Characterization of β-lactamase inhibition by AAI101

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Introduction

β-Lactam antibiotics are the primary treatments for a broad spectrum of bacterial infections. Production of β-lactamases (*e.g.* ESBLs, serine carbapenemases, AmpCs) by multidrug-resistant (MDR) Gram-negative bacteria reduces the clinical efficacy of these drugs by deactivating the β-lactam. MDR is an increasingly common problem associated with longer hospital stays, higher treatment costs, and in some cases increased mortality.[1-3] Development of β-lactamase inhibitors (BLIs) such as tazobactam (Tazo) has helped preserve the clinical value of various β-lactam antibiotics by protecting them against hydrolysis. However, new, more aggressive β-lactamases are emerging that are not susceptible to existing BLIs.[4] New BLIs therefore are required.

AAI101 is an extended-spectrum BLI belonging to the penicillanic acid sulfone class. The purpose of this study was to characterize the spectrum of AAI101 activity against epidemiologically important serine β-lactamases when combined with different classes of β-lactam antibiotics.

Materials and Methods

- 57 isogenic strains, each expressing a unique β-lactamase were prepared from *Escherichia coli* K-12 derivative TOP10/DH10B and DH5α (Table 1).

Table 1. Isogenic strain characteristics

β-Lactam class	n	Expressed β-lactamase
ESBLs	31	Clavulanate-susceptible: 6 TEMs, SHV-12, 8 CTX-Ms, 5 GESs, 4 PERs, VEB-1, BEL-1, BES-1, TLA-2 Clavulanate-resistant: TEM-68, TEM-121, SHV-49 (penicillinase)
Carbapenemases	14	KPC-2, KPC-3; OXA-48, OXA-162, OXA-181, OXA-204, OXA-232; GES-2, GES-5, GES-6, GES-14; IMI-2, NMC-A, SME-1
Non-carbapenemase OXAs	8	OXA-1, OXA-10, OXA-18, OXA-35, OXA-47, OXA-143, OXA-145, OXA-163
pAmpCs	4	CMY-2, DHA-1, FOX-5, ACC-1

- Broth microdilution minimum inhibitory concentrations (MICs) for each strain were obtained for piperacillin (Pip), ceftriaxone (Cro), cefepime (Fep), and meropenem (Mem) ± AAI101 at a fixed concentration of 4, 8, or 16 mg/L; Pip + Tazo 4 mg/L (Pip/Tazo) also was tested.
- Quality control strain *E. coli* ATCC25922 was included in all assay runs.
- Antibiotic susceptibilities were assigned according to 2014 CLSI breakpoints (mg/L) :
  - Pip: S ≤ 16, I = 32-64, R ≥ 128
  - Fep: S ≤ 2, I = 4-8, R ≥ 16
  - Mem: S ≤ 1, I = 2, R ≥ 4
  - Cro: S ≤ 1, I = 2, R ≥ 4

References

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Results

- AAI101 alone lacked intrinsic antibacterial activity against all strains (MIC_{50} >128 mg/L).

ESBL producers

- 6% of strains were susceptible to Pip (Pip^S); up to 97% were Pip^S with AAI101 4-16 mg/L.
- Geometric mean MICs (GMMs) for ESBLs with Pip/Tazo and Pip/AAI101 4 mg/L were 3.42 mg/L and 2.67 mg/L, respectively.
- AAI101 4 mg/L rendered all strains Cro^S or Fep^S (GMMs, 0.06-0.09 mg/L).
- All strains were Mem^S; in some cases addition of AAI101 lowered MICs further.

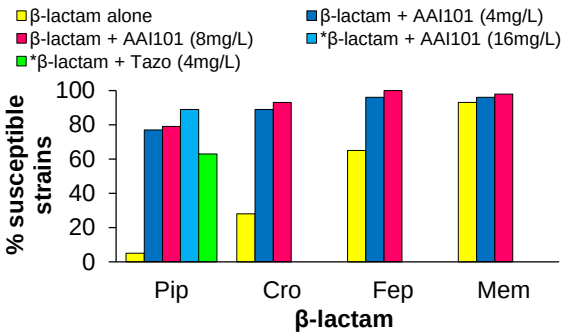
Class A carbapenemase-producers

- No strains were Pip^S; up to 89% were Pip^S with Pip/AAI101 4-16 mg/L.
- 22% were Cro^S (GMM, 6.9 mg/L); up to 67% were Cro^S with Cro/AAI101 4-8 mg/L.
- All but one strain (GES-2) was Fep^S (GMM, 0.73 mg/L); all were susceptible to Fep/AAI101 (GMMs, 0.21 mg/L and 0.06 mg/L for AAI101 4 and 8 mg/L, respectively).
- 56% of strains were Mem^S; up to 89% were susceptible to Mem/AAI101 4-8mg/L.

OXA producers

- 77% of strains (including the OXA-48 producer) were susceptible to Pip/AAI101 16 mg/L.
- 62% were Cro^S (GMM, 0.58mg/L) and 69% were Fep^S (GMM, 2.38 mg/L); 100% were Cro^S and Fep^S with AAI101 8 mg/L (GMMs, 0.06 mg/L and 0.11 mg/L, respectively).
- All strains were Mem^S; in some cases MICs were further reduced with AAI101.

Figure 1. Percentage of strains susceptible to different β-lactam antibiotics ± BLIs (*Only tested with Pip)



Plasmid-encoded AmpC producers

- 75% of strains were Pip^S with Pip/AAI101 16mg/L.
- AAI101 did not increase the number of Cro^S strains but lowered MICs ≥ 2 log_2 dilution steps (GMMs: Cro alone, 11.31 mg/L; Cro/AAI101 4mg/L, 2.80 mg/L; Cro/AAI101 8 mg/L, 2.35 mg/L).
- All strains were Fep^S (GMM, 0.35 mg/L) and Mem^S (MIC_{100} <0.06 mg/L); AAI101 8 mg/L lowered Fep MICs for most strains (GMM, 0.07 mg/L).

Conclusions

- By inhibiting many aggressive β-lactamases of epidemiological concern (e.g. ESBLs, Class A carbapenemases, and OXAs), AAI101 functioned as an extended-spectrum BLI, substantially improving the coverage of Pip, Cro, and Fep towards diverse β-lactamase producers.
- Across the isogenic strain panel the coverage of Pip/AAI101 was comparable to that of Mem and superior to that of Pip/Tazo; coverage of Fep/AAI101 was superior to that of Mem.
- β-Lactam/AAI101 combinations have the potential to replace carbapenems as first-line agents.
- AAI101, in combination with diverse β-lactams, can treat infections caused by MDR Gram-negative pathogens that produce β-lactamases not susceptible to other BLIs.