

# In Vitro Antibacterial Activity of Ceftazidime (CAZ) in Combination with the $\beta$ -Lactamase Inhibitor, NXL104

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P. Levasseur, C. Miossec, A.-M. Girard, L. Lavallade, D. Shlaes\*, M. Black, J. Pace and K. Coleman

Novexel SA, Romainville, France and \*Anti-infectives Consulting, Stonington, CT



Contact Information: premarvarthy.levasseur@novexel.com

## ABSTRACT

**Background.** NXL104 is a novel non  $\beta$ -lactam  $\beta$ -lactamase inhibitor that has been shown *in vitro* and *in vivo* to inhibit both class A and class C enzymes. It is currently in phase 2 of clinical development in combination with CAZ. The objective of the study was to evaluate the antibacterial activities of CAZ/NXL104 combinations against characterized *Enterobacteriaceae* species.

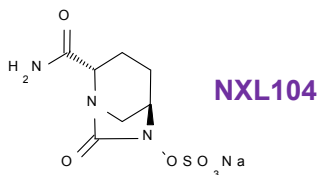
**Methods.** Activity of CAZ/NXL104 combinations against 200 strains of *Enterobacteriaceae*, representing most clinical species, was determined. The panel included 44% CAZ-resistant isolates, most with characterized  $\beta$ -lactamases. Minimal Inhibitory Concentrations (MIC) were determined with NXL104 at fixed weight ratios between 1 and 16, or at fixed concentrations of 2, 4, and 8  $\mu$ g/mL.

**Results.** CAZ/NXL104 combinations were active against all isolates and had significantly lower MICs than CAZ alone, cefotaxime, ceftriaxone, cefepime, or piperacillin/tazobactam (MIC<sub>90</sub>s  $\geq$ 128 $\mu$ g/mL). MIC<sub>50</sub>s and MIC<sub>90</sub>s for CAZ at fixed concentrations of NXL104 were  $\leq$ 0.125-0.5 and 1-4  $\mu$ g/mL, respectively, and 1-2 and 4-16  $\mu$ g/mL, respectively, at fixed ratios of CAZ/NXL104. Potentiation of CAZ by NXL104 was generally 16 to 512-fold for TEM, SHV, CTX-M or KPC producers. Against KPC producers, NXL104 reduced CAZ MICs from  $\geq$ 128 to  $\leq$ 0.25-8  $\mu$ g/mL. Potentiation of 8 to 512-fold was observed against AmpC enzyme producers.

**Conclusion.** The CAZ/NXL104 combination exhibits a broad spectrum activity against resistant *Enterobacteriaceae* isolates, and represents an important new generation of  $\beta$ -lactam/  $\beta$ -lactamase inhibitor combination.

## BACKGROUND

NXL104 is a novel, non- $\beta$ -lactam,  $\beta$ -lactamase inhibitor with a spectrum of activity encompassing both class A and class C  $\beta$ -lactamases. It is currently being tested in human phase 2 trials in combination with ceftazidime (CAZ).



## REFERENCES

1: CLSI Document M100-S18. Clinical Laboratory Standards Institute, Wayne, PA. January, 2008.

## MATERIALS & METHODS

### Bacterial isolates

Bacterial isolates were from the Novexel culture collection, originally collected from a variety of clinical sources; 200 strains of *Enterobacteriaceae* were included in the study (Shown below).

### Susceptibility testing

MIC determination was performed using CLSI methods for antimicrobial susceptibility testing with cation-adjusted Mueller-Hinton (MH) broth<sup>1</sup>.

The following antimicrobials were used: ceftazidime (CAZ), cefotaxime (CTX), cefepime (FEP), piperacillin (PIP), imipenem (IPM) and ciprofloxacin (CIP).

The  $\beta$ -lactamase inhibitor tazobactam (TZB) was used in combination with piperacillin at 4  $\mu$ g/mL fixed concentration; NXL104 was used at different fixed concentrations (2, 4, or 8  $\mu$ g/mL), or at different fixed weight ratios (1/1 to 16/1) with ceftazidime.

Organism	Number	$\beta$ -lactamases
<i>Citrobacter freundii</i>	15	Chromosomal C
<i>Enterobacter aerogenes</i>	1	Chromosomal C
<i>Enterobacter cloacae</i>	15	Chromosomal C; KPC-2, -3; TEM-1
<i>Escherichia coli</i>	52	TEM-1, -3, -5, -7, -8, -12, -16, -24, -43; SHV-1, -3, -4, -5, -38; CTX-M-1, -2, -9, -14, -15, -16; KPC-2; GES-1; PER-1; PSE-4; OXA-1; CMY-2; FOX-1
<i>Klebsiella oxytoca</i>	7	Chromosomal A; OXY-2; TEM-1, -129
<i>Klebsiella pneumoniae</i>	41	TEM-1, -18, -2, -10, -12, -26, -63; SHV-1, -2, -2a, -5, -18; CTX-M-1, -2, -3, -14, -15, -16; KPC-2, -3; PER; OXA-1, ACC-1; CMY-4; DHA-1, -2; FOX-2; MOX-3; VIM-1, -4
<i>Morganella morganii</i>	13	Chromosomal C
<i>Serratia marcescens</i>	13	TEM-1; CTX-M-13
<i>Providencia rettgeri</i>	3	Not determined
<i>Providencia stuartii</i>	14	Not determined
<i>Proteus mirabilis</i>	13	CMY-4
<i>Proteus vulgaris</i>	13	Chromosomal A

## RESULTS

Table 1 shows summary data for all NXL104 combinations against the 200 isolates.

Table 2 shows MICs for a selection of individual isolates, most ceftazidime resistant and most with well-characterized  $\beta$ -lactamases. NXL104 was present at 4  $\mu$ g/mL.

Figure 1 shows the cumulative percentage of strains inhibited by MIC for all antibiotics. Once again, results are presented with NXL104 at 4  $\mu$ g/mL.

## RESULTS (Continued)

Table 1: Summary MICs – All Strains ( $\mu$ g/mL; N = 200)

Antibiotic	MIC $\mu$ g/mL		
	Range	MIC <sub>50</sub>	MIC <sub>90</sub>
Ceftazidime	$\leq$ 0.125 - $>$ 128	8	$>$ 128
Ceftazidime/NXL104 (2 $\mu$ g/mL)	$\leq$ 0.125 - $>$ 128	0.5	4
Ceftazidime/NXL104 (4 $\mu$ g/mL)	$\leq$ 0.125 - $>$ 128	0,25	2
Ceftazidime/NXL104 (8 $\mu$ g/mL)	$\leq$ 0.125 - $>$ 128	$\leq$ 0.125	2
Ceftazidime/NXL104 (1/1)	$\leq$ 0.125 - $>$ 128	1	4
Ceftazidime/NXL104 (2/1)	$\leq$ 0.125 - $>$ 128	1	8
Ceftazidime/NXL104 (4/1)	$\leq$ 0.125 - $>$ 128	1	8
Ceftazidime/NXL104 (8/1)	$\leq$ 0.125 - $>$ 128	1	16
Ceftazidime/NXL104 (16/1)	$\leq$ 0.125 - $>$ 128	2	16
Cefotaxime	$\leq$ 0.125 - $>$ 128	2	$>$ 128
Ceftriaxone	$\leq$ 0.125 - $>$ 128	4	$>$ 128
Cefepime	$\leq$ 0.125 - $>$ 128	0,5	64
Piperacillin/Tazobactam	$\leq$ 0.125 - $>$ 128	4	$>$ 128
Imipenem	$\leq$ 0.125 - 128	0.5	4
Ciprofloxacin	$\leq$ 0.125 - $>$ 128	$\leq$ 0.125	8

Figure1: Cumulative % of Strains Inhibited vs MIC (N = 200)

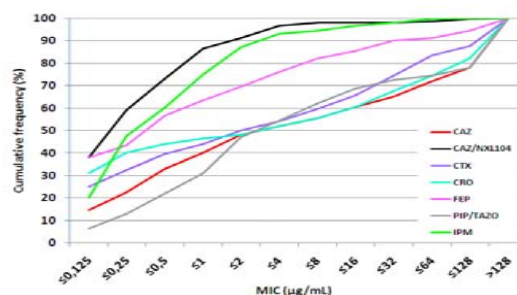


Table 2: MICs of Selected Ceftazidime-Resistant Isolates ( $\mu$ g/mL)

Species	$\beta$ -Lactamase Class		CAZ		CTX	FEP	PIP +TZB (4 $\mu$ g/ml)	IMP	CIP		
	A	B	C	D							
<i>C. freundii</i>		Chrom			32	0.5	16	0.06	4	0.25	0.06
		Chrom			128	0.25	64	0.5	16	0.25	0.06
		Chrom			64	0.5	32	0.5	32	0.25	0.06
	TEM-1	Chrom			256	0.5	64	1	64	0.25	0.06
<i>E. aerogenes</i>		Chrom, FEP-R			256	0.06	32	0.5	128	0.25	0.06
		Chrom			256	4	64	32	128	0.25	0.06
<i>E. cloacae</i>	KPC-2	Chrom			128	1	256	4	256	0.5	0.5
	KPC-3, TEM-1	Chrom	OXA-9		256	8	256	256	256	32	16
<i>E. coli</i>	CTX-M-14, TEM-1				128	1	64	16	32	0.5	128
	CTX-M-15, TEM-1		CMY-2		128	0.25	128	32	2	0.06	64
	KPC-2			OXA-1	64	0.25	16	16	16	128	16
	VEB-1		CMY-2		256	128	256	256	256	0.25	64
	PER-1				256	1	32	8	2	0.06	0.25
	SHV-3				32	0.06	32	8	256	0.5	0.25
	SHV-4				128	0.25	32	4	4	0.06	0.06
	SHV-5				64	0.25	8	1	2	0.25	0.06
	TEM-12				16	0.25	0.25	2	2	0.25	0.06
	TEM-16				256	0.5	4	8	2	1	0.06
TEM-3				64	0.25	128	4	1	0.25	0.06	
TEM-5				32	0.06	2	0.5	1	0.25	0.06	
TEM-8				256	0.25	4	4	2	0.5	0.25	
<i>K. oxytoca</i>	OXY-2, TEM-1				256	4	32	8	16	0.25	32
	TEM-129				256	2	16	32	256	0.06	8
<i>K. pneumoniae</i>	OXY-2, TEM-1				8	1	64	16	256	0.25	32
	TEM-1B, CTX-M-2				128	2	256	128	256	0.25	32
	TEM-1		ACC-1		128	1	16	2	256	0.06	1
	TEM-1		CMY-4		256	0.5	64	2	32	0.5	4
	CTX-M-14			OXA-1	16	1	256	128	16	1	64
	CTX-M-15, TEM-1			OXA-1	256	2	256	256	256	0.06	256
	CTX-M-16			OXA-1	256	1	256	256	256	0.25	0.06
	CTX-M-3				16	0.5	128	32	16	0.06	0.06
	SHV-5, TEM-1		DHA-2		256	2	64	0.5	64	0.5	0.25
	KPC-2				256	1	256	256	256	32	128
KPC-3				256	0.5	64	8	128	0.25	0.25	
SHV-5, TEM-1				256	1	32	2	256	0.25	2	
SHV-1, TEM-1B, CTX-M-3		MOX-2		256	2	32	16	256	0.06	1	
SHV-1, TEM-2, PER				256	4	16	4	256	0.25	1	
SHV-5, TEM-10				128	0.5	32	2	256	0.25	0.06	
SHV-5		VIM-1		256	256	256	256	256	32	8	
<i>S. marcescens</i>				32	0.5	32	4	32	0.5	4	
<i>P. rettgeri</i>	CTX-M-13, TEM-1			4	0.25	256	64	1	0.25	0.06	
<i>P. stuartii</i>				16	0.5	8	4	256	1	0.06	
				8	0.25	2	1	256	1	0.06	
<i>P. mirabilis</i>				4	1	1	1	32	1	0.06	
<i>P. vulgaris</i>				2	1	1	1	8	0.5	1	
<i>M. morganii</i>				256	1	256	256	4	4	8	
		CMY-4		32	0.06	64	1	2	2	0.06	
<i>M. morganii</i>		Chrom		1	0.06	8	1	2	1	0.06	
		Chrom		1	1	256	1	2	1	0.06	

## CONCLUSION

- Ceftazidime MICs were improved considerably against the majority of isolates when combined with NXL104
- Ceftazidime/NXL104 was active against virtually all organisms producing Class A or Class C  $\beta$ -lactamases
- NXL104 also protected ceftazidime against a number of the Class D  $\beta$ -lactamases, but not against Class B enzymes