

# In vitro activity of the ceftazidime / NXL104 combination against *Pseudomonas aeruginosa* clinical isolates

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## REVISED ABSTRACT

NXL104 is a  $\beta$ -lactamase inhibitor under clinical development which displays potent inhibition of a broad spectrum of both class A and class C enzymes. The antibacterial activity of the combination ceftazidime / NXL104 was evaluated against a panel of 126 *Pseudomonas aeruginosa* strains that were collected as consecutive isolates in a French hospital between December 2006 and April 2007. 35% and 26% of isolates were found non-susceptible to ceftazidime and imipenem, respectively; NXL104 was able to protect efficiently ceftazidime from  $\beta$ -lactamase hydrolysis since 94% of isolates were susceptible to the combination. Resistance to ceftazidime was usually due to AmpC production, since only two isolates co-expressed additional beta-lactamases (CARB/OXA genes detected). PFGE indicated high clonal diversity in the strains with intermediate susceptibility or resistance to ceftazidime (29 different pulsotypes in 44 strains). These data show that the addition of NXL104 inhibitor to ceftazidime resulted in lowering of ceftazidime MIC values to susceptibility breakpoints in 82% of the resistant *P. aeruginosa* strains; in this recent panel of isolates, the ceftazidime / NXL104 combination shows better activity than imipenem which is the reference treatment.

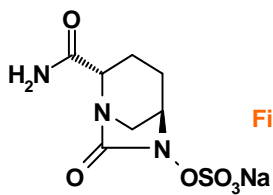


Figure 1 - Chemical structure of NXL104

## BACKGROUND

NXL104 is a novel non- $\beta$ -lactam inhibitor of  $\beta$ -lactamases currently in phase I of clinical development. It displays a broad spectrum inhibition profile for both class A and class C enzymes; both types of enzymes are inactivated very efficiently at low  $IC_{50}$  values, with low turn-over numbers and long covalent intermediate half-lives (1). NXL104 has virtually no intrinsic antibacterial activity, but efficiently protects  $\beta$ -lactams from hydrolysis in a variety of class A and class C producing strains, including ESBL producers. In particular, it has been shown to efficiently restore *in vitro* ceftazidime (CAZ) activity (2-4); protection against acute lethal infections by the CAZ/NXL104 combination has also been demonstrated in murine models (5, 6).

*P. aeruginosa* is a major opportunistic pathogen frequently involved in hospital-acquired infections. The goal of the study was to determine susceptibility profiles of 126 clinical contemporary isolates of *P. aeruginosa* collected in a French hospital to CAZ/NXL104 combination and to comparator agents.

1. Bonnefoy A., J Antimicrob Chemother 2004, 54:410-417
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4. 47<sup>th</sup> ICAAC, 2007: Miossec C., Poster F1-318; Mushtaq S., Poster F1-319, Stachyra T., Poster F1-320
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## MATERIAL AND METHODS

### Bacterial strains and susceptibility testing

- The 126 *P. aeruginosa* strains were collected as consecutive isolates in the South-Paris hospital (Hôpital de Kremlin-Bicêtre, France) between December 2006 and April 2007. They were isolated from patients suffering from significant infections with various underlying diseases.
- MIC determination was performed using reference methods of the Clinical and Laboratory Standards Institute (CLSI) for antimicrobial susceptibility testing with cation-adjusted Mueller-Hinton (MH) broth. MIC was defined as the lowest concentration that inhibited all visual growth. Categorical interpretations were by CLSI breakpoint criteria.
- NXL104 and Tazobactam were used at a fixed concentration of 4  $\mu$ g/mL, with variable concentrations of CAZ and piperacillin (PIP), respectively. Imipenem (IPM), and aztreonam (AZT) were tested alone.

### Chromosomal restriction patterns produced by Pulse-Field Gel Electrophoresis (PFGE)

- Spel-digested DNA was separated by PFGE using CHEF apparatus (BioRad); the gel was stained in ethidium bromide solution and digitized as a tiff file.
- PFGE types were assigned according to the criteria described by Tenover *et al* (7).

### Detection of $\beta$ -lactamase genes

- $\beta$ -lactamase genes were amplified by Polymerase Chain Reaction (PCR) using the appropriate primers for detection of the most common *bla* gene families.
- Primers were designed to amplify the following *bla* gene groups:
  - class A: TEM, SHV, VEB, PER, GES, CARB, CTX-M, KPC
  - class B: IMP, VIM, SPM, SIM
  - class C: *P. aeruginosa* chromosomal AMPC
  - class D: OXA-I, -II, -III subgroups, OXA-5, -9, -20-like, -23-like, -24-like, 48-like, -51-like, -58-like
- The PCRs were performed on lysed cells using the Ready-To-Go reagents according to manufacturer's instructions (Amersham). Briefly, cell lysis was performed at 99°C for 3 min, followed by 30 cycles of amplification (94°C for 1 min, 55°C for 1 min, 72°C for 1 min), and a final extension at 72°C for 5 min.

## RESULTS AND CONCLUSIONS

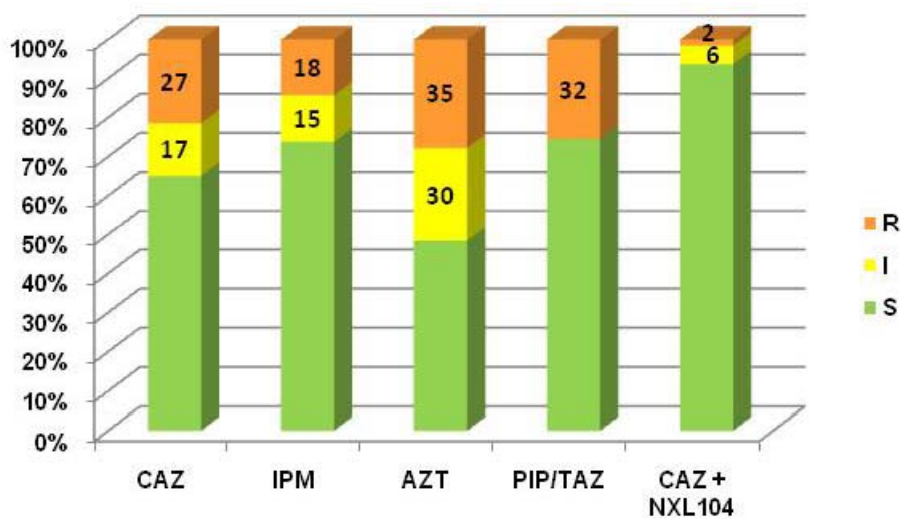


Figure 2 – Susceptibility profile of the 126 *P. aeruginosa* strain panel

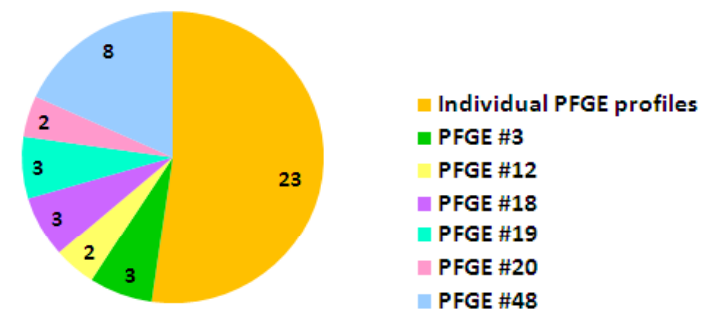


Figure 3 – Clonal diversity within the 44 CAZ-non-susceptible *P. aeruginosa* strains (number of isolates)

Table 1 – MIC values ( $\mu$ g/mL) in strains having non-ampC *bla* genes

Strain ID	CAZ	IPM	CAZ+NXL104	<i>bla</i> gene
391KB45	16	32	4	CARB / OXA-9
391KB8	16	8	16	CARB / OXA-9

• The susceptibility profile of 126 contemporary *P. aeruginosa* isolates was as follows: 65% susceptible to ceftazidime, 74% to imipenem, 48% to aztreonam, 75% to tazocillin, and 94% to ceftazidime / NXL104 combination (Figure 2).

• In this study, the *in vitro* anti-pseudomonal activity of the ceftazidime / NXL104 combination was demonstrated: 82% of ceftazidime-non-susceptible isolates were reversed to susceptibility when ceftazidime was protected from hydrolysis by the presence of NXL104 (Figure 2).

• In most isolates, the non-susceptibility to ceftazidime was most probably due to AmpC expression since additional  $\beta$ -lactamase genes were detected in only 3 strains (Table 1).

• A high clonal diversity was observed in the CAZ-non-susceptible strains: 23 strains had unique PFGE profiles, and 21 strains were scattered in 6 different pulsotypes (Figure 3).

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