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Enhanced activity of cefepime–tazobactam (WCK 4282) against KPC-producing Enterobacteriaceae when tested in media supplemented with human serum or sodium chloride

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ABSTRACT

The aim of this study was to evaluate the in vitro activity of cefepime–tazobactam cation-adjusted Mueller-Hinton broth (CA-MHB) supplemented with 0.85% sodium chloride (NaCl) or 50% human serum in comparison to standard CA-MHB when testing KPC-producing isolates. A total of 209 contemporary *Enterobacteriaceae* clinical isolates carrying bla_{KPC} were tested, and cefepime–tazobactam (tazobactam at fixed 8 mg/L) activity was enhanced 2-fold when tested in CA-MHB supplemented with 0.85% NaCl or 50% human serum ($MIC_{50/90}$, 8/32 mg/L for both media) compared to standard CA-MHB ($MIC_{50/90}$, 16/64 mg/L). Cefepime–tazobactam at a concentration of ≤16 mg/L, which is the pharmacokinetics/pharmacodynamics tentative susceptibility breakpoint based on a high dosing regimen of cefepime–tazobactam (2 g–2 g q8h 90-minute infusion), inhibited 79.4–80.4% of *Enterobacteriaceae* isolates carrying bla_{KPC} in MHB supplemented with 0.85% NaCl or 50% human serum. A similar decrease in MIC values was observed when cefepime alone was tested against a subset of the isolates (n = 54) in CA-MHB supplemented with 50% human serum or 0.85% NaCl; however, imipenem activity against these 54 organisms was similar or 2-fold higher in CA-MHB supplemented with 0.85% of NaCl ($MIC_{50/90}$, 8/16 mg/L) or with 50% human serum (MIC_{50} and MIC_{90}, 16 mg/L) compared standard CA-MHB ($MIC_{50/90}$, 8/16 mg/L). In summary, cefepime–tazobactam MIC values against *Enterobacteriaceae* isolates carrying bla_{KPC} were consistently lower in media supplemented with human serum or NaCl, which better mimics physiological conditions. These results suggest that this carbapenem-sparing candidate agent has potential to be used to treat infections caused by KPC-producing *Enterobacteriaceae*.

1. Introduction

Carbapenems have been used for over 30 years to treat infections caused by isolates producing β-lactamases that hydrolyze broad-spectrum cephalosporins (Zhanel et al., 2007). However, the emergence of and rapid dissemination of genes encoding β-lactamases that hydrolyze these agents (Bush, 2015), namely serine-carbapenemases and metallo-β-lactamases, were described worldwide among pathogens that had previously been susceptible to the carbapenems (Nordmann and Poirel, 2014).

Among carbapenemases, *Klebsiella pneumoniae* carbapenemase (KPC) is the most prevalent enzyme family in the United States (US), and isolates harboring bla_{KPC} have been detected in numerous countries worldwide (Nordmann and Poirel, 2014). Furthermore, KPC-producing organisms are usually resistant to virtually all β-lactams and often display resistance to other antimicrobial classes (Nordmann and Poirel, 2014; Pitout et al., 2015).

Several studies have evaluated alternative therapeutic options that might spare the use of carbapenems for isolates producing β-lactamases (Giamarellou, 2008; Harris et al., 2015; Karaiskos and Giamarellou, 2014; Nguyen et al., 2014; Viale et al., 2015). These strategies aim to reduce the selection of carbapenemase-producing isolates and isolates with reduced permeability specific for carbapenems (Viale et al., 2015). Carbapenem-alternative therapies typically include older β-lactam agents like piperacillin-tazobactam or cefepime.

Cefepime–tazobactam (WCK 4282) is a carbapenem-sparing candidate agent that is currently under clinical development using a high dosage of both agents: 2 grams of cefepime plus 2 grams of tazobactam administered every 8 hours via a 90-minute infusion (Khande et al., 2016; Melchers et al., 2017; Mouton, 2016). Under standard testing conditions, this combination exhibits in vitro activity against isolates producing ESBLs and AmpC-producing isolates resistant to piperacillin-tazobactam (Livermore et al., 2016; Sader et al., 2017). In this study, we compared the activity of cefepime–tazobactam against 209 *Enterobacteriaceae* isolates carrying bla_{KPC} in standard susceptibility testing medium (cation-adjusted Mueller-Hinton broth [CA-MHB]) versus CA-MHB supplemented with 0.85% sodium chloride (NaCl) or

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50% human serum, which offer physiological conditions more alike to those observed in vivo when compared to standard CA-MHB.

2. Materials and methods

2.1. Bacterial isolates

A total of 209 *Enterobacteriaceae* clinical isolates collected during 2009–2015 were evaluated. Most isolates were collected in US hospitals (204 isolates; 97.6%), 2 isolates were collected from China, 2 from Venezuela, and 1 from Argentina. Species identification was confirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry using the Bruker Daltonics MALDI Biotyper (Billerica, Massachusetts, USA) by following manufacturer instructions.

Isolates were initially tested for susceptibility against imipenem, meropenem, and doripenem by broth microdilution method (CLSI, 2015), and isolates with MIC values of ≥2 mg/L for any of the carbapenems were screened for bla_{KPC} using PCR methods and/or by using the Check-MDR CT101 kit (Check-Points, Wageningen, Netherlands), as previously described (Castanheira et al., 2014). Selected amplicons were sequenced, and protein alignments were compared with available sequences. Only isolates carrying a bla_{KPC} were included in the study.

2.2. Antimicrobial susceptibility testing

MIC values for cefepime alone and with tazobactam at a fixed concentration of 8 mg/L were determined using the CLSI broth microdilution method as described in CLSI document M07-A10 (CLSI, 2015). A tentative susceptible breakpoint of ≤16/8 mg/L, based on the results of pharmacokinetic/pharmacodynamic studies (Khande et al., 2016; Mouton, 2016), Melchers et al., 2017 was applied for comparison purposes only.

Cefepime–tazobactam was tested in 3 media types: 1) CA-MHB; 2) CA-MHB supplemented with 50% (*v*/*v*) pooled human serum; and 3) CA-MHB supplemented with 0.85% (*w*/*v*) NaCl. Final MHB nutrient content for each supplemented media type was equivalent to standard CA-MHB, and MIC panels included growth control wells for each media type. Cefepime with and without tazobactam was tested in log2 doubling dilutions ranging from 0.06 to 64 mg/L, and incremental dilutions of 12 mg/L and 24 mg/L were added to the MIC panel to obtain more precise MIC values around the proposed breakpoint of ≤16/8 mg/L. Additionally, a subset of 54 KPC-producing isolates was also tested against cefepime alone and imipenem using the standard testing conditions and with CA-MHB supplemented with 50% (*v*/*v*) pooled human serum or with 0.85% (*w*/*v*) NaCl.

Quality control was performed using *Escherichia coli* ATCC 25922, ATCC 35218 and NCTC 13353, *Klebsiella pneumoniae* ATCC 700603 and ATCC BAA-1705, and *Pseudomonas aeruginosa* ATCC 27853. All MIC results for cefepime and imipenem were within acceptable ranges as published in CLSI documents (CLSI, 2016).

3. Results

A total of 209 *Enterobacteriaceae* clinical isolates harboring bla_{KPC} genes were evaluated as part of this study, including 168 *K. pneumoniae* isolates and another 41 isolates from 9 *Enterobacteriaceae* species or species complexes (Table 1). The majority of the isolates tested were collected in 2013–2015 (207/209), and isolates from prior years were included to increase the number of isolates of less common species. Isolates were received from the following specimen types: pneumonia in hospitalized patients (61 isolates), urinary tract infection (61), skin/soft tissue infection (40), bloodstream infection (36), intra-abdominal infection (3), and other or unknown sites (8).

Cefepime alone displayed limited activity against *Enterobacteriaceae* isolates carrying bla_{KPC} ($MIC_{50/90}$, 24/>64 mg/L), with only 6.7% of the isolates susceptible at ≤2 mg/L (CLSI breakpoint criteria) and 48.8% inhibited at ≤16 mg/L (Table 2). The activity of cefepime–tazobactam (MIC_{50}, 16/8 mg/L and MIC_{90}, 64/8 mg/L) tested in standard CA-MHB was similar to activity of cefepime alone, and 59.8% of these isolates were inhibited by this combination at ≤16/8 mg/L (Table 2).

Table 1
Clinical *Enterobacteriaceae* isolates carrying bla_{KPC} tested in this study.

Bacterial species/group	All years	2009	2012	2013	2014	2015
Klebsiella pneumoniae	168			18	82	68
Other *Enterobacteriaceae*	41	1	1	7	8	24
Citrobacter freundii species complex	3			1		2
Enterobacter aerogenes	1			1		
Enterobacter cloacae species complex	13			2		11
Escherichia coli	9			2	4	3
Klebsiella oxytoca	5				3	2
Proteus mirabilis	1		1			
Proteus penneri	1				1	
Raoultella ornithinolytica	1					1
Serratia marcescens	7	1		1		5
Total	209					

In contrast, when cefepime–tazobactam was tested against *Enterobacteriaceae* isolates carrying bla_{KPC} using CA-MHB supplemented with 0.85% NaCl (MIC_{50}, 8/8 mg/L and MIC_{90}, 32/8 mg/L), the MIC_{50} and MIC_{90} values were 2-fold lower (Table 2) when compared to results of testing with standard CA-MHB, and 80.4% of the isolates were inhibited by cefepime–tazobactam MIC of ≤16/8 mg/L when tested in NaCl-supplemented CA-MHB. MIC_{50} and MIC_{90} values (8/8 mg/L and 32/8 mg/L, respectively) for cefepime–tazobactam tested in CA-MHB supplemented with 50% pooled human serum against *Enterobacteriaceae* isolates harboring bla_{KPC} were also 2-fold lower than the values observed for cefepime–tazobactam tested in standard CA-MHB (16/8 mg/L and 64/8 mg/L, respectively), and 79.4% of the isolates were inhibited at ≤16/8 mg/L (Table 2).

Cefepime exhibited limited activity against *K. pneumoniae* isolates carrying bla_{KPC} (n = 168), and MIC values were similar for this compound tested alone (MIC_{50}, 24 mg/L and MIC_{90}, >64 mg/L) or with tazobactam (MIC_{50}, 16/8 mg/L and MIC_{90}, >64/8 mg/L). When these isolates were tested in media supplemented with 0.85% NaCl (MIC_{50}, 8/8 mg/L and MIC_{90}, 64/8 mg/L) or 50% human serum (MIC_{50}, 8/8 mg/L and MIC_{90}, 32/8 mg/L), cefepime–tazobactam MIC values were at least 2-fold lower than those obtained with the standard CA-MHB (Table 2). A total of 78.0% and 75.6% of the isolates were inhibited by cefepime–tazobactam at ≤16/8 mg/L when this combination was tested with media supplemented with 0.85% NaCl or 50% human serum, respectively (Table 2).

Cefepime alone was more active against other *Enterobacteriaceae* species carrying bla_{KPC} (n = 41; MIC_{50}, 12 mg/L and MIC_{90}, 64 mg/L) when compared to the *K. pneumoniae* isolate subset (MIC_{50}, 24 mg/L and MIC_{90}, >64 mg/L). The cefepime–tazobactam combination was also more active when tested under standard conditions against non-*K. pneumoniae* species (MIC_{50}, 8/8 mg/L and MIC_{90}, 32/8 mg/L) compared to the *K. pneumoniae* subset (MIC_{50}, 16/8 mg/L and MIC_{90}, >64/8 mg/L), with 78.0% of the non-*K. pneumoniae* isolates inhibited by cefepime–tazobactam at ≤16/8 mg/L when tested in standard CA-MHB (Table 2).

Nevertheless, both NaCl and human serum further increased the in vitro potency of cefepime–tazobactam against the subset of non-*K. pneumoniae* isolates just as was observed for the *K. pneumoniae* subset. Cefepime–tazobactam MIC results were ≥2-fold lower when other *Enterobacteriaceae* species were tested in CA-MHB supplemented with 0.85% NaCl (MIC_{50}, 4/8 mg/L and MIC_{90}, 16/8 mg/L) or 50% human serum (MIC_{50}, 4/8 mg/L and MIC_{90}, 12/8 mg/L) compared to standard CA-MHB results (MIC_{50}, 8/8 mg/L and MIC_{90}, 32/8 mg/L). Cefepime–tazobactam inhibited 90.2% and 95.1% of these isolates at ≤16/8 mg/L