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Enhanced Activity of WCK 4282 (Cefepime-Tazobactam) Against KPC-Producing Enterobacteriaceae Collected Worldwide When Tested in Physiological Conditions

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-producers are often resistant to several or all available antimicrobials. WCK 4282 (cefepime-tazobactam [high dose]; FEP-TAZ) displayed *in vivo* efficacy against KPC expressing strains despite elevated FEP-TAZ MICs. *In vitro* activity of FEP-TAZ was evaluated in physiological conditions against KPC-producers.

Methods: KPC-producing Enterobacteriaceae (n=210) were susceptibility (S) tested against FEP-TAZ (TAZ at fixed 8 µg/mL) and FEP by CLSI guidelines using cation-adjusted Mueller-Hinton broth (CA-MHB) ± 50% human serum or 0.85% sodium chloride (NaCl). bla_{KPC} presence was determined by PCR/sequencing.

Results: FEP-TAZ activity against KPC-producing *K. pneumoniae* (KPN; n=167) was enhanced by at least 2- to 4-fold with the addition of serum ($MIC_{50/90}$, 8/32 µg/mL) or NaCl ($MIC_{50/90}$, 8/64 µg/mL) when compared to results for FEP-TAZ tested under standard conditions ($MIC_{50/90}$, 16/>64 µg/mL). FEP-TAZ inhibited 77.8% and 75.4% of the isolates at ≤16 µg/mL (high-dose FEP-TAZ PK-PD tentative breakpoint; **Table**) in the presence of added NaCl or serum, respectively. FEP exhibited limited activity against KPC-producing KPN ($MIC_{50/90}$, 24/>64 µg/mL) with only 21.0% of the isolates S at ≤8 µg/mL. FEP-TAZ activity against KPC-producing KPN was similar to that of FEP alone and inhibited 32.3% of these isolates at ≤8 µg/mL when tested under standard conditions. Against non-KPN, the addition of serum ($MIC_{50/90}$, 4/12 µg/mL) or NaCl ($MIC_{50/90}$, 4/16 µg/mL) lowered the MICs for FEP-TAZ when compared to the reference method (n=43; nine species/complexes) and 90.7%-95.3% of these isolates were inhibited by FEP-TAZ at ≤16 µg/mL in the presence of NaCl or serum. The activity of FEP-TAZ (MIC_{50}, 8 µg/mL) and FEP (MIC_{50}, 12 µg/mL) tested in standard conditions was limited against non-KPN KPC-producers.

Conclusions: FEP-TAZ MIC results for all KPC-producing isolates were consistently lower in MHB supplemented with human serum or NaCl (*in vivo* conditions) and 79.5%-80.5% of the isolates were inhibited by FEP-TAZ at 16 µg/mL.

Organism (no. tested)/ Antimicrobial agent	No. of isolates at MIC (µg/mL; cumulative %):					
	1	2	4	8	16	32
K. pneumoniae (n=167)						
FEP-TAZ	5 (5.4)	9 (10.8)	9 (16.2)	27 (32.3)	25 (55.7)	15 (76.6)
FEP-TAZ + NaCl	9 (10.2)	17 (20.4)	23 (34.1)	39 (57.5)	14 (77.8)	13 (89.2)
FEP-TAZ + Serum	14 (15.6)	12 (22.8)	27 (38.9)	31 (57.5)	18 (75.4)	15 (90.4)
FEP alone	6 (3.6)	6 (7.2)	8 (12.0)	15 (21.0)	18 (44.3)	23 (69.5)
Non-*K. pneumoniae* (n=43)						
FEP-TAZ	1 (5.3)	3 (13.2)	11 (42.1)	6 (57.9)	2 (76.3)	1 (89.5)
FEP-TAZ + NaCl	3 (13.2)	8 (34.2)	9 (57.9)	7 (76.3)	2 (89.5)	0 (94.7)
FEP-TAZ + Serum	4 (34.2)	2 (39.5)	11 (68.4)	3 (76.3)	2 (94.7)	0 (97.4)
FEP alone			5 (13.2)	11 (42.1)	4 (65.8)	3 (78.9)

Introduction

Since its first description in 2001, *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria have been reported for hospitals worldwide. This carbapenem-hydrolyzing β-lactamase is commonly identified in *Klebsiella* spp. clinical isolates, but it can also been found among other Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp. KPC-producing organisms are usually resistant to virtually all β-lactams and often these organisms also display resistance to other antimicrobial classes, limiting the therapeutic options to treat the infections caused by these isolates.

WCK 4282 combines cefepime (FEP) with tazobactam (TAZ) and it is currently under clinical development at 2g/2g q8 hours as well as q12 hours dosage as a 90 min infusion. This combination was demonstrated to be active against several isolates producing extended-spectrum β-lactamases and AmpC-producing isolates under standard testing conditions. In order to understand the basis of *in vivo* efficacy in neutropenic animal models, we evaluated the activity of cefepime-tazobactam when tested in media simulating physiological conditions (supplemented with saline or human serum) against 210 KPC-producing Enterobacteriaceae isolates.

Methods

Bacterial isolates: A total of 210 KPC-producing Enterobacteriaceae clinical isolates collected during 2009-2015 were evaluated. These isolates belonged to the following bacterial species: *Citrobacter freundii* species complex, 2 isolates; *Enterobacter aerogenes*, 2; *Enterobacter asburiae*, 1; *Enterobacter cloacae* species complex, 14; *Escherichia coli*, 9; *Klebsiella oxytoca*, 6; *Klebsiella pneumoniae*, 167; *Proteus mirabilis*, 1; *Proteus penneri*, 1 and *Serratia marcescens*, 7. 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.

Antimicrobial susceptibility testing: MIC values for FEP ± TAZ (using inhibitor at fixed concentration of 8 µg/mL) were determined using the CLSI broth microdilution method as described in CLSI document M07-A10 (2015). FEP-TAZ was tested in three media types: i) standard cation-adjusted Mueller-Hinton broth (CA-MHB); ii) CA-MHB supplemented with 50% pooled human serum; and iii) CA-MHB supplemented with 0.85% NaCl. Additionally, two intermediate dilutions were included: 12 µg/mL between 16 and 8 µg/mL and 24 µg/mL between 16 and 32 µg/mL.

Quality control (QC) was performed using *E. coli* ATCC 25922, ATCC 35218 and NCTC 13353, *Klebsiella pneumoniae* ATCC 700603 and ATCC BAA-1705, and *P. aeruginosa* ATCC 27853. All QC MIC results were within acceptable ranges as published in CLSI documents.

Results

- KPC-producing Enterobacteriaceae isolates displayed elevated FEP ($MIC_{50/90}$, 24/>64 µg/mL) MIC values and 6.7% of the isolates were susceptible at ≤2 µg/mL [CLSI breakpoint criteria] and 49.0% inhibited at ≤16 µg/mL (**Table 1**).
- The activity of FEP-TAZ ($MIC_{50/90}$, 16/64 µg/mL) tested in standard conditions (CA-MHB) was similar to that of FEP alone and 60.0% of these isolates were inhibited by this combination at ≤16 µg/mL (**Table 1** and **Figure 1**).
- When FEP-TAZ was tested against KPC-producing Enterobacteriaceae in the 0.85% NaCl supplemented medium, the MIC_{50} and MIC_{90} values were at least two-fold lower (8 and 32 µg/mL, respectively; **Table 1**) when compared to results of testing under standard conditions and 80.5% of the isolates were inhibited by FEP-TAZ MIC of ≤16 µg/mL when tested in NaCl supplemented medium.
- MIC_{50} and MIC_{90} values (8 and 32 µg/mL, respectively; **Table 1**) for FEP-TAZ tested in CA-MHB supplemented with 50% pooled human serum against Enterobacteriaceae isolates producing KPC enzymes were also at least two-fold lower than the values observed for FEP-TAZ tested in standard conditions and 79.5% of the isolates were inhibited at ≤16 µg/mL when tested in CA-MHB supplemented with 50% human serum.
- FEP exhibited limited activity against KPC-producing *K. pneumoniae* (n=167), and $MIC_{50/90}$ results were similar for this compound tested alone or with TAZ (24/>64 µg/mL and 16/>64 µg/mL, respectively).
- When KPC-producing *K. pneumoniae* were tested in media supplemented with 0.85% NaCl or 50% human serum, FEP-TAZ ($MIC_{50/90}$, 8/64 and 8/32 µg/mL, respectively) MIC values were generally two-fold lower than those obtained with the standard testing conditions. A total of 77.8% and 75.4% of the isolates were inhibited by FEP-TAZ at ≤16 µg/mL when this combination was tested with media supplemented with NaCl or 50% human serum, respectively (**Table 1**).
- The activity of FEP alone was at least two-fold lower against other Enterobacteriaceae species producing KPC (n=43; $MIC_{50/90}$, 12/64 µg/mL) when compared to the *K. pneumoniae* group. FEP-TAZ activity when tested under standard conditions ($MIC_{50/90}$, 8/32 µg/mL; **Table 1**) against non-*K. pneumoniae* species was similar to the activity of FEP alone and 76.7% of the isolates were inhibited FEP-TAZ at ≤16 µg/mL when tested in CA-MHB.
- FEP-TAZ MIC results were two- to four-fold lower when other Enterobacteriaceae species were tested in CA-MHB supplemented with 0.85% NaCl ($MIC_{50/90}$, 4/16 µg/mL) or 50% human serum ($MIC_{50/90}$, 4/12 µg/mL; **Table 1**) when compared to standard CA-MHB results. FEZ-TAZ inhibited 90.7% and 95.3% of these isolates at ≤16 µg/mL when tested in CA-MHB supplemented with NaCl or 50% human serum, respectively.

Figure 1. Comparative activity of FEP and FEP-TAZ tested in different testing conditions against KPC-producing Enterobacteriaceae.

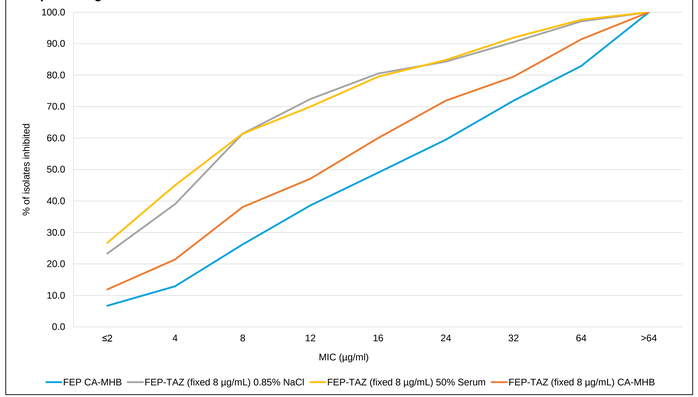

Table 1. Cumulative frequency distribution of MIC results for cefepime (FEP) ± tazobactam (FEP-TAZ) when tested against KPC-producing Enterobacteriaceae isolates.

Organism group (no. tested) Antimicrobial agent	No. of isolates (cumulative percentage) inhibited at MIC (µg/mL) of[a]:															
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	12	16	24	32	64	>64	MIC_{50}	MIC_{90}
Enterobacteriaceae (210)																	
FEP CA-MHB					1 (0.5)	7 (3.8)	6 (6.7)	13 (12.9)	28 (26.2)	26 (38.6)	22 (49.0)	22 (59.5)	26 (71.9)	23 (82.9)	36 (100.0)	24	>64
FEP-TAZ (fixed 8 µg/mL) CA-MHB			2 (1.0)	2 (1.9)	3 (3.3)	6 (6.2)	12 (11.9)	20 (21.4)	35 (38.1)	19 (47.1)	27 (60.0)	25 (71.9)	16 (79.5)	25 (91.4)	18 (100.0)	16	64
FEP-TAZ (fixed 8 µg/mL) 0.85% NaCl			3 (1.4)	3 (2.9)	6 (5.7)	12 (11.4)	25 (23.3)	33 (39.0)	47 (61.4)	23 (72.4)	17 (80.5)	8 (84.3)	13 (90.5)	14 (97.1)	6 (100.0)	8	32
FEP-TAZ (fixed 8 µg/mL) 50% Serum			8 (3.8)	5 (6.2)	11 (11.4)	18 (20.0)	14 (26.7)	39 (45.2)	34 (61.4)	18 (70.0)	20 (79.5)	11 (84.8)	15 (91.9)	12 (97.6)	5 (100.0)	8	32
***Klebsiella pneumoniae* (167)**																	
FEP CA-MHB						6 (3.6)	6 (7.2)	8 (12.0)	15 (21.0)	21 (33.5)	18 (44.3)	19 (55.7)	23 (69.5)	18 (80.2)	33 (100.0)	24	>64
FEP-TAZ (fixed 8 µg/mL) CA-MHB			1 (0.6)	3 (2.4)	5 (5.4)	9 (10.8)	9 (16.2)	27 (32.3)	14 (40.7)	25 (55.7)	20 (67.7)	15 (76.6)	22 (89.8)	17 (100.0)		16	>64
FEP-TAZ (fixed 8 µg/mL) 0.85% NaCl			2 (1.2)	6 (4.8)	9 (10.2)	17 (20.4)	23 (34.1)	39 (57.5)	20 (69.5)	14 (77.8)	6 (81.4)	13 (89.2)	12 (96.4)	6 (100.0)		8	64
FEP-TAZ (fixed 8 µg/mL) 50% Serum	1 (0.6)	1 (1.2)	3 (3.0)	7 (7.2)	14 (15.6)	12 (22.8)	27 (38.9)	31 (57.5)	12 (64.7)	18 (75.4)	10 (81.4)	15 (90.4)	11 (97.0)	5 (100.0)		8	32
Other Enterobacteriaceae (43)																	
FEP CA-MHB					1 (2.3)	1 (4.7)	0 (4.7)	5 (16.3)	13 (46.5)	5 (58.1)	4 (67.4)	3 (74.4)	3 (81.4)	5 (93.0)	3 (100.0)	12	64
FEP-TAZ (fixed 8 µg/mL) CA-MHB			2 (4.7)	1 (7.0)	0 (7.0)	1 (9.3)	3 (16.3)	11 (41.9)	8 (60.5)	5 (72.1)	2 (76.7)	5 (88.4)	1 (90.7)	3 (97.7)	1 (100.0)	8	32
FEP-TAZ (fixed 8 µg/mL) 0.85% NaCl			3 (7.0)	1 (9.3)	0 (9.3)	3 (16.3)	8 (34.9)	10 (58.1)	8 (76.7)	3 (83.7)	3 (90.7)	2 (95.3)	0 (95.3)	2 (100.0)		4	16
FEP-TAZ (fixed 8 µg/mL) 50% Serum			6 (14.0)	2 (18.6)	4 (27.9)	4 (37.2)	2 (41.9)	12 (69.8)	3 (76.7)	6 (90.7)	2 (95.3)	1 (97.7)	0 (97.7)	1 (100.0)		4	12

Conclusions

- **FEP-TAZ tested in CA-MHB supplemented with 0.85% NaCl or 50% serum inhibited 80.5% and 79.5% of the KPC-producing Enterobacteriaceae at ≤16 µg/mL, respectively.**
- **The limited options to treat multidrug resistant KPC-producing Enterobacteriaceae and the dissemination of these isolates worldwide warrant the further development new β-lactam/β-lactamase inhibitor combinations displaying activity against these isolates.**

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