# *In-vitro* activity of diverse β-lactam/AAI101combinations vs. multidrug-resistant Gram-negative clinical strains

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## Introduction

AAI101 is a novel extended-spectrum β-lactamase inhibitor (BLI), belonging to the penicillanic acid sulfone family, which is currently in Phase I clinical trials.

β-Lactam resistance in Gram-negative pathogens principally involves production of a wide diversity of β-lactamases, and may be exacerbated by auxiliary non-β-lactamase mechanisms such as porin mutations and upregulated efflux. Whilst BLIs like tazobactam (Tazo) have helped preserve the clinical value of β-lactams by inhibiting β-lactamases before they can inactivate the antibiotic, emergence of more aggressive β-lactamases, especially when complemented by auxiliary non-β-lactamase resistance mechanisms, have restricted treatment options for infections caused by multidrug-resistant (MDR) Enterobacteriaceae and non-fermentative bacilli.[1]

The purpose of this study was to assess the *in-vitro* activity of AAI101 in combination with different β-lactam antibiotics against 61 Gram-negative clinical isolates representing a variety of β-lactam resistance mechanisms.

## Materials and Methods

- Agar dilution MICs were determined for 61 bacterial strains, each with a defined mechanism of β-lactam resistance (Table 1).

**Table 1. Resistotypes of "challenge panel"***

| Species | Represented β-lactamases (and other resistance mechanism) |
|---|---|
| *E. coli* (n = 22) | TEM-3, TEM-10, 3 CTX-M-2, 3 CTX-M-14, 3 CTX-M-15; 3 KPC, NMC-A; 5 pAmpCs (ACC, 2 CIT/CMYs, DHA, FOX); 2 OXA-48 |
| *K. pneumoniae* (n = 15) | 2 ESBL + porin loss; 11 KPC (including 6 ST258 phenotype); 2 OXA-48 |
| *Enterobacter* spp. (n = 9) | TEM-24; cAmpC, ciAmpC, 2 cdAmpC + porin loss, 3 KPC + cdAmpC; IMI |
| *P. aeruginosa* (n = 6) | PER-1, VEB; 2 cAmpC, 2 upregulated efflux |
| *A. baumannii* (n = 5) | OXA-51, OXA-58, 2 OXA-23, OXA-24/40 |
| Others (n = 4) | K1 hyperproducer (*K. oxytoca*), SME (*S. marcescens*), 2 cAmpC AmpCs (*M. morganii, S. odorifera*) |

*AmpC prefixes: c, chromosome-encoded; cd, chromosome-encoded derepressed; ci, chromosome-encoded inducible; p, plasmid-encoded

- Agents tested were piperacillin (Pip), ceftriaxone (Cro), cefepime (Fep), and meropenem (Mem), alone or combined with AAI101 4, 8, or 16 mg/L; and Pip/Tazo 4 mg/L.
- Control strains *E. coli* ATCC25922 and *P. aeruginosa* ATCC27853 were tested in all assay runs.
- MICs were interpreted according to 2014 CLSI breakpoints (Cro breakpoints for *P. aeruginosa* not assigned, assumed identical to those for *Acinetobacter* spp.).[2]

## Results

- AAI101 generally lacked intrinsic antibacterial activity ($MIC_{50}$ > 128 mg/L).
- Increasing concentrations of AAI101 generally increased the activity of the partnered β-lactam antibiotics.

**KPC producers**: 11 *K. pneumoniae*, 3 *E. coli*, 3 *E. cloacae* + cdAmpC

- All KPC-producing *E. coli* and *Enterobacter* spp. were susceptible ($^S$) to β-lactam/AAI101.
- Generally, KPC-producing *K. pneumoniae* were β-lactam$^S$/AAI101, but ST258 strains were not.

**ESBL producers**: 11 *E. coli*, 2 *K. pneumoniae* + porin loss, 1 *K. oxytoca*, 1 *E. aerogenes*, 2 *P. aeruginosa*

- ESBL-producing Enterobacteriaceae were generally susceptible to Pip/BLI. The geometric mean MIC (GMM) for Pip/Tazo (15.4 mg/L) was reduced to 7.1 mg/L for Pip/AAI101 4 mg/L.

## Results (continued)

- AAI101 increased Cro and Fep coverage of enterobacterial ESBL producers, and rendered the *P. aeruginosa* PER-1 producer Fep$^S$ but not Cro$^S$.

**AmpC producers**: 5 pAmpC (*E. coli*), 8 cAmpC (2 *E. cloacae*, 2 *E. cloacae* + porin loss, 1 *M. morganii*, 1 *S. odorifera*, and 2 *P. aeruginosa*)

- AAI101 had little effect on the coverage of Pip, Cro, or Mem.
- AAI101 increased substantially Fep coverage, from 44% susceptible for Fep alone (GMM, 1.3 mg/L) to 94% susceptible for Fep/AAI101 4 mg/L (GMM, 0.5 mg/L).

**OXA producers**: 2 *E. coli*, 2 *K. pneumoniae*, 5 *A. baumannii*

- No OXA-48 producers were Pip$^S$ or Mem$^S$ ± BLI, but + AAI101 8 mg/L 3/4 were Cro$^S$, and 4/4 Fep$^S$.
- *A. baumannii* producing OXA-51 and OXA-58 were Pip$^S$ (+ AAI101 16 mg/L) and Fep$^S$ (+ AAI101 4 mg/L).

**Efflux**: 2 *P. aeruginosa*

- Both efflux isolates were Pip$^S$ and Mem$^S$; one became Fep$^S$ with AAI101 8 mg/L.

**Table 2. Number of susceptible strains/total strains tested according to 2014 CLSI breakpoints**

| β-Lactam/BLI combination | Class A | Class A + porin loss | Class C | Class C + porin loss | Classes A + C | Class D | Efflux | % Overall (n = 61) |
|---|---|---|---|---|---|---|---|---|
| Pip | 2/32 | 0/2 | 1/11 | 0/2 | 0/3 | 0/9 | 2/2 | 8 |
| Pip/Tazo | 14/32 | 0/2 | 4/11 | 1/2 | 0/3 | 0/9 | 2/2 | 34 |
| Pip/AAI101 (4) | 21/32 | 0/2 | 3/11 | 0/2 | 1/3 | 0/9 | 2/2 | 44 |
| Pip/AAI101 (8) | 20/32 | 1/2 | 8/11 | 0/2 | 2/3 | 0/9 | 2/2 | 54 |
| Pip/AAI101 (16) | 26/32 | 2/2 | 9/11 | 0/2 | 2/3 | 2/9 | 2/2 | 70 |
| Fep | 9/32 | 0/2 | 7/11 | 0/2 | 0/3 | 0/9 | 1/2 | 28 |
| Fep/AAI101 (4) | 25/32 | 0/2 | 11/11 | 1/2 | 3/3 | 5/9 | 1/2 | 75 |
| Fep/AAI101 (8) | 25/32 | 1/2 | 11/11 | 1/2 | 3/3 | 6/9 | 2/2 | 80 |
| Cro | 3/32 | 0/2 | 1/11 | 0/2 | 0/3 | 0/9 | 0/2 | 7 |
| Cro/AAI101 (4) | 18/32 | 0/2 | 2/11 | 0/2 | 0/3 | 2/9 | 0/2 | 36 |
| Cro/AAI101 (8) | 21/32 | 2/2 | 2/11 | 0/2 | 1/3 | 3/9 | 0/2 | 48 |
| Mem | 15/32 | 0/2 | 11/11 | 0/2 | 0/3 | 0/9 | 2/2 | 46 |
| Mem/AAI101 (4) | 23/32 | 0/2 | 11/11 | 0/2 | 2/3 | 0/9 | 2/2 | 62 |
| Mem/AAI101 (8) | 25/32 | 1/2 | 11/11 | 0/2 | 3/3 | 0/9 | 2/2 | 69 |

## Conclusions

- Addition of AAI101 to Pip, Cro, Fep and/or Mem enhanced activity and generally restored susceptibility for a broad variety of MDR Gram-negative isolates, including:
  - ESBL-producing Enterobacteriaceae and *P. aeruginosa;*
  - *K. pneumoniae*, *E. coli,* and *Enterobacter* spp. with acquired class A or class D carbapenemases (KPC, IMI, OXA-48);
  - chromosomally-encoded AmpC-producing Enterobacteriaceae and *P. aeruginosa*;
  - *A. baumannii* with intrinsic or acquired class D carbapenemases; and
  - *P. aeruginosa* strains with upregulated β-lactam efflux.
- AAI101 has the potential to restore the clinical utility of well-established β-lactam for the treatment of infections caused by MDR Gram-negative pathogens.

## References

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