

## Session no. 120: New $\beta$ -lactams in the pipeline (Abstract F-1161)

**Title:** In vitro activity of NXL 104 (formerly AVE1330A), a New  $\beta$ -lactamase Inhibitor, in Combination with Cefpodoxime and Cefixime against 3<sup>rd</sup> Generation Cephalosporin-Resistant Isolates of species of the *Enterobacteriaceae*.

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NXL 104 (formerly AVE1330A)

- a bridged bicyclico[3.2.1]diazabicyclo-octanone



# In vitro activity of AVE1330A, an innovative broad-spectrum non- $\beta$ -lactam $\beta$ -lactamase inhibitor

Bonnefoy et al JAC (2004) 54: 410:417

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	IC <sub>50</sub>		
	AVE1330A (NXL 104)	Clavulanic acid	Tazobactam
TEM-1	8 nM	130 nM	40 nM
P99	80 nM	1x10 <sup>6</sup> nM	5000 nM

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Investigated the combination of ceftazidime/AVE1330A (NXL 104) in a 4:1 ratio.

AVE1330A (NXL 104) restored activity of ceftazidime against TEM-3, TEM-10, SHV-2 and SHV-5 Class A  $\beta$ -lactamases and against Class C enzyme producers.

*In vitro* activity of cefpodoxime, alone and in various combinations with NXL 104, against transformants of *Escherichia coli* expressing characterised  $\beta$ -lactamases.

Expressed $\beta$ -lactamase	Cefpodoxime alone	Cefpodoxime + NXL104 at a fixed concentration of :			Cefpodoxime + NXL 104 in a ratio of:	
		4 $\mu$ g/ml	2 $\mu$ g/ml	1 $\mu$ g/ml	4 : 1	2 : 1
- TEM 1	1	0.03	0.12	0.5	0.5	0.5
- TEM 1	0.5	0.06	0.25	0.25	0.25	0.25
- TEM 2	1	0.25	0.5	0.5	1	0.5
- TEM 3	64	0.25	0.5	0.5	1	1
- TEM 6	16	0.25	0.5	0.5	1	1
- TEM 9	64	0.12	0.25	0.5	1	1
- TEM 9	128	0.5	1	1	2	2
- TEM 10	16	0.25	0.5	0.5	1	1
- OXA 1	4	0.25	0.5	0.5	1	1
- OXA 2	0.5	0.06	0.25	0.5	0.5	0.5
- OXA 3	1	0.25	0.5	0.5	0.5	0.5
- OXA 4	2	0.25	0.5	0.5	0.5	1
- OXA 5	16	0.25	0.5	0.5	1	0.5
- OXA 7	2	0.25	0.5	1	1	1
- SHV 1	0.5	0.12	0.25	0.5	0.5	0.5
- PSE 2	1	0.12	0.25	0.25	1	1
- PSE 2	0.25	0.12	0.12	0.12	0.12	0.25
- PSE 4	0.5	0.25	0.25	0.25	0.5	0.5

*In vitro* activity of cefixime, alone and in various combinations with NXL 104, against transformants of *Escherichia coli* expressing characterised  $\beta$ -lactamases

Expressed $\beta$ -lactamase	Cefixime alone	Cefixime + NXL104 at a fixed concentration of :			Cefixime + NXL 104 in a ratio of:	
		4 $\mu$ g/ml	2 $\mu$ g/ml	1 $\mu$ g/ml	4 : 1	2 : 1
- TEM 1	1	0.03	0.12	0.12	0.25	0.25
- TEM 1	0.5	0.06	0.12	0.12	0.25	0.12
- TEM 2	1	0.06	0.12	0.12	0.25	0.25
- TEM 3	4	0.06	0.12	0.12	0.25	0.25
- TEM 6	8	0.12	0.12	0.25	0.5	0.5
- TEM 9	16	0.06	0.12	0.12	0.5	0.5
- TEM 9	64	0.25	0.25	0.5	1	1
- TEM 10	4	0.12	0.12	0.12	0.5	0.25
- OXA 1	0.5	0.12	0.25	0.25	0.25	0.25
- OXA 2	0.5	0.03	0.12	0.12	0.25	0.12
- OXA 3	1	0.12	0.25	0.25	0.25	0.25
- OXA 4	1	0.12	0.25	0.25	0.25	0.25
- OXA 5	4	0.12	0.12	0.12	0.25	0.25
- OXA 7	1	0.12	0.25	0.25	0.25	0.5
- SHV 1	1	0.06	0.06	0.06	0.25	0.25
- PSE 2	1	0.06	0.12	0.12	0.25	0.12
- PSE 2	0.25	0.03	0.06	0.06	0.12	0.06
- PSE 4	0.5	0.06	0.06	0.06	0.12	0.12

*In vitro* activity of cefpodoxime, alone and in various combinations with NXL 104, against 24 isolates of *Klebsiella* spp. producing SHV-derived ESBL \*.

	MIC µg/ml			% S
	50%	90%	range	
Cefpodoxime alone	32	128	4-≥256	0%
Cefpodoxime + 4 µg/ml NXL104 (fixed concentration)	0.06	0.25	≤0.015-0.25	100%
Cefpodoxime + 2 µg/ml NXL104 (fixed concentration)	0.12	0.5	0.03-1	100%
Cefpodoxime + 1 µg/ml NXL104 (fixed concentration)	0.25	0.5	0.06-2	100%
Cefpodoxime + NXL 104 (4:1 fixed ratio)	0.5	2	0.25-4	95.8%**
Cefpodoxime + NXL 104 (2:1 fixed ratio)	0.5	1	0.12-2	100%

CLSI cefpodoxime breakpoint: susceptible (S) = ≤2 µg/ml

\* = 24 strains include, -- SHV-2 (7), -- SHV-3 (4), -- SHV-4 (3), -- SHV-5 (7), -- SHV-2+5 (1), -- SHV-2+3(1) and -- SHV-3+5 (1)

\*\* = one strain producing an SHV-2 - derived ESBL required an MIC of 4 µg/ml cefpodoxime in a 4:1 ratio with NXL 104.

*In vitro* activity of cefixime, alone and in various combinations with NXL 104, against 24 isolates of *Klebsiella* spp. producing SHV – derived ESBL.

	MIC µg/ml			%S
	50%	90%	range	
Cefixime alone	8	128	2-128	0%
Cefixime + 4 µg/ml NXL104 (fixed concentration)	0.06	0.12	0.03-0.25	100%
Cefixime + 2 µg/ml NXL104 (fixed concentration)	0.12	0.25	0.03-1	100%
Cefixime + 1 µg/ml NXL104 (fixed concentration)	0.12	0.25	0.06-2	95.8%*
Cefixime + NXL 104 (4:1 fixed ratio)	0.5	1	0.25-4	95.8%*
Cefixime + CPD : NXL 104 (2:1 fixed ratio)	0.5	0.5	0.12-2	95.8%*

CLSI cefixime breakpoint: susceptible (S) =  $\leq 1$  µg/ml

\* = one strain producing an SHV-2 - derived ESBL required an MIC of 2 µg/ml cefixime + NXL 104 at a fixed concentration of 1µg/ml, an MIC of 4 µg/ml in a 4:1 ratio with NXL 104 and 2 µg/ml in a 2:1 ratio, with NXL 104.

*In vitro* activity of cefpodoxime and cefixime, alone and in various combinations with NXL 104, against single isolates of *Escherichia coli* producing: i) a CTX-M-1 and ii) a CTX-M-14  $\beta$ -lactamase.

	MIC ( $\mu\text{g/ml}$ ) for:	
	<i>E. coli</i> CTX-M-1	<i>E. coli</i> CTX-M-14
<b>Cefpodoxime</b>		
Cefpodoxime alone	32	$\geq 256$
Cefpodoxime + 4 $\mu\text{g/ml}$ NXL104 (fixed concentration)	0.12	$\leq 0.015$
Cefpodoxime + 2 $\mu\text{g/ml}$ NXL104 (fixed concentration)	0.5	1
Cefpodoxime + 1 $\mu\text{g/ml}$ NXL104 (fixed concentration)	0.5	1
Cefpodoxime + NXL 104 (4:1 fixed ratio)	1	2
Cefpodoxime + NXL 104 (2:1 fixed ratio)	1	2
<b>Cefixime</b>		
Cefixime alone	4	16
Cefixime + 4 $\mu\text{g/ml}$ NXL104 (fixed concentration)	0.06	$\leq 0.015$
Cefixime + 2 $\mu\text{g/ml}$ NXL104 (fixed concentration)	0.12	0.5
Cefixime + 1 $\mu\text{g/ml}$ NXL104 (fixed concentration)	0.12	0.5
Cefixime + NXL 104 (4:1 fixed ratio)	0.25	1
Cefixime + CPD : NXL 104 (2:1 fixed ratio)	0.25	1

Comparative in vitro activity (MIC  $\mu$ g/ml) of cefpodoxime (CPD), alone and in various combinations with NXL 104, against 45 ceftazidime-resistant isolates of species of the *Enterobacteriaceae*.

Species	CPD alone	CPD + 4 $\mu$ g/ml NXL 104			CPD + 2 $\mu$ g/ml NXL 104			CPD + 1 $\mu$ g/ml NXL 104		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range
<i>E. coli</i> (12)	32- $\geq$ 256	0.12	0.5	$\leq$ 0.015-2	0.5	2	0.12-4	1	4	0.25-8
<i>C. freundii</i> (6)	32 $\geq$ 256	-	-	0.12-0.25	-	-	0.25-1	-	-	0.25-2
<i>Klebsiella spp.</i> (12)	8- $\geq$ 256	0.12	0.25	$\leq$ 0.015-0.5	0.25	1	$\leq$ 0.015-1	0.5	1	$\leq$ 0.015-1
<i>E. cloacae</i> (13)	16- $\geq$ 256	2	8	0.12-64	8	16	0.5-128	8	64	0.5-128
<i>M. morgani</i> (2)	128, $\geq$ 256	-	-	0.25, 2	-	-	0.25, 4	-	-	0.5, 2
All isolates (45)	8- $\geq$ 256	0.25	2	$\leq$ 0.015-64	1	8	$\leq$ 0.015-128	1	16	$\leq$ 0.015-128

Species	CPD + NXL 104 (fixed ratio 4:1)			CPD + NXL 104 (fixed ratio 2:1)		
	50%	90%	Range	50%	90%	Range
<i>E. coli</i> (12)	2	4	0.5-8	2	4	0.5-4
<i>C. freundii</i> (6)	-	-	1-4	-	-	0.5-2
<i>Klebsiella spp.</i> (12)	1	2	0.25-2	1	1	0.25-1
<i>E. cloacae</i> (13)	8	16	1-32	4	8	1-32
<i>M. morgani</i> (2)	-	-	2, 4	-	-	1, 4
All isolates (45)	2	8	0.25-32	2	4	0.25-32

Comparative in vitro activity (MIC  $\mu$ g/ml) of cefpodoxime (CPD), alone and in various combinations with NXL 104, against 45 ceftazidime-resistant isolates of species of the *Enterobacteriaceae*.

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% of isolates susceptible to cefpodoxime (MIC  $\leq$  2  $\mu$ g/ml)

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Cefpodoxime alone	0%
Cefpodoxime - 4 $\mu$ g/ml NXL 104	93.3%
Cefpodoxime - 2 $\mu$ g/ml NXL 104	71.1%
Cefpodoxime - 1 $\mu$ g/ml NXL 104	68.9%
Cefpodoxime + NXL 104 in a fixed ratio 4:1	57.8%
Cefpodoxime + NXL 104 in a fixed ratio 2:1	68.9%

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Comparative in vitro activity (MIC  $\mu$ g/ml) of cefixime (CEF), alone and in various combinations with NXL 104, against 45 ceftazidime-resistant isolates of species of the *Enterobacteriaceae*.

Species	CEF alone	CEF + 4 $\mu$ g/ml NXL 104			CEF + 2 $\mu$ g/ml NXL 104			CEF + 1 $\mu$ g/ml NXL 104		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range
<i>E. coli</i> (12)	16 - $\geq$ 256	0.06	0.5	$\leq$ 0.015-4	0.5	2	0.12-8	1	8	0.12-16
<i>C. freundii</i> (6)	8 - $\geq$ 256	-	-	0.06-1	-	-	0.06-2	-	-	0.12-8
<i>Klebsiella spp.</i> (12)	8 - $\geq$ 256	0.12	0.25	$\leq$ 0.015-0.25	0.25	0.5	$\leq$ 0.015-0.5	0.5	1	$\leq$ 0.015-1
<i>E. cloacae</i> (13)	4 - $\geq$ 256	4	16	0.03-64	8	32	0.25-64	16	64	0.25-64
<i>M. morgani</i> (2)	128, $\geq$ 256	-	-	0.12, 2	-	-	0.5, 2	-	-	0.5, 4
All isolates (45)	4 - $\geq$ 256	0.25	8	$\leq$ 0.015-64	0.5	8	$\leq$ 0.015-64	1	32	$\leq$ 0.015-64

Species	CEF + NXL 104 (fixed ratio 4:1)			CEF + NXL 104 (fixed ratio 2:1)		
	50%	90%	Range	50%	90%	Range
<i>E. coli</i> (12)	2	8	0.5-8	1	4	0.25-8
<i>C. freundii</i> (6)	-	-	0.5-8	-	-	0.5-4
<i>Klebsiella spp.</i> (12)	1	2	0.5-2	1	2	0.25-2
<i>E. cloacae</i> (13)	16	16	1-32	8	16	0.5-32
<i>M. morgani</i> (2)	-	-	2, 4	-	-	2, 4
All isolates (45)	2	16	0.5-32	2	8	0.25-32

Comparative in vitro activity (MIC  $\mu$ g/ml) of cefixime (CEF), alone and in various combinations with NXL 104, against 45 ceftazidime-resistant isolates of species of the *Enterobacteriaceae*.

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% of isolates susceptible to cefixime (MIC  $\leq$ 1  $\mu$ g/ml)

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Cefixime alone	0%
Cefixime - 4 $\mu$ g/ml NXL 104	71.1%
Cefixime - 2 $\mu$ g/ml NXL 104	62.2%
Cefixime - 1 $\mu$ g/ml NXL 104	57.8%
Cefixime + NXL 104 in a fixed ratio 4:1	28.9%
Cefixime + NXL 104 in a fixed ratio 2:1	42.2%

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# Summary 1

- NXL 104 is a synthetic  $\beta$ -lactamase inhibitor shown previously to be highly potent against Class A and Class C  $\beta$ -lactamases responsible for ceftazidime-resistance.
- In this study, NXL 104 has been shown to protect both cefpodoxime and cefixime from hydrolysis by Class A and Class C  $\beta$ -lactamases responsible for ceftazidime-resistance among isolates of the *Enterobacteriaceae*.
- The combination of NXL 104 with cefpodoxime and cefixime was highly effective in protecting these cephalosporins from hydrolysis by TEM-3, -6, -9, -10, OXA-1 and OXA-5  $\beta$ -lactamases which hydrolyse the unprotected cephalosporins.

## Summary 2

- NXL 104 was also effective in protecting cefpodoxime and cefixime from hydrolysis by SHV-derived and CTX-ESBLs.
- The optimum  $\beta$ -lactamase inhibitory effect of NXL 104 was achieved in combination with cefpodoxime and cefixime at a fixed concentration of 4  $\mu\text{g}/\text{ml}$ .
- NXL 104 is worthy of further investigation as a component of  $\beta$ -lactamase inhibitor-cephalosporin combinations for use in community infections caused by 3<sup>rd</sup> generation cephalosporin-resistant isolates of the *Enterobacteriaceae*.