Potent Activity of Ceftazidime/NXL104 Tested Against Enterobacteriaceae Isolates Carrying Multiple β-lactamase Enzymes

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AMENDED ABSTRACT

Objectives: Treatment of infections caused by pathogens carrying multiple beta-lactamases (BLs) can be challenging due to the association of beta-lactam-resistance mechanisms with other genetic determinants encoding resistance to non-beta-lactam compounds. NXL104 is a broad spectrum BL-inhibitor and, combined with ceftazidime (CAZ), is undergoing clinical trials to treat hospital infections caused by Gram-negative pathogens, including those with multidrug resistance. The objective of this study was to assess the activity of CAZ/NXL104 against a panel of Enterobacteriaceae (ENT) carrying multiple BL enzymes.

Methods: CAZ activity combined with NXL104 at fixed 4 mg/L was evaluated by CLSI broth microdilution methods against 80 ENT strains (9 species) producing 2 to 4 BLs. BLs included: narrow-spectrum (3 SHV-, 2 OXA-types, TEM-1), ESBLs (4 SHV-, 4 CTX-M-, 2 OXY-variants, OXA-1/30), serine-carbapenemases (3 KPC-, 2 SME-types, NMC-A) and metallo-BLs (VIM-1, VIM-2) all identified by PCR and sequencing. ENT hyperproducing chromosomal AmpC were also tested.

Results: Strains producing 2 (58), 3 (20) and 4 (2) BLs were evaluated. 66 of 80 (82.5%) strains showed CAZ/NXL104 MIC results at ≤2 mg/L. Inhibitory effect was observed despite the number of BLs (Table). Only 5 KPC-producers (1 *C. freundii* and 4 *E. cloacae*) and 5 SHV-12 (all *E. cloacae*) showed CAZ/NXL104 MIC results at 4 mg/L, but MIC reductions were 16- to 128-fold compared to CAZ alone. 2 KPC- and 2 VIM-producers (all *Enterobacter* spp.) had MICs at ≥8 mg/L for CAZ/NXL104, though inhibitory effect (16- and 32-fold decrease) was noted for the KPC-producers. The great majority (85%) of ENT had MICs lowered 4- to 512-fold by NXL104. Four isolates showing no inhibitory effect carried MBL enzymes; one isolate had low/susceptible CAZ MIC value (≤2 mg/L).

Organisms (no. tested)	MIC (mg/L)					MIC reduction with NXL104
	≤2	4	8	16	≥32	
2 beta-lactamases (58)						
CAZ	12	2	1	3	41	None[a]- to >512-fold
CAZ/NXL104	45	10	1	1	2	
3 beta-lactamases (20)						
CAZ	1	3	1	3	11	4- to 512-fold
CAZ/NXL104	19	-	-	-	-	
4 beta-lactamases (2)						
CAZ	-	-	-	-	2	128- to 256-fold
CAZ/NXL104	2	-	-	-	-	

a. Four isolates showing no inhibitory effect carried MBL enzymes; one isolate had low/susceptible CAZ MIC value (≤2 mg/L).

Conclusions: The addition of NXL104 to CAZ resulted in a 4- to 512-fold decrease in MIC for most isolates having multiple BLs. CAZ/NXL104 MIC values were within the CLSI CAZ susceptibility range for the vast majority of ENT tested (>95% of strains), regardless of the number of BLs present or CAZ MIC. These data demonstrate that CAZ/NXL104 could be a significant therapeutic option for treatment of contemporary, most challenging BL-producing ENT species.

INTRODUCTION

The presence of multiple β-lactamases in a single cephalosporin-resistant isolate has been recognized as an alarming fact since early detection of β-lactamases. Although strains reported to produce two to four β-lactamases were commonly characterized, the recent detection of a single *Klebsiella pneumoniae* strain carrying up to eight β-lactamase encoding genes, including a KPC carbapenemase and an inhibitor-resistant TEM variant, emphasizes the ability of these organisms to accumulate multiple resistance determinants. The proliferation of these arrays of enzymes restricts the use of β-lactams as empiric therapeutic option for the treatment of Gram-negative infections, including carbapenems and clinically available β-lactamase inhibitors.

NXL104 is a new non-β-lactam inhibitor of β-lactamases currently in clinical development that displays a broad-spectrum inhibition profile against both class A and class C enzymes, and a variable level of activity against class D enzymes. β-lactamase enzymes are inactivated very efficiently by NXL104 with low MIC_{50} (concentration resulting in 50% inhibition) values, low turnover numbers and form highly stable complexes. NXL104 has virtually no intrinsic antibacterial activity, but efficiently protects β-lactams from hydrolysis in a variety of strains producing class A and class C enzymes, including extended-spectrum β-lactamases (ESBLs).

In this study, we evaluated the activity of ceftazidime with and without NXL104 against 80 Enterobacteriaceae strains producing two or more β-lactamases, including enzymes from Ambler classes A, B, C and D.

MATERIALS AND METHODS

Bacterial strains. Eighty Enterobacteriaceae strains carrying 2 to 4 β-lactamase encoding genes were included in this study. These strains were collected during multicenter surveillance studies and only one isolate per patient from documented infections were included in these surveys. Isolates were collected from bloodstream, respiratory tract and skin and skin structures infections according to common protocols. Species identification was confirmed by standard biochemical tests and the Vitek II System (bioMerieux, Hazelwood, Missouri, USA), when necessary.

Bacterial species producing β-lactamases included: *Enterobacter cloacae* (39), *K. pneumoniae* (16), *Serratia marcescens* (10), *Escherichia coli* (4), *Klebsiella oxytoca* (4), *Citrobacter freundii* (3), *Enterobacter gergoviae* (2), *Enterobacter aerogenes* (1), and *Proteus vulgaris* (1).

Antimicrobial susceptibility testing. All isolates were tested for antimicrobial susceptibility using the 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. Ceftazidime/NXL104 was tested in a fixed 4 mg/L concentration of NXL104. Categorical interpretations were those found in CLSI; M100-S20 and quality control (QC) was performed using *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI documents.

Genotypic detection of resistance. Multiplex PCR approaches were used to detect genes encoding ESBL types, including PER, GES, VEB, CTX-M and oxacillinases (OXA-ESBL), metallo-β-lactamases (IMP, VIM, SPM, GIM, SIM) and serine-carbapenemases (KPC, IMI/NMC-A, SME, OXA-48). TEM and SHV enzymes were amplified in singleplex reactions.

Sequencing analysis. Both strands of PCR amplicons were sequenced and the nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI). Sequences were compared to others available via internet sources (http://www.ncbi.nlm.nih.gov/blast/).

RESULTS

- Eighty Enterobacteriaceae strains producing 2 or more β-lactamases were evaluated. A total of 32 strains carrying 2 or more acquired β-lactamases were summarized in Table 1. The remaining 48 strains producing chromosomal AmpC and one additional β-lactamase carried genes encoding: serine-carbapenemase (24 strains; KPC-2, to -4; SME-2; NMC-A); narrow and extended spectrum β-lactamases (21; OXA-10, OXA-2, CTX-M-14, SHV-7, -11, -12, -30).
- Strains carrying two β-lactamase genes (ESBL and/or narrow-spectrum enzymes; class A or D enzymes) showed ceftazidime MIC reductions of two- to 512-fold when NXL104 was combined with ceftazidime. Ceftazidime/NXL104 MIC_{50} was only 1 mg/L, whereas ceftazidime MIC_{50} was 128 mg/L (Table 2).
- All strains producing three β-lactamase enzymes (Table 1) were inhibited at ≤2 mg/L of ceftazidime/NXL104. The MIC_{50} in the presence of NXL104 was 256-fold lower when compared to the MIC_{50} for ceftazidime alone (Table 2).
- AmpC-producers with one, two or three additional β-lactamases were inhibited at 4 mg/L or less of ceftazidime/NXL104 (Table 2). These strains carried class A and/or D enzymes. Decreases in ceftazidime MIC values in the presence of NXL104 were more limited among strains showing low ceftazidime MIC results (0.25 to 2 mg/L, strains producing SME-2 [3 strains], SHV-30 [1] and KPC-2 [1]).
- All but four carbapenemase-producing isolates, known to have significant AmpC expression were inhibited at 4 mg/L of ceftazidime/NXL104. Two *Enterobacter* spp. strains, displaying ceftazidime/NXL104 at 8 and 16 mg/L, carried KPC genes but showed at least 16-fold decrease in the MIC values in the presence of NXL104. The remaining strains produced VIM-type enzymes (Ambler type B).
- Overall, KPC-producing isolates were inhibited by ceftazidime/NXL104, regardless of the number of additional β-lactamases (two or three). MIC reductions observed, ranged from two- to >256-fold.
- The majority of CTX-M variants exhibited minimal to moderate hydrolytic activity against ceftazidime and MIC results can be within the susceptible range (≤4 mg/L). The presence of NXL104 observed to enhance the activity of ceftazidime in these strains, lowering eight- to 16-fold the MIC_{50} of the inhibitor combination.

Table 1. Isolates producing multiple characterized β-lactamases of various types, including serine-carbapenemases, ESBLs (CTX-M-, SHV-, OXY-variants) and narrow spectrum β-lactamases (oxacillinases, TEM- and SHV-variants).

Organism	Year of isolation	β-lactamases	Category
E. coli	2007	CTX-M-14, OXA-2	Two β-lactamases
K. pneumoniae	2007	CTX-M-14, OXA-2, SHV-5	Three β-lactamases
K. pneumoniae	2007	CTX-M-14, SHV-11	Two β-lactamases
K. pneumoniae	2007	CTX-M-14, SHV-27, TEM-1	Three β-lactamases
K. pneumoniae	2007	CTX-M-15, SHV-1	Two β-lactamases
K. pneumoniae	2007	CTX-M-15, SHV-1, TEM-1	Three β-lactamases
E. coli	2007	CTX-M-15, TEM-1	Two β-lactamases
K. pneumoniae	2001	CTX-M-2, OXA-1, TEM-1	Three β-lactamases
E. coli	2001	CTX-M-2, OXA-2	Two β-lactamases
K. pneumoniae	2001	CTX-M-2, OXA-2, TEM-1	Three β-lactamases
E. cloacae	2001	CTX-M-3, TEM-1	AmpC + two β-lactamases
K. oxytoca	2007	KPC-2, OXA-2, SHV-30	Three β-lactamases/ KPC + two β-lactamase
K. pneumoniae	2007	KPC-2, SHV-1	Two β-lactamases/ KPC + one β-lactamase
K. oxytoca	2007	KPC-2, SHV-40, OXY-1	Three β-lactamases/ KPC + two β-lactamase
K. pneumoniae	2007	KPC-3, SHV-11, TEM-1	Three β-lactamases/ KPC + two β-lactamase
K. pneumoniae	2007	KPC-3, SHV-1, TEM-1	Three β-lactamases/ KPC + two β-lactamase
K. pneumoniae	2007	OXA-10, SHV-11, TEM-1	Three β-lactamases
E. cloacae	2007	OXA-10, SHV-12, TEM-1	AmpC + three β-lactamases
S. marcescens	2007	OXA-10, SHV-30	AmpC + two β-lactamases
S. marcescens	2007	OXA-10, SHV-7	AmpC + two β-lactamases
E. coli	2001	OXA-1, SHV-5	Two β-lactamases
K. pneumoniae	2007	OXA-2, SHV-11, TEM-1	Three β-lactamases
E. cloacae	2007	OXA-2, SHV-30	AmpC + two β-lactamases
K. pneumoniae	2007	OXA-2, SHV-30	Two β-lactamases
E. cloacae	2007	OXA-2, SHV-5	Two β-lactamases
K. pneumoniae	2000	OXA-2, SHV-5, TEM-1	Three β-lactamases
E. cloacae	2007	OXA-2, SHV-7	AmpC + two β-lactamases
E. cloacae	2007	OXA-2, SHV-7, TEM-1	AmpC + three β-lactamases
K. pneumoniae	2007	OXA-2, TEM-1	Two β-lactamases
K. oxytoca	2007	OXA-2, OXY-1a	Two β-lactamases
K. pneumoniae	2007	SHV-12, TEM-1, OXY-1	Three β-lactamases
K. pneumoniae	2007	SHV-31, TEM-1	Two β-lactamases

Table 2. Frequency distributions of ceftazidime/NXL104 when tested against Enterobacteriaceae strains producing multiple β-lactamases.

No. or type of β-lactamase (n)/ Antimicrobial agent	Number of strains inhibited at MIC (mg/L):													MIC_{50}	MIC reduction in the presence of NXL104
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>[a]	
Two β-lactamases (11)															
Ceftazidime/NXL104		1	2	1	5	1	1								1	2- to 512-fold
Ceftazidime					1	2	1				1	2		4	128	
Three β-lactamases (9)															
Ceftazidime/NXL104		2	3		2	2									0.25	4- to >256-fold
Ceftazidime				1			2			1	2	3			64	
AmpC + one β-lactamase (18)															
Ceftazidime/NXL104			2	3	4	4	5								1	2- to >512-fold
Ceftazidime					1	1	1				1	1	3	10	>256	
AmpC + two β-lactamases (7)															
Ceftazidime/NXL104			1	2	4										1	4- to >256-fold
Ceftazidime							1	1	1	1		1		2	16	
AmpC + three β-lactamases (2)															
Ceftazidime/NXL104				1	1										0.5	128- to >256-fold
Ceftazidime											1			1	64	
Carbapenemase + AmpC (29)															
Ceftazidime/NXL104	1	3	5	1	7	4	4	1	1					2	1	None[b] to >256-fold
Ceftazidime			4	1	1			1	3	2	2		6	9	256	
All KPC-producers (27)															
Ceftazidime/NXL104	1	1	2	2	9	5	5	1	1							2- to >256-fold
Ceftazidime			1					1	5	2	2		6	10	1	
KPC + one β-lactamase (24)															
Ceftazidime/NXL104	1	1	2	1	8	4	5	1	1						1	2- to >256-fold
Ceftazidime			1					1	4	2	2		5	9	256	
KPC + two β-lactamases (4)															
Ceftazidime/NXL104	1		2	1											1	32- to >256-fold
Ceftazidime									2				1	1	16	
All CTX-M-producers (13)															
Ceftazidime/NXL104		2	4	2	3	2									0.5	2- to 512-fold
Ceftazidime				1	2	1	2	1		1	2	2		1	8	
CTX-M + 1 β-lactamase (7)															
Ceftazidime/NXL104		1	2	1	2	1									0.5	2- to 512-fold
Ceftazidime					2	1	1				1	2			4	
CTX-M + 2 β-lactamases (6)															
Ceftazidime/NXL104		1	2	1	1	1									0.25	2- to >256-fold
Ceftazidime				1			1	1	1	1	1			1	8	

a. Greater that the last dilution tested (>32 mg/L for ceftazidime/NXL104 and >256 for ceftazidime).
b. Strains presenting no reduction on ceftazidime MIC in the presence of NXL104 were those producing metallo-β-lactamase encoding genes.

CONCLUSIONS

- Ceftazidime/NXL104 demonstrated excellent activity against strains carrying multiple β-lactamases, inhibiting combinations of enzymes that included Ambler class A, C and D.
- The vast majority of the strains showing moderate decreased MIC values in the presence of NXL104 were those displaying a low ceftazidime MIC value (≤4 mg/L).
- Ceftazidime/NXL104 showed no enhanced activity when compared to ceftazidime alone against strains producing metallo-β-lactamase enzymes.
- The presence of different β-lactamase encoding genes within one bacterial cell is known to generate a broad β-lactam resistance profile that can jeopardize the use of all β-lactams, including monobactams and carbapenems. This study highlights the excellent activity of ceftazidime/NXL104 against these strains and the potential for this agent for the clinical empiric therapy of serious infections cause by Gram-negative pathogens.

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