AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Interactions of OP0595, a Novel Triple-Action Diazabicyclooctane, with β-Lactams against OP0595-Resistant *Enterobacteriaceae* Mutants

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OP0595 is a novel diazabicyclooctane which, like avibactam, inhibits class A and C β-lactamases. In addition, unlike avibactam, it has antibacterial activity, with MICs of 0.5 to 4 μg/ml for most members of the family *Enterobacteriaceae*, owing to inhibition of PBP2; moreover, it acts synergistically with PBP3-active β-lactams independently of β-lactamase inhibition, via an "enhancer effect." *Enterobacteriaceae* mutants stably resistant to 16 μg/ml OP0595 were selected on agar at frequencies of approximately 10^{-7}. Unsurprisingly, OP0595 continued to potentiate substrate β-lactams against mutants derived from *Enterobacteriaceae* with OP0595-inhibited class A and C β-lactamases. Weaker potentiation of partners, especially aztreonam, cefepime, and piperacillin—less so meropenem—remained frequent for OP0595-resistant *Enterobacteriaceae* mutants lacking β-lactamases or with OP0595-resistant metallo-β-lactamases (MBLs), indicating that the enhancer effect is substantially retained even when antibiotic activity is lost.

OP0595 is a novel diazabicyclooctane (1, 2) which, like avibactam, inhibits class A and C serine β-lactamases. In addition, unlike avibactam, it strongly binds PBP2 of *Enterobacteriaceae* and has direct antibacterial activity, with MICs of 0.5 to 4 μg/ml for many *Escherichia coli*, *Klebsiella*, and *Enterobacter* isolates (1, 2). Finally, like amdinocillin (3–7), it acts as an "enhancer" of the activity of β-lactams that bind to other penicillin-binding proteins (PBPs), giving synergies that cannot be explained by β-lactamase inhibition (1, 2).

The antibacterial activity of OP0595 is vulnerable to mutational resistance (1), and we sought to explore whether the molecule's other activities were retained against such mutants. These studies will inform the choice of clinical partner(s) for OP0595, which is now in phase I development (8).

MATERIALS AND METHODS

Strains. Parent strains ($n = 82$) (Table 1) were recent submissions to the United Kingdom reference laboratory or were collected in surveys. Extended-spectrum β-lactamase (ESBL) and AmpC cephalosporinase production was inferred from phenotype; carbapenemases were characterized by PCR and sequencing (9, 10).

Selection of OP0595-resistant mutants. OP0595-resistant mutants were selected by applying 0.2 ml of overnight broth culture (approximately 5×10^8 CFU) to Mueller-Hinton agar (Oxoid, Basingstoke, United Kingdom) containing OP0595 (Meiji Seika Pharma, Yokohama, Japan) (16 μg/ml). Morphologically diverse colonies ($n = 5$ per parent) that grew overnight were subcultured to agar with OP0595 (16 μg/ml) and then retained at −80°C.

Susceptibility testing. Mutants and parent strains were recovered on drug-free Mueller-Hinton agar and then subjected to CLSI agar dilution MIC testing (11) with piperacillin (Sigma, Poole, United Kingdom), cefepime (Sequoia Research Products, Pangbourne, United Kingdom), and meropenem (Meiji) alone and with OP0595 (1 to 8 μg/ml). Comparators were ceftazidime (Sigma) alone and with 4 μg/ml avibactam (Meiji); amdinocillin (mecillinam; Sigma), imipenem (Merck, Hoddesdon, United Kingdom), and amikacin (Sigma). MICs were reviewed against EUCAST susceptibility breakpoints for the partner β-lactams, as being generally more conservative than CLSI values.

RESULTS

Selection and phenotypes of OP0595-resistant mutants. OP0595-resistant mutants were readily selected from all 82 parent strains, with frequencies approximately 10^{-7}. Five morphologically diverse mutants were retained per selection and subjected to susceptibility testing. Subsequent exclusions (e.g., for loss of resistance or as suspected contaminants) amounted to just 16 of these 410 (82×5) organisms, and for most parents, the full complement of five mutants was retained.

MICs of OP0595 for mutants rose from the parental values of 0.5 to 4 μg/ml to 8 to >32 μg/ml, with 381/394 (96.7%) values of >32 μg/ml. MIC changes for comparator antibiotics are shown in Table 2 for 93 mutants derived from the 19 β-lactam-susceptible parent strains. Among these 93 mutants, 81 had >128-fold MIC rises for amdinocillin, and 81 had small (mostly 2- to 4-fold) increases for imipenem; shifts for other comparators were scattered around unity (i.e., with similar proportions showing MIC decreases as increases). Analysis is more complex for mutants derived from strains with extended-spectrum β-lactamase (ESBLs), AmpC enzymes, or carbapenemases because many "starting" MICs for the parent organisms were off-scale, precluding calculation of fold change. Nevertheless, amdinocillin remained the sole agent, besides OP0595 itself, for which large MIC increases were widespread (not shown).

OP0595 combinations versus mutants of control strains. OP0595 at concentrations of 2 to 8 μg/ml (1 to 4 μg/ml with

Received 7 September 2015 Returned for modification 8 October 2015
Accepted 5 November 2015
Accepted manuscript posted online 9 November 2015
Citation Livermore DM, Warner M, Mushtaq S, Woodford N. 2016. Interactions of OP0595, a novel triple-action diazabicyclooctane, with β-lactams against OP0595-resistant *Enterobacteriaceae* mutants. Antimicrob Agents Chemother 60:554–560. doi:10.1128/AAC.02184-15.

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TABLE 1 Parent strains used for mutant selection

Resistance mechanism(s)	No. of parent strains used for mutant selection			
	E. coli	*Klebsiella*	*Enterobacter*	*Citrobacter*
None (no AmpC, ESBL, or carbapenemase) (control)	5	5	4	5
ESBL	5	4	2	2
AmpC (plasmid or derepressed)	2	2	5	5
KPC	2	5	2	2
MBLs	1 NDM, 2 VIM	3 NDM, 2 VIM, 2 IMP	2 NDM, 2 VIM	2 NDM, 2 VIM
OXA-48	2	2	2	1

aztreonam) caused widespread potentiation of piperacillin, aztreonam, and cefepime, less so meropenem, against OP0595-selected mutants of cephalosporin- and carbapenem-susceptible control strains (Table 3). At 1 to 4 μg/ml, it reduced the modal MIC of aztreonam from 0.12 μg/ml to ≤0.016 μg/ml and that of piperacillin from 2 μg/ml to 0.25 μg/ml. The modal MICs of unprotected cefepime and meropenem were only 2-fold above the lowest concentration tested, precluding precise estimation of MIC reductions; nevertheless, more than 85% of cefepime MICs were reduced to ≤0.016 μg/ml by OP0595 at 2 to 8 μg/ml, whereas meropenem MICs were less consistently lowered. Potentiation of piperacillin for *Klebsiella pneumoniae* may have partly reflected inhibition of the chromosomal penicillinases that are universal in the species (12), but this cannot explain potentiation for other species, which lack these enzymes, nor potentiation of the other test β-lactams, which are not labile to *K. pneumoniae* penicillinases. Less synergy was seen between ceftazidime and avibactam (4 μg/ml), with MICs generally lowered by only one doubling dilution.

OP0595 combinations versus mutants of strains with class A and C β-lactamases. ESBLs, *K. pneumoniae* carbapenemases (KPCs), and AmpC enzymes are inhibited by diazabicyclooctanes (1, 13, 14), and OP0595 continued to strongly potentiate its partner β-lactams against mutants whenever the partner was a good substrate of the β-lactamase produced—i.e., piperacillin, cefepime, and aztreonam for mutants with ESBLs, piperacillin and aztreonam for those with AmpC, and all four β-lactams for those with KPC enzymes (Table 4). Avibactam (4 μg/ml) likewise potentiated ceftazidime against these organisms, a result in keeping with published data (15–17).

Potentiation was weaker when OP0595 was combined with weak substrates or stable agents. Nevertheless, two- to fourfold reductions in meropenem MICs were widespread for ESBL and AmpC producers, and the geometric mean MIC of cefepime for AmpC producers was reduced from 0.51 μg/ml to ≤0.016 μg/ml by OP0595 at 2 to 8 μg/ml.

OP0595 combinations versus mutants of strains with MBLs. Among the 18 parent strains with IMP, NDM, and VIM metallocarbapenemases, six were susceptible to aztreonam at ≤0.25 μg/ml, indicating the absence of ESBL or AmpC activity that might be inhibited by OP0595. MIC distributions of diazabicyclooctane combinations for mutants of all 18 strains are summarized in Table 5; the MICs for mutants of the aztreonam-susceptible strains are listed in full in Table 6. Potentiation by OP0595 was widespread and, for the aztreonam-resistant organisms, probably largely reflected inhibition of coproduced class A and C enzymes. However, inhibition of secondary enzymes cannot explain the frequent (not universal, even within series) 2- to 16-fold synergy observed between OP0595 and both aztreonam and cefepime for OP0595-resistant mutants of the aztreonam-susceptible strains (Table 6). Potentiation of meropenem was less frequent, being almost exclusive to *E. coli* H112760544 and H112760545. No similar potentiation of ceftazidime by avibactam was seen for mutants of aztreonam-susceptible metallo-β-lactamase (MBL) producers.

TABLE 2 Fold changes in MICs for OP0595-selected mutants ($n = 93$) of the fully susceptible parent control strains ($n = 19$)

Fold change in MIC[a]	No. of mutants with the indicated fold change in MIC[b]								
	OP0595	Piperacillin	Cefepime	Aztreonam	Meropenem	Ceftazidime	Imipenem	Amdinocillin	Amikacin
0.06		1							
0.12		2		5					1
0.25		10	4	10		3			4
0.5		32	24	20	4	21			25
1 (no change)		**37**	**47**	**42**	**46**	**44**	**12**	**1**	**44**
2		9	11	16	32	21	20		11
4		2	5		11	3	37		5
8	2		1				22	1	2
16	5[c]		1				2		1
32	40[c]					1		2	
64	41[c]							3	
128	5[c]							5	
256								17	
>256								64	

[a] Calculated as the MIC for the mutant/MIC for the parent.
[b] The numbers of mutants with no change in the MIC for the different drugs are shown in bold type.
[c] Minimum values for fold change as most MICs for mutants were above the highest concentration of OP0595 tested (32 μg/ml).

TABLE 3 MIC distributions of diazabicyclooctane combinations for 93 OP0595-selected mutants of 19 control strains

MIC (μg/ml) or parameter	No. of mutants with the indicated MICs (μg/ml) for drug alone or in combination[a]: Piperacillin + OP0595				Cefepime + OP0595				Aztreonam + OP0595				Meropenem + OP0595				Ceftazidime	
	0	2	4	8	0	2	4	8	0	1	2	4	0	2	4	8	Alone	+AVI
≤0.016		6	7	5	19	**83**	**86**	**87**	8	**43**	**48**	**50**	17	**48**	**56**	**55**		3
0.03		2	3	10	**39**	10	7	6	11	22	22	22	**59**	33	25	27	1	5
0.06	1	18	20	13	19				27	19	17	17	16	11	12	10	8	26
0.12		24	23	**29**	14				**36**	8	5	3	1	1		1	22	**32**
0.25	3	**26**	**29**	24	2				8	1	1	1					**28**	22
0.5	6	14	10	11					3								23	4
1	12	3	1	1													9	1
2	**26**																	
4	19																1	
8	18																1	
16	6																	
32	1																	
64	1																	
GM[b]	2.77	0.146	0.127	0.124	0.038	≤0.016	≤0.016	≤0.016	0.078	0.028	0.025	0.024	0.030	0.022	0.021	0.021	0.255	0.112

[a] The MIC distribution of drugs alone or in combination with the OP0595 diazabicyclooctane is shown as the number of mutants with the indicated MICs. All MICs are shown in micrograms per milliliter. The MICs of the drugs (e.g., piperacillin, cefepime, etc.) were determined in the presence of OP0595 at 2, 4, and 8 μg/ml (0 for no OP0595). The MIC distribution of ceftazidime alone and with avibactam (+AVI) (4 μg/ml) is shown for comparison. Modal MICs are indicated in bold type.
[b] GM, geometric mean. MICs of >256 μg/ml were counted as 512 μg/ml when calculating the geometric means, and MICs of ≤0.016 were counted as 0.014 μg/ml.

OP0595 combinations versus mutants of strains with OXA-48-like enzymes. MIC distributions of OP0595 combinations for the mutants of organisms with OXA-48-like carbapenemase are shown in Table 7. These mutants, like their parents, were highly resistant to piperacillin, with MICs of >64 μg/ml. This resistance was strongly reversed so that, with OP0595 at 8 μg/ml, the modal piperacillin MIC was reduced to 1 μg/ml, with no value of >8 μg/ml. Resistance to aztreonam, cefepime, and ceftazidime was more variable, probably reflecting the presence or absence of ESBL; nevertheless, potentiation of aztreonam and cefepime by OP0595 and of ceftazidime by avibactam was universal. Potentiation of meropenem by OP0595 was weaker than for other partner β-lactams.

DISCUSSION

OP0595 has MICs of 0.5 to 4 μg/ml for most *E. coli*, *Klebsiella*, *Enterobacter*, and *Citrobacter* isolates (1, 2), but this activity, which reflects attack on PBP2, is vulnerable to mutational resistance (1). Consequently, it is more appropriate to use OP0595 in combinations of drugs than to use it as a single agent, thereby also exploiting its β-lactamase inhibitor and enhancer activities. Evaluation of these activities is, however, complicated by the antibacterial activity, and to elucidate them independently, we studied the behavior of OP0595-resistant mutants.

Unsurprisingly, OP0595's β-lactamase inhibitor activity was not contingent on its antibacterial activity, and the molecule continued to strongly potentiate substrate β-lactams against OP0595-resistant mutants of strains with class A and C β-lactamases (Table 4), which are strongly inhibited (1). Avibactam similarly potentiated ceftazidime against these organisms. Second, and less predictably, OP0595's enhancer activity also proved substantially independent of direct antibacterial activity, with frequent potentiation of aztreonam, cefepime, and piperacillin against mutants of control strains, which lacked β-lactamases (Table 3), and of

TABLE 4 Geometric mean and maximum MICs of diazabicyclooctane combinations for mutants of strains with class A and C β-lactamases

Drug	MIC[a]	Geometric mean or maximum MIC (μg/ml) for drug alone or in combination[b]: Piperacillin + OP0595 0	2	4	8	Cefepime + OP0595 0	2	4	8	Aztreonam + OP0595 0	1	2	4	Meropenem + OP0595 0	2	4	8	Ceftazidime + avibactam 0	4
ESBL (n = 64)	GM	177.2	0.322	0.193	0.160	3.08	≤0.016	≤0.016	≤0.016	23.4	0.101	0.051	0.038	0.044	0.022	0.021	0.021	17.6	0.149
	Max	>256	4	2	1	256	0.125	0.25	0.25	512	2	1	0.5	1	0.125	0.125	0.125	>256	1
AmpC (n = 63)	GM	100.4	0.137	0.093	0.075	0.51	≤0.016	≤0.016	≤0.016	11.1	0.053	0.030	0.024	0.062	0.019	0.018	0.017	48.1	0.152
	Max	>256	1	1	1	32	0.016	0.016	0.016	128	1	0.25	0.12	0.5	0.06	0.06	0.06	>256	1
KPC (n = 53)	GM	213.2	0.897	0.484	0.349	17.8	0.037	0.027	0.024	153.7	0.290	0.127	0.070	8.430	0.053	0.042	0.034	27.4	0.185
	Max	>256	8	4	4	>256	0.125	0.125	0.125	512	4	2	1	64	0.25	0.25	0.25	>256	1

[a] GM, geometric mean; Max, maximum. MICs of >256 μg/ml were counted as 512 μg/ml when calculating geometric means, and MICs of ≤0.016 were counted as 0.014 μg/ml.
[b] The geometric mean and maximum MIC of drugs alone or in combination with the OP0595 diazabicyclooctane are shown. All MICs are shown in micrograms per milliliter with and without OP0595 at 2, 4, and 8 μg/ml (0 for no OP0595). The geometric mean and maximum MIC of ceftazidime alone and with avibactam (4 μg/ml) are shown for comparison.

TABLE 5 MIC distributions for 86 OP0595-selected mutants of 18 MBL-producing strains

MIC (μg/ml) or parameter[a]	No. of mutants with the indicated MICs (μg/ml) for drug alone or in combination[b]:																	
	Piperacillin + OP0595				Cefepime + OP0595				Aztreonam + OP0595				Meropenem + OP0595				Ceftazidime + avibactam	
	0	2	4	8	0	2	4	8	0	1	2	4	0	2	4	8	0	4
≤0.016						3	2	3		**21**	**31**	**37**		2	4	4		
0.03			1	1		1	2	2	8	19	22	22		2	2	1		
0.06		1						2	3	13	15	12				1		
0.12		1	1	2	1	7	7	5	**15**	18	6	3		2	1	2		
0.25		1	2	3		3	9	9	3	1	3	6	1	5	3	2		
0.5		5	6	8	1	9	6	5	1	6	4	3	2	3	2	3		3
1		4	3	3	2	4	2	5	1	2	2	3	7	5	6	8		4
2		4	6	3	5	**17**	**16**	14	2	2	2		11	18	19	18		4
4		3	2	4	5	10	12	11	3	2			**25**	**22**	**24**	**20**	1	2
8	2	7	8	7	9	12	12	**15**	3	1	1		15	9	8	10		3
16	4	9	11	10	16	10	10	7	10				15	8	6	7	1	7
32	5	**23**	**18**	**20**	**17**	3	1	1	11	1			6	6	7	7	8	9
64	2	13	14	11	9	4	4	5	10				3	3	3	2	11	7
128	5	6	7	6	11	1	1	1	9				1	1	1	1	7	6
256	7	5	5	6	2	2	2	1	2								5	5
>256	**61**	4	2	2	8				5								**53**	**36**
GM	280	18.8	15.6	13.3	26.4	2.54	2.25	1.97	4.68	0.072	0.045	0.036	5.75	2.96	2.83	2.70	236	74.6
%S	2.3	30.2	33.7	36.0	4.7	31.4	32.6	36.0	36.0	93.0	96.5	100	24.4	43.0	43.0	45.3	0	8.1

[a] The MICs of piperacillin, cefepime, etc., are shown. The geometric mean (GM) and percent susceptible (%S) at current EUCAST susceptibility breakpoints for the unprotected compounds are shown. The current EUCAST susceptibility breakpoints for the unprotected compounds are 1 μg/ml for aztreonam, cefepime, and ceftazidime, 2 μg/ml for meropenem, and 8 μg/ml for piperacillin. MICs of >256 μg/ml were counted as 512 μg/ml when calculating geometric means, and MICs of ≤0.016 were counted as 0.014 μg/ml.
[b] The MIC distribution of drugs alone or in combination with the OP0595 diazabicyclooctane is shown as the number of mutants with the indicated MICs. All MICs are shown in micrograms per milliliter. The MICs of the drugs (e.g., piperacillin, cefepime, etc.) were determined in the presence of OP0595 at 2, 4, and 8 μg/ml (0 for no OP0595). The MIC distribution of ceftazidime alone and with avibactam (4 μg/ml) is shown for comparison. Modal MICs are indicated in bold type.

both cefepime and aztreonam against mutants of aztreonam-susceptible MBL producers, which were inferred to lack enzymes likely to be inhibited by OP0595. Potentiation of meropenem was weaker or absent probably because it, unlike aztreonam and cefepime, has significant affinity for PBP2 (18). There was no similar enhancer effect for avibactam.

Mutant-to-mutant variation in the extent of the enhancer effect (Tables 3 and 6) and in MIC shifts for β-lactams besides amdinocillin (Table 2) may reflect diversity in the underlying mechanisms. Aside from OP0595 resistance, their most consistent trait was sharply increased resistance to amdinocillin, which solely targets PBP2, and smaller MIC increases for imipenem, which primarily targets PBP2 (18, 19). Recent studies show that amdinocillin selects diverse mutations, mostly upregulating the stringent response and increasing cellular levels of guanosine tetraphosphate (20), and preliminary data suggest similar patterns among the present OP0595-selected mutants (21). Changes to *pbp2* itself are rare or absent. It is unclear how ppGpp-induced changes protect against PBP2-active agents (20, 22), but it is most plausible that they are somehow compensatory. Notably, amdinocillin- and OP0595-resistant mutants grow as stable round forms under amdinocillin or OP0595 challenge (1, 6, 23), indicating that PBP2 itself remains vulnerable, an observation keeping with the retention of the enhancer effect.

The ease with which OP0595-resistant mutants could be selected begs the question of whether they will be a clinical problem. This cannot be answered definitively, but again, insight is provided by the recent work of Thulin et al. (20) with amdinocillin, which showed that that, whereas laboratory-selected amdinocillin-resistant mutants were diverse, amdinocillin-resistant clinical isolates of *E. coli* consistently had a *cysB* mutation, implying that many other mutant types might be less competitive. This view was supported by competition and growth rate studies, and it is hard to believe that bacteria retain full virulence once their cell shape is grossly distorted by continued attack on PBP2. There has been little accumulation of amdinocillin-resistant *E. coli* in Scandinavia, where the drug is widely used (24), but this may reflect good stewardship and use mostly in low-risk cystitis patients.

An obvious route to lower the risk of OP0595 resistance in *Enterobacteriaceae* would be to combine it with aztreonam, ensuring activity against MBL producers irrespective of the enhancer effect. Nevertheless, the enhancer effect with cefepime and piperacillin is striking and often allowed retention of activity, even at EUCAST's low breakpoints, against OP0595-resistant mutants of strains with MBLs or OXA-48-like enzymes. Such combinations would allow better anti-*Pseudomonas* coverage than aztreonam-OP0595. Last, although potentiation of meropenem for OP0595 was weaker than for PBP3-directed partners, meropenem has the broadest antibacterial spectrum of the four partners tested here, meaning that it continues to merit consideration.

ACKNOWLEDGMENTS

This work was funded by Meiji Seika Pharma Co., Ltd.

D.M.L. has served on advisory boards or served as an ad hoc consultant for Accelerate, Achaogen, Adenium, Alere, Allecra, Altermune, AstraZeneca, Auspherix, Basilea, BioVersys, Cubist, Curetis, Cycle, Discuva, GSK, Meiji, Pfizer, Roche, Shionogi, Tetraphase, VenatoRx, and Wockhardt. D.M.L. has been paid for lectures by AOP Orphan, AstraZeneca, Bruker, Curetis, Merck, Pfizer, and Leo. D.M.L. has shareholdings in Dechra, GSK, Merck, Perkin Elmer, and Pfizer amounting to <10% of portfolio value. M.W., S.M., and N.W. have no personal interests to declare. However, the Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit of Public Health England (PHE)

TABLE 6 MICs for mutants of aztreonam-susceptible (i.e., ESBL- and AmpC-negative) MBL producers

Strain[a]	MIC (μg/ml) for drug alone or in combination[b]																	
		Piperacillin + OP0595				Cefepime + OP0595				Aztreonam + OP0595				Meropenem + OP0595				Ceftazidime	
	OP0595	0	2	4	8	0	2	4	8	0	1	2	4	0	2	4	8	Alone	+AVI
E. coli H112760544 (VIM)																			
Parent	1	>256	≤0.016	≤0.016	≤0.016	64	≤0.016	≤0.016	≤0.016	0.125	≤0.016	≤0.016	≤0.016	8	≤0.016	≤0.016	≤0.016	>256	256
Mut 1	>32	>256	64	64	64	128	16	16	8	0.125	≤0.016	≤0.016	≤0.016	4	0.25	2	1	>256	256
Mut 2	>32	>256	64	64	32	64	16	16	8	0.125	0.03	0.03	0.03	4	1	2	1	>256	128
Mut 3	>32	>256	4	4	4	16	2	2	2	0.03	≤0.016	≤0.016	≤0.016	1	0.25	0.25	0.125	128	32
Mut 4	>32	>256	64	32	32	16	8	4	4	0.03	≤0.016	≤0.016	≤0.016	1	0.5	0.5	0.5	64	32
Mut 5	8	>256	0.06	0.03	0.03	128	≤0.016	≤0.016	≤0.016	0.125	≤0.016	≤0.016	≤0.016	16	≤0.016	≤0.016	≤0.016	>256	128
E. coli H112760545 (VIM)																			
Parent	1	>256	≤0.016	≤0.016	≤0.016	64	≤0.016	≤0.016	≤0.016	0.125	≤0.016	≤0.016	≤0.016	8	≤0.016	≤0.016	≤0.016	>256	256
Mut 1	>32	>256	32	32	32	16	4	4	4	0.03	≤0.016	≤0.016	≤0.016	1	0.25	0.125	0.125	256	128
Mut 2	>32	>256	8	2	4	64	2	2	2	0.125	≤0.016	≤0.016	≤0.016	2	0.125	≤0.016	≤0.016	256	32
Mut 3	32	>256	16	2	0.125	128	0.5	0.25	0.03	0.125	≤0.016	≤0.016	≤0.016	4	≤0.016	≤0.016	≤0.016	>256	128
Mut 4	>32	>256	32	32	32	16	4	4	4	0.03	≤0.016	≤0.016	≤0.016	1	0.25	0.25	0.25	128	64
Mut 5	>32	>256	64	64	64	128	16	16	16	0.125	0.03	0.03	0.03	4	2	2	2	>256	256
Klebsiella H090380495 (IMP)																			
Parent	1	64	≤0.016	≤0.016	≤0.016	32	≤0.016	≤0.016	≤0.016	0.125	≤0.016	≤0.016	≤0.016	16	≤0.016	≤0.016	≤0.016	>256	0.25
Mut 1	>32	32	0.5	0.5	0.5	16	1	2	1	0.25	≤0.016	≤0.016	≤0.016	4	0.125	≤0.016	≤0.016	>256	16
Mut 2	>32	64	0.5	0.25	0.25	64	2	2	2	0.25	0.03	0.03	0.03	4	4	4	4	>256	16
Mut 3	>32	32	2	2	2	32	8	8	8	0.25	0.03	0.03	0.03	8	4	4	4	>256	128
Mut 4	>32	16	1	1	1	8	2	2	2	0.125	0.03	0.03	0.03	2	2	2	2	>256	32
Mut 5	>32	16	0.5	0.5	0.5	64	2	2	1	0.125	<0.016	<0.016	<0.016	8	2	2	2	>256	16
Citrobacter H124560395 (NDM)																			
Parent	4	>256	64	≤0.016	≤0.016	64	8	≤0.016	≤0.016	0.25	0.125	≤0.016	≤0.016	16	2	≤0.016	≤0.016	>256	>256
Mut 1	>32	128	16	16	16	8	4	4	4	0.03	≤0.016	≤0.016	≤0.016	2	2	2	2	>256	>256
Mut 2	>32	128	32	32	32	32	8	8	8	0.125	0.06	0.03	0.03	4	4	4	4	>256	>256
Mut 3	>32	>256	64	64	64	32	16	16	8	0.125	0.06	≤0.016	≤0.016	16	16	32	16	>256	>256
Mut 4	>32	128	32	32	32	16	8	8	8	0.125	0.03	0.03	0.03	4	4	4	4	>256	>256
Mut 5	>32	256	32	32	32	32	8	8	4	0.06	≤0.016	≤0.016	≤0.016	4	4	4	4	>256	>256
Klebsiella H101620268 (IMP)																			
Parent	2	32	≤0.016	≤0.016	≤0.016	16	≤0.016	≤0.016	≤0.016	0.03	≤0.016	≤0.016	≤0.016	8	≤0.016	≤0.016	≤0.016	256	256
Mut 1	>32	8	0.5	0.5	0.5	8	1	0.25	0.25	0.03	≤0.016	≤0.016	≤0.016	1	0.5	1	0.5	64	2
Mut 2	>32	16	1	0.5	0.5	16	2	2	2	0.03	≤0.016	≤0.016	≤0.016	8	8	4	4	128	32
Mut 3	>32	32	1	0.5	0.5	16	4	4	4	0.06	0.03	0.03	0.03	8	8	4	4	256	64
Mut 4	>32	8	0.5	0.5	0.25	8	4	4	4	0.03	≤0.016	≤0.016	≤0.016	8	4	4	4	64	16
Mut 5	>32	32	2	2	1	32	16	16	16	0.06	0.03	0.03	0.03	16	16	16	16	256	64
Citrobacter H132820363 (NDM)																			
Parent	1	>256	≤0.016	≤0.016	≤0.016	32	≤0.016	≤0.016	≤0.016	0.06	≤0.016	≤0.016	≤0.016	4	≤0.016	≤0.016	≤0.016	>256	>256
Mut 1	>32	>256	16	8	0.5	32	4	4	4	0.125	≤0.016	≤0.016	≤0.016	4	2	2	2	>256	>256
Mut 2	>32	>256	64	64	32	32	16	8	8	0.125	0.03	0.03	0.03	8	8	8	8	>256	>256
Mut 4	>32	256	16	16	16	16	4	4	4	0.125	0.03	0.03	0.03	4	4	4	4	>256	>256
Mut 5	>32	>256	16	8	0.5	64	4	4	4	0.125	≤0.016	≤0.016	≤0.016	4	2	2	2	>256	>256

[a] The strain (e.g., *E. coli* H112760544) and the metallocarbapenemase it possesses (IMP, NDM, or VIM) are shown. The parent strain and the five mutants (Mut 1 to Mut 5) studied here are given.

[b] The MICs of drugs alone or in combination with the OP0595 diazabicyclooctane at 2, 4, and 8 μg/ml (0 for no OP0595) are shown. All MICs are shown in micrograms per milliliter. The MIC of ceftazidime alone and with avibactam (+AVI) (4 μg/ml) is shown for comparison.

TABLE 7 MIC distributions for 35 OP0595-selected mutants of 7 OXA-48 carbapenemase-producing strains

MIC (μg/ml) or parameter[a]	No. of mutants with the indicated MICs (μg/ml) for drug alone or in combination[b]:																	
	Piperacillin + OP0595				Cefepime + OP0595				Aztreonam + OP0595				Meropenem + OP0595				Ceftazidime + avibactam	
	0	2	4	8	0	2	4	8	0	1	2	4	0	2	4	8	0	4
≤0.016						5	6	7		6	8	8						
0.03						6	**11**	**11**	1	**10**	10	11	1	2	2	1		2
0.06						**12**	7	8	2	9	**14**	**14**	1			2		4
0.12				1	2	9	9	7	7	9	3	2	1	3	4	7	1	**15**
0.25				1	4	3	2	2	5	1			3	6	6	4	5	7
0.5		1	4	7	4				**9**				6	3	3	1	6	7
1		5	8	10	3				1				2	1		5	**9**	
2		6	**10**	**12**	**9**									4	4	2	6	
4		**11**	**10**	3	3								2	3	4	4	3	
8		8	2	1	3				1				3	6	6	6		
16		3	1		1				3				6	3	4	2	2	
32		1			1				2				6	4	2	1	3	
64	4				3				2				4					
128	4				2				2									
256	7																	
>256	**20**																	
GM	300	3.84	2.04	1.20	2.54	0.059	0.049	0.045	0.96	0.048	0.038	0.036	3.76	1.67	1.45	0.96	1.37	0.19
%S	0	88.6	97.1	100	37.1	100	100	100	71.4	100	100	100	40.0	54.3	54.3	62.9	60	100

[a] The MICs of piperacillin, cefepime, etc., are shown. The geometric mean (GM) and percent susceptible (%S) at current EUCAST susceptibility breakpoints for the unprotected compounds are shown. The current EUCAST susceptibility breakpoints for the unprotected compounds are 1 μg/ml for aztreonam, cefepime, and ceftazidime, 2 μg/ml for meropenem, and 8 μg/ml for piperacillin. MICs of >256 μg/ml were counted as 512 μg/ml when calculating geometric means, and MICs of ≤0.016 were counted as 0.014 μg/ml.
[b] The MIC distribution of drugs alone or in combination with the OP0595 diazabicyclooctane is shown as the number of mutants with the indicated MICs. All MICs are shown in micrograms per milliliter. The MICs of the drugs (e.g., piperacillin, cefepime, etc.) were determined in the presence of OP0595 at 2, 4, and 8 μg/ml (0 for no OP0595). The MIC distribution of ceftazidime alone and with avibactam (4 μg/ml) is shown for comparison. Modal MICs are indicated in bold type.

has received financial support for conference attendance, lectures, and research projects or contracted evaluations from numerous sources, including Achaogen, Allecra Antiinfectives, Amplex, AstraZeneca, Becton Dickinson, British Society for Antimicrobial Chemotherapy, Cepheid, Check-Points, Cubist, United Kingdom Department of Health, United Kingdom Food Standards Agency, Glaxo SmithKline, Henry Stewart Talks, IHMA, Melinta, Merck Sharpe & Dohme, Meiji, Momentum Biosciences, Nordic, Norgine, Rempex, Rokitan, Smith & Nephew, Trius, VenatoRx, and Wockhardt.

FUNDING INFORMATION

Meiji Seika Pharma provided funding to David Livermore.

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