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Antimicrobial Activity of Ceftaroline Combined With NXL104 Tested Against a Collection of Organisms Expressing Multiple β-Lactamases

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Amended Abstract

Objective: To evaluate the activity of ceftaroline combined with NXL104 (fixed 4 mg/L; CPT104) against Enterobacteriaceae (ENT) with various types of β-lactamases (BL), with most strains carrying multiple BLs. Ceftaroline is a novel, parenteral cephalosporin with broad-spectrum activity against Gram-positive (including MRSA and MDRSP) and -negative organisms. Ceftaroline has limited activity against extended-spectrum β-lactamase (ESBL)- and AmpC-producing strains. NXL104 is a novel non-beta-lactam BL inhibitor that inhibits Ambler class A, C, and D enzymes (eg, ESBL, KPC, and AmpC).

Methods: CPT104 and comparators were tested for susceptibility (S) by CLSI broth microdilution methods against 148 clinical strains of ENT producing KPC + AmpC (26 strains), ESBL + AmpC (27), KPC + ESBL (7), multiple ESBLs (37 strains), SME or NMC-A carbapenemases (7), KPC (12), CTX-M (9), plasmidic AmpC (15), and metallo-BL (MBL; 8). Isolates were collected from 1999-2008 from global surveillance programs.

Results: CPT104 exhibited potent inhibitory effects against all BL types except MBLs. All isolates were inhibited at CPT104 MIC ≤4 mg/L except the MBL-producing strains (Table). CPT104 was highly active against ENT producing KPC (MIC_{90}, 0.5 mg/L), KPC + AmpC (MIC_{90}, 2 mg/L), and KPC + ESBL (MIC_{100}, 1 mg/L). CPT104 was more active than meropenem (MIC_{90}, >8 mg/L) against these 3 groups. ENT-producing CTX-M (highest CPT104 MIC, 0.5 mg/L) and those producing more than one ESBL type (including CTX-M, SHV, TEM, OXA and OXY; MIC_{90}, 1 mg/L) were also very S to CPT104. The highest CPT104 MIC observed among plasmidic AmpC was 0.5 mg/L. All strains producing SME or NMC-A were also inhibited at ≤0.5 mg/L of CPT104. CPT104 (MIC, >32 mg/L) and all β-lactam compounds tested showed limited activity against MBL-producing ENT.

Abstract Table

BL type (no. tested)	Cumulative % inhibited at CPT104 MIC (mg/L): ≤0.06	0.12	0.25	0.5	1	2	4	>4
KPC + AmpC (26)	3.8	3.8	11.5	30.8	73.1	96.1	100.0	-
ESBL + AmpC (27)	0.0	14.8	37.0	55.6	74.0	96.3	100.0	-
KPC + ESBL (7)	0.0	0.0	14.3	57.1	100.0	-	-	-
Multiple ESBLs (37)	37.8	62.2	86.5	89.2	97.3	100.0	-	-
SME/NMC-A (7)	0.0	14.3	42.9	100.0	-	-	-	-
KPC (12)	0.0	0.0	16.7	91.7	100.0	-	-	-
CTX-M (9)	11.1	67.7	88.9	100.0	-	-	-	-
Plasmidic AmpC (15)	20.0	60.0	86.7	100.0	-	-	-	-
MBL (8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0

Conclusions: Results of this study clearly demonstrated that NXL104, when combined with ceftaroline, effectively lowers ceftaroline MIC for ENT that produce the most clinically significant BLs, except MBLs. CPT104 was highly active against ENT that produce KPC, various ESBL types, and AmpC (chromosomally derepressed or plasmid mediated), as well as those producing more than one of these BL types (see Table). CPT104 represents a promising therapeutic option for treatment of infections caused by multidrug-resistant ENT.

Introduction

The emergence and spread of resistance mechanisms in Gram-negative bacilli have complicated the treatment of serious nosocomial infections. Gram-negative pathogens have developed varying mechanisms of resistance to β-lactam that were once effective for their treatment. Among β-lactam resistance mechanisms, β-lactamases are most worrisome because of their potential to acquire mutations that can broaden their spectrum of hydrolysis against different β-lactams, as well as their ability to disseminate.

Ceftaroline is a novel broad-spectrum cephalosporin that possesses both Gram-positive and -negative activity with extended activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-resistant *Streptococcus pneumoniae* (PRSP). Similar to other cephalosporins, ceftaroline is less active against some β-lactamase-producing organisms. However, when combined with NXL104, a potent non-β-lactam β-lactamase inhibitor, the spectrum of activity of ceftaroline is expanded against strains producing class A β-lactamases, such as TEM, SHV, CTX-M, and KPC enzymes, class C cephalosporinases, and class D oxacillinases with narrow or extended spectrum of activity.

In this study, we evaluated the activity of ceftaroline combined with NXL104 (CPT104) against 148 Enterobacteriaceae strains carrying different β-lactamases, most of them carrying multiple enzymes.

Materials and Methods

Bacterial Isolates. A total of 148 β-lactamase-producing Enterobacteriaceae strains identified during the 1999-2008 period in two surveillance studies (SENTRY Antimicrobial Surveillance Program and MYSTIC Program) were evaluated. Only one isolate per patient from documented infections was included in the study. Isolates were collected from bloodstream, respiratory tract, and skin structure infections according to defined protocols. Species identification was confirmed by standard biochemical tests, the Vitek System (bioMerieux; Hazelwood, Missouri), or 16S rRNA sequencing, when necessary.

Susceptibility Testing. All strains were tested for antimicrobial susceptibility using the reference broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI; M07-A8, 2009). Ceftaroline was combined with NXL104 at a fixed concentration of 4 mg/L (CPT104). Quality control (QC) was performed using *Escherichia coli* ATCC 25922, *S. aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI document M100-S20 (2010).

Genotypic Detection of β-lactamase Genes. Different PCR approaches were used to detect extended-spectrum β-lactamase (ESBL), plasmidic AmpC, and carbapenemase-encoding genes. Generic primers were used to detect PER, GES, VEB, CTX-M, and oxacillinases (OXA-ESBL), TEM- and SHV-encoding genes. Plasmidic AmpC genes were detected as described elsewhere. Isolates showing reduced susceptibility to imipenem or meropenem (MIC, ≥2 mg/L) were tested for the presence of carbapenemase genes, including bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{SME}, bla_{GES} variants and for bla_{IMI}, bla_{NMC-A}, bla_{OXA-48}, combined in two amplification reactions.

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). Sequences were compared with others available via internet sources (http://www.ncbi.nlm.nih.gov/blast/).

Results

- Ceftaroline MIC values were greatly reduced in the presence of NXL104. Against strains producing multiple β-lactamases, including serine-carbapenemases, MIC reductions of 4- to 8192-fold were noted when NXL104 was combined with ceftaroline (Tables 1 and 2)
- Overall, CPT104 demonstrated excellent activity against β-lactamase-producing strains (all MIC results ≤4 mg/L), except for those producing metallo-β-lactamases (MβL; Table 3)
- Chromosomal AmpC-producing strains carrying KPC carbapenemases displayed a CPT104 MIC_{50} value of 1 mg/L and 96.1% of these strains were inhibited at ≤2 mg/L (highest CPT104 MIC, 4 mg/L). Meropenem was the most active comparator agent (MIC_{50}, 4 mg/L), displaying a susceptibility rate of only 23.0% (Table 3) using current EUCAST breakpoints
- CPT104 inhibited all AmpC producers carrying additional β-lactamases (one or more narrow-spectrum or ESBL-types) at ≤4 mg/L, and 96.3% were inhibited at ≤2 mg/L. Meropenem inhibited 96.3% of these strains at ≤8 mg/L). Only 74.0% of these strains were susceptible to cefepime (Table 3)
- KPC-producing *Klebsiella pneumoniae* and *E. coli* strains carrying additional β-lactamase-encoding genes were inhibited by CPT104, with all strains having CPT104 MIC values of ≤1 mg/L. Comparator agents showed limited activity against these strains (0.0% to 28.6% susceptibility; Table 3)
- Enterobacteriaceae strains producing multiple β-lactamases (including narrow-spectrum and ESBL-types) were inhibited by CPT104 at ≤2 mg/L and meropenem at ≤4 mg/L (CLSI current susceptible breakpoint; Table 3)
- SME-producing *Serratia marcescens* and NMC-A-producing *Enterobacter cloacae* displayed elevated meropenem MIC results (>8 mg/L) and lower MIC values for CPT104, ceftaroline, and cefepime (all ≤2 mg/L; Table 3)
- Strains producing KPC-carbapenemases had ceftaroline MIC decreases of 128- to 1024-fold (Table 2) in the presence of NXL104, and all MIC results for CPT104 were ≤1 mg/L. This subset of strains demonstrated high resistance rates to comparator agents (only 25.0% susceptible to cefepime and meropenem [0.0% at ≤1 mg/L]; Table 3)
- CPT104 coverage was most similar to that of meropenem when testing against CTX-M-producing strains and those strains carrying plasmidic AmpC enzymes (MIC_{50}, ≤0.12 mg/L for both compounds; Table 3)
- CPT104 and all β-lactams tested demonstrated limited activity against MβL-producing strains (Table 3)

Table 1. AmpC-producing Enterobacteriaceae (*Enterobacter cloacae*, *Serratia marcescens*, *Citrobacter freundii*, *Enterobacter gergoviae*, *Enterobacter aerogenes*, and *Proteus vulgaris*) Carrying β-lactamases

No. of enzymes[a]	Carbapenemases KPC	SME	NMC-A	MβL	ESBL CTX-M	SHV	GES	Non-ESBL SHV	TEM	OXA	No. of strains	Ceftaroline MIC reductions (log_2)[b,c]
2					X						2	4- to 128-fold
2						X					15	16- to 1024-fold
2							X				1	512-fold
2										X	1	512-fold
3					X					X	1	1024-fold
3						X				X	5	32- to 2048-fold
4						X				X	2	128- to 512-fold
2	X										26	128- to 1024-fold[d]
2		X									6	4- to 16-fold
2			X								1	512-fold
2				X							7	No effect

a. Including derepressed AmpC
b. In the presence of NXL104
c. Off scale results were represented by the immediately higher dilution step
d. No inhibitory effect was observed with one KPC-2-producing *E. aerogenes* strain that showed ceftaroline alone MIC at ≤0.12 mg/L

Table 2. *Klebsiella* spp., *Escherichia coli*, *Proteus mirabilis*, and *Salmonella* spp. Strains Producing β-lactamases

No. of enzymes	Carbapenemases KPC	MBL	ESBL CTX-M	SHV	OXA	OXY	Non-ESBL SHV	TEM	OXA	Plasmidic AmpC	No. of strains	Ceftaroline MIC reductions (log_2)[a,b]
1			X								9	1024- to 2048-fold
1										X	15	32- to 512-fold
2			X						X		3	256- to 2048-fold
2			X				X				5	512- to 8192-fold
2			X					X			7	512- to 8192-fold
2				X					X		3	32- to 8192-fold
2								X	X		2	64- to 2048-fold
2					X				X		1	2048-fold
2				X				X			1	4096-fold
2							X	X			1	512-fold
2							X		X		2	32- to 8192-fold
3			X	X					X		1	8192-fold
3			X		X			X			1	4096-fold
3			X	X				X			1	8192-fold
3			X				X	X			4	512- to 4096-fold
3			X					X	X		1	512-fold
3				X				X	X		1	256-fold
3							X	X	X		2	2048- to 8192-fold
3				X		X		X			1	512-fold
1	X										12	128- to 1024-fold
1		X									1	No effect
2	X						X				1	512-fold
3	X			X		X					1	1024-fold
3	X			X					X		1	512-fold
3	X						X		X		1	256-fold
3	X						X	X			3	512- to 1024-fold

a. In the presence of NXL104
b. Off scale results were represented by the immediately higher dilution step

Table 3. MIC Distribution for Ceftaroline/NXL104 (CPT104) and Comparators Alone Against β-lactamase-producing Enterobacteriaceae Grouped According to Types of Enzyme(s)

β-lactamase type (no. tested)	No. of strains (Cumulative % inhibited) at MIC (mg/L): ≤0.12	0.25	0.5	1	2	4	8	16	32	>[a]	MIC 50%	MIC 90%	% Susc.[b]
KPC + AmpC (26)													
CPT104	3.8	11.5	30.8	73.1	96.1	100.0	-	-	-	-	1	2	96.1
Ceftaroline	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	100.0	>128	>128	3.8
Cefepime	3.8	3.8	3.8	3.8	11.5	19.2	46.1	-	100.0	>16	>16	>16	19.2
Meropenem	3.8	3.8	3.8	3.8	23.0	50.0	73.0	-	-	100.0	4	>8	50.0
Piperacillin/tazobactam	0.0	0.0	0.0	0.0	3.8	3.8	3.8	3.8	7.7	100.0	>32	>32	3.8
ESBL + AmpC (27)													
CPT104	14.8	37.0	55.6	74.0	96.3	100.0	-	-	-	-	0.5	2	96.3
Ceftaroline	0.0	0.0	0.0	3.7	3.7	3.7	3.7	22.2	22.2	100.0	>128	>128	3.7
Cefepime	0.0	0.0	0.0	7.4	14.8	48.1	74.0	92.6	-	100.0	8	16	74.0
Meropenem	74.04	77.8	88.9	96.3	96.3	96.3	96.3	-	-	100.0	≤0.12	≤0.12	96.3
Piperacillin/tazobactam	0.0	0.0	0.0	0.0	7.4	18.5	33.3	37.0	51.8	100.0	32	>32	37.0
KPC + ESBL (7)													
CPT104	0.0	14.3	57.1	100.0	-	-	-	-	-	-	0.5	1	100.0
Ceftaroline	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	>128	>128	0.0
Cefepime	0.0	0.0	0.0	14.3	14.3	14.3	14.3	42.9	-	100.0	>16	>16	14.3
Meropenem	0.0	0.0	0.0	0.0	0.0	28.6	28.6	-	-	100.0	>8	>8	28.6
Piperacillin/tazobactam	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	>32	>32	0.0
Multiple ESBLs (37)													
CPT104	62.2	86.5	89.2	97.3	100.0	-	-	-	-	-	0.12	1	100.0
Ceftaroline	0.0	0.0	0.0	0.0	0.0	2.7	5.4	8.1	13.5	100.0	>128	>128	0.0
Cefepime	2.7	5.4	5.4	16.2	29.7	43.2	51.3	59.4	-	100.0	8	>16	51.3
Meropenem	91.9	97.3	100.0	-	-	-	-	-	-	-	≤0.12	≤0.12	100.0
Piperacillin/tazobactam				2.7	16.2	32.4	54.0	59.5	70.3	100.0	8	>32	59.5
SME/NMC-A (7)													
CPT104	14.3	42.9	100.0	-	-	-	-	-	-	-	0.5	0.5	100.0
Ceftaroline	0.0	0.0	0.0	42.9	100.0	-	-	-	-	-	2	2	100.0
Cefepime	71.4	100.0	-	-	-	-	-	-	-	-	≤0.12	≤0.12	100.0
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	>8	>8	0.0
Piperacillin/tazobactam	0.0	0.0	0.0	0.0	71.4	85.7	85.7	85.7	85.7	100.0	2	2	85.7
KPC (12)													
CPT104	0.0	16.7	91.7	100.0	-	-	-	-	-	-	0.5	0.5	100.0
Ceftaroline	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	>128	>128	0.0
Cefepime	0.0	0.0	0.0	0.0	8.3	16.7	25.0	25.0	-	100.0	>8	>8	25.0
Meropenem	0.0	0.0	0.0	0.0	16.7	25.0	50.0	91.7	-	100.0	8	16	25.0
Piperacillin/tazobactam	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	>32	>32	0.0
CTX-M (9)													
CPT104	67.7	88.9	100.0	-	-	-	-	-	-	-	0.12	1	100.0
Ceftaroline	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	>128	>128	0.0
Cefepime	0.0	0.0	0.0	0.0	11.1	11.1	11.1	22.2	-	100.0	>16	>16	11.1
Meropenem	100.0	-	-	-	-	-	-	-	-	-	≤0.12	≤0.12	100.0
Piperacillin/tazobactam	0.0	0.0	0.0	0.0	0.0	11.1	33.3	66.7	77.8	100.0	16	>32	66.7
Plasmidic AmpC (15)													
CPT104	60.0	86.7	100.0	-	-	-	-	-	-	-	0.12	0.5	100.0
Ceftaroline	0.0	0.0	0.0	0.0	0.0	0.0	26.7	53.3	80.0	100.0	16	>128	0.0
Cefepime	6.7	26.7	60.0	60.0	73.3	93.3	93.3	93.3	-	100.0	0.5	4	93.3
Meropenem	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	≤0.12	≤0.12	100.0
Piperacillin/tazobactam	0.0	0.0	0.0	0.0	0.0	0.0	40.0	46.7	53.3	100.0	32	>32	46.7
MBL (8)													
CPT104	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	>32	>32	0.0
Ceftaroline	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	>128	>128	0.0
Cefepime	0.0	0.0	0.0	12.5	12.5	25.0	25.0	50.0	-	100.0	16	>16	25.0
Meropenem	0.0	0.0	0.0	12.5	25.0	37.5	62.5	-	-	100.0	8	>8	37.5
Piperacillin/tazobactam	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	37.5	100.0	>32	>32	0.0

a. > than the highest dilution tested
b. A breakpoint of ≤2 mg/L was applied for ceftaroline and CPT104 for comparison purposes only

Conclusions

- CPT104 demonstrated excellent in vitro activity against these very challenging sets of isolates producing β-lactamases of different classes (except MβLs), including isolates producing up to 4 different β-lactamases
- In general, ceftaroline MIC reductions were variable, but MIC values were at least 32-fold lower when NXL104 was combined with ceftaroline than when ceftaroline was tested alone for all groups (except MβL-producing strains)
- Many β-lactamase groups have been highlighted by the epidemiologic impact and antimicrobial therapy limitations that their dissemination can cause. CPT104 appears to present broader antimicrobial coverage and greater activity than any β-lactam currently available for clinical use against organisms carrying β-lactamases from classes A, C, and D

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