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Common Clinical Substitutions Enhance the Carbapenemase Activity of OXA-51-Like Class D β-Lactamases from *Acinetobacter* spp.

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Carbapenem resistance in *Acinetobacter baumannii* is a growing threat to the effective treatment of nosocomial infections. Acquired carbapenemases (e.g., OXA-23, OXA-24, and their variants) are a major source of carbapenem resistance (1). *A. baumannii* has a chromosomal carbapenem-hydrolyzing class D β-lactamase (CHDL) called OXA-51. There are nearly 100 clinical variants of OXA-51 documented (2–4), and mutations in bla_{OXA-51} are sometimes associated with an increase in the MIC values of carbapenems (5).

Starting with the bla_{OXA-51} gene from *A. baumannii* (GenBank accession number AJ309734), we synthesized the I129L and L167V variants of OXA-51. These substitutions occur in the active site and are associated with large increases in carbapenem MICs (4, 5). The mature form (residues 26 to 274) of all three genes were cloned into pET-24a, expressed, and purified (>95%) as described previously for OXA-24/40 (6). OXA-51 displays high K_m and low k_{cat} values for ampicillin (Table 1; methods are described in reference 7). OXA-51's affinity for carbapenems appears to be tighter (K_m, 5 to 150 μM), although not as tight as that seen for OXA-23 or OXA-24/40 (doripenem K_m, 10 to 30 nM) (7–9). Carbapenem K_m values (and K_s values) were much lower for both mutants, suggesting greatly increased affinity that approaches or equals that seen with OXA-24/40 and OXA-23.

To illuminate the structural basis of these results, we generated a model of OXA-51 using the PHYRE2 protein fold recognition server (Fig. 1) (10). The general fold of the predicted structure matches quite well OXA-24-doripenem (68% sequence identity) and shows especially strong overlap for active-site residues, including I129 (V130 in OXA-24/40) and L167 (L168). The δ methyl group of I129 is predicted to clash strongly with the hydroxyethyl group present on all carbapenems, explaining the relatively low affinity of those β-lactams for OXA-51 compared to that for OXA-24/40. Attempts to relieve this clash by rotation of the I129 side chain were unsuccessful, with further clashes forming between the δ methyl group and the side chain of L167 or between the γ_2 methyl group of I129 and the doripenem hydroxyethyl group. Modeling the L167V mutation using PyMOL (11) relieves the former clash by providing space to accommodate the δ methyl group after rotating it toward V167. Modeling the I129L mutation eliminates the γ_2 methyl group and thus allows L129 to rotate in the other direction without causing that group to clash with the hydroxyethyl moiety. The lower K_m for both variants with respect to substrates containing α-hydroxyethyl groups can thus be explained by mutation-induced remodeling of the active site to better accommodate that group (2). The OXA-51 model also predicts the presence of the hydrophobic bridge found in other class D carbapenemases. The presence of a tryptophan in OXA-51 (W222) in place of the methionine found in the OXA-24/40 and OXA-23 bridge (M223 and M221) may further explain kinetic differences among CHDLs (12). The homologous W222 in the OXA-51 model shows much less conformational flexibility and therefore accounts for the very weak binding of ampicillin (K_m, >10 mM compared to <500 μM for OXA-24/40 and OXA-23). Interestingly, there are known clinical variants of OXA-51-like enzymes with substitutions for W222 that are predicted to increase the flexibility at this position (e.g., W222G in OXA-79 and W222L in OXA-200) (13).

In conclusion, it appears that selective pressure caused by treatment with carbapenems is leading to the emergence of another

TABLE 1 OXA-51 K_m and k_{cat} values for β-lactam substrates

CHDL and antimicrobial	K_m (μM)[d]	K_s (μM)[a]	k_{cat} (s^{-1})	k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)
OXA-51				
Ampicillin	>10,000[b]		>25	
Imipenem	105 ± 3	>79	0.660 ± 0.005	0.0063 ± 0.0002
Doripenem	4.7 ± 0.2	>4.1	0.0730 ± 0.0009	0.016 ± 0.001
Cefotaxime	NA		<0.02	
Ceftriaxone	NA		<0.02	
Aztreonam	NA		<0.02	
Cefoxitin	NA		<0.02	
OXA-51 I129L				
Ampicillin	9,100 ± 900[b]		160 ± 6	0.018 ± 0.002
Imipenem	<2	0.610 ± 0.094	0.330 ± 0.008	0.54 ± 0.08[c]
Doripenem	<2	0.110 ± 0.010	0.140 ± 0.003	1.4 ± 0.2[c]
Cefotaxime	NA		<0.02	
Ceftriaxone	NA		<0.02	
Aztreonam	NA		<0.02	
Cefoxitin	NA		<0.02	
OXA-51 L167V				
Ampicillin	8,000 ± 600[b]		470 ± 12	0.058 ± 0.004
Imipenem	<2	0.190 ± 0.040	0.150 ± 0.003	0.81 ± 0.20[c]
Doripenem	<2	<0.05	0.032 ± 0.002	>0.70[c]
Cefotaxime	NA		<0.02	
Ceftriaxone	NA		<0.02	
Aztreonam	NA		<0.02	
Cefoxitin	NA		<0.02	

[a] K_s values were determined using ampicillin as a reporter substrate (14).
[b] For substrates with high K_m values, a 0.2-mm path length cuvette was used.
[c] k_{cat}/K_s ($\mu M^{-1} \cdot s^{-1}$).
[d] NA, not applicable.

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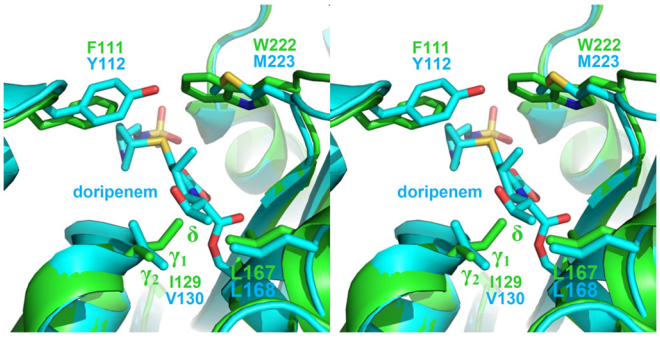

FIG 1 Stereodiagram of OXA-24/40-doripenem (cyan) aligned with a model of OXA-51 (green). The sequence of the mature OXA-51 enzyme (residues 26 to 274) was submitted to the PHYRE2 server. The resulting Protein Data Bank file was aligned with the structure of OXA-24/40-doripenem (Protein Data Bank accession no. 3PAE) using PyMOL.

dangerous mechanism of resistance to those key "last-resort" drugs.

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