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Activity of the novel extended-spectrum β-lactamase inhibitor AAI101 in combination with cefepime against ESBL-producing Enterobacteriaceae collected from US and European hospitals during 2014/2015

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ABSTRACT

Background: AAI101 is a novel extended-spectrum β-lactamase inhibitor (BLI) active against ESBLs and a broad array of other β-lactamases. AAI101 combined with cefepime has completed Phase 2 clinical development. Cefepime/AAI101 was granted Qualified Infectious Disease Product and Fast Track designations by the United States Food and Drug Administration. Emergence of new extended-spectrum β-lactamases (ESBLs) amongst Enterobacteriaceae has compromised the clinical efficacy of β-lactam/β-lactamase inhibitor combinations such as piperacillin-tazobactam (PIP/TAZ). This study assessed the in vitro activity of cefepime/AAI101 against Enterobacteriaceae isolated from patients in the USA and Europe during 2014/2015.

Materials/methods: *E. coli*, *K. pneumoniae*, and *Enterobacter* isolates (n = 1,696) were collected during 2014/2015 from the USA (50%) and France, Germany, Italy, Spain and the UK (10% each). ESBLs were identified by genotyping. MICs were determined by broth microdilution following CLSI methodology.

Results: MIC_{90} data for cefepime/AAI101 and β-lactam comparators are shown in the Table. Against *K. pneumoniae* cefepime/AAI101 activity was far superior to ceftolozane-tazobactam (TOL/TAZ) or PIP/TAZ, and similar to ceftazidime-avibactam (CAZ/AVI). Against *E. coli* cefepime/AAI101 activity was superior to PIP/TAZ, particularly ESBL-producing isolates, and similar to CAZ/AVI or TOL/TAZ. cefepime/AAI101 activity *vs. Enterobacter* isolates was much greater than that of PIP/TAZ or TOL/TAZ, and was similar to CAZ/AVI.

Pathogen (n)	MIC_{90} (mg/L)					
	cefepime	cefepime/ AAI101[4*]	cefepime/ AAI101[8*]	CAZ/AVI [4*]	TOL/TAZ [4*]	PIP/TAZ [4*]
K. pneumoniae (799)	>64	0.5	0.5	0.5	8	>128
- ESBL *K. pneumoniae* (87)	>64	0.5	0.5	0.5	16	>128
E. coli (697)	16	0.12	0.12	0.25	0.5	8
- ESBL *E. coli* (103)	>64	0.25	0.12	0.25	1	64
E. aerogenes (100)	0.5	0.25	0.25	0.5	4	64
E. cloacae (100)	16	2	1	0.5	16	128

CAZ, ceftazidime, AVI, avibactam, TOL, ceftolozane; TAZ, tazobactam; PIP, piperacillin; *BLI at fixed conc. in mg/L

Conclusions: Addition of AAI101, a potent β-lactamase inhibitor, to cefepime renders this cephalosporin active against ESBL-producing *K. pneumoniae* and *E. coli*, and other Enterobacteriaceae. Improved susceptibility of ESBL-producing Enterobacteriaceae to cefepime/AAI101 compared to PIP/TAZ suggests that cefepime/AAI101 may be useful in hospitals where resistance to PIP/TAZ is significant.

INTRODUCTION

AAI101 is an new extended-spectrum β-lactamase inhibitor belonging to the penicillanic acid sulfone class (Figure 1), whose mechanism of action towards β-lactamases is distinguishable from that of tazobactam [1].

In this study, the activity of cefepime/AAI101 against *Escherichia coli, Klebsiella pneumoniae*, and *Enterobacter* spp. was evaluated, with isolates coming from US medical centres across the country and from medical centres representing the five principal West European healthcare markets.

MATERIALS & METHODS

- A collection of 1,696 isolates of Enterobacteriacaeae collected during 2014/2015 from USA (50%) and France, Germany, Spain Italy and the UK (10% each) were tested.
- MICs for cefepime/AAI101 [at fixed AAI101 concentrations of 4 or 8 mg/L] and comparator antibiotics were determined by broth microdilution using CLSI methodology [2]. AAI101, avibactam and ceftolozane were provided by Allecra Therapeutics SAS, and other antibacterials were purchased from commercial sources.
- Susceptibility was determined according to CLSI breakpoints [3] except for ceftazidime/avibactam, for which FDA breakpoints were used.
- For comparative purposes, susceptibility of AAI101 combinations with cefepime was determined using CLSI breakpoints for cefepime alone [3].

RESULTS

Figure 1. Chemical structure of AAI101

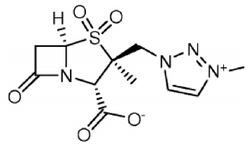


Table 1. Overview MIC_{90} [mg/L] (% susceptibility) by pathogen

Pathogen	cefepime	cefepime/ AAI101 (4)	cefepime/ AAI101 (8)	ceftazidime	ceftazidime/ avibactam	ceftolozane/ tazobactam	meropenem	piperacillin/ tazobactam
E. coli **n=697**	16 (85.8%)	0.12 (99.9%)	0.12 (99.9%)	16 (86.7%)	0.25 (100%)	0.5 (98.1%)	0.03 (99.6%)	8 (92.4%)
K. pneumoniae **n=799**	>64 (80.9%)	0.5 (92.9%)	0.5 (93.7%)	>64 (80.4%)	0.5 (99.6%)	8 (87.5%)	0.12 (92.7%)	>128 (83.1%)
E. aerogenes **n=100**	0.5 (97.0%)	0.25 (100%)	0.25 (100%)	64 (73.0%)	0.5 (100%)	4 (84.0%)	0.12 (100%)	64 (77.0%)
E. cloacae **n=100**	16 (79.0%)	2 (94.0%)	1 (96.0%)	>64 (58.8%)	0.5 (98.0%)	16 (71.0%)	0.12 (97.0%)	128 (68.0%)

Key: susceptible; intermediate or susceptible dose-dependent; resistant

Table 2. Summary MIC and susceptibility data for all Enterobacteriaceae (n=1996)

Drug	Percentage			MIC (mg/L)			
	Susceptible	Intermediate	Resistant	MIC_{50}	MIC_{90}	Min	Max
Cefepime	83.7	3.2	13.0	0.06	32	0.015	> 64
Cefepime/AAI101 [4]	96.2	1.4	2.4	0.06	0.25	0.015	> 64
Cefepime/AAI101 [8]	96.8	1.3	1.9	0.06	0.25	0.015	> 64
Ceftazidime	81.2	1.9	16.9	0.25	64	0.03	> 64
Ceftazidime/Avibactam [4]	99.7	-	-	0.12	0.5	≤ 0.015	> 64
Ceftolozane/Tazobactam [4]	90.7	2.0	7.3	0.25	2	0.06	> 32
Ciprofloxacin	76.5	1.4	22.1	0.03	> 16	0.004	> 16
Gentamicin	89.0	0.5	10.5	0.5	16	0.12	> 32
Meropenem	96.2	0.2	3.6	0.03	0.06	0.008	> 8
Piperacillin/Tazobactam [4]	85.7	4.7	9.6	2	64	0.12	> 128

Table 3. Summary MIC and susceptibility for all ESBL-only producing Enterobacteriaceae (n=191)

Drug	Percentage			MIC (mg/L)			
	Susceptible	Intermediate	Resistant	MIC_{50}	MIC_{90}	Min	Max
Cefepime	9.4	18.8	71.7	32	> 64	0.12	> 64
Cefepime/AAI101 [4]	98.4	1.1	0.5	0.06	0.25	0.015	16
Cefepime/AAI101 [8]	99.5	0.5	0.0	0.06	0.25	0.015	4
Ceftazidime	17.3	35.6	47.1	16	> 64	0.25	> 64
Ceftazidime/Avibactam [4]	100	-	-	0.12	0.25	≤ 0.015	2
Ceftolozane/Tazobactam [4]	79.6	7.8	12.6	0.5	8	0.12	> 32
Ciprofloxacin	13.6	5.2	81.2	> 16	> 16	0.008	> 16
Gentamicin	52.9	0.5	46.6	1	> 32	0.12	> 32
Meropenem	99.5	0.5	0.0	0.03	0.06	0.008	4
Piperacillin/Tazobactam [4]	69.6	11.0	19.4	8	>128	0.5	> 128

Table 4. Summary MIC and susceptibility data for all *E. coli* (n=697)

Drug	Percentage			MIC (mg/L)			
	Susceptible	Intermediate	Resistant	MIC_{50}	MIC_{90}	Min	Max
Cefepime	85.8	4.0	10.2	0.06	16	0.015	> 64
Cefepime/AAI101 [4]	99.9	0.0	0.1	0.06	0.12	0.015	> 64
Cefepime/AAI101 [8]	99.9	0.0	0.1	0.06	0.12	0.015	32
Ceftazidime	86.7	2.7	10.6	0.25	16	0.06	> 64
Ceftazidime/Avibactam [4]	100.0	-	-	0.12	0.25	≤ 0.015	2
Ceftolozane/Tazobactam [4]	98.1	0.6	1.3	0.25	0.5	0.06	> 32
Ciprofloxacin	68.0	0.7	31.3	0.015	> 16	0.004	> 16
Gentamicin	86.2	0.3	13.5	0.5	32	0.12	> 32
Meropenem	99.6	0.1	0.3	0.015	0.03	0.008	8
Piperacillin/Tazobactam [4]	92.4	3.3	4.3	2	8	≤ 0.12	> 128

Table 5. Summary MIC and susceptibility data for all *K. pneumoniae* (n=799)

Drug	Percentage			MIC (mg/L)			
	Susceptible	Intermediate	Resistant	MIC_{50}	MIC_{90}	Min	Max
Cefepime	80.9	1.9	17.3	0.06	> 64	0.015	> 64
Cefepime/AAI101 [4]	92.9	2.8	4.4	0.06	0.5	0.015	> 64
Cefepime/AAI101 [8]	93.7	2.6	3.6	0.06	0.5	0.015	> 64
Ceftazidime	80.4	1.3	18.4	0.25	> 64	0.03	> 64
Ceftazidime/Avibactam [4]	99.6	-	-	0.12	0.5	≤ 0.015	> 64
Ceftolozane/Tazobactam [4]	87.5	1.8	10.8	0.25	8	0.06	> 32
Ciprofloxacin	80.0	2.1	17.9	0.03	> 16	0.004	> 16
Gentamicin	90.0	0.6	9.4	0.25	8	0.12	> 32
Meropenem	92.7	0.1	7.1	0.03	0.12	0.008	> 8
Piperacillin/Tazobactam [4]	83.1	2.9	14.0	4	> 128	0.25	> 128

Table 6. Summary MIC and susceptibility data for ESBL-only producing *K. pneumoniae* (n=87)

Drug	Percentage			MIC (mg/L)			
	Susceptible	Intermediate	Resistant	MIC_{50}	MIC_{90}	Min	Max
Cefepime	5.7	12.6	81.6	64	>64	0.03	>64
Cefepime/AAI101 [4]	96.6	2.3	1.1	0.12	0.5	0.03	16
Cefepime/AAI101 [8]	98.9	1.1	0.0	0.12	0.5	0.03	4
Ceftazidime	5.7	8.0	86.2	64	>64	0.25	>64
Ceftazidime/Avibactam [4]	100.0	-	-	0.25	0.5	≤0.015	2
Ceftolozane/Tazobactam [4]	60.9	13.8	25.3	1	16	0.12	>32
Ciprofloxacin	16.1	10.3	73.6	>16	>16	0.008	>16
Gentamicin	44.8	1.1	54.0	32	>32	0.25	>32
Meropenem	98.9	0.0	1.1	0.03	0.12	0.015	4
Piperacillin/Tazobactam [4]	50.6	14.9	34.5	16	>128	1	>128

Table 7. Summary MIC and susceptibility data for KPC-only producing *K. pneumoniae* (n=29)

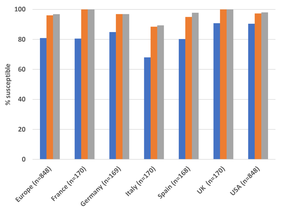
Drug	Percentage			MIC (mg/L)			
	Susceptible	Intermediate	Resistant	MIC_{50}	MIC_{90}	Min	Max
Cefepime	0.0	0.0	100	> 64	> 64	16	>64
Cefepime/AAI101 [4]	0.0	13.8	86.2	32	> 64	4	> 64
Cefepime/AAI101 [8]	6.9	13.8	79.3	32	> 64	2	> 64
Ceftazidime	0.0	0.0	100	> 64	> 64	32	> 64
Ceftazidime/Avibactam [4]	96.6	-	-	1	8	0.06	16
Ceftolozane/Tazobactam [4]	0.0	0.0	100	> 32	> 32	32	> 32
Ciprofloxacin	3.4	0.0	96.6	> 16	> 16	0.5	> 16
Gentamicin	79.3	10.3	10.3	2	> 32	0.25	>32
Meropenem	0.0	0.0	100	> 8	> 8	4	> 8
Piperacillin/Tazobactam [4]	3.4	0	96.6	> 128	> 128	1	> 128

Table 8. Summary MIC and susceptibility data for all *E. cloacae* (n=100)

Drug	Percentage			MIC (mg/L)			
	Susceptible	Intermediate	Resistant	MIC_{50}	MIC90	Min	Max
Cefepime	79.0	9.0	12.0	0.12	16	0.03	>64
Cefepime/AAI101 [4]	94.0	2.0	4.0	0.12	2	0.03	>64
Cefepime/AAI101 [8]	96.0	1.0	3.0	0.12	1	0.03	>64
Ceftazidime	58.0	1.0	41.0	0.5	>64	0.12	>64
Ceftazidime/Avibactam [4]	98.0	-	-	0.25	0.5	0.03	>64
Ceftolozane/Tazobactam [4]	71.0	9.0	20.0	0.5	16	0.25	>32
Ciprofloxacin	89.0	1.0	10.0	0.03	2	0.008	>16
Gentamicin	92.0	2.0	7.0	0.25	0.5	0.12	>32
Meropenem	97.0	1.0	2.0	0.03	0.12	0.008	>8
Piperacillin/Tazobactam [4]	68.0	14.0	18.0	4	128	1	>128

Tables 2 – 8: the "Intermediate" category corresponds to "Susceptible Dose-Dependent" for cefepime and cefepime/AAI101. Some ESBL-only producing isolates coproduced original-spectrum β-lactamases (OSBLs).

Figure 2. Percentage susceptibility for cefepime and AAI101 combinations against Enterobacteriaceae (n=1696) by country/region



RESULTS

- Addition of 4 mg/L or 8 mg/L of AAI101 to cefepime restored the MIC_{90} to the susceptible category for the combined panel of Enterobactariaceae. The susceptibility rate of cefepime/AAI101 was comparable to that of meropenem. Cefepime/AAI101 was more active than ceftolozane/tazobactam and piperacillin/tazobactam (**Table 2**).
  - Against a subset of genotyped, ESBL-only producing Enterobacteriaceae showing > 70% cefepime resistance, addition of AAI101 shifted almost all isolates to the susceptible category (**Table 3**).
- Cefepime/AAI101 susceptibility rates for *E. coli* were comparable to those of ceftolozane/tazobactam, meropenem and ceftazidime/avibactam, and were higher than that of piperacillin/tazobactam (**Table 4**).
- Cefepime/AAI101 susceptibility rates for *K. pneumoniae* were comparable to those of meropenem, and higher than those of piperacillin/tazobactam and ceftolozane tazobactam. Ceftazidim/avibactam was the most potent drug tested (**Table 5**).
  - Against a subset of genotyped, ESBL-only producing *K. pneumoniae* isolates with > 80% cefepime resistance, addition of AAI101 shifted almost all isolates to the susceptible category (**Table 6**).
  - Against a subset of genotyped, KPC-only producing *K. pneumoniae* isolates with 100% cefepime resistance, ceftazidime/avibactam was the only effective drug (**Table 7**).

RESULTS (continued)

- Against *E. cloacae* cefepime/AAI101, meropenem and ceftazidime/avibactam showed comparable high susceptibility rates. Ceftolozane/tazobactam and piperacillin/tazobactam susceptibility rates were below 75% (**Table 8**). *E. aerogenes* isolates were fully susceptible to cefepime/AAI101, ceftazidime/avibactam and meropenem whereas MIC_{90}s were below the susceptible breakpoint for ceftolozane/tazobactam and piperacillin/tazobactam (**Table 1**).
- Susceptibility to cefepime +/- AAI101 by country/region are shown in **Fig 2**.

SUMMARY

- AAI101 restores the activity of cefepime against *K. pneumoniae*, *E. coli* and *E. cloacae*. For *E. aerogenes*, cefepime alone showed good activity.
- The activity of AAI101 was most obvious when tested against Enterobacteriaceae expressing ESBLs only. When combined with cefepime at a fixed concentration of 8 mg/L, all of the isolates were in the non-resistant categories.
- Towards this collection of recent clinical isolates of Enterobacteriaceae, cefepime/AAI101 clearly outperformed piperacillin/tazobactam, was much more active than ceftolozane/tazobactam and was as active as meropenem.
- Ceftazidime/avibactam was the most potent compound tested, likely due to its ability to inhibit KPC-producing isolates.
- Ciprofloxacin MIC_{90} were in the susceptible category only for *E. aerogenes*.
- Gentamicin MIC_{90}s were in the susceptible category for *K. pneumoniae* (all isolates) and *Enterobacter* spp. but not for ESBL-producing *K. pneumoniae*.
- Geographic differences in the activity of cefepime and cefepime/AAI101 were observed. Overall, the difference between Europe and the US is small, with some regional differences in Europe

CONCLUSIONS

- **AAI101 is a novel extended-spectrum β-lactamase inhibitor with particular activity against ESBLs.**
- **AAI101, in combination with cefepime, demonstrated very good activity against a recent clinical panel of Enterobacteriaceae collected from Europe and the US.**
- **Cefepime-AAI101 may be useful in hospitals where resistance to piperacillin-tazobactam is significant.**

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