

Protection of cephalosporins by oxapenem AM-112,
a novel, broad-spectrum β -lactamase inhibitorC E Jamieson¹, P A Lambert¹ & I N Simpson²¹Aston University, Birmingham, B4 7ET; ²Micron Research, Cambridge CB3 7ES, UKP A Lambert
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INTRODUCTION

β -Lactamases, particularly Class A and Class C enzymes, compromise the clinical efficacy of cephalosporins. Despite the development of cephalosporins with increased β -lactamase stability, (1st, 2nd, 3rd and 4th generation cephalosporins), the β -lactamase problem has increased over the last 30 years due to:

1. Rapid spread of plasmid-mediated enzymes, particularly TEM and SHV Class A β -lactamases, within and between bacterial species. These enzymes possess potent activity against penicillins and 1st generation cephalosporins.
2. Induction and derepression of chromosomally mediated Class C β -lactamases, conferring resistance to most cephalosporins, irrespective of generation.
3. Mutation of TEM and SHV β -lactamases resulting in extended spectrum β -lactamases (ESBLs), capable of hydrolysing many 3rd and 4th generation cephalosporins.
4. Plasmid-carriage of some Class C β -lactamases.

Established β -lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam are effective inhibitors of Class A and some ESBL enzymes and form potent combinations with amoxicillin, piperacillin and ampicillin respectively, but possess little activity against Class C enzymes.

Oxapenem AM-112 is a novel, β -lactamase inhibitor with a potent activity against Class A, C and D β -lactamases. However, selection of a β -lactam partner is an important consideration in the development of a β -lactamase inhibitor; and is influenced by many factors including antibacterial spectrum, β -lactamase stability profile, pharmacokinetics, formulation and cost of goods.

OBJECTIVE

To investigate the potential of AM-112, relative to clavulanic acid (CLAV), to protect a variety of 1st, 2nd, 3rd and 4th generation cephalosporins against β -lactamase attack in MIC tests using a panel of bacterial isolates producing representative Class A and Class C β -lactamases.

METHODS

Bacterial strains. Seven Gram-negative, bacterial strains producing a representative variety of Class A and Class C β -lactamases were selected as assay strains (Table 1).

Susceptibility tests. Minimum inhibitory concentrations (MICs) were determined by broth microdilution in accordance with NCCLS guidelines. For combination studies, AM-112 and CLAV were incorporated in the wells at a final fixed concentration of 4 mg/L.

RESULTS

Antibacterial activity of clavulanic acid and AM-112
CLAV displayed MICs of 32 - >64 mg/L against all assay strains (Table 1). AM-112 was marginally more active, displaying MICs of 8 - 16 mg/L against *E. cloacae* P99, *E. coli* SHV-5 and *C. diversus* 2046E, but 32 - >64 mg/L against the remaining strains.

Antibacterial activity of cephalosporins alone and in combination with CLAV or AM-112

- MIC results for each of the cephalosporins alone and in the presence of CLAV or AM-112 at a fixed concentration of 4 mg/L are shown in Figures 1 - 8.
- All of the cephalosporins displayed poor activity (MICs \geq 32 mg/L) against *E. cloacae* P99 and *E. cloacae* Hennessey, which produce Class C β -lactamases.
- All cephalosporins, except cefotaxime (MIC 2 mg/L), were inactive against *E. faecalis* SFZ (MICs \geq 32 mg/L).

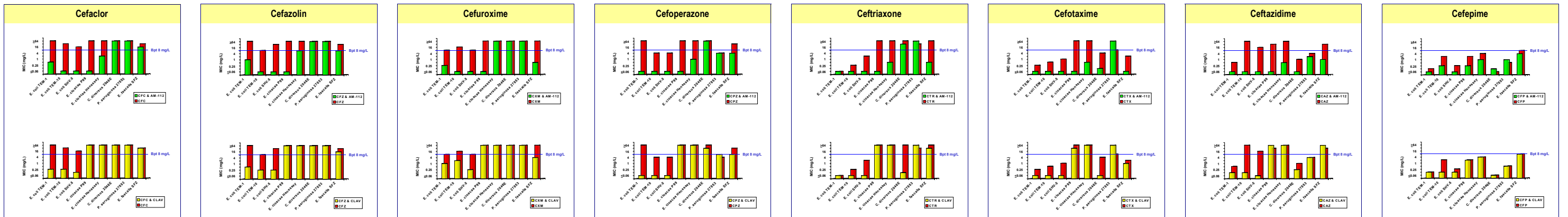
Table 1. Details of bacterial strains used

Bacterial strain	β -lactamase Class	MIC (mg/L)	
		CLAV	AM-112
<i>Escherichia coli</i> J53 TEM-1	A	32	32
<i>Escherichia coli</i> J53 TEM-10	A (ESBL)	32	16
<i>Escherichia coli</i> J53 SHV-5	A (ESBL)	32	8
<i>Enterobacter cloacae</i> P99	C (derepressed)	32	16
<i>Enterobacter cloacae</i> Hennessey	C (inducible)	32	64
<i>Citrobacter diversus</i> 2046E	C (derepressed)	32	16
<i>Pseudomonas aeruginosa</i> 27853	C (inducible)	>64	>64
<i>Enterococcus faecalis</i> SFZ	Negative	>64	64

CONCLUSIONS

- Although successive generations of cephalosporins possess increasing stability to a broader range of β -lactamases, all cephalosporins remain labile to some β -lactamases, and particularly Class C enzymes.
- Clavulanic acid offers significant protection to Class A β -lactamases but not Class C β -lactamases.
- AM-112, a novel oxapenem β -lactamase inhibitor, offers significant protection against both Class A and Class C β -lactamases. Often, the level of AM-112 protection exceeds that of clavulanic acid against Class A β -lactamases.
- The level of protection and improvement in antibacterial spectrum offered by AM-112 varies between cephalosporins.
- Surprisingly, combinations of AM-112 and some cephalosporins show synergistic activity against β -lactamase negative *E. faecalis*, suggesting complementary inhibition of the penicillin binding proteins.
- AM-112 combined with either ceftazidime or cefepime offers the most potent and broadest antibacterial spectrum of the β -lactam/ β -lactamase inhibitor combinations tested. In the presence of AM-112 at 4 mg/L, all of the panel strains were fully susceptible to ceftazidime and cefepime, using the NCCLS breakpoints.

Figures 1—8. MICs of eight cephalosporins alone and in the presence of AM-112 at 4 mg/L (Figs 1a—8a, top row) or CLAV at 4 mg/L (Figs 1b—8b, bottom row)



Cefaclor (CFC) Figure 1a & b

- CFC was inactive against all isolates except *E. coli* SHV-5 (MIC 16 mg/L).
- Addition of CLAV reduced the CFC MIC \geq 64-fold against the three isolates producing Class A β -lactamases, but had no effect on the remaining isolates.
- AM-112 reduced the CFC MIC \geq 250-fold against all three isolates with Class A β -lactamases and also reduced the CFC MIC 32-fold from >64 mg/L to \leq 2 mg/L against both isolates of *E. cloacae*.

Cefazolin (CFZ) Figure 2a & b

- CFZ was inactive against all isolates except *E. coli* TEM-10 (MIC 8 mg/L).
- As with CFC, addition of CLAV reduced the cefazolin MIC against the three isolates producing Class A β -lactamases, but had no effect on the remaining isolates.
- AM-112 reduced the CFZ MIC against all three isolates with Class A β -lactamases, but also both *E. cloacae* isolates (at least 8-fold).
- AM-112 also reduced the CFZ MIC against *E. faecalis* SFZ from 32 mg/L to 8 mg/L.

Cefuroxime (CXM) Figure 3a & b

- CXM was moderately active (MICs 8 - 16 mg/L) against the three *E. coli* isolates with Class A β -lactamases, but inactive against the rest.
- Addition of CLAV reduced the CXM MIC \geq 8-fold against all three *E. coli* isolates, but had no effect on the remaining β -lactamase producing isolates.
- AM-112 also reduced the CXM MIC \geq 32-fold against all three isolates of *E. coli*, but also *E. cloacae* P99 (>64 mg/L to <0.06 mg/L) and *E. faecalis* SFZ (64 mg/L to 0.5 mg/L).

Cefoperazone (CPZ) Figure 4a & b

- CPZ exhibited an unusual antibacterial profile with good activity (MIC 4 mg/L) against *P. aeruginosa* 27853 and *E. coli* isolates with ESBLs, but was inactive against *E. coli* TEM-1 and the remaining isolates.
- CLAV reduced the CPZ MIC \geq 64-fold against all three *E. coli* isolates, but had no effect on the remaining β -lactamase producing isolates.
- AM-112 also reduced the CPZ MIC against all three isolates of *E. coli*, both isolates of *E. cloacae* P99 (at least 64-fold) and *E. faecalis* SFZ (8-fold).

Ceftriaxone (CTR) Figure 5a & b

- CTR exhibited a similar profile to CXM but was more active against the three *E. coli* isolates.
- Addition of CLAV reduced the CTR MIC \geq 4-fold against *E. coli* isolates and *C. diversus* 2046E.
- AM-112 reduced the CTR MIC against all isolates except *P. aeruginosa*, and *C. diversus* 2046E, by at least 32-fold.

Cefotaxime (CTX) Figure 6a & b

- CTX exhibited moderate/good activity (MIC <8 mg/L) against all isolates except *E. cloacae*.
- CLAV reduced the CTX MIC against all three *E. coli* isolates and *C. diversus* 2046E by at least 4-fold, increased the CTX MIC 8-fold against *P. aeruginosa* 27853, but had no effect on the remaining isolates.
- AM-112 reduced the CTX MIC at least 4-fold against all isolates except *P. aeruginosa*, where, in common with CLAV, some antagonism was observed.

Ceftazidime (CAZ) Figure 7a & b

- CAZ exhibited good activity (MIC 4 mg/L) against *P. aeruginosa* 27853. CAZ also exhibited good activity against *E. coli* TEM-1 and *C. diversus* 2046E but moderate or poor activity against the remaining isolates.
- CLAV reduced the CAZ MIC against both isolates of *E. coli* with ESBLs (at least 500-fold), but had no effect on the remaining isolates.
- AM-112 reduced the CAZ MIC against all isolates except *P. aeruginosa* 27853. The lowest MIC observed was 2 mg/L.

Cefepime (CPM) Figure 8a & b

- CPM exhibited a similar profile to CAZ, but with a lower MIC (8 mg/L) against *E. faecalis* SFZ.
- CLAV reduced the CPM MIC against all isolates, except *E. faecalis* SFZ. However, reductions in MIC against isolates producing Class C β -lactamases were \leq 8-fold.
- AM-112 reduced the CPM MIC against all isolates except *E. faecalis* SFZ.
- Reductions in MIC were more marked than for CLAV against isolates with Class A (> 500-fold) and Class C (64-fold) β -lactamases. The maximum MIC observed was 2 mg/L.