

## ABSTRACT

## Background:

NXL104 is a new  $\beta$ -lactamase inhibitor that inhibits class A and class C enzymes. Its activity was evaluated in combination with the 3<sup>rd</sup> generation cephalosporins ceftazidime (CAZ), and ceftriaxone (CRO), or piperacillin (PIP) against 316 anaerobes. It is currently being tested in phase 2 clinical trials for the treatment of complicated intra-abdominal infections.

## Methods:

Minimal inhibitory concentrations (MIC) were determined using the agar dilution method (CLSI M11A7). NXL104 or tazobactam (TZB) were added to the  $\beta$ -lactams at 4 $\mu$ g/mL fixed concentration. A triple combination (3C) of NXL104 with an 8/1 ratio of CAZ and metronidazole (MTR) was also tested.

## Results:

The activities of CAZ, CRO, and PIP in combination with NXL104 were enhanced against many of the bacteria. CAZ/NXL104 MIC<sub>50</sub>s were 8 to 16-fold lower than those of CAZ against Gram-negative anaerobes. The combination of CAZ/NXL104 with MTR was additive or synergistic for most strains. The antibiotic resistance rates against all anaerobic strains were: CAZ (37.7%), CRO (31%), CAZ/NXL104 (15.2%), CRO/NXL104 (5.4%), and MTR (4.1%). No resistant strains could be observed with PIP/TZB, PIP/NXL104, or the triple combination MTR/CAZ/NXL104.

## Conclusions:

The triple combination of MTR/CAZ/NXL104 demonstrated potent antibacterial activity against anaerobes representing most clinical species; it appears appropriate for the treatment of polymicrobial infections since CAZ/NXL104 exhibits potent activity against  $\beta$ -lactamase producing *Enterobacteriaceae*.

## INTRODUCTION

The aim of this study was to determine the antimicrobial activity of a new  $\beta$ -lactamase inhibitor NXL104 against a wide range of anaerobic bacteria from human origin by determining the minimal inhibitory concentration (MIC) values of this new drug and comparators against recent clinical isolates. The anti-anaerobic activity of the new drug NXL104 was determined as a single antimicrobial agent and further studied in combination with either piperacillin or a third generation cephalosporin (ceftriaxone and ceftazidime). A triple combination of ceftazidime +NXL104 + metronidazole was investigated.

## MATERIALS AND METHODS

## Strains

The 316 strains were clinical isolates, together with appropriate reference and control strains (*B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, *Eggerthella lenta* ATCC 43055 and *Clostridium difficile* ATCC 700057).

## Susceptibility testing

Each isolate was tested with an agar dilution method according to approved M11-A7 standards of the CLSI using Brucella blood agar (Difco) with 5% lysed horse blood.

A Mast multipoint inoculator was used to deliver inocula of approx. 10<sup>5</sup> CFU per spot.

Plates were incubated in an anaerobic chamber (Don Whitley) and the minimum inhibitory concentrations (MICs) read after 48 hours of incubation at 35°C.

When combined, tazobactam and NXL104 were used at a fixed 4 mg/L concentration. A combination of ceftazidime /metronidazole in an 8/1 ratio was added in combination with NXL104 (4 mg/L fixed concentration) and labelled in this report as triple combination.

Breakpoints for anaerobes: CLSI. As no breakpoints were available for ceftazidime, ceftriaxone breakpoints were used.

## RESULTS

The activities of NXL104 alone and combined with piperacillin (PIP), ceftazidime (CAZ), ceftriaxone (CRO) and the comparator agents against isolates tested are shown in tables 1 to 4 (MIC Range, MIC<sub>50</sub> & MIC<sub>90</sub> values).

## Activity of NXL104 alone

The activity of NXL104 alone was poor against Gram-negative anaerobic bacteria and *Clostridium difficile*. In contrast, NXL104 demonstrated some intrinsic activity against other Gram-positive anaerobes inhibiting 45 of 145 strains at 16 mg/L. *Clostridium perfringens* was the most susceptible species (MICs 1 - 64 mg/L).

Activity of NXL104 in combination with  $\beta$ -lactams*Bacteroides fragilis* Group (Table 1)

With the addition of 4 mg/L NXL104, only 5 strains (3.9%) were resistant to ceftriaxone + NXL104 and seventeen (13.3%) to ceftazidime + NXL104, with all strains inhibited by ceftazidime (32 mg/L) + NXL104 (4 mg/L) + metronidazole (4 mg/L).

No resistance could be detected to metronidazole, piperacillin plus tazobactam, or piperacillin plus NXL104. Twenty-two strains (14.2%) were resistant to amoxicillin + clavulanic acid (co-amoxiclav) but remained susceptible to imipenem, and 35.4% were resistant to clindamycin.

## Other Gram-negative anaerobes (Table 2)

As 76.4% of the strains of *Prevotella* produced a  $\beta$ -lactamase and were resistant to amoxicillin, most were also intermediate or resistant to ceftriaxone and ceftazidime. No resistance could be detected to piperacillin plus NXL104, ceftriaxone plus NXL104, the triple combination and imipenem. The fusobacteria were susceptible to all  $\beta$ -lactams with the exception of amoxicillin for one  $\beta$ -lactamase producing *F. necrophorum*.

## All Gram-negative anaerobes

The resistance rates against all Gram-negative anaerobic strains of the antibiotics tested were: ceftazidime (50.3%), ceftriaxone (46%), clindamycin (29.2%) piperacillin (27.5%) co-amoxiclav (11.6%), ceftazidime plus NXL104 (11.1%) and ceftriaxone plus NXL104 (2.9%).

No strains were resistant to piperacillin plus tazobactam, piperacillin plus NXL104, metronidazole, imipenem or the triple combination.

## Gram-positive anaerobic bacilli (Tables 3 and 4)

*C. perfringens* was susceptible to all penicillins alone or combined with a  $\beta$ -lactamase inhibitor. Like clavulanic acid and tazobactam, NXL104 was able to decrease the MICs of penicillins and cephalosporins.

For *C. difficile*, in most cases, clavulanic acid, tazobactam, and NXL104 had no impact when associated with  $\beta$ -lactams.

Considering all Gram-positive anaerobes, the 145 strains were all inhibited by coAmoxiclav, piperacillin plus tazobactam, piperacillin plus NXL104, ceftriaxone, ceftriaxone plus NXL104, the triple combination and imipenem.

Table 1: Activities of NXL104 and other agents against the *B. fragilis* group

Microorganism	N	Antibacterial agent	MIC (mg/L)		
			Range	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Bacteroides fragilis</i>	98	NXL104	2 - >64	>64	>64
		Amoxicillin	0.25 - >64	64	>64
		CoAmoxiclav	50.06 - >64	0.25	32
		Piperacillin	0.5 - >128	16	>128
		Piperacillin + tazom	50.06 - 32	0.25	4
		Piperacillin + NXL104	50.06 - 64	2	32
		Ceftriaxone	1 - >128	16	128
		Ceftriaxone + NXL104	50.06 - >64	0.5	8
		Ceftazidime	0.5 - >128	64	>128
		Ceftazidime + NXL104	50.06 - >64	4	32
		Triple combination*	0.125 - 32/4	2/0.25	8/1
		Metronidazole	50.06 - 4	1	2
Imipenem	50.03 - 8	0.25	2		
Clindamycin	50.06 - >64	0.5	>64		
Other <i>Bacteroides</i> of the <i>fragilis</i> group	29	NXL104	64 - >64	>64	>64
		Amoxicillin	0.06 - 64	>64	>64
		CoAmoxiclav	50.06 - >64	16	32
		Piperacillin	0.125 - >128	32	>128
		Piperacillin + tazo	50.06 - 16	2	8
		Piperacillin + NXL104	50.06 - 64	4	16
		Ceftriaxone	1 - >128	>128	>128
		Ceftriaxone + NXL104	50.06 - >64	8	16
		Ceftazidime	0.5 - >128	64	>128
		Ceftazidime + NXL104	50.06 - >64	32	>64
		Triple combination*	1/0.125 - 32/4	4/0.5	8/1
		Metronidazole	50.06 - 2	0.5	1
Imipenem	50.03 - 4	0.5	1		
Clindamycin	50.06 - >64	>64	>64		
All <i>Bacteroides</i>	127	NXL104	2 - >64	>64	>64
		Amoxicillin	0.06 - 64	64	>64
		CoAmoxiclav	50.06 - >64	16	32
		Piperacillin	0.125 - >128	16	>128
		Piperacillin + tazo	50.06 - 32	0.5	4
		Piperacillin + NXL104	50.06 - 64	4	32
		Ceftriaxone	50.06 - >128	64	>128
		Ceftriaxone + NXL104	50.06 - >64	0.5	16
		Ceftazidime	0.5 - >128	128	>128
		Ceftazidime + NXL104	50.06 - >64	8	64
		Triple combination*	0.125 - 32/4	2/0.25	8/1
		Metronidazole	50.06 - 4	0.5	2
Imipenem	50.03 - 8	0.25	2		
Clindamycin	50.06 - >64	0.5	>64		

Table 3: Activities of NXL104 and other agents against *Clostridia*

Microorganism	N	Antibacterial agent	MIC (mg/L)		
			Range	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>C. perfringens</i>	25	NXL104	1 - >64	4	>64
		Amoxicillin	50.06 - 4	50.06	0.5
		CoAmoxiclav	50.06 - >64	50.06	0.5
		Piperacillin	50.06 - 16	0.125	4
		Piperacillin + tazo	50.06 - 4	50.06	2
		Piperacillin + NXL104	50.06 - 2	50.06	2
		Ceftriaxone	50.06 - 16	0.5	2
		Ceftriaxone +NXL104	50.06 - 4	50.06	2
		Ceftazidime	0.5 - >128	64	>128
		Ceftazidime +NXL104	50.06 - 64	50.06	32
		Triple combination*	50.06 - 16/2	50.06	2/0.25
		Metronidazole	0.125 - 2	0.5	1
Imipenem	50.03 - 0.5	0.125	0.5		
Clindamycin	50.06 - 16	50.06	2		
<i>C. difficile</i>	41	NXL104	>64	>64	>64
		Amoxicillin	0.5 - 4	1	2
		CoAmoxiclav	50.06 - 1	1	2
		Piperacillin	8 - 64	16	32
		Piperacillin + tazo	4 - 64	16	32
		Piperacillin + NXL104	1 - 32	16	32
		Ceftriaxone	16 - >128	32	128
		Ceftriaxone +NXL104	32 - >128	32	64
		Ceftazidime	64 - >128	128	>128
		Ceftazidime +NXL104	32 - >64	64	64
		Triple combination*	1/0.125 - 32/4	4/0.5	8/1
		Metronidazole	0.125 - 2	0.25	0.5
Imipenem	2 - 16	8	8		
Clindamycin	50.06 - >64	8	64		
Other <i>Clostridia</i>	12	NXL104	16 - >64	>64	>64
		Amoxicillin	0.25 - 16	0.5	16
		CoAmoxiclav	50.06 - 2	0.5	1
		Piperacillin	0.5 - >128	2	16
		Piperacillin + tazo	50.06 - 64	1	16
		Piperacillin + NXL104	0.25 - 32	2	16
		Ceftriaxone	50.06 - 32	8	32
		Ceftriaxone +NXL104	50.06 - 32	4	32
		Ceftazidime	0.25 - >128	128	>128
		Ceftazidime +NXL104	50.06 - >64	32	64
		Triple combination*	50.06 - 4/0.5	0.5/0.06	4/0.5
		Metronidazole	50.06 - 0.5	0.25	0.25
Imipenem	0.125 - 4	0.25	2		
Clindamycin	50.06 - >64	1	16		

\*: NXL104 (4 mg/L) + ceftazidime and metronidazole in a 4/1 ratio

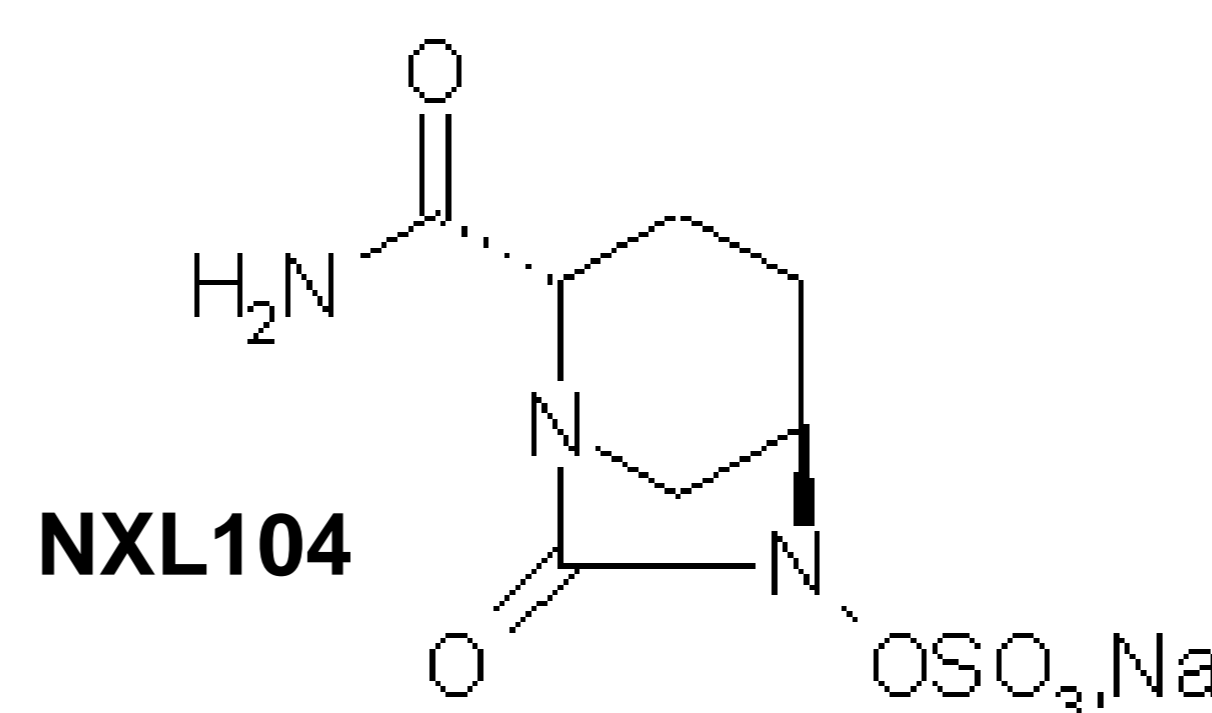


Table 2: Activities of NXL104 and other agents against Gram-negative anaerobes

Microorganism	N	Antibacterial agent	MIC (mg/L)		
			Range	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Prevotella</i> spp.	35	NXL104	16 - >64	>64	>64
		Amoxicillin	50.06 - >64	16	>64
		Coamoxiclav	50.06 - 32	0.125	4
		Piperacillin	0.5 - >128	8	64
		Piperacillin + tazo	50.06 - 4	0.5	2
		Piperacillin + NXL104	50.06 - >64	1	8
		Ceftriaxone	1 - >128	16	128
		Ceftriaxone + NXL104	50.06 - 16	0.25	4
		Ceftazidime	50.06 - >128	16	>128
		Ceftazidime + NXL104	50.06 - >64	4	16
		Triple combination*	50.06 - 8/2	2/0.5	4/1
		Metronidazole	0.125 - 2	0.5	1
Imipenem	50.03 - 2	0.12	0.5		
Clindamycin	50.06 - >64	0.25	1		
<i>Fusobacterium</i> spp.	8	NXL104	32 - >64		
		Amoxicillin	0.06 - 16		
		Coamoxiclav	50.06 - 0.5		
		Piperacillin	50.06 - 32		
		Piperacillin + tazo	50.06 - 4		
		Piperacillin + NXL104	50.06 - 16		
		Ceftriaxone	50.06 - 2		
		Ceftriaxone + NXL104	50.06 - 2		
		Ceftazidime	0.125 - 32		
		Ceftazidime + NXL104	50.06 - 2		
		Triple combination*	50.06 - 1/0.25		
		Metronidazole	50.06 - 2		
Imipenem	50.03 - 0.5				
Clindamycin	50.06 - >64				
All Gram-negatives	171	NXL104	2 - >64	>64	>64
		Amoxicillin	50.06 - >64	0.25	32
		Coamoxiclav	50.06 - >64	0.25	32
		Piperacillin	50.06 - >128	16	>128
		Piperacillin + tazo	50.06 - 64	0.5	4
		Piperacillin + NXL104	50.06 - 64	2	32
		Ceftriaxone	50.06 - >128	32	>128
		Ceftriaxone + NXL104	50.06 - >128	0.5	16
		Ceftazidime	50.06 - >128	32	>128
		Ceftazidime + NXL104	50.06 - >64	4	64
		Triple combination*	0.125 - 32/8	2/0.5	8/2
		Metronidazole	50.06 - 4	0.5	2
Imipenem	50.03 - 8	0.125	2		
Clindamycin	50.06 - >64	0.5	>64		

Table 4: Activities of NXL104 and other agents against anaerobes

Microorganism	N	Antibacterial agent	MIC (mg/L)		
			Range	MIC <sub>50</sub>	MIC <sub>90</sub>
All Gram-positive anaerobes	145	NXL104	1 - >64	64	>64
		Amoxicillin	50.06 - 16	0.25	2
		Coamoxiclav	50.06 - 2	0.125	2
		Piperacillin	50.06 - >128	0.5	32
		Piperacillin + tazo	50.06 - 64	0.25	16
		Piperacillin + NXL104	50.06 - 32	0.25	16
		Ceftriaxone	50.06 - 32	1	64
		Ceftriaxone +NXL104	50.06 - 16	0.25	32
		Ceftazidime	0.125 - >128	8	>128
		Ceftazidime +NXL104	50.06 - >64	2	64
		Triple combination*	50.06 - 16/2	5/0.06	4/0.5
		Metronidazole	50.06 - >64	0.5	8
Imipenem	50.03 - 16	0.125	8		
Clindamycin	50.06 - >64	0.5	16		
All anaerobes	316	NXL104	1 - >64	>64	>64
		Amoxicillin	0.06 - >64	2	>64
		Coamoxiclav	50.06 - >64	0.25	4
		Piperacillin	50.06 - >128	8	>128
		Piperacillin + tazo	50.06 - 64	0.5	16
		Piperacillin + NXL104	50.06 - 64	2	16
		Ceftriaxone	50.06 - >128	16	>128
		Ceftriaxone +NXL104	50.06 - >64	0.5	32
		Ceftazidime	50.06 - >128	32	>128
		Ceftazidime +NXL104	50.06 - >64	4	64
		Triple combination*	50.06 - 32/4	2/0.25	4/0.5
		Metronidazole	50.06 - >64	0.5</	