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, and 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 β-lactams before they can reach their targets. MDR pathogens are 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 such as tazobactam (Tazo) has helped preserve the clinical value of 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 with a broader spectrum of activity are therefore required. AAI101 is an novel extended-spectrum β-lactamase inhibitor (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.

Materials and Methods

- Fifty-seven isogenic strains, each expressing a unique β-lactamase, were prepared from *Escherichia coli* K-12 derivatives 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; GES-2, GES-5, GES-6, GES-14; IMI-2, NMC-A, SME-1; OXA-48, OXA-162, OXA-181, OXA-204, OXA-232
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 fixed BLI concentrations of 4, 8, or 16 mg/L; and for Pip + Tazo 4 mg/L (Pip/Tazo).
- 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
  - Cro: S ≤ 1, I = 2, R ≥ 4
  - Fep: S ≤ 2, I = 4-8, R ≥ 16
  - Mem: S ≤ 1, I = 2, R ≥ 4

*S, susceptible; I, intermediate or susceptible-dose dependent; R, resistant

References

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Results

- AAI101 alone lacked intrinsic antibacterial activity against all strains (MIC_{100} >128 mg/L).

ESBLs (n = 31)

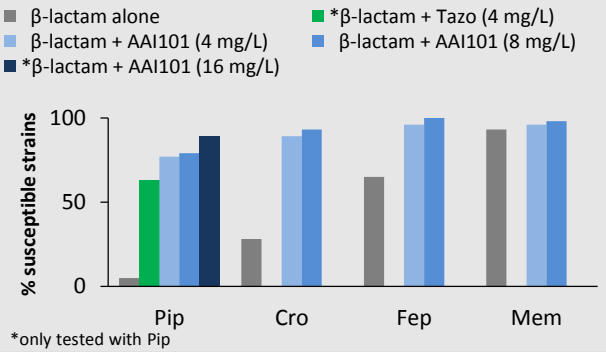
- Pip alone was inactivated by all but 1 enzyme (CTX-M-93).
- AAI101 4-16 mg/L protected Pip from all but 1 enzyme (SHV-49, MIC = 32 mg/L).
- The geometric mean MIC (GMM) of 3.42 mg/L for Pip/Tazo was reduced to 2.67 mg/L for Pip/AAI101 4 mg/L.
- AAI101 4 mg/L rendered all strains Cro^S or Fep^S.
- Addition of AAI101 also lowered some MICs to Mem.

Class A carbapenemases (n = 9)

- Pip alone was inactivated by all carbapenemases, but AAI101 up to 16 mg/L protected Pip in the presence of all but one enzyme (SME-1).
- 2/9 were Cro^S; up to 6 became Cro^S with Cro/AAI101 8 mg/L.
- Only one enzyme (GES-2) inactivated Fep, this was reversed by AAI101 4 mg/L.
- 4 enzymes inactivated Mem alone; only the SME-1 producer remained resistant with AAI101.

OXAs (n = 13: 5 carbapenemases and 8 non-carbapenemases)

- 10/13 enzymes (including OXA-48) were inhibited by Pip + AAI101 16 mg/L, but OXA-1, OXA-10, and OXA-143 producers remained resistant to Pip.
- All strains became Cro^S and Fep^S with AAI101 8 mg/L (GMMs, 0.06 and 0.11 mg/L, respectively).
- Addition of AAI101 also lowered some MICs to Mem.



*only tested with Pip

Plasmid-encoded AmpCs (n = 4)

- AAI101 16 mg/L protected Pip from inactivation by all but one enzyme (FOX-5).
- AAI101 lowered MICs ≥2 log_2 dilution steps (GMMs: Cro alone, 11.31 mg/L; Cro/AAI101 4 mg/L, 2.80 mg/L; Cro/AAI101 8 mg/L, 2.35 mg/L).
- All strains were Fep^S and Mem^S; AAI101 8 mg/L lowered Fep MICs for most strains (GMM, 0.07 mg/L).

Conclusions

- AAI101 is an extended spectrum BLI with broader and more potent activity than tazobactam:
  - Combined with piperacillin, AAI101 was a more potent inhibitor of ESBLs.
  - AAI101 was active against many class A carbapenemases and OXAs, including KPC-2, KPC-3, and OXA-48.
- AAI101 substantially improved the coverage of piperacillin, ceftriaxone, and cefepime towards diverse β-lactamase producers.
- AAI101 combinations may offer a therapeutic alternative to carbapenems as first-line agents for treatment of MDR Gram-negative infections.