Spectrum of Activity of Ceftaroline/NXL104 and β-Lactam Comparator Agents Tested Against Methicillin-Resistant *Staphylococcus aureus* Carrying Different SCC*mec* Types and Gram-Negative Bacilli With Well-Characterized Resistance Mechanisms

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ECCMID 2010

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Amended Abstract

Objective: To determine the spectrum of activity and potency of ceftaroline combined with a fixed 4 mg/L concentration of NXL104 (CPT104) and comparator antimicrobial agents tested against selected molecularly characterized MRSA carrying different SCC*mec* types and well-characterized Gram-negative strains harbouring different beta-lactamase (BL)-encoding genes. Ceftaroline is a novel parenteral broad-spectrum cephalosporin with 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: Susceptibility testing for all antimicrobials was performed by CLSI broth microdilution method (M07-A8, 2009) on a total of 250 strains categorized as follows: MRSA (100 MRSA subcategorized by SCC*mec* type [types I-IV and type IV subtypes]); E-ESBL (50 Enterobacteriaceae [ENT] with ESBLs); E-AMPC (49 ENT with AmpC enzymes [28 chromosomal, 21 plasmidic]); E-CARB (31 ENT with carbapenemases [25 KPC, 5 OXA-48, 1 SME]); ACB (10 *Acinetobacter baumannii* [3 wild-type, 7 OXA-23, -24, and -58]); and PSA (10 *Pseudomonas aeruginosa* [2 wild-type, others include AmpC, MexX, OMP, and metallo-BL producers]).

Results: All MRSA strains, except one, were inhibited at ≤2/4 mg/L of CPT104. The ceftaroline MIC was not affected by addition of NXL104. CPT104 was also very active against a wide variety of ENT with ESBL, chromosomal/plasmidic AmpC, and carbapenemases (highest MIC, 4 mg/L). Ceftaroline was not active against the vast majority of these selected ENT. Although CPT104 demonstrated activity against wild-type ACB and PSA, activity was low against BL-producing strains. Ceftazidime and piperacillin/tazobactam (P/T) were inactive against all six organism categories. Imipenem was active against E-ESBL and E-AMPC but had very limited or no activity against other categories.

Abstract Table

	MIC_{50}/MIC_{90} (mg/L)					
Antimicrobial	**MRSA**	**E-ESBL**	**E-AMPC**	**E-CARB**	**ACB**	**PSA**
Ceftaroline	1/2	>64/>64	32/>64	>64/>64	>64/>64	>64/>64
CPT104[a]	1/2	0.06/0.25	0.12/0.5	0.5/2	16/>32	16/>32
Ceftazidime	>16/>16	>16/>16	>16/>16	>16/>16	16/>16	>16/>16
P/T	64/>64	32/>64	32/>64	>64/>64	>64/>64	64/>64
Imipenem	>8/>8	0.12/0.5	0.5/1	8/>8	>8/>8	8/>8

a. Concentrations reported in the table for CPT104 refer to the concentration of ceftaroline

Conclusions: CPT104 was very active against MRSA regardless of SCC*mec* type or subtype and all ENT regardless of BL type, but had limited intrinsic activity against ACB and PSA expressing a variety of BLs. CPT104 had a wider spectrum of activity against these resistant Gram-positive and -negative categories than the four comparators. CPT104 represents a potential therapeutic option for empiric therapy in settings where MRSA and BL-positive ENT predominate.

Introduction

Ceftaroline is a novel parenteral broad-spectrum cephalosporin with potent activity against Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant (MDR) *Streptococcus pneumoniae*. Ceftaroline is also active against most Enterobacteriaceae species but, like all cephalosporins, has limited activity against extended-spectrum β-lactamase (ESBL)- and AmpC-hyperproducing strains.

NXL104 is a novel non-β-lactam β-lactamase inhibitor that inhibits Ambler classes A (eg, ESBL, KPC), C (AmpC), and D (OXA-like) enzymes. In the present study, we evaluated the spectrum of activity and potency of ceftaroline combined with NXL104 (CPT104) and comparator antimicrobial agents tested against well-characterized Gram-negative strains harboring various β-lactamase-encoding genes. A selected group of molecularly characterized MRSA carrying different SCC*mec* types was also evaluated.

Materials and Methods

Bacterial Isolates. A total of 250 organisms were tested:

1. Enterobacteriaceae with selected β-lactamase types (130)
   - ESBLs (50)
   - Chromosomal and plasmidic AmpC enzyme types (49)
   - Serine carbapenemases (31)
2. *Acinetobacter* spp.(10)
   - Wild-type (3)
   - Carbapenemase producers (7)
3. *Pseudomonas aeruginosa* (10)
   - Wild-type (2)
   - AmpC hyperproducers +/- overexpression of efflux pump (3)
   - Metallo-β-lactamase (MβL) producers (4)
   - GES (1)
4. *S. aureus* strains carrying different SCC*mec* types from various geographic regions each representing predominant national or regional clones (100 total)
   - SCC*mec* type I (19 strains)
   - SCC*mec* type II (20 strains)
   - SCC*mec* type III (20 strains)
   - SCC*mec* type IV (41 strains)

Susceptibility Testing. All isolates were tested for antimicrobial susceptibility using the reference broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI) (M07-A8, 2009). Cation-adjusted Mueller-Hinton broth was used in validated panels. Ceftaroline was combined with NXL104 at a fixed concentration of 4 mg/L (CPT104). Categorical interpretations for all antimicrobials were those found in M100-S20 (2010), and quality control (QC) was performed using *Escherichia coli* ATCC 25922, *S. aureus* ATCC 29213, and *P. aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI documents.

Results

- Among ESBL-producing Enterobacteriaceae, CPT104 MIC results ranged from ≤0.015/4 to 1/4 mg/L. The majority (13 of 21; 62%) of CTX-M-producing strains exhibited a CPT104 MIC of 0.06/4 mg/L (MIC ranged from 0.03/4 to 1/4 mg/L). One *Serratia marcescens* (CTX-M-2) and one *Enterobacter* spp. (CTX-M-like) had CPT104 MICs of 1/4 mg/L, while all other CTX-M-producing strains were inhibited at ≤0.25/4 mg/L (Table 1)
- SHV-producing Enterobacteriaceae exhibited a broad range of CPT104 MIC values (≤0.015/4 to 1/4 mg/L), and the highest MIC values (0.5/4 to 1/4 mg/L) were observed among *Enterobacter* spp. All *Klebsiella* spp. strains were inhibited at ≤0.25/4 mg/L, and the only SHV-12-positive *E. coli* tested had a CPT104 MIC of 0.03/4 mg/L (Table 1)
- CPT104 MIC values were generally low (≤0.25/4 mg/L) among TEM-producing strains and the vast majority of isolates in this group produced an ESBL in addition to the TEM enzyme (Table 1)
- The chromosomally stably derepressed AmpC producer group contained *Citrobacter* spp., *Enterobacter* spp., and *Serratia* spp., and showed CPT104 MIC_{50} of 0.12/4 and MIC_{90} of 0.5/4 mg/L, similar to those of plasmidic AmpC producers (MIC_{50}, 0.12/4 mg/L and MIC_{90}, 0.25/4 mg/L). The plasmidic AmpC group consisted mostly of *E. coli* isolates (Table 1)
- Wild-type strains of *Acinetobacter* spp. exhibited CPT104 values of 1/4 or 2/4 mg/L. All OXA-producing strains showed CPT104 MIC values of ≥16/4 mg/L. The addition of the NXL104 component to ceftaroline resulted in a modest reduction of the ceftaroline MIC to 16/4 mg/L in 2 strains (an OXA-24- and an OXA-58-producing strain)
- Among *P. aeruginosa*, CPT104 MIC results varied from 4/4 to >32/4 mg/L. Wild-type strains showed the lowest CPT104 MIC values (4/4 and 16/4 mg/L), and the highest CPT104 MIC results were observed among the MβL-producing strains (>32/4 mg/L; Table 1)
- Ceftazidime and piperacillin/tazobactam showed very limited activity against all six organism/enzyme categories. Imipenem was active against ESBL- and AmpC-producing Enterobacteriaceae, but had very limited or no activity against other resistance mechanism categories (Table 2)
- Carbapenemase-producing Enterobacteriaceae strains were also susceptible to CPT104 (MIC_{50}, 0.5/4 mg/L), but exhibited MIC values slightly higher than those of ESBL- or AmpC-producing strains (MIC_{50}, 0.12/4 mg/L)
- All MRSA strains except one, were inhibited at ≤2/4 mg/L of CPT104 (MIC_{50}, 1/4 mg/L). Furthermore, ceftaroline MIC was not affected by addition of NXL104, and MIC values for ceftaroline and CPT104 were essentially identical or only varied +/- 1 doubling dilution step (Tables 1 and 2). The lowest CPT104 MIC values were observed among strains with SCC*mec* type IV (MIC mode, 0.5/4 mg/L), while the highest MIC values were found among MRSA with SCC*mec* type I (MIC mode, 2/4 mg/L; Table 1)

Table 1. Frequency of Occurrence of CPT104 MIC Values Among the Organism Groups Evaluated

	No. of strains (cumulative %) inhibited at CPT104 MIC[a] (mg/L) of:												
Organism (no. tested)	**≤0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**>32**
Enterobacteriaceae (All; 130)	3 (2)	5 (6)	32 (31)	38 (60)[b]	21 (76)	15 (88)	10 (95)	3 (98)	3 (100)	-	-	-	-
ESBL-producing (50)	3 (6)	3 (12)	21 (54)	13 (80)	6 (92)	1 (94)	3 (100)	-	-	-	-	-	-
CTX-M (21)	-	1 (5)	13 (67)	2 (76)	3 (90)	0 (90)	2 (100)	-	-	-	-	-	-
OXY (7)	-	-	4 (57)	3 (100)	-	-	-	-	-	-	-	-	-
SHV (12)	2 (17)	1 (25)	2 (42)	3 (67)	2 (83)	1 (92)	1 (100)	-	-	-	-	-	-
TEM (10)	1 (10)	1 (20)	2 (40)	5 (90)	1 (100)	-	-	-	-	-	-	-	-
AmpC-producing (49)	-	2 (4)	10 (24)	21 (67)	11 (90)	4 (98)	1 (100)	-	-	-	-	-	-
Chromsomal (28)	-	-	3 (11)	13 (57)	7 (82)	4 (96)	1 (100)	-	-	-	-	-	-
Plasmid CMY (21)	-	2 (10)	7 (43)	8 (81)	4 (100)	-	-	-	-	-	-	-	-
Carbapenemase-producing (31)	-	-	1 (3)	4 (16)	4 (29)	10 (61)	6 (81)	3 (90)	3 (100)	-	-	-	-
KPC (25)	-	-	-	3 (12)	4 (28)	9 (64)	5 (84)	2 (92)	2 (100)	-	-	-	-
OXA-48 (5)	-	-	1 (20)	1 (40)	0 (40)	0 (40)	1 (60)	1 (80)	1 (100)	-	-	-	-
SME (1)	-	-	-	-	-	1 (100)	-	-	-	-	-	-	-
Acinetobacter spp. (10)	-	-	-	-	-	-	2 (20)	1 (30)	0 (30)	0 (30)	2 (50)	0 (50)	5 (100)
Wild-type (3)	-	-	-	-	-	-	2 (67)	1 (100)	-	-	-	-	-
OXA-23 (3)	-	-	-	-	-	-	-	-	-	-	-	-	3 (100)
OXA-24 (2)	-	-	-	-	-	-	-	-	-	-	1 (50)	0 (50)	1 (50)
OXA-58 (2)	-	-	-	-	-	-	-	-	-	-	1 (50)	0 (50)	1 (50)
P. aeruginosa (10)	-	-	-	-	-	-	-	-	1 (10)	0 (10)	5 (60)	0 (60)	4 (100)
Wild-type (2)	-	-	-	-	-	-	-	-	1 (50)	0 (50)	1 (100)	-	-
AmpC/MexX/OMP (3)	-	-	-	-	-	-	-	-	-	-	3 (100)	-	-
MBL IMP/VIM/SPM (4)	-	-	-	-	-	-	-	-	-	-	-	-	4 (100)
GES (1)	-	-	-	-	-	-	-	-	-	-	1 (100)	-	-
S. aureus (100)	-	-	-	-	-	28 (28)	55 (83)	29 (99)	1 (100)	-	-	-	-
SCC*mec* I (19)	-	-	-	-	-	-	1 (5)	17 (95)	1 (100)	-	-	-	-
SCC*mec* II (20)	-	-	-	-	-	2 (10)	15 (85)	3 (100)	-	-	-	-	-
SCC*mec* III (20)	-	-	-	-	-	2 (10)	12 (70)	6 (100)	-	-	-	-	-
SCC*mec* IV (41)	-	-	-	-	-	24 (59)	17 (100)	-	-	-	-	-	-

a. Ceftaroline combined with NXL104 at a fixed concentration of 4 mg/L. MIC value indicates ceftaroline concentration
b. Underlined value indicates the mode

Table 2. Comparison of In vitro Activity of CPT104 and Selected Antimicrobial Agents Tested Against a Well-characterized Collection of β-lactamase-producing Enterobacteriaceae, Nonfermentative Gram-negative Bacilli, and Methicillin-resistant *S. aureus*

Antimicrobial agent	**MIC_{50}**	**MIC_{90}**	**Range**	**% susceptible/ resistant[a]**
Enterobacteriaceae				
ESBL-producing[b] (50)				
CPT104	0.06	0.25	≤0.015 – 1	-/- / -
Ceftaroline	>64	>64	0.06 – >64	- / -
Ceftazidime	>16	>16	0.25 – >16	40.0 / 54.0
Cefepime	4	>16	≤0.12 – >16	56.0 / 38.0
Ampicillin/sulbactam	>16	>16	4 – >16	4.0 / 86.0
Piperacillin/tazobactam	32	>64	≤0.5 – >64	44.0 / 46.0
Imipenem	0.12	0.5	≤0.06 – 8	98.0 / 0.0
Amikacin	4	16	1 – >32	90.0 / 8.0
AmpC-producing[c] (49)				
CPT104	0.12	0.5	0.03 – 1	- / -
Ceftaroline	32	>64	0.25 – >64	- / -
Ceftazidime	>16	>16	2 – >16	4.1 / 95.9
Cefepime	1	2	≤0.12 – 4	100.0 / 0.0
Ampicillin/sulbactam	>16	>16	>16	0.0 / 100.0
Piperacillin/tazobactam	32	>64	1 – >64	42.9 / 30.6
Imipenem	0.5	1	0.12 – 4	100.0 / 0.0
Amikacin	2	8	1 – >32	95.9 / 2.0
Carbapenemase-producing[d] (31)				
CPT104	0.5	2	0.06 – 4	- / -
Ceftaroline	>64	>64	1 – >64	- / -
Ceftazidime	>16	>16	≤0.12 – >16	9.6 / 83.9
Cefepime	>16	>16	0.25 – >16	25.8 / 61.3
Ampicillin/sulbactam	>16	>16	>16	0.0 / 100.0
Piperacillin/tazobactam	>64	>64	1 – >64	3.2 / 90.3
Imipenem	8	>8	0.25 – >8	38.7 / 32.3
Amikacin	16	>32	1 – >32	58.1 / 32.3
Acinetobacter spp. (10)				
CPT104	16	>32	1 – >32	- / -
Ceftaroline	>64	>64	1 – >64	- / -
Ceftazidime	16	>16	2 – >16	30.0 / 50.0
Cefepime	>16	>16	0.5 – >16	30.0 / 70.0
Ampicillin/sulbactam	>16	>16	1 – >16	30.0 / 60.0
Piperacillin/tazobactam	>64	>64	≤0.5 – >64	30.0 / 70.0
Imipenem	>8	>8	0.25 – >8	30.0 / 70.0
Amikacin	4	>32	0.5 – >32	50.0 / 30.0
P. aeruginosa (10)				
CPT104	16	>32	4 – >32	- / -
Ceftaroline	>64	>64	8 – >64	- / -
Ceftazidime	>16	>16	2 – >16	20.0 / 60.0
Cefepime	>16	>16	2 – >16	10.0 / 60.0
Ampicillin/sulbactam	>16	>16	>16	- / -
Piperacillin/tazobactam	64	>64	4 – >64	60.0 / 40.0
Imipenem	8	>8	1 – >8	20.0 / 50.0
Amikacin	>32	>32	4 – >32	30.0 / 60.0
MRSA (100)				
CPT104	1	2	0.5 – 4	- / -
Ceftaroline	1	2	0.5 – 4	- / -
Cefepime	>16	>16	8 – >16	4.0 / 96.0
Ceftazidime	>16	>16	>16	0.0 / 100.0
Imipenem	>8	>8	≤0.12 – >8	42.0 / 52.0
Vancomycin	1	1	0.5 – 2	100.0 / 0.0

a. Criteria as published by the CLSI [2010]. β-lactam susceptibility should be directed by the oxacillin test results
b. Includes: *Enterobacter aerogenes* (1 strain), *Enterobacter cloacae* (4 strains), *Escherichia coli* (18 strains), *Klebsiella oxytoca* (7 strains), *Klebsiella pneumoniae* (16 strains), *Proteus mirabilis* (2 strains), *Salmonella typhimurium* (1 strain), and *Serratia marcescens* (1 strain)
c. - = breakpoint criteria not available
d. Includes: *Citrobacter freundii* (14 strains), *Enterobacter aerogenes* (4 strains), *Enterobacter cloacae* (7 strains), *Escherichia coli* (14 strains), *Klebsiella pneumoniae* (2 strains), *Proteus mirabilis* (2 strains), *Salmonella newport* (1 strain), *Serratia marcescens* (3 strains), Group C *Salmonella* (1 strain), and Group D *Salmonella* (1 strain)
e. Includes: *Citrobacter freundii* (2 strains), *Enterobacter cloacae* (2 strains), *Escherichia coli* (4 strains), *Klebsiella oxytoca* (3 strains), *Klebsiella pneumoniae* (19 strains), and *Serratia marcescens* (1 strain)

Conclusions

- CPT104 was very active against ESBL-producing strains independent of the type of enzyme. CPT104 MIC values for ESBL-producing strains were similar to the ceftaroline MIC values exhibited by wild-type strains of the same species
- CPT104 was also very active against both chromosomal- and plasmid-mediated AmpC-producing strains (MIC_{50}, 0.12/4 mg/L)
- Carbapenemase-producing Enterobacteriaceae strains were also susceptible to CPT104 (MIC_{50}, 0.5/4 mg/L), but exhibited MIC values slightly higher than those of ESBL- or AmpC-producing strains (MIC_{50}, 0.12/4 mg/L)
- CPT104 showed good in vitro activity against a small number of wild-type *Acinetobacter* spp. strains tested (MIC, 1/4 - 2/4 mg/L), but limited activity against OXA-producing MDR *Acinetobacter* spp. and the collection *P. aeruginosa* strains
- CPT104 was very active against MRSA, with the lowest MIC values being observed among the more common community-associated USA300 isolates possessing SCC*mec* type IV cassette (USA300-like)
- CPT104 represents a potential therapeutic option for empiric therapy in settings where β-lactamase-producing Enterobacteriaceae and MRSA may predominate

References

1. Castanheira M, Mendes RE, Rhomberg PR, Jones RN (2008). Rapid emergence of bla_{CTX-M} among Enterobacteriaceae in U.S. Medical Centers: molecular evaluation from the MYSTIC Program (2007). *Microb Drug Resist* 14: 211-216.
2. Clinical and Laboratory Standards Institute (2009). *M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: eighth edition* Wayne, PA: CLSI.
3. Clinical and Laboratory Standards Institute (2010). *M100-S20. Performance standards for antimicrobial susceptibility testing: 20th informational supplement*. Wayne, PA: CLSI.
4. Endimiani A, Choudhary Y, Bonomo RA (2009). In vitro activity of NXL104 in combination with beta-lactams against *Klebsiella pneumoniae* isolates producing KPC carbapenemases. *Antimicrob Agents Chemother* 53: 3599-3601.
5. Livermore DM, Mushtaq S, Warner M, Miossec C, Woodford N (2008). NXL104 combinations versus Enterobacteriaceae with CTX-M extended-spectrum beta-lactamases and carbapenemases. *J Antimicrob Chemother* 62: 1053-1056.
6. Stachyra T, Levasseur P, Pechereau MC, Girard AM, Claudon M, Miossec C, Black MT (2009). *In vitro* activity of the β-lactamase inhibitor NXL104 against KPC-2 carbapenemase and Enterobacteriaceae expressing KPC carbapenemases. *J Antimicrob Chemother* 64: 326-329.

Acknowledgment

Supported by Forest Laboratories, Inc.