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β-lactam Activity Tested in Combination with β-lactamase Inhibitor Candidates against Enterobacteriaceae Producing Class A, B and D Carbapenemases

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ABSTRACT

Background: Carbapenemase enzymes have challenged antimicrobial therapy worldwide. This study assessed the *in vitro* activity of β-lactams tested with and without a novel β-lactamase inhibitor (BLI) FPI-1465, which does not have direct antimicrobial inhibitory activity against these carbapenemase-producing isolates.

Methods: 27 molecularly characterized isolates were selected. Isolates produced the following carbapenemases: VIM-like (5 isolates); IMP-like (4); NDM-1 (1); KPC (3); OXA-48-like (10 OXA-48 and 2 OXA-181); and SME (2). Aztreonam, ceftazidime and meropenem were tested for susceptibility alone and in combination with FPI-1465 (at fixed 4 μg/mL) per CLSI specifications (M07-A9). Piperacillin-tazobactam was tested as a comparator.

Results: Isolates were non-susceptible to aztreonam, except for one VIM-12 and two IMP-like producers. The AZT-FPI-1465 combination ($MIC_{50/90}$, 0.03/1 μg/mL) showed a MIC_{50} result 4,096-fold lower than aztreonam tested alone ($MIC_{50/90}$, 128/256 μg/mL). The aztreonam-BLI combination inhibited all NDM, KPC, IMP, OXA, VIM and SME producers at ≤0.015, ≤0.12, ≤1, ≤0.25, ≤0.5 and ≤1 μg/mL, respectively, except for one OXA-48-producing *K. pneumoniae* (MIC, >32 μg/mL). Overall, CAZ-FPI-1465 (MIC_{50}, 0.12 μg/mL) was 1,024-fold more active than ceftazidime tested alone (MIC_{50}, 128 μg/mL). Meropenem combined with FPI-1465 (MIC_{50}, 0.06 μg/mL) had a MIC_{50} result 128-fold lower than meropenem tested alone (MIC_{50}, 8 μg/mL). All three combinations inhibited KPC-producing isolates at ≤0.12 μg/mL. Piperacillin-tazobactam was inactive ($MIC_{50/90}$, >64/>64 μg/mL; 11.1% susceptible).

Conclusions: AZT-FPI-1465 showed greatest potency and inhibited all carbapenemase producers at ≤1 μg/mL, but one. The BLI candidate rescued the aztreonam activity, increasing the susceptibility rates from 11.1%, when tested alone, to 96.3% for the combination. These data warrant further development of this BLI.

INTRODUCTION

A decline in the susceptibility rates for carbapenems among clinical isolates of Enterobacteriaceae has been observed during the last decade. This emerging resistance phenotype has been mostly due to the production of KPC carbapenemase enzymes in United States hospitals and KPC, OXA-48, and VIM and NDM metallo-β-lactamases (MBL) among European and surrounding countries, as well as in other regions worldwide. These carbapenemase enzymes will confer resistance to a variety of narrow- and extended-spectrum β-lactam agents, which still remain at the forefront of antimicrobial chemotherapy. In addition, isolates carrying such carbapenemase-encoding genes are often resistant to other clinically prescribed antimicrobial classes and eventually only susceptible to other classes, such as polymyxins.

β-lactamase-inhibitor (BLI) compounds (tazobactam, clavulanate and sulbactam) have been clinically developed as a strategy for inactivating the β-lactamase enzymes. Although combinations of β-lactam-BLI compounds have proven effective for treating infections caused by Enterobacteriaceae that produce certain enzymes, these BLI compounds do not inhibit the hydrolytic activities of carbapenemases. Therefore, several newer BLI molecules have been developed. While these do inhibit Class A and D carbapenemases, these compounds are generally inactive against Class B enzymes. In this study, the synergistic activities of extended-spectrum β-lactam agents combined with a new investigational BLI compound (FPI-1465; see poster F-1188 for structure) were assessed against a panel of well-defined carbapenemase producers.

MATERIALS AND METHODS

Bacterial isolates. A total of 27 molecularly characterized carbapenemase producers recovered from hospitalized patients with documented infections were included in this study (**Table 1**). These isolates were submitted as part of the SENTRY Antimicrobial Surveillance Program and consisted of 19 *Klebsiella pneumoniae*, four *Serratia marcescens*, two *Enterobacter aerogenes* and one each *Escherichia coli* and *K. oxytoca*. Species identification was performed by the participating SENTRY Program medical center site and confirmed by the monitoring laboratory (JMI Laboratories, North Liberty, Iowa, USA) by Vitek® 2 (bioMérieux, Hazelwood, Missouri, USA), and supported when necessary 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.

Screening for ESBL enzymes. Selected isolates were characterized for the β-lactamase content using the microarray based reaction Check-MDR CT101 kit (Check-points, Wageningen, Netherlands) or customized multiplex PCR assays. Detected β-lactamase-encoding genes were confirmed by sequencing analysis using the Lasergene® software package (DNAStar; Madison, Wisconsin, USA).

Antimicrobial susceptibility testing. Carbapenemase producers selected for this study were tested for susceptibility against the co-drugs aztreonam, ceftazidime and meropenem alone and in combination with FPI-1465 at a fixed concentration of 4 μg/mL. FPI-1465 was also tested alone to confirm the absence of direct antimicrobial activity against these isolates. The β-lactam and β-lactam-FPI-1465 combinations and piperacillin-tazobactam (comparator) were tested by broth microdilution methods according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (M07-A9, 2012). Validation of the co-drugs and piperacillin-tazobactam MIC values was performed by concurrent testing of CLSI-recommended quality control (QC) reference strains (*E. coli* ATCC 25922 and 35218, and *Pseudomonas aeruginosa* ATCC 27853). All QC results were within published acceptable ranges. In addition, the inoculum density was monitored by colony counts to assure an adequate number of cells for each testing event. MIC interpretations were based on the CLSI M100-S23 (2013) and European Committee on Antimicrobial Susceptibility Testing (EUCAST; 2013) breakpoint criteria. The β-lactam susceptible breakpoint was used for MIC interpretation of respective β-lactam-FPI-1465 combination.

RESULTS

- Overall, only 11.1 and 7.4% of tested isolates were susceptible to aztreonam ($MIC_{50/90}$, 128/256 μg/mL) when applying the CLSI and EUCAST breakpoint criteria, respectively (**Table 2**). When combined with FPI-1465, the aztreonam ($MIC_{50/90}$, 0.03/1 μg/mL) MIC_{50} and MIC_{90} results decreased 4,096- and 256-fold, respectively, which was reflected in an increased susceptibility rate (96.3%).
- The aztreonam-FPI-1465 combination inhibited all NDM, KPC, IMP, OXA, VIM and SME producers at ≤0.015, ≤0.12, ≤1, ≤0.25, ≤0.5 and ≤1 μg/mL, respectively. One exception was observed for an OXA-48-producing *K. pneumoniae*, which showed a markedly elevated aztreonam-FPI-1465 MIC value at >32 μg/mL (**Table 3**).
- Ceftazidime-FPI-1465 (MIC_{50}, 0.12 μg/mL) was 1,024-fold more active than ceftazidime when tested alone (MIC_{50}, 128 μg/mL). However, five Class B (two IMP and three VIM variants) and one Class D (OXA-48) carbapenemase producers showed ceftazidime-FPI-1465 MIC results above the CLSI breakpoint for susceptibility (**Tables 2** and **3**).
- Meropenem combined with FPI-1465 ($MIC_{50/90}$, 0.06/8 μg/mL) had a MIC_{50} result 128-fold lower than meropenem when tested alone (MIC_{50}, 8 μg/mL), and this combination inhibited 73.1% of isolates at either the CLSI or EUCAST susceptible breakpoints (**Tables 2** and **3**). Isolates not inhibited at these breakpoints were MBL (VIM and IMP variants) and OXA-48 producers (**Table 3**).
- All Class A carbapenemase (KPC and SME variants) producers were inhibited at ≤1 μg/mL by the three combinations tested. Piperacillin-tazobactam ($MIC_{50/90}$, >64/>64 μg/mL; 11.1% susceptible) was inactive when tested against this collection of carbapenemase production (**Tables 2** and **3**).

Table 1. Enterobacteriaceae clinical isolates selected for this study.

Country	Organism	β-lactamase enzyme[a]	Enzyme Class[b]	Country	Organism	β-lactamase enzyme[a]	Enzyme Class[b]
Germany	*Klebsiella pneumoniae*	KPC-2	A	Greece	*Klebsiella oxytoca*	VIM-12	B
Poland	*Klebsiella pneumoniae*	KPC-2	A	Argentina	*Klebsiella pneumoniae*	OXA-48	D
Italy	*Klebsiella pneumoniae*	KPC-3	A	Argentina	*Klebsiella pneumoniae*	OXA-48	D
USA	*Serratia marcescens*	SME-2	A	Argentina	*Klebsiella pneumoniae*	OXA-48	D
USA	*Serratia marcescens*	SME-2	A	India	*Klebsiella pneumoniae*	OXA-181	D
Australia	*Serratia marcescens*	IMP-4	B	India	*Klebsiella pneumoniae*	OXA-181	D
Poland	*Serratia marcescens*	IMP-19	B	Turkey	*Klebsiella pneumoniae*	OXA-48	D
Turkey	*Klebsiella pneumoniae*	IMP-1	B	Turkey	*Klebsiella pneumoniae*	OXA-48	D
Philippines	*Klebsiella pneumoniae*	IMP-26	B	Turkey	*Klebsiella pneumoniae*	OXA-48	D
India	*Klebsiella pneumoniae*	NDM-1	B	Turkey	*Klebsiella pneumoniae*	OXA-48	D
Greece	*Klebsiella pneumoniae*	VIM-26	B	Turkey	*Klebsiella pneumoniae*	OXA-48	D
Greece	*Klebsiella pneumoniae*	VIM-1	B	Turkey	*Klebsiella pneumoniae*	OXA-48	D
Greece	*Enterobacter aerogenes*	VIM-1	B	Turkey	*Escherichia coli*	OXA-48	D
Greece	*Enterobacter aerogenes*	VIM-1	B				

a. Represent enzyme deduced by the nucleotide sequence of detected β-lactamase-encoding gene.
b. Carbapenemase Class according to Bush and Jacoby (2010) classification.

Table 2. MIC distribution and antimicrobial activity of β-lactam tested alone and in combination with FPI-1465 (fixed concentration of 4 μg/mL).

β-lactam/FPI-1465	Number (cumulative %) inhibited at MIC (μg/mL)[a]															
	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>[b]
Aztreonam	-[c]	-	-	-	2(7.4)	0(7.4)	0(*7.4*)	0(7.4)	1(**11.1**)	1(14.8)	0(14.8)	3(25.9)	2(33.3)	10(70.4)	7(96.3)	1(100.0)
Aztreonam-FPI-1465	11(40.7)	3(51.9)	2(59.3)	3(70.4)	3(81.5)	1(85.2)	3(96.3)	0(96.3)	0(**96.3**)	0(96.3)	0(96.3)	0(96.3)	-	-	-	1(100.0)
Ceftazidime	-	-	-	-	0(0.0)	0(0.0)	2(7.4)	0(7.4)	0(**7.4**)	0(7.4)	0(7.4)	1(11.1)	3(22.2)	8(51.9)	6(74.1)	7(100.0)
Ceftazidime-FPI-1465	6(25.0)	4(41.7)	1(45.8)	3(58.3)	2(66.7)	0(66.7)	0(66.7)	2(75.0)	0(**75.0**)	2(75.0)	2(75.0)	2(75.0)	-	-	-	1(100.0)
Meropenem	-	-	-	-	1(3.7)	1(7.4)	4(*22.2*)	0(*22.2*)	3(33.3)	5(51.9)	4(66.7)	2(74.1)	4(88.9)	2(96.3)	1(100.0)	
Meropenem-FPI-1465	6(23.1)	4(38.5)	3(50.0)	1(53.9)	1(57.7)	2(65.4)	2(**73.1**)	0(*73.1*)	2(80.8)	3(92.3)	1(96.2)	0(96.2)	-	-	-	1(100.0)
Piperacillin-tazobactam[d]	-	-	-	-	-	0(0.0)	0(0.0)	2(7.4)	1(11.1)	0(*11.1*)	0(**11.1**)	2(18.5)	0(18.5)	-	-	22(100.0)

a. Dilution ranges for β-lactam agents tested alone were 0.25 - 256 μg/mL. The schedule for meropenem, aztreonam and ceftazidime combinations was 0.015 - 32 μg/mL Simple and double underline results represent MIC_{50} and MIC_{90} values for a given β-lactam or β-lactam-FPI-1465 combination, respectively. Bold and italic values represent the percentage susceptibility when applying CLSI and EUCAST breakpoint criteria, respectively. The β-lactam breakpoints were applied for interpreting the respective β-lactam-FPI-1465-combination MIC results.
b. MIC values read >256, >32 and >64 μg/mL for the β-lactam tested alone, the β-lactam-FPI-1465 combinations and piperacillin-tazobactam, respectively.
c. Concentration not tested.
d. The comparator piperacillin-tazobactam was tested in the following dilution range: 0.5 - 64 μg/mL.

Table 3. MIC results obtained for β-lactams tested alone and in combination with FPI-1465, and piperacillin-tazobactam against carbapenemase producers.

		β-lactam-FPI-1465 combination MIC (μg/mL)										
		Aztreonam			Ceftazidime			Meropenem				
Organism	Enzyme[a]	Alone	Combination	Synergy[b]	Alone	Combination	Synergy[b]	Alone	Combination	Synergy[b]	P/T[c]	
K. pneumoniae	NDM-1	128	≤0.015	≥8192	>256	NT[d]	NA[d]	16	NT	NA	>64	
K. pneumoniae	OXA-181	256	0.12	2048	256	0.12	2048	4	0.06	64	>64	
K. pneumoniae	OXA-181	256	0.12	2048	>256	0.12	≥4096	64	4	16	>64	
K. pneumoniae	IMP-1	≤0.25	≤0.015	NQ[d]	256	0.06	4096	16	8	2	32	
S. marcescens	IMP-19	128	1	128	128	>32	≤2	8	8	1	>64	
S. marcescens	IMP-4	≤0.25	0.06	≤4	128	>32	≤2	16	8	2	2	
K. pneumoniae	IMP-26	128	0.03	4096	>256	NT	NA	16	≤0.015	≥1024	>64	
K. pneumoniae	KPC-2	128	≤0.015	≥8192	32	≤0.015	≥2048	4	≤0.015	≥256	>64	
K. pneumoniae	KPC-2	128	0.12	1024	256	0.03	8192	64	0.03	2048	>64	
K. pneumoniae	KPC-3	128	≤0.015	≥8192	256	≤0.015	≥16384	32	0.03	1024	>64	
K. pneumoniae	OXA-48	8	0.25	32	128	2	64	≤0.25	≤0.015	NQ	32	
E. coli	OXA-48	64	≤0.015	≥4096	64	≤0.015	≥4096	0.5	≤0.015	≥32	>64	
K. pneumoniae	OXA-48	128	≤0.015	≥8192	64	≤0.015	≥4096	1	0.06	16	>64	
K. pneumoniae	OXA-48	256	0.03	8192	128	0.03	4096	1	0.03	32	>64	
K. pneumoniae	OXA-48	32	≤0.015	≥2048	128	2	64	1	0.12	8	>64	
K. pneumoniae	OXA-48	256	0.03	8192	128	≤0.015	≥8192	1	0.03	32	>64	
K. pneumoniae	OXA-48	256	≤0.015	≥16384	256	≤0.015	≥16384	4	0.06	64	>64	
K. pneumoniae	OXA-48	>256	≤0.015	≥32768	64	0.03	2048	8	≤0.015	≥512	>64	
K. pneumoniae	OXA-48	128	0.06	2048	128	0.12	1024	8	0.5	16	>64	
K. pneumoniae	OXA-48	128	>32	≤2	128	>32	≤2	32	16	2	>64	
S. marcescens	SME-2	64	1	64	1	0.25	4	64	0.5	128	4	
S. marcescens	SME-2	32	1	32	1	0.25	4	128	0.25	512	2	
K. pneumoniae	VIM-1	32	≤0.015	≥2048	>256	0.03	≥16384	8	≤0.015	≥512	>64	
E. aerogenes	VIM-1	128	0.25	512	>256	>32	NQ	64	4	16	>64	
E. aerogenes	VIM-1	256	0.5	512	>256	>32	NQ	128	>32	≤2	>64	
K. oxytoca	VIM-12	4	≤0.015	≥256	256	>32	≤4	8	1	8	>64	
K. pneumoniae	VIM-26	256	0.25	1024	>256	NT	NA	256	1	256	>64	

a. Represent enzyme deduced by the nucleotide sequence of detected β-lactamase-encoding gene.
b. Synergy represents the β-lactam MIC value divided by the β-lactam-FPI-1465 combination MIC value.
c. P/T, piperacillin-tazobactam.
d. NT, not tested; NA, not applicable; NQ, not quantifiable.

CONCLUSIONS

- Among the combinations evaluated, aztreonam-FPI-1465 demonstrated highest potency and inhibited all carbapenemase producers at ≤1 μg/mL, except for one OXA-48-producing isolate. Other combinations tested were less active when tested against MBL isolates.
- These *in vitro* MIC results illustrate the potent activity of broad-spectrum β-lactam agents when combined with FPI-1465 against this collection of carbapenemase-producing Enterobacteriaceae. These results warrant the further development of FPI-1465 or analogue compounds in combination with a β-lactam for treatment of multidrug-resistant Gram-negative bacilli.

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