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In Vitro Antibacterial Activity of WCK 5999: A New Carbapenem/β-lactamase Inhibitor Combination against ESBL-Phenotype and Carbapenemase Producing Enterobacteriaceae from a Worldwide Surveillance Program (2015)

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Amended Abstract*

Background: WCK 5999 is a new carbapenem/β-lactamase inhibitor combination comprising meropenem (MEM) and the novel broader-spectrum β-lactamase inhibitor, WCK 4234, with enhanced activity against Class D carbapenemases. The *in vitro* antibacterial activity of MEM-WCK 4234 using both fixed 4 (F4) and fixed 8 (F8) µg/mL of WCK 4234 was evaluated against Enterobacteriaceae (ENT) subgroups expressing resistance to β-lactams including ESBL-phenotype, ceftazidime (CAZ) non-susceptible (NS), MEM NS and carbapenem-resistant ENT (CRE).

Methods: MEM-WCK 4234 (F4 and F8) and comparator compound MIC values were determined using a reference broth microdilution method against ENT subgroups collected during a 2015 worldwide surveillance program.

Results: MEM-WCK 4234 (F4 and F8) displayed potent activity (MIC_{50}/MIC_{90} values of ≤0.03/≤0.25 µg/mL) against 1,142 ENT isolates displaying either an ESBL-phenotype or CAZ NS (**Table**). The MEM-WCK 4234 MIC_{50} (F4 and F8) against MEM NS *K. pneumoniae* (KPN) and CRE was 0.12 µg/mL compared to 32 µg/mL for MEM alone. Applying CLSI breakpoint interpretive criteria, S rates against ESBL-phenotype *E. coli* (EC) and KPN ranged 6.7-29.2% for CAZ, 68.1-98.6% for MEM and 35.0-83.7% for piperacillin-tazobactam (P/T). CAZ and P/T S rates against MEM NS KPN and CRE were very low (0.8-2.6%) whereas MEM-WCK 4234 (F4 and F8) combinations retained activity (81.7-84.3% S) with MIC_{50} values of 0.12 µg/mL.

Conclusions: WCK 5999 is a potent new antibacterial combination against ENT displaying an ESBL phenotype, CAZ NS, MEM NS and CRE. 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
EC / ESBL-phenotype (503)	≤0.015/0.03 (99.2%)[b]	≤0.015/0.03 (99.2%)[b]	16/>32 (29.2%)	0.03/0.06 (98.6%)	4/64 (83.7%)
KPN / ESBL-phenotype (417)	0.03/0.25 (94.7%)[b]	0.03/0.25 (95.0%)[b]	>32/>32 (6.7%)	0.06/>32 (68.1%)	64/>64 (35.0%)
Enterobacter spp. / CAZ NS (222)	0.03/0.03 (100%)[b]	≤0.015/0.03 (100%)[b]	>32/>32 (0.0%)	0.06/0.25 (92.8%)	64/>64 (30.6%)
KPN / MEM NS (134)	0.12/32 (83.6%)[b]	0.12/>32 (84.3%)[b]	>32/>32 (0.8%)	32/>32 (0.0%)	>64/>64 (2.3%)
ENT / CRE (153)	0.12/32 (81.7%)[b]	0.12/32 (82.4%)[b]	>32/>32 (2.0%)	32/>32 (2.0%)	>64/>64 (2.6%)

a. According to CLSI breakpoints.
b. % inhibited at ≤1 µg/mL MEM.

*Abstract has been updated with additional isolates tested 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 (**Figure 1**), with enhanced activity against Class D carbapenemases.

According to the current CLSI breakpoint criteria for Enterobacteriaceae published in the M100-S26 document, meropenem susceptible, intermediate and resistant breakpoints are ≤1, 2 and ≥4 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 1,456 contemporary (2015) Enterobacteriaceae obtained from 134 medical centers in 32 countries as part of a worldwide surveillance program. The Enterobacteriaceae were divided into subgroups based on resistance to β-lactams including ESBL-phenotype, ceftazidime-non-susceptible, meropenem-non-susceptible and carbapenem-resistant Enterobacteriaceae (CRE).

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) isolates 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, *E. coli* NCTC 13353, *K. pneumoniae* ATCC 700603 and *K. pneumoniae* ATCC BAA-1705.

Organism collection: Enterobacteriaceae isolates displaying an ESBL-phenotype (n=947), ceftazidime (n=222) or meropenem non-susceptibility (n=134) or carbapenem-resistance (n=153) were selected for testing as part of the 2015 SENTRY worldwide surveillance program. Isolates were collected from 134 medical institutions worldwide, including Europe (EU; 38 medical centers), United States (USA; 64), Latin America (LA; eight) and Asia-West Pacific (APAC) regions (excluding China, 14) and China (10).

All organisms were isolated 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: An ESBL-screen-positive phenotype was defined according to CLSI: i.e., a MIC of ≥2 µg/mL for ceftazidime and/or ceftriaxone and/or aztreonam. Ceftazidime and meropenem non-susceptibility was defined as an MIC value of ≥8 and ≥2 µg/mL (CLSI), respectively. Carbapenem-resistant Enterobacteriaceae (CRE) was defined as an MIC, ≥4 µg/mL (CLSI) to imipenem (excluding *P. mirabilis* and indole-positive Proteeae), meropenem or doripenem.

Results

- Meropenem-WCK 4234 combinations (fixed 4 and 8 µg/mL) were highly active and similar in activity against each of the resistant Enterobacteriaceae subgroups tested (**Tables 1** and **2**).
- With the exception of colistin ($MIC_{50/90}$, 0.12/0.25 µg/mL; 99.4% susceptible [EUCAST]), meropenem-WCK 4234 (fixed 4 and 8 µg/mL) was the most potent agent ($MIC_{50/90}$, ≤0.015/0.03 µg/mL) tested against 503 ESBL-phenotype *E. coli*, inhibiting 99.2% of isolates at the CLSI susceptibility breakpoint MIC of ≤1 µg/mL for meropenem (**Tables 1** and **2**).
- Meropenem-WCK 4234 combinations (fixed 4 and 8 µg/mL; **Tables 1** and **2**; **Figure 2**) were highly active against ESBL-phenotype *K. pneumoniae* isolates (94.7-95.0% inhibited at ≤1 µg/mL) and were 2- to ≥256-fold more active than meropenem alone ($MIC_{50/90}$, 0.06/>32 µg/mL; 68.1/71.9% susceptible [CLSI/EUCAST]) as well as ≥256-fold more active than ceftazidime ($MIC_{50/90}$, >32/>32 µg/mL; 6.7/1.9% susceptible), cefepime ($MIC_{50/90}$, 64/>64 µg/mL; 10.1/7.9% susceptible) and piperacillin-tazobactam ($MIC_{50/90}$, 64/>64 µg/mL; 35.0/25.2% susceptible; **Table 2**).
- Against meropenem non-susceptible *K. pneumoniae*, meropenem-WCK 4234 (fixed 4 and 8 µg/mL) was the most active agent tested ($MIC_{50/90}$, 0.12/≥32 µg/mL; 83.6-84.3% inhibited at ≤1 µg/mL, **Tables 1** and **2**) followed by colistin ($MIC_{50/90}$, 0.25/>8 µg/mL; 71.4% susceptible [EUCAST]), amikacin ($MIC_{50/90}$, 16/>32 µg/mL; 54.1/40.6% susceptible [CLSI/EUCAST]) and gentamicin ($MIC_{50/90}$, >8/>8 µg/mL; 39.8/39.1% susceptible[CLSI/EUCAST]). Susceptibilities to ceftazidime, cefepime and piperacillin-tazobactam ranged from 0.7-2.3% (CLSI) to 0.0-0.8% (EUCAST; **Table 2**).
- The highest meropenem-WCK 4234 (fixed 4 and 8 µg/mL) MIC against ESBL-phenotype *K. oxytoca* was 0.03 µg/mL (100% strains inhibited at 0.03 µg/mL) compared to 8 µg/mL for meropenem alone (**Table 1**). ESBL-phenotype *K. oxytoca* susceptibilities (CLSI/EUCAST) to ceftazidime, cefepime and piperacillin-tazobactam were 74.1/63.0%, 77.8/55.6% and 29.6/22.2%, respectively (**Table 2**).
- All (100.0%) of ceftazidime non-susceptible *Enterobacter* spp. isolates had meropenem-WCK 4234 (fixed 4 and 8 µg/mL) MIC values ≤1 µg/mL ($MIC_{50/90}$, ≤0.03/0.03 µg/mL) compared to 92.8% for meropenem alone (**Table 1**). β-lactam comparator compound susceptibilities (CLSI/EUCAST) were 60.8/48.6% for cefepime and 30.6/18.5% for piperacillin-tazobactam (**Table 2**).
- Meropenem-WCK-4234 (fixed 4 and 8 µg/mL) was the most active compound tested against a collection of 153 CRE isolates ($MIC_{50/90}$, 0.12/32 µg/mL; 81.7-82.4% inhibited at ≤1 µg/mL) followed by colistin ($MIC_{50/90}$, 0.12/>8 µg/mL; 71.7% susceptible [EUCAST]), amikacin ($MIC_{50/90}$, 16/>32 µg/mL; 59.2/47.4% susceptible [CLSI/EUCAST]) and gentamicin ($MIC_{50/90}$, 8/>8 µg/mL; 40.8/38.8% susceptible [CLSI/EUCAST]). Ceftazidime, cefepime and piperacillin-tazobactam susceptibilities were low against CRE and ranged from 2.0-2.6% (CLSI) to 1.3% (EUCAST; **Table 2**).

Figure 1. Compound structure of WCK 4234.

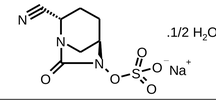


Table 1. Activity and cumulative % distribution for meropenem and meropenem-WCK 4234 combinations against ESBL-phenotype, ceftazidime-non-susceptible and carbapenemase producing Enterobacteriaceae from a worldwide surveillance program (2015).

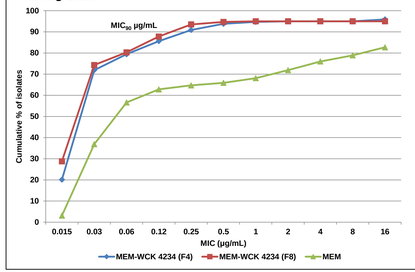
Organism (no. tested)	Cumulative % inhibited at MIC (µg/mL) of: ≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	$MIC_{50/90}$ (µg/mL)
E. coli													
ESBL-phenotype (503)													
Meropenem-WCK 4234 (F4)	78.1	98.4	99.2	99.2	99.2	99.2	99.2	99.2	99.2	99.2	100.0		≤0.015/0.03
Meropenem-WCK 4234 (F8)	86.9	98.4	98.6	98.8	99.2	99.2	99.2	99.2	99.2	99.2	100.0		≤0.015/0.03
Meropenem	35.8	89.9	96.6	97.8	98.2	98.2	98.6	98.6	98.8	99.2	99.6	100.0	0.03/0.06
K. pneumoniae													
ESBL-phenotype (417)													
Meropenem-WCK 4234 (F4)	20.1	71.9	79.4	85.6	90.9	93.8	94.7	95.0	95.0	95.0	95.9	97.1	0.03/0.25
Meropenem-WCK 4234 (F8)	28.8	74.3	80.3	87.8	93.5	94.7	95.0	95.0	95.0	95.0	95.0	96.6	0.03/0.25
Meropenem	3.1	36.9	56.6	62.8	64.7	65.9	68.1	71.9	76.0	78.9	82.7	85.9	0.06/>32
K. pneumoniae													
meropenem-non-susceptible (134)													
Meropenem-WCK 4234 (F4)	3.0	21.6	35.8	55.2	71.6	80.6	83.6	84.3	84.3	84.3	87.3	91.0	0.12/32
Meropenem-WCK 4234 (F8)	7.5	26.1	38.8	61.9	79.9	83.6	84.3	84.3	84.3	84.3	84.3	89.6	0.12/>32
Meropenem							0.0	11.9	24.6	33.6	46.3	56.0	32/>32
K. oxytoca													
ESBL-phenotype (27)													
Meropenem-WCK 4234 (F4)	33.3	100.0											0.03/0.03
Meropenem-WCK 4234 (F8)	74.1	100.0											≤0.015/0.03
Meropenem	11.1	74.1	88.9	92.6	92.6	96.3	96.3	96.3	96.3	100.0			0.03/0.12
***Enterobacter* spp.**													
ceftazidime-non-susceptible (222)													
Meropenem-WCK 4234 (F4)	49.5	95.0	96.8	97.7	99.1	99.5	100.0						0.03/0.03
Meropenem-WCK 4234 (F8)	52.3	94.6	96.8	98.2	99.5	99.5	100.0						≤0.015/0.03
Meropenem	0.9	30.2	72.5	86.9	90.5	91.9	92.8	94.1	97.7	98.2	100.0		0.06/0.25
Enterobacteriaceae													
carbapenem-resistant (153)													
Meropenem-WCK 4234 (F4)	6.5	25.5	36.6	52.9	68.6	77.8	81.7	83.0	83.0	83.0	88.2	91.5	0.12/32
Meropenem-WCK 4234 (F8)	11.8	28.1	37.3	58.2	76.5	81.0	82.4	83.0	83.0	83.0	85.6	90.2	0.12/32
Meropenem						0.0	2.0	4.6	21.6	32.7	49.0	58.8	32/>32

Table 2. Activity of meropenem-WCK 4234 combinations and comparator antimicrobials tested against ESBL-phenotype, ceftazidime-non-susceptible and carbapenemase producing Enterobacteriaceae from a worldwide surveillance program during 2015.

Organism (no. tested) / antimicrobial agent	MIC (µg/mL) MIC_{50}	MIC_{90}	%S / %I / %R CLSI[a]	EUCAST[a]
E. coli				
ESBL-phenotype (503)				
Meropenem-WCK 4234 (F4)[b]	≤0.015	0.03	-[b] / - / -	- / - / -
Meropenem-WCK 4234 (F8)[b]	≤0.015	0.03	- / - / -	- / - / -
Meropenem	0.03	0.06	98.6 / 0.0 / 1.4	98.6 / 0.6 / 0.8
Ceftazidime	16	>32	29.2 / 9.5 / 61.2	10.1 / 19.1 / 70.8
Cefepime	16	>64	24.7 / 18.3 / 57.1[c]	18.9 / 14.7 / 66.4
Piperacillin-tazobactam	4	64	83.7 / 7.4 / 8.9	76.5 / 7.2 / 16.3
Amikacin	4	8	97.4 / 1.6 / 1.0	92.0 / 5.4 / 2.6
Gentamicin	≤1	>8	59.2 / 1.0 / 39.8	59.0 / 0.2 / 40.8
Levofloxacin	>4	>4	29.2 / 2.8 / 68.0	28.0 / 1.2 / 70.8
Colistin	0.12	0.25	- / - / -	99.4 / - / 0.6
K. pneumoniae				
ESBL-phenotype (417)				
Meropenem-WCK 4234 (F4)	0.03	0.25	- / - / -	- / - / -
Meropenem-WCK 4234 (F8)	0.03	0.25	- / - / -	- / - / -
Meropenem	0.06	>32	68.1 / 3.8 / 28.1	71.9 / 7.0 / 21.1
Ceftazidime	>32	>32	6.7 / 4.3 / 89.0	1.9 / 4.8 / 93.3
Cefepime	64	>64	10.1 / 10.1 / 79.9[c]	7.9 / 6.0 / 86.1
Piperacillin-tazobactam	64	>64	35.0 / 17.7 / 47.2	25.2 / 9.8 / 65.0
Amikacin	4	>32	80.8 / 6.0 / 13.2	74.1 / 6.7 / 19.2
Gentamicin	>8	>8	40.5 / 2.6 / 56.8	39.1 / 1.4 / 59.5
Levofloxacin	>4	>4	31.5 / 4.8 / 63.7	27.6 / 3.8 / 68.5
Colistin	0.12	8	- / - / -	88.2 / - / 11.8
K. pneumoniae				
meropenem-non-susceptible (134)				
Meropenem-WCK 4234 (F4)	0.12	32	- / - / -	- / - / -
Meropenem-WCK 4234 (F8)	0.12	>32	- / - / -	- / - / -
Meropenem	32	>32	0.0 / 11.9 / 88.1	11.9 / 21.6 / 66.4
Ceftazidime	>32	>32	0.8 / 0.8 / 98.5	0.0 / 0.8 / 99.2
Cefepime	>64	>64	0.7 / 3.7 / 95.5[c]	0.0 / 2.2 / 97.8
Piperacillin-tazobactam	>64	>64	2.3 / 3.8 / 94.0	0.8 / 1.5 / 97.7
Amikacin	16	>32	54.1 / 17.3 / 28.6	40.6 / 13.5 / 45.9
Gentamicin	>8	>8	39.8 / 6.0/ 54.1	39.1 / 0.8 / 60.2
Levofloxacin	>4	>4	7.5 / 3.0 / 89.5	6.0 / 1.5 / 92.5
Colistin	0.25	>8	- / - / -	71.4 / - / 58.6
K. oxytoca				
ESBL-phenotype (27)				
Meropenem-WCK 4234 (F4)	0.03	0.03	- / - / -	- / - / -
Meropenem-WCK 4234 (F8)	≤0.015	0.03	- / - / -	- / - / -
Meropenem	0.03	0.12	96.3 / 0.0 / 3.7	96.3 / 3.7 / 0.0
Ceftazidime	1	>32	74.1 / 3.7 / 22.2	63.0 / 11.1 / 25.9
Cefepime	1	8	77.8 / 14.8 / 7.4[c]	55.6 / 29.6 / 14.8
Piperacillin-tazobactam	>128	>128	29.6 / 0.0 / 70.4	22.2 / 7.4 / 70.4
Amikacin	2	4	100.0 / 0.0 / 0.0	96.3 / 3.7 / 0.0
Gentamicin	0.5	>8	81.5 / 3.7 / 14.8	81.5 / 0.0 / 18.5
Levofloxacin	0.06	>4	88.9 / 0.0 / 11.1	85.2 / 3.7 / 11.1
Colistin	0.12	0.5	- / - / -	96.3 / - / 3.7
***Enterobacter* spp.**				
ceftazidime-non-susceptible (222)				
Meropenem-WCK 4234 (F4)	0.03	0.03	- / - / -	- / - / -
Meropenem-WCK 4234 (F8)	≤0.015	0.03	- / - / -	- / - / -
Meropenem	0.06	0.25	92.8 / 1.4 / 5.9	94.1 / 4.1 / 1.8
Ceftazidime	>32	>32	0.0 / 5.0 / 95.0	0.0 / 0.0 / 100.0
Cefepime	2	64	60.8 / 14.4/ 24.8[c]	48.6 / 23.0 / 28.4
Piperacillin-tazobactam	64	>64	30.6 / 48.2 / 21.2	18.5 / 12.2 / 69.4
Amikacin	1	4	96.8 / 1.4 / 1.8	95.5 / 1.4 / 3.2
Gentamicin	≤1	>8	77.9 / 3.6 / 18.5	76.1 / 1.8 / 22.1
Levofloxacin	≤0.12	>4	81.5 / 5.4 / 13.1	77.0 / 4.5 / 18.5
Colistin	0.12	>8	- / - / -	87.3 / - / 12.7
Enterobacteriaceae				
carbapenem-resistant (153)				
Meropenem-WCK 4234 (F4)	0.12	32	- / - / -	- / - / -
Meropenem-WCK 4234 (F8)	0.12	32	- / - / -	- / - / -
Meropenem	32	>32	2.0 / 2.6 / 95.4	4.6 / 28.1 / 67.3
Ceftazidime	>32	>32	2.0 / 0.0 / 98.0	1.3 / 0.7 / 98.0
Cefepime	>64	>64	2.6 / 7.2 / 90.2[c]	1.3 / 3.9 / 94.8
Piperacillin-tazobactam	>64	>64	2.6 / 5.9 / 91.4	1.3 / 1.3 / 97.4
Amikacin	16	>32	59.2 / 15.8 / 25.0	47.4 / 11.8 / 40.8
Gentamicin	8	>8	40.8 / 11.2 / 48.0	38.8 / 2.0 / 59.2
Levofloxacin	>4	>4	17.8 / 3.3 / 78.9	13.2 / 4.6 / 82.2
Colistin	0.12	>8	- / - / -	71.7 / - / 28.3

a. Criteria as published by the CLSI [2016] and EUCAST [2016] and "-" = no breakpoint available for interpretation.
b. WCK 4234 at fixed concentrations of 4 (F4) and 8 (F8) µg/mL.
c. Intermediate interpreted as susceptible-dose dependent.

Figure 2. Comparison of meropenem and meropenem-WCK 4234 (fixed 4 and 8 µg/mL) cumulative % inhibition distributions against 417 ESBL-phenotype *K. pneumoniae* isolates collected during 2015.



Conclusions

- **Meropenem-WCK 4234 (WCK 5999) represents a potent new antibacterial combination against susceptible and drug-resistant Enterobacteriaceae isolates including ESBL-phenotype, ceftazidime-non-susceptible, meropenem-non-susceptible and carbapenem-resistant strains.**
- **Meropenem-WCK 4234 (fixed 4 and 8 µg/mL) combinations demonstrated greater potency than meropenem alone, ceftazidime, cefepime and piperacillin-tazobactam when tested against contemporary (2015) Enterobacteriaceae isolates demonstrating an ESBL-phenotype, ceftazidime non-susceptibility, meropenem non-susceptibility or carbapenem-resistance collected during a worldwide surveillance program.**
- **WCK 5999 may represent a valuable treatment option for 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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