

In vitro activity of AVE1330A, a novel β -lactamase inhibitor, in combination with aztreonam or ceftazidime against ceftazidime-resistant isolates of species of the *Enterobacteriaceae*

J. Shackcloth¹, L. Williams¹, J. Northwood¹, A. Bryskier² and D. Felmingham¹

¹G.R. Micro Ltd, 7–9 William Road, London NW1 3ER UK; ²Sanofi Aventis, Paris cedex 75012, France

INTRODUCTION

β -lactam antimicrobials are important antibacterial agents because collectively they offer clinical efficacy against a wide range of pathogenic species and have a very low toxicity when used in humans.¹

However, the effectiveness of this class of antimicrobials is under considerable threat from the evolution of resistance, particularly that mediated by the Class A extended spectrum β -lactamases (ESBL) and the Class C de-repressed, chromosomally located cephalosporinases.^{2,3}

Thus, the therapeutic value of third generation cephalosporins such as ceftazidime, and the monobactam, aztreonam, is now compromised.

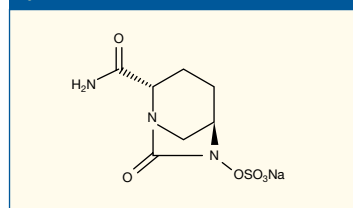
As β -lactamases have evolved by mutation and selection to include an increasing number of β -lactams within their hydrolytic spectrum, the pharmaceutical industry has responded by pursuing two research strategies:

1. Structural modification of existing molecules to block β -lactamase active-site binding.
2. The development of β -lactamase inhibitors to protect existing β -lactams from hydrolysis.

Three β -lactamase inhibitors are available, currently, in antibacterial combinations, – clavulanic acid, sulbactam and tazobactam. However, these compounds have a limited spectrum of inhibitory activity confined, generally, to Class A β -lactamases and even within this class inhibitor-resistant enzymes are now recognised. Importantly, none of these β -lactamase inhibitors is effective against strains of species of the *Enterobacteriaceae* producing Class C de-repressed, chromosomally located, cephalosporinases.^{4,5,6,7}

AVE1330A is an investigational, non β -lactam, β -lactamase inhibitor (Figure 1).⁸

Figure 1. Chemical structure of AVE1330A



In this study, the ability of AVE1330A to protect ceftazidime and aztreonam against hydrolysis by a variety of β -lactamases, was investigated.

MATERIALS AND METHODS

Bacterial isolates

Bacterial isolates used in this study were derived from clinical material examined recently by colleagues from hospitals and reference centres worldwide. Isolates were maintained with minimal sub-culture, deep-frozen at -70°C as a dense suspension in a high protein matrix of undiluted horse serum.

Antimicrobials

The following antimicrobials were used: AVE1330A (Lot no 92985, Aventis Pharma), amoxicillin (Lot no. 112K0481, Sigma), clavulanic acid (Lot no. BRL 14151, GlaxoSmithKline), aztreonam (Lot no. 6016E, ICM Biomedical), ceftazidime (Lot no. 2251042, Sigma) and ceftazidime (Lot no. 13K0525, Sigma).

Determination of minimum inhibitory concentrations (MIC)

MIC were determined using methods for antimicrobial susceptibility testing published by the Clinical Laboratory Standards Institute (CLSI), formerly the NCCLS.⁹

RESULTS AND DISCUSSION

The results of this study are presented in Tables 1–3.

Ceftazidime-resistant strains of species of the *Enterobacteriaceae* (Table 1)

A total of 45 ceftazidime-resistant strains of species of the *Enterobacteriaceae* were examined (*Escherichia coli* (12), *Citrobacter freundii* (6), *Klebsiella pneumoniae* (11), *Klebsiella oxytoca* (1), *Enterobacter cloacae* (13) and *Morganella morganii* (2)).

The addition of AVE1330A, at a fixed concentration of 4 mg/L, to ceftazidime and aztreonam was highly effective in protecting each antimicrobial against the hydrolytic activity of β -lactamases produced by ceftazidime-resistant strains of *Escherichia coli*. MIC of ceftazidime and aztreonam in combination with AVE1330A were in the range ≤ 0.03 –1 mg/L compared to 16 – 64 mg/L for ceftazidime, alone, and, ≤ 0.06 –0.25 mg/L compared to 4 – 64 mg/L for aztreonam, alone.

A similar picture was seen with strains of *Citrobacter freundii* (MIC range: ceftazidime = 32 – 128 mg/L; ceftazidime + AVE1330A = 0.25 – 1 mg/L; aztreonam = 4 – 64 mg/L; aztreonam + AVE1330A = 0.12 – 0.25 mg/L).

Eleven (11) strains of ceftazidime-resistant *Klebsiella pneumoniae* were highly susceptible to the combination of ceftazidime + AVE1330A (MIC range: ≤ 0.03 –1 mg/L) and to that of aztreonam + AVE1330A (MIC range: ≤ 0.06 –0.5 mg/L).

The activity of aztreonam + AVE1330A (fixed concentration, 4 mg/L) against a single strain of *Klebsiella oxytoca* (MIC: 0.25 mg/L) was 16-fold greater than that of the combination of AVE1330A with ceftazidime (MIC: 4 mg/L), although both combinations were considerably more active than either aztreonam or ceftazidime, alone.

The combinations of both aztreonam and ceftazidime, with a fixed concentration of 4 mg/L AVE1330A, were both highly effective against the majority of the 13 strains of *Enterobacter cloacae* tested with MIC in the range of ≤ 0.06 –1 mg/L and 0.5–8 mg/L, respectively. One (1) strain required a MIC of 2 mg/L ceftazidime + AVE1330A compared to 1 mg/L aztreonam + AVE1330A and one (1) required a MIC of 8 mg/L ceftazidime + AVE1330A compared to 0.5 mg/L aztreonam + AVE1330A. Two (2) strains of ceftazidime-resistant *Morganella morganii* were both highly susceptible to the combination of either aztreonam or ceftazidime with AVE1330A, at a fixed concentration of 4 mg/L (MIC, $\leq 0.06/0.25$ and $0.12/0.25$, respectively).

Fixed ratio combinations of ceftazidime and AVE1330A at 4:1 and 4:2, respectively, demonstrated similar inhibitory activity against the β -lactamase producing isolates. However, MIC were generally 2–8 fold higher when compared to those achieved with ceftazidime plus a 4mg/L fixed concentration of AVE1330A.

Escherichia coli transformants expressing TEM, OXA, SHV and PSE β -lactamases (Table 2)

AVE1330A was highly effective in protecting both aztreonam and ceftazidime against the hydrolytic activity of transformants of *Escherichia coli* expressing TEM 3, TEM 6, TEM 9, TEM 10 and OXA 5 enzymes which were active against aztreonam and ceftazidime when the two antimicrobials were tested alone.

Klebsiella spp. expressing characterised extended spectrum β -lactamases (ESBL) (Table 3)

In combination with AVE1330A, at a fixed concentration of 4 mg/L, both aztreonam and ceftazidime were protected from hydrolysis by a range of extended spectrum β -lactamases (ESBL) of the SHV-type.

Fixed ratio combinations of ceftazidime and AVE1330A at 4:1 and 4:2, respectively, demonstrated similar inhibitory activity against the β -lactamase producing isolates. However, MIC were generally 2–8 fold higher when compared to those achieved with ceftazidime plus a 4mg/L fixed concentration of AVE1330A.

Table 1. The comparative in vitro activity (mg/L) of ceftazidime and aztreonam + AVE1330A (at a fixed concentration of 4 mg/L), and ceftazidime in a 4:1 and 4:2 ratio, respectively, with AVE1330A against ceftazidime-resistant strains of species of the *Enterobacteriaceae*.

Species	Isolate ID	AMX	AMX-C (2:1)	AMX-C (4mg/L)	AMX-AVE (4mg/L)	FOX	AZT	AZT-AVE (4mg/L)	CAZ	CAZ-AVE (4mg/L)	CAZ-AVE 4:1	CAZ-AVE 4:2
<i>E.coli</i>	C16/3/08	>64	32	>64	4	>64	16	0.12	32	0.25	1	1
<i>E.coli</i>	C20/2/06	>64	16	32	≤ 0.06	32	64	≤ 0.06	16	0.5	2	1
<i>E.coli</i>	C20/3/06	>64	16	32	≤ 0.06	32	64	0.12	16	≤ 0.03	1	1
<i>E.coli</i>	C20/4/42	>64	16	32	≤ 0.06	32	64	≤ 0.06	16	0.25	2	1
<i>E.coli</i>	C22/1/24	>64	16	16	≤ 0.06	32	8	≤ 0.06	>128	1	8	4
<i>E.coli</i>	C23/2/08	>64	32	>64	2	16	4	≤ 0.06	32	0.25	2	1
<i>E.coli</i>	C23/3/22	>64	32	>64	1	32	4	≤ 0.06	128	0.5	2	2
<i>E.coli</i>	C3/2/21	>64	32	>64	16	>64	8	0.25	128	0.5	NT	NT
<i>E.coli</i>	C32/1/33	>64	16	32	≤ 0.06	16	64	≤ 0.06	32	0.25	0.5	0.5
<i>E.coli</i>	C32/3/24	>64	8	4	≤ 0.06	4	64	≤ 0.06	16	≤ 0.03	1	1
<i>E.coli</i>	C33/2/37	>64	16	64	2	4	16	≤ 0.06	16	0.25	0.25	0.25
<i>E.coli</i>	C4/2/39	>64	16	>64	≤ 0.06	64	>64	≤ 0.06	>128	≤ 0.03	16	8
<i>C.frendii</i>	C20/3/35	>64	32	>64	4	>64	8	0.12	128	0.25	1	1
<i>C.frendii</i>	C23/4/25	>64	32	>64	4	>64	64	0.25	128	0.5	2	2
<i>C.frendii</i>	C24/2/41	>64	32	>64	>64	64	64	0.12	>128	1	4	2
<i>C.frendii</i>	C34/4/09	>64	64	>64	4	>64	16	0.12	128	0.5	2	1
<i>C.frendii</i>	C4/4/06	>64	64	>64	16	>64	4	0.12	32	0.5	0.5	0.5
<i>C.frendii</i>	C7/2/44	>64	64	>64	4	>64	32	0.12	128	0.5	2	1
<i>K.pneumoniae</i>	C20/1/22	>64	8	8	4	4	>64	0.12	128	0.5	2	1
<i>K.pneumoniae</i>	C20/2/27	>64	4	2	≤ 0.06	2	64	≤ 0.06	128	≤ 0.03	1	1
<i>K.pneumoniae</i>	C20/2/32	>64	8	4	4	8	>64	0.25	>128	1	4	4
<i>K.pneumoniae</i>	C20/3/10	>64	16	64	16	4	>64	0.25	>128	1	4	4
<i>K.pneumoniae</i>	C24/2/20	>64	32	>64	32	8	2	0.12	128	1	4	4
<i>K.pneumoniae</i>	C4/2/11	>64	16	16	4	8	>64	0.5	>128	1	2	2
<i>K.pneumoniae</i>	C4/2/27	>64	16	16	4	8	>64	0.25	>128	1	4	4
<i>K.pneumoniae</i>	C4/2/28	>64	8	8	1	8	>64	0.12	128	0.5	2	2
<i>K.pneumoniae</i>	C4/2/30	>64	32	64	≤ 0.06	8	>64	0.12	>128	0.5	2	2
<i>K.pneumoniae</i>	C5/2/11	>64	16	32	≤ 0.06	16	>64	0.12	>128	0.5	4	2
<i>K.pneumoniae</i>	C5/2/39	>64	8	16	4	4	>64	0.12	128	0.5	2	2
<i>K.oxytoca</i>	C19/1/41	>64	16	16	16	4	>64	0.25	>128	4	4	4
<i>E.cloacae</i>	C18/4/15	>64	64	>64	64	>64	>64	1	>128	1	4	4
<i>E.cloacae</i>	C21/2/17	>64	64	>64	16	>64	32	0.25	128	0.5	2	2
<i>E.cloacae</i>	C22/2/30	>64	>64	>64	64	>64	64	1	128	2	4	4
<i>E.cloacae</i>	C24/2/43	>64	64	>64	16	>64	32	0.25	128	0.5	2	2
<i>E.cloacae</i>	C24/3/02	>64	64	>64	16	64	4	0.12	16	0.5	2	0.5
<i>E.cloacae</i>	C30/2/40	>64	64	>64	32	>64	32	0.5	128	1	4	2
<i>E.cloacae</i>	C31/2/42	>64	64	>64	16	>64	64	0.25	128	1	4	4
<i>E.cloacae</i>	C32/3/29	>64	64	>64	32	>64	0.5	≤ 0.06	16	0.25	4	2
<i>E.cloacae</i>	C32/4/36	>64	64	>64	>64	64	0.5	128	8	8	8	8
<i>E.cloacae</i>	C33/4/13	>64	64	>64	32	>64	64	0.5	128	1	4	2
<i>E.cloacae</i>	C4/2/26	>64	32	>64	≤ 0.06	>64	>64	≤ 0.06	>128	0.5	4	4
<i>E.cloacae</i>	C7/1/44	>64	64	>64	32	>64	4	1	32	1	4	2
<i>E.cloacae</i>	C8/4/10	>64	64	>64	16	>64	32	0.5	128	0.5	2	2
<i>M.morganii</i>	C34/2/28	>64	>64	>64	8	16	2	≤ 0.06	16	0.12	0.5	0.25
<i>M.morganii</i>	C34/4/06	>64	>64	>64	32	16	8	0.25	64	0.25	2	0.5

Antimicrobial abbreviations: AMX = amoxicillin, AMX-C (2:1) = amoxicillin + clavulanic acid in a 2:1 ratio; AMX-C (4 mg/L) = amoxicillin + clavulanic acid at a fixed concentration of 4 mg/L; AMX-AVE = amoxicillin + AVE1330A at a fixed concentration of 4 mg/L; FOX = ceftiofur, AZT = aztreonam, AZT-AVE = aztreonam + AVE1330A at a fixed concentration of 4mg/L; CAZ = ceftazidime, CAZ-AVE = ceftazidime + AVE 1330A at a fixed concentration of 4mg/L; CAZ-AVE = ceftazidime + AVE 1330A at a ratio of 4:1; CAZ-AVE = ceftazidime + AVE 1330A at a ratio of 4:2

Table 2. The comparative in vitro activity (mg/L) of ceftazidime and aztreonam + AVE1330A (at a fixed concentration of 4 mg/L) against *Escherichia coli* transformants expressing various β -lactamase types.

Species	Plasmid type	AMX	AMX-C (2:1)	AMX-C (4mg/L)	AMX-AVE (4mg/L)	FOX	AZT	AZT-AVE (4mg/L)	CAZ	CAZ-AVE (4mg/L)	CAZ-AVE 4:1	CAZ-AVE 4:2
<i>E.coli</i>	TEM 1	>64	16	32	4	0.5	≤ 0.06	≤ 0.06	0.12	0.06	NT	NT
<i>E.coli</i>	TEM 1	>64	16	16	2	4	0.12	≤ 0.06	0.25	0.12	NT	NT
<i>E.coli</i>	TEM 2	>64	32	64	32	4	0.12	≤ 0.06	0.5	0.25	NT	NT
<i>E.coli</i>	TEM 3	>64	8	8	4	8	4	≤ 0.06	16	0.12	NT	NT
<i>E.coli</i>	TEM 6	>64	16	32	32	8	16	0.12	>128	0.5	NT	NT
<i>E.coli</i>	TEM 9	>64	16	16	16	4	64	≤ 0.06	>128	0.5	NT	NT
<i>E.coli</i>	TEM 9	>64	16	16	16	4	64	≤ 0.06	>128	0.5	NT	NT
<i>E.coli</i>	TEM 10	>64	16	16	16	8	32	0.12	128	0.5	NT	NT
<i>E.coli</i>	OXA 1	>64	32	>64	32	8	0.12	0.12	0.25	0.25	NT	NT
<i>E.coli</i>	OXA 2	>64	16	32	32	4	≤ 0.06	≤ 0.06	0.25	0.12	NT	NT
<i>E.coli</i>	OXA 3	>64	16	16	16	4	0.12	0.12	0.5	0.25	NT	NT
<i>E.coli</i>	OXA 4	>64	32	64	32	4	0.12	≤ 0.06	0.25	0.25	NT	NT
<i>E.coli</i>	OXA 5	>64	8	8	8	4	16	0.12	64	0.5	NT	NT
<i>E.coli</i>	OXA 7	>64	32	>64	>64	4	2	0.5	0.5	0.25	NT	NT
<i>E.coli</i>	SHV 1	>64	8	8	2	4	0.12	≤ 0.06	0.25	0.12	NT	NT
<i>E.coli</i>	PSE 2	>64	32	64	>64	2	0.5	0.12	0.25	0.25	NT	NT
<i>E.coli</i>	PSE 2	16	4	4	4	0.5	≤ 0.06	≤ 0.06	0.12	0.06	NT	NT
<i>E.coli</i>	PSE 4	>64	16	16	>64	4	≤ 0.06	≤ 0.06	0.12	0.12	NT	NT

Antimicrobial abbreviations: AMX = amoxicillin, AMX-C (2:1) = amoxicillin + clavulanic acid in a 2:1 ratio; AMX-C (4 mg/L) = amoxicillin + clavulanic acid at a fixed concentration of 4 mg/L; AMX-AVE = amoxicillin + AVE1330A at a fixed concentration of 4 mg/L; FOX = ceftiofur, AZT = aztreonam, AZT-AVE = aztreonam + AVE1330A at a fixed concentration of 4mg/L; CAZ = ceftazidime, CAZ-AVE = ceftazidime + AVE 1330A at a fixed concentration of 4mg/L; CAZ-AVE = ceftazidime + AVE 1330A at a ratio of 4:1; CAZ-AVE = ceftazidime + AVE 1330A at a ratio of 4:2

Table 3. The comparative in vitro activity (mg/L) of ceftazidime and aztre