*Journal of Antimicrobial Chemotherapy* (2008) **62**, 1053–1056
doi:10.1093/jac/dkn320
Advance Access publication 9 August 2008

JAC

# NXL104 combinations versus Enterobacteriaceae with CTX-M extended-spectrum β-lactamases and carbapenemases

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*Received 29 April 2008; returned 12 June 2008; revised 7 July 2008; accepted 14 July 2008*

***Background*: The β-lactamase landscape is changing radically, with CTX-M types now the most prevalent extended-spectrum β-lactamases (ESBLs) worldwide, except maybe in the USA. In addition, there are growing numbers of Enterobacteriaceae with KPC and metallo-carbapenemases. We examined whether combinations of oxyimino-cephalosporins with NXL104, a novel non-β-lactam β-lactamase inhibitor, overcame these resistances.**

***Methods*: NXL104 was tested at 4 mg/L in combination with cefotaxime and ceftazidime versus: (i) *Escherichia coli* transconjugants and wild-type Enterobacteriaceae with CTX-M ESBLs; (ii) Enterobacteriaceae with ertapenem resistance contingent on combinations of impermeability and ESBLs or AmpC; and (iii) Enterobacteriaceae with KPC, SME, metallo- or OXA-48 carbapenemases.**

***Results*: MICs of cefotaxime + NXL104 were ≤1 mg/L for most Enterobacteriaceae with CTX-M, KPC or OXA-48 enzymes and were ≤2 mg/L for those that also had ertapenem resistance contingent on combinations of β-lactamase and impermeability. MICs of the ceftazidime + NXL104 combination were ≤4 mg/L, except for a single *Enterobacter aerogenes* with KPC and AmpC enzymes together with porin loss, which required an MIC of 32 mg/L. The major gap was that NXL104 could not potentiate cephalosporins against Enterobacteriaceae with IMP or VIM metallo-enzymes.**

***Conclusions*: Oxyimino-cephalosporin + NXL104 combinations have potential against strains with the prevalent ESBLs and non-metallo-carbapenemases.**

Keywords: β-lactamase inhibitors, ESBLs, KPC carbapenemase, metallo-β-lactamases

## Introduction

NXL104 (Novexel) is a novel-structure β-lactamase inhibitor (Figure 1). It was shown previously to inhibit AmpC and TEM/SHV-type extended-spectrum β-lactamases (ESBLs), potentiating cephalosporins against producers.[1]

However, the β-lactamase landscape is now changing radically. CTX-M enzymes, rather than TEM and SHV mutants, are increasingly the predominant ESBLs in Europe and Asia,[2,3] with this replacement perhaps now beginning also in North America.[4] Other ESBLs are locally frequent, for example, PER enzymes in Turkey and Argentina[5,6] and VEB types in the East Asia. In addition, there is a slow emergence of carbapenem resistance in Enterobacteriaceae. Some of this is due to porin loss—often unstable—in ESBL- and AmpC-producing strains,[7] more worryingly, though, true carbapenemases are also gradually accumulating. Specifically, (i) *Klebsiella pneumoniae* clones with KPC carbapenemases are spreading across the USA, Colombia and Israel[8,9] with scattered reports from Europe and China; (ii) there are clusters of Enterobacteriaceae isolates with metallo-carbapenemases in southern Europe and Asia; and (iii) there are multiple reports of Enterobacteriaceae with OXA-48, a weak carbapenemase in Turkey.

We assessed the activity of NXL104 combined with cefotaxime and ceftazidime against these increasingly important resistance types.

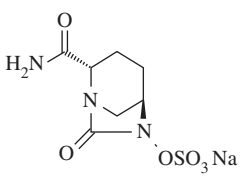


**Figure 1.** Structure of NXL104.

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**Table 1.** MICs (mg/L) for *E. coli* transconjugants with CTX-M and PER enzymes

| Strain | Mechanism | Cefotaxime | Cefotaxime + NXL104 | Ceftazidime | Ceftazidime + NXL104 | Piperacillin/tazobactam | Imipenem |
|---|---|---|---|---|---|---|---|
| DH5α | R−recipient | 0.031 | ≤0.015 | 0.25 | 0.06 | 0.5 | 0.12 |
| DH5α | CTX-M-15 | 8 | ≤0.015 | 2 | 0.06 | 2 | 0.12 |
| DH5α | CTX-M-9 | 16 | ≤0.015 | 2 | 0.25 | 1 | 0.12 |
| DH5α | CTX-M-14 | 32 | ≤0.015 | 2 | 0.06 | 1 | 0.12 |
| J53-1 | R−recipient | 0.062 | 0.03 | 0.5 | 0.12 | 1 | 0.12 |
| J53-1 PER-1 | PER-1 | >256 | 0.06 | >256 | 0.5 | 2 | 0.5 |

**Table 2.** MIC distributions of cefotaxime and ceftazidime and their combinations with NXL104 for clinical isolates

| | MIC (mg/L) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.008 | 0.015 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | ≥256 |
| Cefotaxime alone versus ertapenem-susceptible isolates with CTX-M ESBLs | | | | | | | | | | | | | | | | |
| group 1 CTX-M | | | | | | | | | | | | | | | 1 | 20 |
| group 2 CTX-M | | | | | | | | | | | | | | | | 5 |
| group 8 CTX-M | | | | | | | | | | | | | | | | 1 |
| group 9 CTX-M | | | | | | | | | | | | 1 | 5 | 7 | 5 | 11 |
| group 25 CTX-M | | | | | | | | | | | | | 1 | | | |
| Cefotaxime + 4 mg/L NXL104 versus ertapenem-susceptible isolates with CTX-M ESBLs | | | | | | | | | | | | | | | | |
| group 1 CTX-M | 4 | | 10 | 5 | 2 | | | | | | | | | | | |
| group 2 CTX-M | 2 | | 1 | 1 | 1 | | | | | | | | | | | |
| group 8 CTX-M | | | | | | | 1 | | | | | | | | | |
| group 9 CTX-M | 3 | | 8 | 5 | 6 | 6 | 1 | | | | | | | | | |
| group 25 CTX-M | | | | | 1 | | | | | | | | | | | |
| Ceftazidime alone versus ertapenem-susceptible isolates with CTX-M ESBLs | | | | | | | | | | | | | | | | |
| group 1 CTX-M | | | | | | | | | 1 | 2 | 1 | 3 | 5 | 9 | | |
| group 2 CTX-M | | | | | | | | | | | 2 | 2 | 1 | | | |
| group 8 CTX-M | | | | | | | | | | | 1 | | | | | |
| group 9 CTX-M | | | | | | | | 3 | 11 | 4 | 5 | | 2 | 1 | | 3 |
| group 25 CTX-M | | | | | | | | | | 1 | | | | | | |
| Ceftazidime + 4 mg/L NXL104 versus ertapenem-susceptible isolates with CTX-M ESBLs | | | | | | | | | | | | | | | | |
| group 1 CTX-M | 4 | | | | 3 | 8 | 3 | 3 | | | | | | | | |
| group 2 CTX-M | 1 | | | | | 3 | | 1 | | | | | | | | |
| group 8 CTX-M | | | | | | 1 | | | | | | | | | | |
| group 9 CTX-M | 1 | | | | 4 | 11 | 12 | 1 | | | | | | | | |
| group 25 CTX-M | | | | | | | 1 | | | | | | | | | |
| Cefotaxime alone versus ertapenem-resistant *K. pneumoniae* and *Enterobacter* spp. isolates with β-lactamase and impermeability | | | | | | | | | | | | | | | | |
| AmpC | | | | | | | | | | | | | | 1 | 1 | 3 |
| ESBL | | | | | | | | | | | | | | | 1 | 11 |
| Cefotaxime + 4 mg/L NXL104 versus ertapenem-resistant *K. pneumoniae* and *Enterobacter* spp. isolates with β-lactamase and impermeability | | | | | | | | | | | | | | | | |
| AmpC | | | | | 1 | | 1 | 1 | 2 | | | | | | | |
| ESBL | | | 1 | | 1 | 5 | 1 | 4 | | | | | | | | |
| Ceftazidime alone versus ertapenem-resistant *K. pneumoniae* and *Enterobacter* spp. isolates with β-lactamase and impermeability | | | | | | | | | | | | | | | | |
| AmpC | | | | | | | | | | | 1 | | | | | 4 |
| ESBL | | | | | | | | | | | | | | | 3 | 9 |

*Continued*

**Table 2.** *Continued*

| | MIC (mg/L) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.008 | 0.015 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | ≥256 |
| Ceftazidime + 4 mg/L NXL104 versus ertapenem-resistant *K. pneumoniae* and *Enterobacter* spp. isolates with β-lactamase and impermeability | | | | | | | | | | | | | | | | |
| AmpC | | | | | | | 1 | 1 | 2 | | | | | 1 | | |
| ESBL | | | 1 | | | | 2 | 3 | 5 | 1 | | | | | | |
| Imipenem versus ertapenem-resistant *K. pneumoniae* and *Enterobacter* spp. isolates with β-lactamase and impermeability | | | | | | | | | | | | | | | | |
| AmpC | | | | | | | 1 | 1 | | 1 | 1 | | | 1 | | |
| ESBL | | | | | | 1 | 2 | 4 | 2 | 1 | 1 | 1 | | | | |

## Materials and methods

The organisms tested comprised: (i) *Escherichia coli* transconjugants with CTX-M enzymes or carbapenemases ($n = 5$); (ii) carbapenem-susceptible Enterobacteriaceae isolates with CTX-M enzymes, mostly CTX-M-15, collected in a recent UK survey ($n = 57$); (iii) *Klebsiella* spp. isolates substantially resistant to ertapenem (MICs ≥4 mg/L) owing to combinations of ESBLs with impermeability and *Enterobacter* spp. resistant to ertapenem owing to combinations of ESBL or AmpC and impermeability ($n = 17$); and (iv) Enterobacteriaceae isolates with KPC, SME, IMP, VIM and OXA-48 carbapenemases, collected worldwide ($n = 23$).

MICs of cefotaxime and ceftazidime (both Sigma, Poole, Dorset, UK) were mostly determined in combination with 4 mg/L NXL104 by the CLSI (formerly the NCCLS) method on Mueller–Hinton agar (Oxoid, Basingstoke, UK).[10] Anomalous results were re-checked with cefotaxime and ceftazidime Etests® (AB Biodisk, Solna, Sweden) on Mueller–Hinton agar with or without 4 mg/L NXL104; this method was also used for all tests with metallo-β-lactamase producers, undertaken subsequently to the main batch of organisms. The agar dilution and Etest methods gave identical results, ± one doubling dilution, when applied to the same isolate.

## Results and discussion

MICs for Enterobacteriaceae transconjugants with the three most prevalent CTX-M ESBLs in Europe (i.e. CTX-M-9, 14 and -15, also PER-1) are shown in Table 1, whereas those for carbapenem-susceptible clinical isolates with various CTX-M enzymes are shown in Table 2, sorted by the enzyme group.

The potentiation of cefotaxime by NXL104 generally exceeded 1000-fold for isolates with CTX-M enzymes, with MICs reduced from ≥128 to 0.03 to 0.12 mg/L. MICs of ceftazidime for isolates

**Table 3.** MIC distribution of NXL104 combination for isolates with carbapenemases

| | MIC (mg/L) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.015 | 0.03 | 0.06 | 0.12 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | ≥128 |
| *K. pneumoniae* (6) and *Enterobacter cloacae* (1) with KPC enzymes | | | | | | | | | | | | | | |
| cefotaxime | | | | | | | | | | | | | 1 | 6 |
| cefotaxime + NXL104 | 4 | | | | | 2 | 1 | | | | | | | |
| ceftazidime | | | | | | | | | | | | | | 7 |
| ceftazidime + NXL104 | 4 | | | | | | | 1 | 1 | | | 1 | | |
| *S. marcescens* (1) with SME-1 | | | | | | | | | | | | | | |
| cefotaxime | | | | | | 1 | | | | | | | | |
| cefotaxime + NXL104 | | | | 1 | | | | | | | | | | |
| ceftazidime | | | | | | 1 | | | | | | | | |
| ceftazidime + NXL104 | | | | | | 1 | | | | | | | | |
| *K. pneumoniae* (3) and *E. coli* (1) with OXA-48 | | | | | | | | | | | | | | |
| cefotaxime | | | | | | | | | | | | | | 4 |
| cefotaxime + NXL104 | | | | | | 1 | 3 | | | | | | | |
| ceftazidime | | | | | | | | | | | 1 | 1 | | 2 |
| ceftazidime + NXL104 | | | | | | 1 | 2 | 1 | | | | | | |
| *Klebsiella* spp. (7) and *E. coli* (4) with IMP and VIM metallo-β-lactamases | | | | | | | | | | | | | | |
| cefotaxime | | | | | | | | | | | | | | 11 |
| cefotaxime + NXL104 | | | | | | | | | | | | | | 11 |
| ceftazidime | | | | | | | | | | | | | | 11 |
| ceftazidime + NXL104 | | | | | | | | | | | | | | 11 |

with CTX-M-15 enzyme were reduced from (generally) ≥64 to 0.25–1 mg/L; those for isolates with other CTX-M types, which generally confer lower levels of ceftazidime resistance than CTX-M-15, were reduced from 2–8 to 0.12–1 mg/L.

When tested against ertapenem-resistant (MIC >2 mg/L) Enterobacteriaceae isolates that had combinations of CTX-M or AmpC enzymes together with impermeability, NXL104 reduced cefotaxime MICs to ≤2 mg/L and those of ceftazidime to ≤4 mg/L. Imipenem MICs ranged up to 64 mg/L for these organisms (Table 2).

MIC data for Enterobacteriaceae isolates and transconjugants with carbapenemases are shown in Table 3. NXL104 reduced cefotaxime MICs from >128 to ≤0.5 mg/L for most isolates with KPC enzymes and those of ceftazidime from ≥128 to ≤4 mg/L. The sole exception was *Enterobacter aerogenes* E624, which had KPC-4 enzyme, derepressed AmpC and porin loss, where the cefotaxime and ceftazidime MICs were reduced to 1 and 32 mg/L, respectively. In view of the good activity of the cefotaxime + NXL104 combination, it seems likely that the high MIC of ceftazidime + NXL104 reflected this combination of factors, rather than any specific trait of the KPC-4 enzyme.

A *Serratia marcescens* isolate with SME-1 carbapenemase was not substantially resistant to cefotaxime and ceftazidime, as is typical of the phenotype; nevertheless, the cefotaxime MIC was reduced 4-fold in the presence of NXL104. Good synergy was seen for four Turkish isolates with OXA-48 enzyme—one *E. coli* and three *K. pneumoniae*—in which MICs of cefotaxime and ceftazidime generally were reduced from >64 to ≤2 mg/L. NXL104 achieved no significant reduction of the MICs of cefotaxime and ceftazidime for metallo-β-lactamase producers with IMP and VIM enzymes.

Taken as a whole, the activity of cephalosporin + NXL104 combinations against isolates and transconjugants with ESBLs and non-metallo-carbapenemases was impressive. Such combinations may provide a future alternative to carbapenems in infections due to ESBL, which are growing problems worldwide, and may also provide a route to overcoming isolates with carbapenem resistance contingent on KPC β-lactamases—another growing problem. The utility of these combinations depends, of course, on satisfactory pharmacokinetics, where phase I data show that a single 500 mg dose of NXL104 gave a $C_{max}$ of 10 mg/L with a $t_{1/2}$ of 1.8 h.[11] The lack of activity against metallo-β-lactamase producers is a limitation but might be overcome by combining NXL104 with a monobactam, as these compounds are stable to class B enzymes.

## Funding

We are grateful to Novexel for financial support of these studies.

## Transparency declarations

C. M. is an employee of Novexel and has stock options in the company. D. M. L. has acted as an advisor to Novexel and has received research finance from them and from numerous other pharmaceutical companies. He has shareholdings in GlaxoSmithKline, who were the original manufacturer of ceftazidime and other companies including Schering-Plough, Dechra, Pfizer and AstraZeneca and, as Enduring Attorney, manages shareholdings for a close relative in GlaxoSmithKline and Eco Animal Health. The remaining authors have none to declare.

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