Activity of PF-1 and PF-2, Novel Siderophore Conjugated Beta-lactams, Tested Against Enterobacteriaceae Strains Carrying Emerging Resistance Mechanisms

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ABSTRACT

Objectives: To evaluate the activity of two novel conjugated siderophore beta-lactam agents (PF-1 and PF-2) and comparator agents against Enterobacteriaceae (ENT) producing emerging β-lactamases, including CTX-M, NDM-1, KPC and OXA-48/-181 or carrying known plasmid-borne fluoroquinolone resistance (PMQR) genes, *qnr* and *aac(6')-Ib-cr*.

Methods: PF-1 and -2 (with and without the iron chelator 2,2'-dipyridyl) and comparators were tested for susceptibility using CLSI broth microdilution methods against 111 clinical strains of ENT (13 species [including 53 *K. pneumoniae*, 27 *E. coli*, 10 *E. cloacae* among others]) producing CTX-M-14 (12 strains), CTX-M-15 (12), KPC-2 to -4 (24), NDM-1 (12), OXA-48/181 (19), *qnr* (20), *aac(6')-Ib-cr* (3). Isolates were collected from 2001-2009 from global surveillance programs. Resistance mechanisms were identified by PCR and sequencing.

Results: PF-1 and -2 displayed potent activity against all groups tested (MIC_{50} results, <=0.25 mg/L). Overall, in the presence of 2,2'-dipyridyl (a siderophore chelator; Table) MIC results were either identical or slightly lower (two- to four-fold) when compared to the compounds tested alone. PF-1 and -2 were the most active antimicrobial agents tested against NDM-1-producing strains, which were highly resistant to nearly all compounds tested except for tigecycline and polymyxin B (MIC_{50}, 0.5 and <=0.5 mg/L, respectively). Against KPC-producing strains, PF-1 and -2 MIC_{50} results (0.25 and 0.12 mg/L) were the lowest among all agents tested. Enterobacteriaceae strains producing CTX-M enzymes displayed the lowest MIC_{50} results for PF-1 and -2 (<=0.06 to <=0.03 mg/L) among all groups. PF-1 and -2 also demonstrated potent activity against all OXA-48/-181 strains and most PMQR strains tested. Among all comparator agents, tigecycline was the only antimicrobial agent demonstrating 100% susceptibility rates against these tested strains.

Resistance group (no. tested)		No. of isolates at MIC (mg/L) with chelator:								
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	≥8
NDM-1 strains (12)	PF-1	-	2	4[a]	1	3	0	1	1	0
	PF-2	3	4	1	1	1	0	1	1	0
KPC strains (34)	PF-1	-	13	3	3	1	3	4	3	4
	PF-2	11	5	1	2	2	7	1	2	3
CTX-M strains (24)	PF-1	-	14	2	3	2	1	0	1	1
	PF-2	14	0	5	2	1	0	1	1	0
OXA-48/-181 strains (19)	PF-1	-	9	3	4	0	1	2	0	0
	PF-2	10	0	4	2	0	1	2	0	0
PMQR strains (22)	PF-1	-	10	2	1	2	5	1	0	1
	PF-2	11	1	0	3	5	1	0	1	0

a. MIC_{50} values are underlined

Conclusions: Overall, PF-1 and -2 inhibited >90% of all tested strains at <=2 mg/L. PF-2 seems to be slightly more active compared to PF-1 against these selected resistant strains. The presence of 2,2'-dipyridyl did not appreciably impact MIC results. Both PF-1 and -2 retained activity (MIC_{50}, <=0.03 to 0.25 mg/L) against strains carrying resistance mechanisms (including NDM-1) that are rapidly disseminating among contemporary Gram-negative enteric bacilli.

INTRODUCTION

The emergence and spread of resistance mechanisms in Gram-negative bacilli have complicated the treatment of serious nosocomial infections. β-lactam antimicrobial agents that once were highly effective therapeutic options against Gram-negative pathogens can now be evaded by various resistance determinants. Enterobacteriaceae strains susceptible only to carbapenems, tigecycline and polymyxin B are now common worldwide and the rise of carbapenemases, such as KPCs and NDM-1, are a source of great concern among infectious disease/microbiology professionals.

Among β-lactam resistance mechanisms, β-lactamases are most worrisome because of their potential of acquiring mutations that can broaden their spectrum of hydrolysis against different β-lactams, as well as their ability to disseminate. The genes encoding β-lactamases are associated with mobile genetic elements that allow rapid spread in the clinical setting. Furthermore, these genetic structures often carry other genes encoding resistance against aminoglycosides and fluoroquinolones, among others.

In this study, we evaluated the activity of conjugated siderophore β-lactam agents PF-1 and PF-2 alone and in combination with the chelator agent 2,2'-dipyridyl, and comparator antimicrobial agents against 111 Enterobacteriaceae strains producing emerging resistance mechanisms, including β-lactamases (NDM-1, KPC, CTX-M, OXA-48/-181 enzymes) and plasmid mediated quinolone resistance genes (*qnr* and *aac(6')-Ib-cr*).

MATERIALS AND METHODS

Organism Collection: A total of 111 Enterobacteriaceae strains belonging to 13 bacterial species identified during the 2001-2009 period in two surveillance studies (SENTRY Antimicrobial Surveillance Program and MYSTIC Programme) were evaluated. These strains produced β-lactamase enzymes or plasmid-mediated quinolone resistance (PMQR) genes, listed in Table 1.

Only one isolate per patient from documented infections were included in the study. Isolates were collected from bloodstream, respiratory tract and skin structure infections according to defined protocols. Local species identification was confirmed by standard biochemical tests, the Vitek 2 System (bioMerieux, Hazelwood, Missouri, USA) or 16S rRNA sequencing, when necessary.

Susceptibility Testing: Isolates were susceptibility tested against PF-1 and PF-2 with and without 2,2'-dipyridyl and comparator agents by reference broth microdilution methods as described by Clinical and Laboratory Standards Institute (CLSI) M07-A8 (2009) and CLSI interpretations were based on M100-S21. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were concurrently tested for quality assurance; all results were within the published ranges.

Genotypic Detection of Resistance Genes. Different PCR approaches were used to detect β-lactamases and PMQR genes. PCR amplicons were sequenced on both strands and the nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, Wisconsin, USA). Sequences were compared to others available via internet sources (http://www.ncbi.nlm.nih.gov/blast/).

RESULTS

- The activity of conjugated siderophore β-lactam agents PF-1 and PF-2 with and without 2,2'-dipyridyl and comparators against Enterobacteriaceae strains producing emerging resistance mechanisms (Table 1) is summarized in Tables 2 and 3. PF-1 ± 2,2'-dipyridyl and PF-2 alone inhibited all NDM-1-producers at ≤4 mg/L. PF-2 with 2,2'-dipyridyl inhibited all these strains at ≤2 mg/L (Table 2).
- Against NDM-1-producers, MIC_{50} values for PF-1 and -2 ± 2,2'-dipyridyl varied from 0.006 to 0.12 mg/L (Table 3). NDM-1-producing strains were highly resistant to nearly all comparator agents tested (Table 3), except for tigecycline (100.0% susceptible).
- MIC_{50} results for the investigational compounds (PF-1 and -2) against KPC-producers (0.12 to 0.25 mg/L; both ± 2,2'-dipyridyl; Table 3) were the lowest among all agents tested.
- Against these KPC-producing strains, MIC_{90} values for PF-2 ± 2,2'-dipyridyl (4 mg/L, respectively) were two- to four-fold lower when compared to PF-1 tested ± 2,2'-dipyridyl (16 and 8 mg/L, respectively; Table 3).
- Strains producing CTX-M enzymes displayed the lowest MIC_{50} results for PF-1 and PF-2 ± 2,2'-dipyridyl among all groups analyzed (range, ≤0.03 to ≤0.06 mg/L; Table 3). However, low susceptibility rates were noted for most comparators, except for amikacin, meropenem, and tigecycline (87.5, 91.7 and 100.0% susceptible, respectively).
- Against strains producing OXA-48 or OXA-181, the activity of PF-2 ± 2,2'-dipyridyl (MIC_{50}, ≤0.03 mg/L for both; Table 3) was at least two-fold greater than the activity of PF-1 ± 2,2'-dipyridyl (MIC_{50}, 0.06 and 0.12 mg/L, respectively). MIC_{90} values were 2 mg/L for both compounds with or without chelator.
- PMQR strains were susceptible to most non-fluoroquinolone antimicrobial agents, with lowest susceptibility rates (36.4% susceptible) for ciprofloxacin, levofloxacin, tetracycline and trimethoprim/ sulfamethoxazole.
- PF-1 inhibited 21 of 22 PMQR strains at 4 mg/L, whereas compound PF-2 inhibited all strains at this concentration (Table 2). The presence of 2,2'-dipyridyl, in general, generated two- to four-fold lower MIC values.
- $MIC_{50/90}$ values for these investigational compounds against PMQR strains were: ≤0.03/1, 0.12/1 mg/L for PF-1 ± 2,2'-dipyridyl, and ≤0.03/1, ≤0.03/1 mg/L for PF-2 ± 2,2'-dipyridyl, respectively.

Table 1. Gram-negative bacilli strains carrying resistance mechanisms tested against conjugated siderophore β-lactam agents PF-1 and PF-2 and comparator antimicrobial agents.

Resistance mechanism (no.)	Bacterial species (no. of strains tested)
NDM-1 (12)	*E. coli* (5), *E. cloacae* (2), *K. pneumoniae* (5)
KPC	
KPC-2 (19)	*E. cloacae* (3), *E. gergoviae* (1), *Klebsiella oxytoca* (2), *K. pneumoniae* (12), and *S. marcescens* (1)
KPC-3 (14)	*C. freundii* (2), *E. coli* (2), *E. cloacae* (2), *E. gergoviae* (1), *K. oxytoca* (1), *K. pneumoniae* (5), and *S. marcescens* (1)
KPC-4 (1)	*E. cloacae* (1)
CTX-M	
CTX-M-14 (12)	*E. coli* (5), *K. pneumoniae* (5), *Proteus mirabilis* (2)
CTX-M-15 (10)	*C. freundii* (1), *E. coli* (5), *K. pneumoniae* (4), *P. mirabilis* (2)
OXA-48/181[a] (19)	*E. coli* (5), *E. cloacae* (2), *K. pneumoniae* (12)
PMQR[b]	
qnr (19)	*E. coli* (4), *E. aerogenes* (1), *K. oxytoca* (1), *K. pneumoniae* (9), and *Salmonella* spp. (4)
aac(6')-Ib-cr (3)	*E. coli* (1), *K. pneumoniae* (1), *Salmonella* spp. (1)

a. This group also included the recently described OXA-163.
b. PMQR = plasmid-mediated quinolone resistance.

Table 2. MIC distributions for compounds PF-1 and PF-2 with and without 2,2'-dipyridyl when tested against 111 Enterobacteriaceae strains carrying resistance mechanisms.

Organism group/ Antimicrobial agent	No. of isolates at MIC (mg/L):											
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	≥64
NDM-1-producing strains												
PF-1	-[a]	2	4	1	3	0	1	1	0	0	0	0
PF-1 with 2,2'-dipyridyl	2	4	2	0	2	0	1	1	0	0	0	0
PF-2	3	4	1	1	1	0	1	1	0	0	0	0
PF-2 with 2,2'-dipyridyl	5	2	1	1	1	1	1	0	0	0	0	0
KPC-producing strains												
PF-1	-	13	3	3	1	3	4	3	0	4	0	0
PF-1 with 2,2'-dipyridyl	7	6	3	1	4	2	4	3	1	3	0	0
PF-2	11	5	1	2	2	7	1	2	3	0	0	0
PF-2 with 2,2'-dipyridyl	12	3	2	2	3	4	3	2	3	0	0	0
CTX-M-producing strains												
PF-1	-	14	2	3	2	1	0	1	1	0	0	0
PF-1 with 2,2'-dipyridyl	11	3	3	3	2	0	0	1	1	0	0	0
PF-2	14	0	5	2	1	0	1	1	0	0	0	0
PF-2 with 2,2'-dipyridyl	12	5	2	2	1	0	1	1	0	0	0	0
OXA-48/-181-producing strains[b]												
PF-1	-	9	3	4	0	1	2	0	0	0	0	0
PF-1 with 2,2'-dipyridyl	9	2	2	3	0	0	2	1	0	0	0	0
PF-2	10	0	4	2	0	1	2	0	0	0	0	0
PF-2 with 2,2'-dipyridyl	11	0	4	1	0	1	2	0	0	0	0	0
PMQR[c] strains												
PF-1	-	10	2	1	2	5	1	0	1	0	0	0
PF-1 with 2,2'-dipyridyl	12	0	1	3	2	3	1	0	0	0	0	0
PF-2	11	1	0	3	5	1	0	1	0	0	0	0
PF-2 with 2,2'-dipyridyl	12	1	1	3	4	0	1	0	0	0	0	0

a. "-" = Not tested at this concentration.
b. This group also included the recently described OXA-163.
c. PMQR = plasmid-mediated quinolone resistant.

Table 3. Activity of PF-1 and PF-2 with and without 2,2'-dipyridyl and comparator antimicrobial agents when tested against 111 Enterobacteriaceae strains carrying prevalent resistance mechanisms.

Organism group/ Antimicrobial agent	MIC (mg/L) 50%	90%	% Susc[a, b, c]	Antimicrobial agent	MIC (mg/L) 50%	90%	% Susc[a, b, c]
NDM-1-producing strains				OXA-48/-181-producing strains			
PF-1	0.12	2	NA	PF-1	0.12	2	NA
PF-1 with 2,2'-dipyridyl	0.06	2	NA	PF-1 with 2,2'-dipyridyl	0.06	2	NA
PF-2	0.06	2	NA	PF-2	≤0.03	2	NA
PF-2 with 2,2'-dipyridyl	0.06	1	NA	PF-2 with 2,2'-dipyridyl	≤0.03	2	NA
Ciprofloxacin	64	256	8.3	Ciprofloxacin	2	256	47.4
Ceftazidime	>16	>16	0	Ceftazidime	16	>16	42.1
Cefepime	>16	>16	8.3	Cefepime	>16	>16	47.4
Aztreonam	>16	>16	0	Aztreonam	>16	>16	42.1
Piperacillin/Tazobactam	>64	>64	0	Piperacillin/Tazobactam	>64	>64	0
Meropenem	8	>8	16.7	Meropenem	1	>8	57.9
Amikacin	>32	>32	0	Amikacin	4	>32	68.4
T/S[f]	>2	>2	8.3	T/S[d]	>2	>2	47.4
Tetracycline	>8	>8	16.7	Tetracycline	8	>8	47.4
Tigecycline[e]	0.5	1	100.0	Tigecycline[e]	0.5	2	100.0
Polymyxin B	≤0.5	≤0.5	NA	Polymyxin B	≤0.5	1	NA
KPC-producing strains				PMQR[f] strains			
PF-1	0.25	16	NA	PF-1	0.12	1	NA
PF-1 with 2,2'-dipyridyl	0.25	8	NA	PF-1 with 2,2'-dipyridyl	≤0.03	1	NA
PF-2	0.12	4	NA	PF-2	≤0.03	0.5	NA
PF-2 with 2,2'-dipyridyl	0.12	4	NA	PF-2 with 2,2'-dipyridyl	≤0.03	0.5	NA
Ciprofloxacin	8	128	29.4	Ciprofloxacin	2	256	36.4
Ceftazidime	>16	>16	5.9	Ceftazidime	16	>16	40.9
Cefepime	>16	>16	20.6	Cefepime	1	>16	68.2
Aztreonam	>16	>16	0	Aztreonam	1	>16	50.0
Piperacillin/Tazobactam	>64	>64	0	Piperacillin/Tazobactam	≤8	>64	68.2
Meropenem	8	>8	2.9	Meropenem	≤0.12	≤0.12	95.5
Amikacin	≤4	32	67.6	Amikacin	≤4	8	100.0
T/S[d]	>2	>2	17.6	T/S[d]	>4	>4	36.4
Tetracycline	≤4	>8	58.8	Tetracycline	>8	>8	36.4
Tigecycline[e]	0.5	2	100.0	Tigecycline[e]	0.25	1	95.5
Polymyxin B	≤0.5	>4	NA	Polymyxin B	≤0.5	1	NA
CTX-M-producing strains							
PF-1	≤0.06	1	NA				
PF-1 with 2,2'-dipyridyl	≤0.06	0.5	NA				
PF-2	≤0.03	0.5	NA				
PF-2 with 2,2'-dipyridyl	≤0.03	0.5	NA				
Ciprofloxacin	32	256	29.2				
Ceftazidime	4	>16	58.3				
Cefepime	16	>16	41.7				
Aztreonam	16	>16	41.7				
Piperacillin/Tazobactam	4	>64	62.5				
Meropenem	≤0.12	≤0.12	91.7				
Amikacin	2	32	87.5				
T/S[d]	>2	>2	12.5				
Tetracycline	>8	>8	16.7				
Tigecycline[e]	0.25	1	100.0				
Polymyxin B	≤0.5	>4	NA				

a. % Susc = % Susceptible.
b. Using CLSI M100-S21 (2011) breakpoint criteria.
c. NA = not available.
d. T/S= Trimethoprim/sulfamethoxazole.
e. US-FDA breakpoints were applied [Tygacil Product Insert, 2005].
f. PMQR = plasmid-mediated quinolone resistant.

CONCLUSIONS

- PF-1 and PF-1 ± 2,2'-dipyridyl retained good activity (MIC_{50}, ≤0.03 to 0.25 mg/L) against these sub-sets of challenge strains carrying well defined resistance mechanisms that are disseminated among contemporary Gram-negative enteric bacilli, including NDM-1-producing strains.
- PF-2 was slightly more active compared to PF-1 against these resistant strains. Furthermore, the presence 2,2'-dipyridyl effected slightly the activity of these siderophore β-lactam agents PF-1 and PF-2.
- The rapid dissemination of resistance mechanisms among Gram-negative bacteria highlights the pressing need for new antimicrobial agents to treat infections caused by these organisms. The present results demonstrated that PF-1 and PF-2 are valuable candidates for further clinical development.

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