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Omadacycline Is Not a Substrate for Clinically Relevant β-Lactamase Enzymes

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INTRODUCTION

- Omadacycline was recently (October 2018) approved by the Food and Drug Administration for treating acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia in adults
- Omadacycline is a novel aminomethylcycline class antimicrobial; therefore, we hypothesized that omadacycline should not be a hydrolysable substrate of β-lactamase enzymes
- In this study, crude extracts from isogenic strains expressing potent extended-spectrum β-lactamases (ESBLs) and carbapenemases commonly produced by *Enterobacteriaceae* were prepared and incubated with omadacycline, and stability was evaluated via absorbance assay

MATERIALS AND METHODS

Bacterial isolates

- A total of 17 isogenic strains containing recombinant or wild-type plasmids carrying β-lactamase genes were selected for the study and are listed in Table 1

Antimicrobial susceptibility testing

- To confirm that the β-lactamase enzymes were active, β-lactam MICs were obtained via broth microdilution assays in cation-adjusted Mueller-Hinton broth following guidelines in the Clinical and Laboratory Standards Institute (CLSI) M07 (2018) document
- *Escherichia coli* ATCC 25922 was used as a negative control, and a recombinant *E. coli* carrying *tet*(X), known to modify tetracycline derivatives that include omadacycline, was utilized as a positive control in the susceptibility testing and hydrolysis assays

Hydrolysis assays

- Crude extracts from the respective strains were prepared and contained the encoded β-lactamases or Tet(X)
- The absorbance of omadacycline, ampicillin, ceftriaxone, and imipenem were measured in the presence of each crude extract in an Ultrospec™ 3300 pro UV/visible spectrophotometer using the most appropriate wavelength for each antimicrobial
- The absorbances of the β-lactams, ampicillin, ceftriaxone, imipenem, and omadacycline were monitored during exposure to crude extract over time
  - β-lactam absorbances were monitored at T_0 and at T_2 minutes
  - Omadacycline absorbances were monitored at T_0 and at T_2 and T_4 hours
  - β-lactam and omadacycline absorbances over time were also measured in the absence of crude extract, i.e., 10 mM HEPES buffer alone, to monitor for any potential degradation over the test period
- Modification of the omadacycline and/or β-lactam molecules was defined as a difference between initial and final absorbance for any given drug over time (delta absorbance) that was greater than the negative and blank controls

RESULTS

Table 1 Omadacycline and comparator MIC values against the crude extract source strains: ESBL-, carbapenemase-, and monooxygenase-producers

Enzyme class	Isolate ID	Species	Enzyme	MIC (µg/mL)		
				OMC	CAZ	CPT
ESBL	QC199	*E. coli*	TEM-2	1	0.25	0.5
	QC193	*E. coli*	SHV-2	1	32	>32
	3134J	*K. pneumoniae*	SHV-12	4	4	16
	QC279	*E. coli*	CTX-M-15	0.5	16	>32
Carbapenemase	12649J	*E. coli*	KPC-3	0.5	>32	>32
	12646J	*E. coli*	KPC-2	0.5	2	8
	12147J	*E. coli*	VIM-1	0.5	>32	>32
	3027J	*E. coli*	VIM-2	0.5	2	4
	24D	*P. aeruginosa*	VIM-6	16	>32	>32
	12282J	*E. coli*	NDM-1	0.25	>32	>32
	12071J	*E. coli*	OXA-48	0.5	1	16
	12072J	*E. coli*	OXA-48	1	0.5	2
Monooxygenase	14057J	*E. coli*[a]	TetX	32	0.5	0.5

ESBL, extended-spectrum β-lactamase; OMC, omadacycline; CAZ, ceftazidime; CPT, ceftaroline.
[a] *E. coli* carrying the *tet*(X) recombinant vector. This isolate exhibited tigecycline, tetracycline, minocycline, and doxycycline MIC results of 8, >16, 16, and >8 µg/mL, respectively.

Table 2 Ampicillin and ceftriaxone absorbance values in crude extracts containing β-lactamases

Isolate	Enzyme[a]	Absorbance at		Δ Absorbance ($T_2 - T_0$)	
		$T_{0\ min}$	$T_{2\ min}$	Per minute	Per minute/mg of protein
Ampicillin					
QC 199	TEM-2	2.857	2.5354	-0.1608	-0.091
QC 193	SHV-2	2.968	2.6756	-0.1462	-0.075
3134J	SHV-12	2.693	2.2234	-0.2348	-0.327
ATCC 25922	– control	2.572	2.6718	0.0499	0.021
Blank[b]	– control	2.839	2.833	-0.0030	NA
Ceftriaxone					
QC 279	CTX-M-15	2.931	2.7228	-0.1041	-0.047
ATCC 25922	– control	2.915	2.9674	0.0262	0.011
Blank[b]	– control	2.739	2.7388	-0.0002	NA

NA, not applicable. Ampicillin absorbance measured at the appropriate wavelength, i.e., 228 nm. Ceftriaxone absorbance measured at the appropriate wavelength, i.e., 241 nm.
[a] Enzyme present in crude extract.
[b] Contains substrate solution in 10 mM HEPES buffer only.

Table 3 Imipenem absorbance values in crude extracts containing β-lactamases

Isolate	Enzyme[a]	Absorbance at		Δ Absorbance ($T_2 - T_0$)	
		$T_{0\ min}$	$T_{2\ min}$	Per minute	Per minute/mg of protein
12649J	KPC-3	1.388	0.314	-0.537	-0.212
12646J	KPC-2	1.589	1.537	-0.026	-0.011
12147J	VIM-1	2.599	2.575	-0.012	-0.018
3027J	VIM-2	2.524	2.35	-0.087	-0.088
24D	VIM-6	2.625	1.873	-0.376	-0.119
12282J	NDM-1	2.580	2.498	-0.041	-0.024
12071J	OXA-48	1.575	1.527	-0.024	-0.008
12072J	OXA-48	1.589	1.562	-0.014	-0.005
ATCC 25922	– control	2.528	2.514	-0.007	-0.002
Blank[a]	– control	2.614	2.608	-0.003	NA

NA, not applicable. Imipenem measured at the appropriate wavelength, i.e., 299 nm.
[a] Enzyme present in crude extract.
[b] Contains imipenem solution in 10 mM HEPES buffer only.

Table 4 Omadacycline absorbance values in crude extracts containing β-lactamases and monooxygenase Tet(X)

Isolate	Enzyme[a]	Absorbance at			Δ Absorbance ($T_4 - T_0$)	MIC[b] (µg/mL)
		$T_{0\ hs}$	$T_{2\ hs}$	$T_{4\ hs}$		
QC 199	TEM-2	2.155	2.134	2.081	-0.074	1
QC 193	SHV-2	2.155	2.076	2.013	-0.142	1
3134J	SHV-12	2.155	2.148	2.129	-0.026	4
QC 279	CTX-M-15	2.155	2.084	2.033	-0.122	0.5
12649J	KPC-3	2.170	2.057	2.030	-0.140	0.5
12646J	KPC-2	2.170	1.999	1.904	-0.266	0.5
12147J	VIM-1	2.155	2.116	2.065	-0.090	0.5
3027J	VIM-2	2.155	2.157	2.084	-0.071	0.5
24D	VIM-6	2.155	2.065	1.969	-0.186	16
12282J	NDM-1	2.155	2.042	2.006	-0.149	0.25
12071J	OXA-48	2.170	2.097	2.040	-0.130	0.5
12072J	OXA-48	2.170	2.154	2.086	-0.084	1
14057J	TetX	2.155	1.635	1.419	-0.736	32
ATCC 25922	– control	2.155	2.108	2.094	-0.061	0.5
Blank[c]	– control	2.155	2.196	2.148	-0.007	NA

NA, not applicable. Omadacycline measured at the appropriate wavelength, i.e., 376 nm.
[a] Enzyme present in crude extract.
[b] Omadacycline MIC results.
[c] Contains omadacycline solution in 10 mM HEPES buffer only.

RESULTS *(continued)*

- The delta absorbance (per milligram of protein) for ampicillin and ceftriaxone in the presence of ESBL enzymes ranged from -0.37 to -0.047, indicating hydrolysis
- The delta absorbance for these same agents after exposure to the negative control *E. coli* ATCC 25922 strain crude extract was minimal (0.011) (Table 2)
- Similarly, the delta absorbance (per milligram of protein) for imipenem was up to -0.005 over a 2-minute period (Table 3)
- Omadacycline had delta absorbance values lower than -0.3 after incubation with all 12 β-lactamase enzymes during a period of 4 hours.; however, when omadacycline was exposed to the positive control enzyme Tet(X), a delta absorbance of -0.74 was observed over time (Table 4)

CONCLUSIONS

- Omadacycline is not a substrate of β-lactamase enzymes, including clinically important ESBLs and carbapenemases, which are the primary β-lactam resistance mechanisms in *Enterobacteriaceae* clinical isolates
- The hydrolysis of control β-lactam agents confirmed the presence of β-lactamase enzymes in each crude extract, while the alteration of omadacycline by Tet(X) confirmed the experiment's ability to detect the monooxygenation modification in this molecule

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