In vitro activity of cefepime-AAI101 *vs.* drug-resistant *Klebsiella pneumoniae* clinical isolates

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Introduction

AAI101 is a novel zwitterionic extended-spectrum β-lactamase inhibitor (BLI) belonging to the penicillanic acid sulfone family. *In vitro* studies using Gram-negative clinical isolates with defined mechanisms of β-lactam resistance and isogenic strains of *E. coli* each expressing a single defined class A, C, or D β-lactamase established that AAI101 synergizes the antibacterial activity of diverse β-lactam classes.[1,2] Mouse models have demonstrated the superior potency of AAI101 to tazobactam (Tazo) when one or the other BLI is coadministered with a β-lactam to animals with a systemic or localized tissue (thigh) infection.[3,4] A combination of AAI101 and cefepime (Fep) is in Phase II trials for infections caused by multidrug-resistant Gram-negative pathogens.

As producers of extended-spectrum β-lactamases (ESBLs) and KPC carbapenemases, *Klebsiella pneumoniae* represents a particular therapeutic challenge. KPC-2 is the most common KPC isozyme, though outbreaks of *Klebsiella* expressing KPC-3 have occurred in some localities.[5] Spread of KPC-producing *K. pneumoniae* reportedly is associated with multilocus sequence type 258 (ST258) and single locus variants thereof, which jointly comprise *K. pneumoniae* clonal cluster 258 (CC258).

This study compared the activity of AAI101 with that of tazobactam on cefepime MICs towards a challenge panel of predominantly cefepime-resistant *K. pneumoniae* strains expressing ESBLs or carbapenemases, and belonging to diverse sequence types.

Materials and Methods

- AAI101, weight-purity 96%, was supplied by Allecra Therapeutics SAS (St-Louis, France); cefepime and tazobactam were obtained from commercial suppliers.
- Broth microdilution MICs were obtained according to CLSI protocols.[6] Cefepime was examined as doubling dilutions over the range 0.5-32 μg/mL; tazobactam was tested at a fixed concentration of 4 μg/mL, whereas AAI101 was tested at fixed concentrations of 4 μg/mL and 8 μg/mL.
- *In vitro* testing with higher AAI101 concentrations is supported by the longer half-life of AAI101 compared to that of tazobactam.[7] MIC testing with a fixed concentration of 8 μg/mL of AAI101 correlates best with *in vivo* efficacy using humanized dosing of cefepime-AAI101,[4] reflecting the greater exposure achievable for a given dose of AAI101 compared to an equimolar dose of tazobactam.
- Geometric mean (geomean) MICs were calculated as described by Caspers *et al.*[8]
- Breakpoints for cefepime-BLI combinations have not been assigned; therefore, CLSI breakpoint assignments (S = S-DD or R) followed those for cefepime alone.
- A largely cefepime-resistant challenge panel of 106 *K. pneumoniae* clinical strains, from patients hospitalized in the Middle East, Europe, and the USA during 2007-2013, was recruited from the culture collection of the Tel Aviv Sourasky Medical Center.
- β-Lactamases in strains comprising the challenge panel were identified by PCR and gene sequencing. Sequence type was established by MLST or PFGE; ST258 clones also were identified by detection of the *pilv-1* allele. Quality control strains *E. coli* ATCC25922 and *Ps. aeruginosa* ATCC27853 were included in all assay runs.
- The β-lactamase and sequence type distributions for the 106 *K. pneumoniae* clinical isolates surveyed were as follows:

24 non-KPC producers

9 **ST258** ESBL (6 **CTX-M-2**, 3 **CTX-M-25**) producers from the Middle East

15 **non-CC258** isolates from the Middle East:
- 5 **non-CTX-M ESBL** producers
- 10 **OXA-48-like carbapenemase** producers

21 KPC-2 and 61 KPC-3 producers

41 **ST258** isolates from the Middle East, Greece, the USA, and Columbia

15 **non-ST258 CC258** isolates from the Middle East and Italy

26 **non-CC258** isolates from the Middle East, Greece, the USA, and Columbia

References

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Results

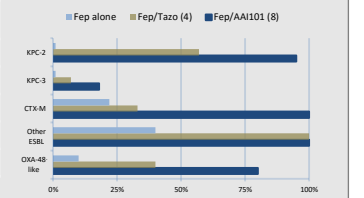
- Cefepime alone up to 8 μg/mL (CLSI S-DD breakpoint) inhibited growth of 5/106 strains (4.7%).
- AAI101 alone up to 128 μg/mL did not inhibit growth of any *K. pneumoniae* strain examined.
- AAI101 proved a more potent inhibitor of CTX-Ms, KPCs, and OXA-48s than tazobactam.

Table: Effect of tazobactam and AAI101 on cefepime susceptibility* and geometric mean MICs (μg/mL)

β-Lactamase	Fep	Fep-Tazo (4 μg/mL)	Fep-AAI101 (4 μg/mL)	Fep-AAI101 (8 μg/mL)
KPC-2 (n = 21) *Geomean MIC*	**0** (0%) *51 μg/mL*	**12** (57%) *10 μg/mL*	**16** (76%) *3.1 μg/mL*	**20** (95%) *1.8 μg/mL*
KPC-3 (n = 61) *Geomean MIC*	**0** (0%) *61 μg/mL*	**4** (7%) *44 μg/mL*	**8** (13%) *37 μg/mL*	**11** (18%) *30 μg/mL*
CTX-M (n = 9) *Geomean MIC*	**2** (22%) *32 μg/mL*	**3** (33%) *18 μg/mL*	**7** (78%) *1.9 μg/mL*	**9** (100%) *0.73 μg/mL*
Other ESBL (n = 5) *Geomean MIC*	**2** (40%) *12 μg/mL*	**5** (100%) *0.57 μg/mL*	**5** (100%) *0.57 μg/mL*	**5** (100%) *0.50 μg/mL*
OXA-48-like (n=10) *Geomean MIC*	**1** (10%) *39 μg/mL*	**4** (40%) *16 μg/mL*	**9** (90%) *2.3 μg/mL*	**8** (80%) *2.1 μg/mL*

*CLSI Fep S-DD breakpoint = 8 μg/mL

Figure: Effect of tazobactam and AAI101 on cefepime susceptibility*



- AAI101 concentrations of 4 μg/mL (or 8 μg/mL) combined with cefepime up to 8 μg/mL inhibited growth *in vitro* of most ESBL, KPC-2, and OXA-48-like producers.
  - A dosage effect for AAI101 was noted, broader coverage and lower cefepime MICs generally being achieved with the higher AAI101 concentration
- Cefepime-AAI101 was far more effective at inhibiting *K. pneumoniae* producing KPC-2 than KPC-3.
  - The isozymes differ by a single amino acid in their active sites (KPC-2: His274; KPC-3: Tyr274),[9] which may confer different reactivities towards AAI101, though this remains to be studied.
  - KPC-2-producing *K. pneumoniae* reportedly are also more sensitive to avibactam than KPC-3 producers.[10]
  - In this challenge panel most KPC-2 producers belonged to non-CC258 sequence types, whereas KPC-3 producers were mostly CC258s. Nonetheless, difference in β-lactamase type rather than sequence type is the more plausible explanation for reduced susceptibility of Fep-AAI101 towards KPC-3 producers, since all CTX-M producers were ST258s and all of them were susceptible to Fep-AAI101.

Conclusions

- **AAI101, a novel zwitterionic extended-spectrum β-lactamase inhibitor, proved highly effective at protecting the activity of cefepime against a challenge panel of largely cefepime-resistant clinical isolates of *Klebsiella pneumoniae* representing the high end of the enterobacterial resistance spectrum.**
- **AAI101 was a more potent inhibitor than tazobactam, and afforded protection against a broader spectrum of β-lactamases.**
- **AAI101 expanded the spectrum of cefepime to include nearly all ESBL-, KPC-2-, and OXA-48-like-producing *K. pneumoniae*.**
- **The combination of AAI101 and cefepime is a carbapenem-sparing potential treatment option for infections suspected to be caused by ESBL- and many other β-lactamase-producing pathogens.**