

# NXL104, a Novel $\beta$ -lactamase Inhibitor, Restores the Bactericidal Activity of Ceftazidime Against ESBL and AmpC Producing Strains of *Enterobacteriaceae*.

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## Poster F-127

### 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  $\beta$ -lactamases. Ceftazidime (CAZ) is a third generation cephalosporin whose clinical utility has been eroded by the spread of  $\beta$ -lactamases. The aim of the study was to demonstrate by time kill assays that the combination of CAZ with NXL104 restored the bactericidal activity of CAZ in strains refractive to CAZ alone due to  $\beta$ -lactamases.

**Methods:** Polymerase chain reaction (PCR) amplification was performed on 9 CAZ-R *Enterobacteriaceae* strains for detection of  $\beta$ -lactamase genes; isoelectric focusing was used to confirm their expression. The strains exhibited MICs in a range of 32-256  $\mu$ g/ml for CAZ and 1-4  $\mu$ g/ml for CAZ/NXL104. Kill kinetics were determined in Mueller Hinton broth at 37°C. An overnight broth culture was diluted to achieve an inoculum of  $\sim 10^6$  cfu/ml. The test antibiotic CAZ/NXL104 in 4:1 ratio was added at the appropriate concentrations. A culture without antibiotic was included as control. Samples were taken immediately and at timed intervals thereafter. The viable bacterial count was determined by spiral plating. A  $3 \log_{10}$  reduction in the original viable count was considered bactericidal.

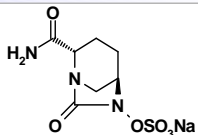
**Results:** Derepressed AmpC expression was shown in 3 *Enterobacter cloacae* and 1 *Citrobacter freundii* strains; another *C. freundii* isolate had an inducible AmpC. 4 *Klebsiella pneumoniae* strains expressed a class A and/or a class C enzyme. CAZ/NXL104 was bactericidal against the 3 AmpC strains of *E. cloacae* and the 2 AmpC strains of *C. freundii* within 5 hours of exposure at 2-4 fold MIC. All 4 strains of *K. pneumoniae* producing both AmpC and/or class A enzymes were rapidly killed by the CAZ/NXL104 combination at 2-fold MIC.

**Conclusion:** Against strains of *Enterobacteriaceae* which exhibited resistance to CAZ by confirmed  $\beta$ -lactamase production, NXL104 restored the rapid bactericidal activity of CAZ.

### BACKGROUND

Resistance to  $\beta$ -lactam antimicrobials is a particularly serious threat as they are active against a wide range of bacterial pathogens and have very low toxicity to humans (1). In Gram-negatives, the most important mechanism of resistance to  $\beta$ -lactams is the enzymatic cleavage of the  $\beta$ -lactam ring by  $\beta$ -lactamases. These enzymes are classified into four classes based on their amino acid sequences. Class A, C, and D  $\beta$ -lactamases use an active site serine to hydrolyse the  $\beta$ -lactam ring common to  $\beta$ -lactam antimicrobials. Class B enzymes are metallo- $\beta$ -lactamases. The currently marketed  $\beta$ -lactamase inhibitors (clavulanic acid, tazobactam, and sulbactam) have a limited spectrum of clinical utility as their inhibitory activity is confined, generally, to class A and some class D  $\beta$ -lactamases. 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  $\beta$ -lactamases (2, 3), which represent most enzymes of major clinical significance. The aim of the study was to demonstrate by time kill assays that the combination of ceftazidime (CAZ) with NXL104 restored the bactericidal activity of CAZ in strains refractive to CAZ alone due to  $\beta$ -lactamases.

### STRUCTURE OF NXL 104



### METHOD

#### Compounds

The following antimicrobials were used: Ceftazidime pentahydrate (CAZ), Tazocillin (4 g/500mg Piperacillin/Tazobactam) (Tazo)

The following  $\beta$ -lactam inhibitors were used: Clavulanate lithium (CA), NXL104 (NXL). CAZ/CA and CAZ/NXL104 combinations were prepared at 4:1 w:w ratio.

#### Bacterial Isolates

The nine strains of ceftazidime - resistant (CAZ-R) *Enterobacteriaceae* species included: 2 *Citrobacter freundii*, 3 *Enterobacter cloacae*, and 4 *Klebsiella pneumoniae*.

#### MIC determination

MICs, for CAZ, CAZ/NXL, CAZ/CA, and Tazo, were determined using CLSI (formerly the NCCLS) methods for antimicrobial susceptibility testing with Mueller-Hinton (MH) broth. MIC was defined as the lowest concentration which inhibited all visual growth.

#### Detection and identification of expressed $\beta$ -lactamases

$\beta$ -lactamase genes were amplified by Polymerase Chain Reaction using the appropriate primers for detection of the most common  $\beta$ -lactamases; amplified fragments were sequenced.

Crude cell extracts were prepared by sonication for IsoElectric Focusing and detection of enzyme activity with nitrocefin.

#### Bactericidal activity determination by Time/Kill Kinetics

#### Inoculum preparation:

Bacteria were grown overnight in cation adjusted MH broth at 37°C. A 1/100 dilution of the overnight culture was seeded in prewarmed MH broth and incubated for 2 hours or until exponential growth commenced. Bacterial inocula were prepared from the exponential culture and diluted to achieve the desired starting inoculum of  $\sim 10^6$  CFU/ml in the flask. The initial viable count of the inoculum was verified by plating serial two-fold dilutions on MH agar plates.

#### Time/Kill assay:

Experiments were performed in 20 ml volumes of cation adjusted MH broth with initial inoculum of  $\sim 10^6$  CFU/ml Test antibiotics, alone and in combination with the  $\beta$ -lactamase inhibitors, were added at appropriate concentrations. The CAZ/NXL104 concentrations tested were 1, 2, 4, 8, and 16 fold the MIC for each species. Timed samples over a 24-hour period were enumerated and the viable bacterial count determined by serial two-fold dilutions. A culture without antibiotic was included as control.

#### Bactericidal activity:

A  $3 \log_{10}$  reduction in the original viable count was considered to be a bactericidal effect. This is denoted by a solid black horizontal line on each time/kill curve graph (Figures 1 - 3).

### RESULTS

**Table 1:** The *in vitro* activity of ceftazidime alone, and in combination with  $\beta$ -lactam inhibitors (4:1 ratio) and tazocillin (piperacillin/tazobactam, 8:1 ratio) against the strains of *Enterobacteriaceae* species.

**Table 2:** Characterisation of  $\beta$ -lactamases expressed by CAZ-R strains of *Enterobacteriaceae* species.

**Figures 1, 2, and 3:** Bactericidal activity of CAZ/NXL104 (4:1 ratio) against CAZ-R *Enterobacteriaceae* species.

### RESULTS

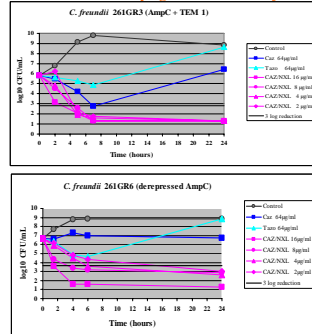
**Table 1. MICs ( $\mu$ g/mL) of CAZ, CAZ/NXL 104 (4:1), CAZ/CA (4:1), and Tazocillin (8:1) against strains of *Enterobacteriaceae* species.**

Strain	CAZ	CAZ/NXL104 (4:1)	CAZ/CA (4:1)	Tazocillin (8:1)
<i>C. freundii</i> 261GR3	64	2	>32	64
<i>C. freundii</i> 261GR6	>32	2	>32	8
<i>E. cloacae</i> P99	>64	4	>32	64
<i>E. cloacae</i> 293GR8	>128	4	>32	64
<i>E. cloacae</i> 293GR38	>64	4	>32	64
<i>K. pneumoniae</i> 283 KB4	>256	4	>32	128
<i>K. pneumoniae</i> 283KB5	32	1	>32	16
<i>K. pneumoniae</i> 283IP10	>256	4	4	>256
<i>K. pneumoniae</i> 283IP35	32	4	8	>256

**Table 2. Characterisation of  $\beta$ -lactamases expressed in strains of *Enterobacteriaceae* species**

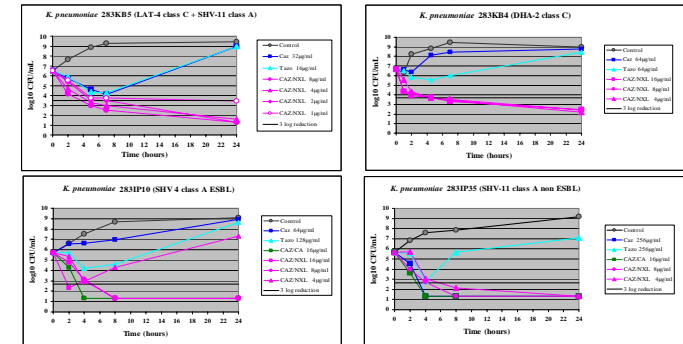
Strain	$\beta$ -lactamase gene	Molecular Class
<i>C. freundii</i> 261GR3	TEM-1 & AmpC	Class A & Class C
<i>C. freundii</i> 261GR6	Derepressed AmpC	Class C
<i>E. cloacae</i> P99	Derepressed AmpC	Class C
<i>E. cloacae</i> 293GR8	Derepressed AmpC	Class C
<i>E. cloacae</i> 293GR38	Derepressed AmpC	Class C
<i>K. pneumoniae</i> 283 KB4	DHA-2	Class C
<i>K. pneumoniae</i> 283KB5	LAT-4 & SHV-11 variant	Class A & Class C
<i>K. pneumoniae</i> 283IP10	SHV-4	Class A ESBL
<i>K. pneumoniae</i> 283IP35	SHV-11	Class A non ESBL

**Figure 1. CAZ/NXL 104 (4:1 ratio): Bactericidal activity against *Citrobacter freundii***



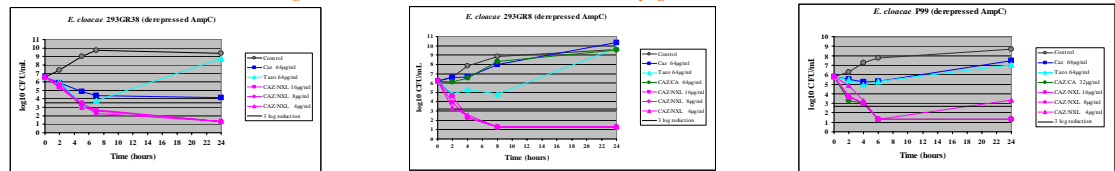
**CAZ/NXL 104:** Cidal within 6 hours at 2 fold MIC (2  $\mu$ g/mL) for both strains of *C. freundii*.  
**CAZ alone or Tazo:** Not effective at concentrations up to 64  $\mu$ g/mL.

**Figure 2. CAZ/NXL 104 (4:1 ratio): Bactericidal activity against *Klebsiella pneumoniae***



**CAZ/NXL104:** Cidal within 4-8 hours at 1-2 fold MIC for 3 strains of *K. pneumoniae* (MIC 4  $\mu$ g/mL strains 283KB4, 283IP10 and 283IP35) and at 4 fold MIC for strain 283KB5 (MIC 1  $\mu$ g/mL).  
**CAZ alone or Tazo:** Not effective up to 64  $\mu$ g/ml against strains 283IP10, 283KB4, and 283KB5.  
**CAZ/CA:** Cidal against Class A producing strains 283IP10 and 283IP35.

**Figure 3. CAZ/NXL 104 (4:1 ratio): Bactericidal activity against *Enterobacter cloacae***



**CAZ/NXL 104:** Cidal within 6 hours for the 3 *E. cloacae* strains, at the MIC for 293GR38 and 293GR8 (2 and 4  $\mu$ g/mL, respectively), and at 2 fold MIC (4  $\mu$ g/mL) for strain P99.  
**CAZ alone, CAZ/CA, or Tazo:** Not effective at concentrations up to 64  $\mu$ g/mL.

### CONCLUSION

- NXL104 restored the *in vitro* activity of ceftazidime against isolates of *Enterobacteriaceae* species resistant to ceftazidime caused by class A ESBLs and class C enzyme production.
- NXL104 rapidly restored the bactericidal activity of ceftazidime against ceftazidime resistant isolates of the species of *Enterobacteriaceae*.
- NXL104 has clinical potential to extend the usefulness of ceftazidime for treatment of infections caused by  $\beta$ -lactam-resistant *Enterobacteriaceae* species when resistance is due to the expression of a wide range of both class A and class C  $\beta$ -lactamases.

### REFERENCES

- Livermore, D. *Scand. J Infect. Dis* 1996, 101(Suppl), 33-43
- Robbins M *et al* in Proceedings of Abstracts 45<sup>th</sup> ICAAC, Abstract F-1162.
- Levasseur P *et al* in Proceedings of Abstracts 45<sup>th</sup> ICAAC, Abstract F-1164.