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Activity of β-lactam Agents Tested in Combination with Novel β-lactamase Inhibitor Compounds against Enterobacteriaceae Producing Extended-spectrum β-lactamases

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ABSTRACT

Background: Enterobacteriaceae producing extended-spectrum β-lactamases (ESBL) have become prevalent in both nosocomial and community settings. This study assessed the synergistic effects of β-lactams combined with a new investigational β-lactamase inhibitor (BLI) FPI-1465, which does not have direct antimicrobial inhibitory activity against the tested panel.

Methods: 21 molecularly characterized isolates were selected. These isolates produced one or a combination of the following ESBLs: CTX-M-14, -15 and -59; OXA-2-, -10- or -30-like; PER-4; CMY-2; DHA-1; SHV-7 or -30. Aztreonam, ceftazidime and meropenem were tested for susceptibility alone and in combination with FPI-1465 at a fixed concentration of 4 μg/mL using frozen-form panels per CLSI specifications (M07-A9). Piperacillin-tazobactam was tested as comparator.

Results: Aztreonam had MIC_{50} and MIC_{90} values of 8 and 128 μg/mL, respectively, when tested against this collection; while aztreonam tested in combination with FPI-1465 ($MIC_{50/90}$, ≤0.015/0.5 μg/mL) showed MIC results ≥256-fold lower than this β-lactam tested alone. Ceftazidime combined with FPI-1465 ($MIC_{50/90}$, 0.03/1 μg/mL) exhibited MIC values 256- to 1024-fold lower than ceftazidime tested alone ($MIC_{50/90}$, 32/256 μg/mL). Meropenem was very active against these ESBL-producing isolates ($MIC_{50/90}$, ≤0.25/0.5 μg/mL; 90.5% susceptible) and FPI-1465 still lowered (four-fold) the meropenem MIC_{90} results ($MIC_{50/90}$, 0.06/0.12 μg/mL). Piperacillin-tazobactam had limited activity ($MIC_{50/90}$, 8/>64 μg/mL; 71.4% susceptible).

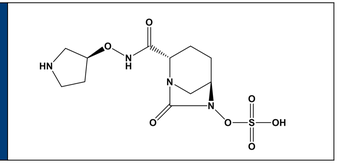
Conclusions: The FPI-1465 BLI compound showed great synergistic effects when combined with aztreonam and ceftazidime, reflecting increased susceptibility rates for these β-lactams from 47.6 and 38.1%, respectively, to ≥95.2%, when applying current CLSI breakpoints. Synergy was less pronounced for the meropenem-FPI-1465 combination. These results warrant further development of this BLI compound.

INTRODUCTION

The production of β-lactamase enzymes among Enterobacteriaceae remains the main resistance mechanism against this important and vastly prescribed class of antimicrobial agents. Clinical isolates of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBL) have now become prevalent in both nosocomial and community settings, and have challenged the treatment of infections caused by these pathogens. CTX-M variants are currently the most common ESBL enzymes detected in *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates and $bla_{CTX\text{-}M\text{-}15}$ comprises the most common CTX-M-encoding gene. The success of $bla_{CTX\text{-}M\text{-}15}$ dissemination is primarily attributed to the dissemination of IncF plasmids carrying this gene and also the ability of *E. coli* isolates associated with multilocus sequence type (MLST) 131 to acquire these plasmids.

Most commonly found ESBL enzymes hydrolyze narrow- and extended-spectrum penicillins and cephalosporins, and monobactams. Carbapenems still remain effective choices for the empirical and directed treatment of infections caused by ESBL-producing pathogens. Additional strategies include the use of compounds designed to bind reversibly or irreversibly to the β-lactamase active site, and as a consequence, rescuing the β-lactam activity. In this regard, several β-lactamase inhibitor (BLI) compounds have been developed and clavulanate, sulbactam and tazobactam are clinically available in combination with broader-spectrum penicillins. In this study, the synergistic effects of extended-spectrum β-lactam agents combined with a new investigational BLI compound (FPI-1465; **Figure 1**) were assessed.

Figure 1. Chemical structure of BLI compound FPI-1465.



MATERIALS AND METHODS

Bacterial isolates. A total of 21 molecularly characterized ESBL-producing enteric bacilli 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 five *K. pneumoniae*, five *Serratia marcescens*, six *Proteus mirabilis*, three *E. coli*, one *Enterobacter aerogenes* and one *Morganella morganii*. Species identification was performed by the participating SENTRY medical 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) according to 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 (**Table 1**). Detected β-lactamase-encoding genes were confirmed by sequencing analysis using the Lasergene® software package (DNAStar; Madison, Wisconsin, USA).

Antimicrobial susceptibility testing. ESBL 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 fixed concentration of 4 μg/mL. FPI-1465 was also tested alone to confirm the absence of direct antimicrobial activity against ESBL producers. 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

- **Table 2** shows the MIC distribution and antimicrobial activity of β-lactams and β-lactam-FPI-1465 combinations. Overall, aztreonam-FPI-1465 ($MIC_{50/90}$, ≤0.015/0.5 μg/mL) and ceftazidime-FPI-1465 ($MIC_{50/90}$, 0.03/1 μg/mL) showed MIC_{90} values 256-fold lower than the co-drugs tested alone ($MIC_{50/90}$, 8/128 μg/mL and $MIC_{50/90}$, 32/256 μg/mL, respectively).
- The aztreonam antimicrobial coverage increased from 47.6% susceptible when tested alone to 95.2% susceptible when tested in combination with FPI-1465 by applying the CLSI criteria, or from 23.8 to 95.2% susceptible according to EUCAST breakpoints (**Table 2**).
- Similarly, ceftazidime inhibited 38.1% and 14.3% of ESBL producers at the CLSI (≤4 μg/mL) and EUCAST (≤1 μg/mL) breakpoint criteria, respectively; while the ceftazidime-FPI-1465 combination demonstrated a susceptibility rate of 95.2%, regardless of breakpoint (**Table 2**).
- Aztreonam-FPI-1465 ($MIC_{50/90}$, ≤0.015/0.5 μg/mL) and ceftazidime-FPI-1465 ($MIC_{50/90}$, 0.03/1 μg/mL) showed MIC_{50} and MIC_{90} values ≥256- and ≥128-fold lower than piperacillin/tazobactam ($MIC_{50/90}$, 8/>64 μg/mL), respectively (**Table 2**).
- Meropenem was very active against these ESBL-producing isolates ($MIC_{50/90}$, ≤0.25/0.5 μg/mL; 90.5% susceptible) and FPI-1465 further lowered (four-fold) the meropenem MIC_{90} results ($MIC_{50/90}$, 0.06/0.12 μg/mL). This increased potency reflected in a 95.2% coverage rate by using either the CLSI or EUCAST criteria (**Table 2**).
- **Table 3** displays the MIC results obtained for the co-drugs tested alone and in combination with FPI-1465. Aztreonam, ceftazidime and meropenem combined with FPI-1465 inhibited all ESBL producers at ≤1, ≤1 and ≤0.12 μg/mL, respectively. One exception was found for each β-lactam-FPI-1465 combination with MIC values of 4, 8 and >32 μg/mL.
- Synergistic effects were most pronounced when FPI-1465 was combined with aztreonam or ceftazime, where the potency of these β-lactam co-drugs increased at least 16-fold when tested against 95.2 and 85.7% of the isolates selected for this study (**Table 3**).

Table 1. Enterobacteriaceae clinical isolates selected for this study.

Country	Organism	β-lactamase enzyme(s)[a]	Enzyme Class[b]
USA	*Escherichia coli*	FOX-5	C
USA	*Escherichia coli*	CMY-2	C
USA	*Escherichia coli*	CTX-M-14	A
USA	*Klebsiella pneumoniae*	CTX-M-15	A
USA	*Klebsiella pneumoniae*	CTX-M-15	A
USA	*Klebsiella pneumoniae*	CTX-M-15	A
USA	*Klebsiella pneumoniae*	CTX-M-15/OXA-1/30	A/D
USA	*Klebsiella pneumoniae*	CTX-M-15/OXA-1/30	A/D
USA	*Morganella morganii*	DHA-1	C
USA	*Serratia marcescens*	SHV-7/OXA-10	A/D
USA	*Serratia marcescens*	SHV-30/OXA-10	A/D
USA	*Serratia marcescens*	SHV-30/OXA-10	A/D
USA	*Serratia marcescens*	OXA-2	D
USA	*Serratia marcescens*	OXA-2	D
USA	*Proteus mirabilis*	CMY-2	C
USA	*Proteus mirabilis*	DHA-1	C
USA	*Proteus mirabilis*	CMY-2	C
Brazil	*Proteus mirabilis*	CTX-M-14	A
Chile	*Proteus mirabilis*	CTX-M-15	A
Turkey	*Proteus mirabilis*	PER-4/OXA-10	A/D
Greece	*Enterobacter aerogenes*	CTX-M-59/OXA-2	A/D

a. Represent enzymes deduced by the nucleotide sequence of detected β-lactamase-encoding gene.
b. Enzyme 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).

	Number (cumulative %) inhibited at MIC (μg/mL)[a]															
β-lactam-FPI-1465	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>[b]
Aztreonam	-[c]	-	-	-	1(4.8)	0(4.8)	*4(23.8)*	2(33.3)	**3(47.6)**	1(52.4)	2(61.9)	1(66.7)	1(71.4)	5(95.2)	1(100.0)	
Aztreonam-FPI-1465	12(57.1)	0(57.1)	1(61.9)	3(76.2)	2(85.7)	1(90.5)	1(95.2)	0(95.2)	**0(95.2)**	1(100.0)	-	-	-	-	-	-
Ceftazidime	-	-	-	-	0(0.0)	1(4.8)	*2(14.3)*	1(19.1)	**4(38.1)**	0(38.1)	2(47.6)	4(66.7)	1(71.4)	1(76.2)	3(90.5)	2(100.0)
Ceftazidime-FPI-1465	6(28.6)	5(52.4)	1(57.1)	2(66.7)	1(71.4)	2(81.0)	3(95.2)	0(95.2)	**0(95.2)**	0(95.2)	0(95.2)	0(95.2)	-	-	-	1(100.0)
Meropenem	-	-	-	-	17(81.0)[c]	2(90.5)	**0(90.5)**	0(90.5)	1(95.2)	0(95.2)	1(100.0)	-	-	-	-	-
Meropenem-FPI-1465	6(28.6)	4(47.6)	8(85.7)	2(95.2)	0(95.2)	0(95.2)	**0(95.2)**	0(95.2)	1(100.0)	-	-	-	-	-	-	-
Piperacillin-tazobactam[d]	-	-	-	-	-	1(4.8)	3(19.1)	4(38.1)	2(47.6)	5(71.4)	**0(71.4)**	0(71.4)	1(76.2)	-	-	5(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 co-drug 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 selected ESBL 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]
E. aerogenes	CTX-M-59/OXA-2	128	0.5	256	>256	0.12	≥4096	≤0.25	0.06	≤4	>64
K. pneumoniae	CTX-M-15/OXA-1/30	256	0.12	2048	256	0.12	2048	4	0.12	32	>64
K. pneumoniae	CTX-M-15/OXA-1/30	128	0.25	512	128	1	128	≤0.25	0.06	≤4	64
K. pneumoniae	CTX-M-15	128	≤0.015	≥8192	256	≤0.015	≥16384	≤0.25	≤0.015	NQ[d]	>64
K. pneumoniae	CTX-M-15	128	≤0.015	≥8192	256	≤0.015	≥16384	0.5	≤0.015	≥32	>64
K. pneumoniae	CTX-M-15	64	≤0.015	≥4096	32	0.03	1024	≤0.25	≤0.015	NQ	4
P. mirabilis	CTX-M-15	8	≤0.015	≥512	1	≤0.015	≥64	≤0.25	0.06	≤4	1
P. mirabilis	CTX-M-14	1	≤0.015	≥64	0.5	0.03	16	≤0.25	0.03	≤8	1
E. coli	CTX-M-14	4	≤0.015	≥256	1	≤0.015	≥64	≤0.25	≤0.015	NQ	1
E. coli	FOX-5	2	≤0.015	≥128	32	≤0.015	≥2048	≤0.25	≤0.015	NQ	>64
E. coli	CMY-2	32	≤0.015	≥2048	32	≤0.015	≥2048	≤0.25	≤0.015	NQ	8
M. morganii	DHA-1	2	≤0.015	≥128	16	0.06	256	≤0.25	0.03	≤8	2
P. mirabilis	DHA-1	1	≤0.015	≥64	64	0.03	2048	≤0.25	0.06	≤4	2
P. mirabilis	CMY-2	1	≤0.015	≥64	4	0.03	128	≤0.25	0.03	≤8	≤0.5
P. mirabilis	CMY-2	≤0.25	≤0.015	NQ	4	0.03	128	≤0.25	0.06	≤4	2
P. mirabilis	PER-4/OXA-10	16	8	2	>256	>32	NQ	≤0.25	0.12	≤2	8
S. marcescens	OXA-2	1	0.06	16	2	0.25	8	≤0.25	0.03	≤8	4
S. marcescens	OXA-2	16	0.25	64	16	1	16	16	4	4	8
S. marcescens	SHV-7/OXA-10	128	1	128	32	1	32	≤0.25	0.06	≤4	2
S. marcescens	SHV-30/OXA-10	4	0.12	32	4	0.5	8	0.5	0.06	8	8
S. marcescens	SHV-30/OXA-10	4	0.12	32	4	0.5	8	≤0.25	0.06	≤4	8

a. Represent enzymes 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. NQ, not quantifiable.

CONCLUSIONS

- The investigational FPI-1465 BLI compound showed synergistic effects when combined with aztreonam and ceftazidime, which reflected in an increased and potent antimicrobial coverage (% susceptibility) for these β-lactam co-drugs (95.2% susceptible, when applying current CLSI or EUCAST breakpoints).
- These preliminary *in vitro* results show FPI-1465 as a potent inhibitor of hydrolytic activities of CTX-M, plasmid AmpC and other ESBL enzymes currently prevalent in several Enterobacteriaceae species worldwide. These results warrant further investigations for the development of FPI-1465 or derivative molecules in combination with safe broader-spectrum β-lactams.

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REFERENCES

1. Bush K, Jacoby GA (2010). Updated functional classification of β-lactamases. *Antimicrob Agents Chemother* 54: 969-976.
2. Castanheira M, Farrell SE, Deshpande LM, Mendes RE, Jones RN (2013). Prevalence of β-lactamase encoding genes among Enterobacteriaceae bacteremia isolates collected in 26 USA hospitals: Report from the SENTRY Antimicrobial Surveillance Program (2010). *Antimicrob Agents Chemother* 57: 3012-3020.
3. Castanheira M, Sader HS, Jones RN (2010). Antimicrobial susceptibility patterns of KPC-producing or CTX-M-producing Enterobacteriaceae. *Microb Drug Resist* 16: 61-65.
4. Clinical and Laboratory Standards Institute (2012). *M07-A9. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard: Ninth edition*. Wayne, PA, USA.
5. Clinical and Laboratory Standards Institute (2013). *M100-S23. Performance standards for antimicrobial susceptibility testing: 23rd Informational Supplement.* Wayne, PA, USA.
6. European Committee on Antimicrobial Susceptibility Testing (2013). Breakpoint tables for interpretation of MICs and zone diameters. Version 3.0, January 2013. Available at: http://www.eucast.org/clinical_breakpoints/. Accessed January 1, 2013.
7. Pitout JD, Laupland KB (2008). Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis* 8: 159-166.