]) MEM-WCK 4234 (F4)	MEM-WCK 4234 (F8)	CAZ	MEM	P/T
ACB (639)	2/16 (75.3%)[b]	2/8 (83.9%)[b]	>32/>32 (30.6%)	32/>32 (37.2%)	>64/>64 (28.5%)
PSA (1,291)	0.25/16 (85.2%)[b]	0.25/16 (85.5%)[b]	2/32 (81.7%)	0.5/16 (76.0%)	4/64 (79.0%)
PSA / CAZ NS (235)	8/>32 (48.9%)[b]	4/>32 (50.2%)[b]	32/>32 (0.0%)	16/>32 (30.6%)	64/>64 (7.2%)
PSA / MDR (251)	8/>32 (41.8%)[b]	8/>32 (43.4%)[b]	32/>32 (23.1%)	16/>32 (15.9%)	64/>64 (16.7%)
PSA / XDR (170)	16/>32 (29.4%)[b]	16/>32 (31.2%)[b]	32/>32 (13.5%)	16/>32 (9.4%)	64/>64 (5.3%)

a. According to CLSI breakpoints
b. % inhibited at ≤ 4 μg/mL based on 2 g TID MEM dose

*Abstract has been updated with additional isolates after its submission.

Introduction

Over the past decade, Gram-negative infections have been increasing in prevalence worldwide, along with antimicrobial resistance; and there have been associated increases in morbidity and mortality. Empirical and targeted therapies to treat infections with these organisms are becoming increasingly limited. WCK 5999 represents a new carbapenem/β-lactamase inhibitor combination in clinical development comprising meropenem and the novel broader-spectrum β-lactamase inhibitor, WCK 4234, with enhanced activity against Class D carbapenemases.

According to the current CLSI breakpoint criteria for *Pseudomonas aeruginosa* and *Acinetobacter* spp. published in the CLSI M100-S26 document, meropenem susceptible, intermediate and resistant breakpoints are ≤2, 4 and ≥8 μg/mL, respectively. In this study, we evaluated the *in vitro* antibacterial activity of meropenem combined with WCK 4234 using both fixed 4 and fixed 8 μg/mL against a collection of 1,291 contemporary (2015) *P. aeruginosa* and 639 *Acinetobacter baumannii calcoaceticus* species complex (*A. baumannii*) isolates obtained from 134 medical centers located in 32 countries as part of the SENTRY worldwide surveillance program. The *P. aeruginosa* isolates were further subdivided in to groups based on resistance phenotypes including ceftazidime-non-susceptible, meropenem-non-susceptible, multidrug-resistant (MDR) and extensively drug-resistant.

Methods

Susceptibility testing: Minimum inhibitory concentration (MIC) values were determined for meropenem-WCK 4234 combinations (fixed 4 and 8 μg/mL) and comparator agents using the Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution method (M07-A10). Quality control (QC) reference strains were tested daily and the inoculum density monitored by colony counts. QC ranges and interpretive criteria for comparator compounds were as published in CLSI M100-S26 and EUCAST v6.0 (2016) documents. The tested QC reference strains included the following: *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *K. pneumoniae* ATCC BAA-1705 and *P. aeruginosa* ATCC 27853.

Organism collection: *P. aeruginosa* (1,291) and *A. baumannii* (639) isolates were selected for testing from the 2015 SENTRY worldwide surveillance program. These isolates were collected during 2015 from 134 medical institutions worldwide, including Europe (EU; 38 medical centers), United States (USA; 64), Latin America (LA; eight) and the Asia-West Pacific (APAC) region (excluding China, 14) and China (10). The *P. aeruginosa* isolates were further subdivided into ceftazidime-non-susceptible (n=235), meropenem-non-susceptible (n=310), MDR (n=251) and XDR (N=170) groups for additional analysis.

All isolates were obtained from documented infections and only one isolate per patient-infection episode was included in the surveillance collection. Species identifications were confirmed by Matrix-Assisted Laser Desorption Time of Flight Mass Spectrometry (MALDI-TOF MS), using the Bruker Daltonics MALDI Biotyper (Billerica, MA, USA).

Resistant subsets: Ceftazidime- and meropenem-non-susceptibility are defined as an MIC value of ≥16 and ≥4 μg/mL (based on a 2g TID meropenem dose), respectively (CLSI). Isolates were further categorized as multidrug-resistant (MDR) and extensively drug-resistant (XDR) according to criteria published by Magiorakos *et al.* (2012): i.e., MDR = non-susceptible to ≥1 agent in ≥3 antimicrobial classes, and XDR = non-susceptible to ≥1 agent in all but ≤2 antimicrobial classes. Class representatives used in the analysis were: ceftazidime, meropenem, piperacillin-tazobactam, levofloxacin, gentamicin and colistin (six classes), for *P. aeruginosa*.

Results

- Meropenem-WCK 4234 combinations (fixed 4 and 8 μg/mL) were very active against 639 *A. baumannii* isolates with $MIC_{50/90}$ values of 2/16 and 2/8 μg/mL, respectively, compared to meropenem tested alone ($MIC_{50/90}$, 32/>32 μg/mL; **Table 1** and **Figure 2**).
- With the exception of colistin ($MIC_{50/90}$, ≤0.5/1 μg/mL; 99.4% susceptible), meropenem-WCK 4234 (fixed 4 and 8 μg/mL) was the most potent antibacterial tested against 639 *A. baumannii* isolates, inhibiting 75.3 and 83.9% of isolates, respectively, at an MIC of ≤4 μg/mL (based on a 2g TID meropenem dose; **Tables 1** and **2**). Susceptibility to meropenem tested alone was low (37.2% susceptible) as was susceptibility to amikacin (44.4/41.5% susceptible [CLSI/EUCAST]), gentamicin (39.7/39.7% susceptible) and levofloxacin (31.8/30.8% susceptible).
- Against 1,291 *P. aeruginosa,* meropenem-WCK 4234 (fixed 4 and 8 μg/mL) was consistently more active ($MIC_{50/90}$, 0.25/16 μg/mL; 85.2-85.5% inhibited at ≤4 μg/mL) than meropenem alone ($MIC_{50/90}$, 0.5/16 μg/mL; 76.0% susceptible) at each of the drug dilutions tested (**Table 1**).
- The improved *in vitro* activity of meropenem-WCK 4234 combinations (fixed 4 and 8 μg/mL) relative to meropenem alone against *P. aeruginosa* isolates was also evident against ceftazidime-non-susceptible (48.9-50.2% inhibited at ≤4 μg/mL), meropenem-non-susceptible (38.4-39.7% inhibited at ≤4 μg/mL), MDR (41.8-43.4% inhibited at ≤4 μg/mL) and XDR strains (29.4-31.1% inhibited at ≤4 μg/mL; **Tables 1** and **2** and **Figure 3**).
- Only amikacin (39.4-73.5% susceptible) and colistin (100.0% susceptible) were more active than meropenem-WCK 4234 (fixed 4 and 8 μg/mL) combinations (29.4-50.2% inhibited at ≤4 μg/mL) against the resistant *P. aeruginosa* subgroups tested. Cefepime, gentamicin, levofloxacin and piperacillin-tazobactam susceptibilities were lower against these resistant organism groups and ranged 15.3-46.5%, 23.5-54.7%, 5.3-38.2% and 5.3-40.5%, respectively.

Figure 1. Compound structure of WCK 4234.

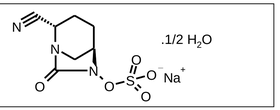


Figure 2. Comparison of meropenem and meropenem-WCK 4234 (fixed 4 and 8 μg/mL) cumulative % inhibition distributions against 639 *Acinetobacter baumannii* isolates collected during 2015.

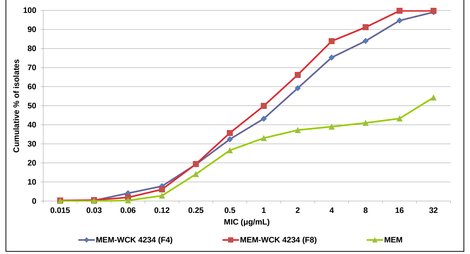


Table 1. Activity and cumulative % distributions for meropenem and meropenem-WCK 4234 combinations against *A. baumannii* and *P. aeruginosa* isolates including ceftazidime-non-susceptible, meropenem-non-susceptible MDR and XDR resistant strains from a worldwide surveillance program during 2015.

Organism (no.)[a]	Cumulative % inhibited at MIC (μg/mL) of: ≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	$MIC_{50/90}$ (μg/mL)
***A. baumannii* (639)**													
Meropenem-WCK 4234 (F4)[a]	0.2	0.3	4.1	7.8	19.2	32.4	43.2	59.2	75.3	84.0	94.7	99.1	2/16
Meropenem-WCK 4234 (F8)[a]	0.3	0.5	1.9	6.1	19.4	35.7	49.9	66.2	83.9	91.2	98.3	99.8	2/8
Meropenem	-	0.0	0.3	2.8	14.1	26.6	33.0	37.2	39.0	41.0	43.3	54.3	32/>32
***P. aeruginosa* (1,291)**													
Meropenem-WCK 4234 (F4)	3.9	6.7	17.1	33.5	52.3	66.2	73.7	80.4	85.2	89.2	95.5	97.0	0.25/16
Meropenem-WCK 4234 (F8)	4.5	7.2	16.5	34.4	53.8	66.7	73.5	79.6	85.5	89.2	94.7	97.1	0.25/16
Meropenem	0.4	1.5	8.7	19.8	40.0	58.0	70.5	76.0	81.9	86.4	93.5	96.1	0.5/16
ceftazidime non-susceptible (235)													
Meropenem-WCK 4234 (F4)	2.6	3.8	7.2	13.2	19.6	27.7	31.9	40.0	48.9	58.7	78.3	83.8	8/>32
Meropenem-WCK 4234 (F8)	3.0	5.1	8.1	13.2	20.0	28.5	33.2	40.9	50.2	60.4	75.7	84.7	4/>32
Meropenem	-	0.0	0.9	3.0	6.4	16.6	26.0	30.6	38.7	48.5	69.4	80.9	16/>32
meropenem non-susceptible (310)													
Meropenem-WCK 4234 (F4)	-	0.0	0.3	0.6	1.0	2.6	3.2	18.4	38.4	55.2	81.3	87.4	8/>32
Meropenem-WCK 4234 (F8)	0.3	0.3	0.6	1.0	2.3	2.9	3.9	20.0	39.7	55.2	77.7	88.1	8/>32
Meropenem	-	-	-	-	-	-	-	0.0	24.5	43.2	72.9	83.9	16/>32
MDR (251)													
Meropenem-WCK 4234 (F4)	1.6	1.6	3.2	4.8	9.6	14.3	17.9	27.1	41.8	55.8	77.3	84.5	8/>32
Meropenem-WCK 4234 (F8)	2.0	3.2	4.0	5.6	10.0	15.5	18.7	29.1	43.4	56.6	77.7	89.2	8/>32
Meropenem	-	0.0	0.4	0.8	2.8	6.8	11.6	15.9	27.1	41.8	66.9	80.1	16/>32
XDR (170)													
Meropenem-WCK 4234 (F4)	0.6	0.6	1.2	2.4	5.3	8.8	12.9	19.4	29.4	44.7	70.0	78.2	16/>32
Meropenem-WCK 4234 (F8)	1.2	2.4	2.4	2.9	5.9	10.6	12.9	21.2	31.1	46.4	66.5	79.4	16/>32
Meropenem	-	-	0.0	0.6	1.2	3.5	5.8	9.4	17.1	27.6	55.9	71.8	16/>32

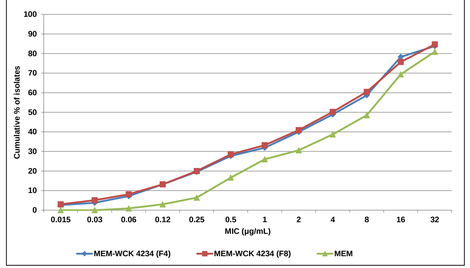
a. F4 = fixed 4 μg/mL and F8 = fixed 8 μg/mL

Table 2. Activity of meropenem-WCK 4234 combinations and comparator antimicrobials tested against *A. baumannii* and *P. aeruginosa* isolates including ceftazidime-non-susceptible, meropenem-non-susceptible, MDR and XDR strains from a worldwide surveillance program during 2015.

Organisms (no. tested) antimicrobial agent	MIC (μg/mL) MIC_{50}	MIC_{90}	%S / %I / %R CLSI[a]	EUCAST[a]	Organisms (no. tested) antimicrobial agent	MIC (μg/mL) MIC_{50}	MIC_{90}	%S / %I / %R CLSI[a]	EUCAST[a]
***A. baumannii* (639)**					**meropenem-non-susceptible (310)**				
Meropenem-WCK 4234 (F4)[b]	2	16	- / - / -	- / - / -	Meropenem-WCK 4234 (F4)	8	>32	- / - / -	- / - / -
Meropenem-WCK 4234 (F8)[b]	2	8	- / - / -	- / - / -	Meropenem-WCK 4234 (F8)	8	>32	- / - / -	- / - / -
Meropenem	32	>32	37.2 / 1.7 / 61.0	37.2 / 3.8 / 59.0	Meropenem	16	>32	0.0 / 24.5 / 75.5	0.0 / 43.2 / 56.8
Imipenem	>8	>8	39.5 / 2.1 / 58.5	39.5 / 3.3 / 57.2	Imipenem	8	>8	7.8 / 10.4 / 81.9	18.1 / 40.8 / 41.1
Ceftazidime	>32	>32	30.6 / 3.9 / 65.4	- / - / -	Ceftazidime	16	>32	47.2 / 12.3 / 40.5	47.2 / - / 52.8
Cefepime	64	>64	29.6 / 5.9 / 64.5[c]	- / - / -	Cefepime	16	64	46.5 / 17.7 / 35.8	46.5 / - / 53.5
Piperacillin-tazobactam	>64	>64	28.5 / 6.2 / 65.3	- / - / -	Piperacillin-tazobactam	32	>64	40.5/ 32.0 / 27.5	40.5 / - / 59.5
Amikacin	>32	>32	44.4 / 3.9 / 51.7	41.5 / 2.8 / 55.6	Amikacin	8	>32	73.5 / 5.8 / 20.7	62.8 / 10.7 / 26.5
Gentamicin	>8	>8	39.7 / 3.3 / 57.0	39.7 / - / 60.3	Gentamicin	4	>8	54.7 / 8.7 / 36.6	54.7 / - / 45.3
Levofloxacin	>4	>4	31.8 / 6.2 / 62.1	30.8 / 0.9 / 68.2	Levofloxacin	>4	>4	38.2 / 10.4 / 51.5	19.4 / 18.8 / 61.8
Colistin	≤0.5	1	94.8 / - / 5.2	94.8 / - / 5.2	Colistin	1	1	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
***P. aeruginosa* (1,291)**					**MDR (251)**				
Meropenem-WCK 4234 (F4)	0.25	16	- / - / -	- / - / -	Meropenem-WCK 4234 (F4)	8	>32	- / - / -	- / - / -
Meropenem-WCK 4234 (F8)	0.25	16	- / - / -	- / - / -	Meropenem-WCK 4234 (F8)	8	>32	- / - / -	- / - / -
Meropenem	0.5	16	76.0 / 5.9 / 18.1	76.0 / 10.4 / 13.6	Meropenem	16	>32	15.9 / 11.2 / 72.9	15.9 / 25.9 / 58.2
Imipenem	1	>8	74.5 / 4.3 / 21.2	78.8 / 10.9 / 10.2	Imipenem	8	>8	19.9 / 7.2 / 72.9	27.1 / 27.9 / 45.0
Ceftazidime	2	32	81.7 / 4.7 / 13.6	81.7 / - / 18.3	Ceftazidime	32	>32	23.1 / 17.9 / 59.0	23.1 / - / 76.9
Cefepime	2	32	81.6 / 8.2 / 10.1[c]	81.6 / - / 18.4	Cefepime	16	>64	21.1 / 29.5 / 49.4[c]	21.1 / - / 78.9
Piperacillin-tazobactam	4	64	79.0 / 11.4 / 9.6	79.0 / - / 21.0	Piperacillin-tazobactam	64	>64	16.7 / 42.6 / 40.6	16.7 / - / 83.3
Amikacin	4	16	92.2 / 1.8 / 6.1	87.1 / 5.0 / 7.8	Amikacin	8	>32	65.3 / 7.2 / 27.5	50.6 / 14.7 / 34.7
Gentamicin	2	>8	84.4 / 4.0 / 11.6	84.4 / - / 15.6	Gentamicin	8	>8	36.7 / 13.9 / 49.4	36.7 / - / 63.3
Levofloxacin	0.5	>4	74.6 / 6.1 / 19.3	64.5 / 10.1 / 24.4	Levofloxacin	>4	>4	19.1 / 13.5 / 67.3	8.0 / 11.2 / 80.9
Colistin	≤0.5	1	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0	Colistin	1	1	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0
ceftazidime-non-susceptible (235)					**XDR (170)**				
Meropenem-WCK 4234 (F4)	8	>32	- / - / -	- / - / -	Meropenem-WCK 4234 (F4)	16	>32	- / - / -	- / - / -
Meropenem-WCK 4234 (F8)	4	>32	- / - / -	- / - / -	Meropenem-WCK 4234 (F8)	16	>32	- / - / -	- / - / -
Meropenem	16	>32	30.6 / 8.1 / 61.3	30.6 / 17.9 / 51.5	Meropenem	16	>32	9.4 / 7.6 / 82.9	9.4 / 18.2 / 72.4
Imipenem	8	>8	33.2 / 5.1 / 61.7	38.3 / 21.3 / 40.4	Imipenem	>8	>8	14.1 / 4.1 / 81.8	18.2 / 27.1 / 54.7
Ceftazidime	32	>32	0.0 / 25.5 / 74.5	0.0 / 0.0 / 100.0	Ceftazidime	32	>32	13.5 / 20.0 / 66.5	13.5 / - / 86.5
Cefepime	32	>64	18.3 / 30.6 / 51.1[c]	18.3 / - / 81.7	Cefepime	32	>64	15.3 / 24.7 / 60.0[c]	15.3 / - / 84.7
Piperacillin-tazobactam	64	>64	7.2 / 43.4 / 49.4	7.2 / - / 92.8	Piperacillin-tazobactam	64	>64	5.3 / 51.8 / 42.9	5.3 / - / 94.7
Amikacin	8	>32	68.9 / 6.0 / 25.1	59.1 / 9.8 / 31.1	Amikacin	16	>32	52.4 / 9.4 / 38.2	39.4 / 12.9 / 47.6
Gentamicin	4	>8	51.5 / 7.2 / 41.3	51.5 / - / 48.5	Gentamicin	>8	>8	23.5 / 14.1 / 62.4	23.5 / - / 76.5
Levofloxacin	>4	>4	34.9 / 9.8 / 55.3	23.0 / 11.9 / 65.1	Levofloxacin	>4	>4	5.3 / 51.8 / 42.9	5.3 / - / 94.7
Colistin	1	1	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0	Colistin	1	1	100.0 / 0.0 / 0.0	100.0 / 0.0 / 0.0

a. Criteria as published by the CLSI [2016] and EUCAST [2016] and "-" = no breakpoint available for interpretation.
b. F4 = fixed 4 μg/mL and F8 = fixed 8 μg/mL.
c. Intermediate interpreted as susceptible-dose dependent.

Figure 3. Comparison of meropenem and meropenem-WCK 4234 (fixed 4 and 8 μg/mL) cumulative % inhibition distributions against 235 ceftazidime-non-susceptible *P. aeruginosa* isolates collected during 2015.



Conclusions

- **Meropenem-WCK 4234 (WCK 5999) is a new antibacterial combination with notable *in vitro* activity over meropenem and other comparator agents against *A. baumannii* isolates. Against this important organism group, only colistin was more active than WCK 5999.**
- **Meropenem-WCK 4234 (fixed 4 and 8 μg/mL) combinations demonstrated enhanced *in vitro* activity over meropenem alone against *P. aeruginosa* isolates. The improved activity of WCK 5999 over meropenem alone was also observed against ceftazidime-non-susceptible, meropenem-non-susceptible, MDR and XDR *P. aeruginosa* organism groups.**
- **WCK 5999 may represent a valuable option for the treatment of Gram-negative infections, including those caused by various resistant organism groups. These data support the continued clinical development of this promising new antibacterial combination.**

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