

In Vitro Activity of NXL104/Ceftazidime Against β -Lactamase Producing Anaerobic Bacteria

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ABSTRACT

Background: NXL104 is a novel β -lactamase (β -L) inhibitor that potentiates β -lactams against many β -L producing organisms. This study tested NXL104 at 4 μ g/ml combined with ceftazidime (CAZ) against 413 strains of β -L producing anaerobes.

Methods: MICs were determined using the CLSI agar dilution method. Strains of *Bacteroides*, *Prevotella* and others were selected based on previously determined susceptibility to amoxicillin/clavulanate (A/C), Metronidazole (MET) was added at 3 different concentrations to the combination to determine possible synergy. Other comparators were ampicillin/sulbactam, and piperacillin/tazobactam (P/T), imipenem and clindamycin.

Results: NXL104 alone showed activity (MIC_{50/90} at 8/128 μ g/ml) only against *Bilophila*. CAZ had MICs \leq 16 μ g/ml to 15 of 67 *B. fragilis*, and 19 of 46 *Prevotella* spp. For *B. fragilis* and *B. vulgatus*, the A/C susceptible (S) group had CAZ/NXL104 MIC_{50/90} 4/16 and 16/32 μ g/ml respectively. MICs for the A/C-resistant (R) strains were 16/64 and 64/128 μ g/ml respectively. Most of the *Parabacteroides distans* group were non-S to A/C, and CAZ/NXL104 MIC_{50/90} was 16/128 μ g/ml. Addition of MET at 1/2 its MIC reduced all CAZ/NXL104 MICs by 1 to 2 dilutions. MICs for the indole-positive *Bacteroides* species were similar for the A/C-S and R groups with most strains at \geq 128 μ g/ml. MIC_{50/90} for *Prevotella* and *Porphyromonas* spp were 1/4 μ g/ml and for imipenem-S strains of *Bilophila*, \leq 0.06/8 μ g/ml. 8 of 10 strains of *Fusobacterium* except 2 *F. mortiferum* were inhibited by \leq 2 μ g/ml. NXL104 did not increase the activity of CAZ against β L producing *C. clostridioforme* group, *Desulfovibrio* spp, P/T-R strains of *Veillonella*, and cephalosporin-R strains of *E. lenta*.

Conclusions: Although the activity of CAZ/NXL104 against members of the *B. fragilis* group was poor, its activity against the predominant *B. fragilis* strains and *Prevotella* species was good. MET at 1/2 its MIC enhanced the activity of CAZ/NXL104 substantially thus making this a potentially powerful combination for treating mixed infections.

INTRODUCTION

With the continuing emergence of resistance to commonly used antimicrobial agents, alternative therapies are needed to treat serious infections. NXL104 is a novel β -lactamase inhibitor with demonstrated activity against AmpC, TEM/SHV, and CTX-M β -lactamases as well as non-metallo-carbapenemases^{3,9}. To explore its activity further, we tested it alone and in combination with ceftazidime against 407 clinical strains of anaerobic bacteria that had various levels of previously determined β -lactamase inhibitor combination MICs, with an emphasis on less susceptible strains. Included were *Bacteroides* species, *Prevotella* species, *Porphyromonas* *somerae*, *Bilophila wadsworthia*, *Desulfovibrio* species, *Campylobacter* species, *Sutterella wadsworthensis*, *Selenomonas infelix*, *Acidimicrococcus fermentans*, *Veillonella* species, *Clostridium clostridioforme* group species and *Eggerthella lenta*. Comparator drugs included amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, metronidazole, clindamycin and imipenem.

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MATERIALS AND METHODS

The test strains had been collected from patients with a variety of infection types, primarily intra-abdominal sources. They were identified by standard methods^{4, 6}. For strains that could not be identified by conventional techniques, partial sequencing of 16S rDNA was performed as described elsewhere¹¹. The strains were stored at -70 $^{\circ}$ C in 20% skim milk.

The antimicrobial agents were reconstituted according to their manufacturers' instructions or according to instructions provided in the CLSI M11-A7 document¹. Stock solutions were stored at -70 $^{\circ}$ C.

The agar dilution test was conducted according to the procedures in CLSI M11-A7¹. Prior to testing, the strains were taken from the freezer and transferred at least twice on supplemented Brucella blood agar to assure purity and good growth. On the day of testing, inocula were prepared in the anaerobic chamber from 48h plates by suspending organisms into Brucella broth to the turbidity of the 0.5 McFarland standard. The inocula were pipetted into the wells of a Steers replicator, removed from the anaerobic chamber and applied to the test plates for a final inoculum of \sim 10⁶ cfu/spot. Control strains including *Bacteroides fragilis* ATCC 25285, *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were included each day of testing. All plates were incubated in the anaerobic chamber at 37 $^{\circ}$ C for 44h before they were removed from the chamber and examined for growth. The MIC was the drug concentration that completely inhibited growth or resulted in a major reduction of growth as compared to the drug-free growth control¹.

RESULTS

Table 1 shows the MIC distribution and cumulative percent at each value for the *Bacteroides* and *Prevotella* species for the β -lactamase inhibitor combinations and imipenem. Table 2 shows the MIC distributions of *Bacteroides* and *Prevotella* with CAZ/NXL104, MET alone and CAZ/NXL104 in combination with MET at various concentrations relative to the MET MIC.

NXL104 alone showed activity of \leq 4 μ g/ml against 9 of 20 strains of *Bilophila wadsworthia*, whereas CAZ/NXL104 MICs were \leq 1 μ g/ml for 13 of the 20 strains.

Within the amoxicillin/clavulanate susceptible group, *B. fragilis* was the most susceptible of the *Bacteroides* group to ceftazidime alone and the combination with NXL104 with CAZ/NXL104 MIC_{50/90} of 4/16 μ g/ml. *B. vulgatus* was also quite susceptible with MIC_{50/90} at 16/32 μ g/ml. Among the amoxicillin/clavulanate-resistant group, the CAZ/NXL104 MIC_{50/90}s were 16/64 and 64/128 μ g/ml, respectively. Most of the *Parabacteroides distans* group showed elevated MICs to all the β -lactamase inhibitor combinations and to imipenem, and the CAZ/NXL104 MIC_{50/90} was 16/128 μ g/ml. The indole-positive strains, including *B. thetaiotaomicron*, *B. ovatus* and *B. uniformis*, were more resistant to CAZ and CAZ/NXL104, regardless of susceptibility to the other β -lactamase inhibitor combinations, with most strains at \geq 128 μ g/ml. For *Prevotella* and *Porphyromonas* spp., the MIC_{50/90} was 1/4 μ g/ml. For many of the *Bacteroides* and *Prevotella* strains, the addition of metronidazole at 1/2 its MIC resulted in a further reduction in the MIC of CAZ/NXL104 by one or more dilutions.

RESULTS (CONT.)

Among the other organisms, 8 of 10 strains of *Fusobacterium* (the exceptions were 2 strains of *F. mortiferum*) were inhibited by \leq 2 μ g/ml of CAZ/NXL104. NXL104 did not increase the activity of ceftazidime against β -lactamase producing strains of the *Clostridium clostridioforme* group, *C. butyricum*, *Desulfovibrio* spp., penicillin-resistant strains of *Veillonella* and *Campylobacter* or the cephalosporin-resistant strains of *Eggerthella lenta*. All strains except one strain each of *Selenomonas* and *Sutterella* were susceptible to metronidazole. Clindamycin-resistance was present in 52% of the *Bacteroides fragilis* group species, 30% of the *Parabacteroides* species and 28% of *Bacteroides fragilis*. Imipenem-resistance was present in only five strains of *Bacteroides* species, but many showed elevated MICs of 2-4 μ g/ml.

STRUCTURE OF NXL104

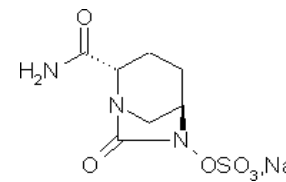


Table 1. MIC distributions of selected Gram-negative anaerobes with β -lactamase inhibitor combinations and imipenem (%)

Organism (No)	MIC (ppm)											
	\leq 0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
B. caecae (15)												
CAZ/NXL104												
A/C												
AS												
PFT/TAZO												
IMPENEM												
B. fragilis (48)												
CAZ/NXL104												
A/C												
AS												
PFT/TAZO												
IMPENEM												
B. caecae (51)												
CAZ/NXL104												
A/C												
AS												
PFT/TAZO												
IMPENEM												
B. distans/thetaiotaomicron (25)												
CAZ/NXL104												
A/C												
AS												
PFT/TAZO												
IMPENEM												
B. distans/thetaiotaomicron (52)												
CAZ/NXL104												
A/C												
AS												
PFT/TAZO												
IMPENEM												
B. caecae (50)												
CAZ/NXL104												
A/C												
AS												
PFT/TAZO												
IMPENEM												
Prevotella spp (43)												
CAZ/NXL104												
A/C												
AS												
PFT/TAZO												
IMPENEM												

Table 2. MIC distributions of selected Gram-negative anaerobes with ceftazidime/NXL104, metronidazole (MET) alone and CAZ/NXL104 in combination with metronidazole at various concentrations relative to the metronidazole MIC (%)

Organism	N	MIC μ g/ml											
		\leq 0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Bacteroides caecae													
CAZ/NXL104	15												
MET	15												
CAZ/NXL104+MET	15												
B. fragilis													
CAZ/NXL104	48												
MET	48												
CAZ/NXL104+MET	48												
B. caecae													
CAZ/NXL104	51												
MET	51												
CAZ/NXL104+MET	51												
B. distans/thetaiotaomicron													
CAZ/NXL104	25												
MET	25												
CAZ/NXL104+MET	25												
B. distans/thetaiotaomicron													
CAZ/NXL104	52												
MET	52												
CAZ/NXL104+MET	52												
B. caecae													
CAZ/NXL104	50												
MET	50												
CAZ/NXL104+MET	50												
Prevotella and Porphyromonas species													
CAZ/NXL104	43												
MET	43												
CAZ/NXL104+MET	43												

CONCLUSION

In combination with metronidazole, Ceftazidime/NXL104 is a potentially powerful combination for the treatment of serious aerobic-anaerobic polymicrobial infections.

DISCUSSION

Resistance to β -lactam antibiotics can occur through a variety of mechanisms, the most common being β -lactamase production due to the presence of genes such as *cepA*, *crxA* or *cfiA*, some of them coding for carbapenemase^{2,3,7,8,10}. Other mechanisms include reduced penetration of the antibiotic into the periplasmic space by loss of porins, or alterations in the penicillin binding proteins; efflux pumps may also play an important role in certain strains¹². Some strains contain several resistance mechanisms and can be multi-resistant to a wide range of β -lactam antibiotics. Thus, the MIC range for β -lactam antibiotics against *Bacteroides* is quite variable and cannot always be explained by any one mechanism.

Strains selected for this study included those with reduced susceptibility to the standard β -lactamase inhibitor combinations to see if NXL104 would show enhanced activity in counteracting their β -lactamases. *B. fragilis* was remarkably susceptible to the CAZ/NXL104 combination, with 71 of 78 strains susceptible to \leq 16 μ g/ml. There was a relationship with the level of the MICs of other β -lactamase inhibitor combinations in some but not all of the strains. Other species in the *Bacteroides fragilis* group had higher MICs, but the addition of metronidazole at 1/2 its MIC reduced the CAZ/NXL104 MICs by 1-3 dilutions.

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