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# Structural Basis for Enhancement of Carbapenemase Activity in the OXA-51 Family of Class D $\beta$ -Lactamases

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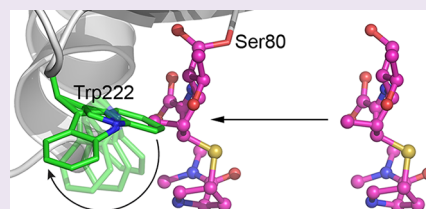
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## S Supporting Information

**ABSTRACT:** Class D  $\beta$ -lactamases of *Acinetobacter baumannii* are enzymes of the utmost clinical importance, producing resistance to last resort carbapenem antibiotics. Although the OXA-51-like enzymes constitute the largest family of class D  $\beta$ -lactamases, they are poorly studied and their importance in conferring carbapenem resistance is controversial. We present the detailed microbiological, kinetic, and structural characterization of the eponymous OXA-51  $\beta$ -lactamase. Kinetic studies show that OXA-51 has low catalytic efficiency for carbapenems, primarily due to the low affinity of the enzyme for these substrates. Structural studies demonstrate that this low affinity results from the obstruction of the enzyme active site by the side chain of Trp222, which presents a transient steric barrier to an incoming carbapenem substrate. The Trp222Met substitution relieves this steric hindrance and elevates the affinity of the mutant enzyme for carbapenems by 10-fold, significantly increasing the levels of resistance to these antibiotics. The ability of OXA-51 to evolve into a robust carbapenemase as the result of a single amino acid substitution may, in the near future, elevate the ubiquitous enzymes of the OXA-51 family to the status of the most deleterious *A. baumannii* carbapenemases, with dire clinical consequences.



Over the past decade *Acinetobacter baumannii* has evolved from a commensal microorganism of little clinical importance into a major nosocomial pathogen. This remarkable transformation resulted from the ability of *A. baumannii* to rapidly spread and survive for a long time in the clinical setting and its propensity to accumulate and exploit various antibiotic resistance mechanisms.<sup>1–3</sup> Due to its utmost clinical importance, *A. baumannii* was included by the Infectious Diseases Society of America (IDSA) to the list of the six clinically most important bacteria that are responsible for the majority of nosocomial infections.<sup>4</sup> Carbapenem antibiotics such as imipenem and meropenem were the drugs of choice for treatment of *A. baumannii* infections. However, the emergence and rapid spread of carbapenem resistant isolates has dramatically reduced available therapeutic options. Currently, up to 40–70% of clinical *A. baumannii* isolates are resistant to carbapenems.<sup>5–9</sup> As these isolates are commonly resistant to the majority of other antibiotics of various classes, mortality rates from such multidrug-resistant pathogens often exceeds 50%. The major mechanism of resistance to carbapenem antibiotics in *A. baumannii* is production by bacteria of  $\beta$ -lactamases, enzymes capable of cleaving the four-membered ring of the drugs rendering them inactive. Among the known molecular classes of  $\beta$ -lactamases (A, B, C, and D), class D enzymes constitute the predominant mechanism of resistance to carbapenems in *Acinetobacter*. Class D enzymes are also referred to as oxacillinases or OXA-type  $\beta$ -lactamases, as the early representatives of this class exhibited high catalytic activity

for the  $\beta$ -lactam antibiotic oxacillin. Class D enzymes capable of conferring resistance to carbapenem antibiotics have been named CHDLs (from carbapenem-hydrolyzing class D  $\beta$ -lactamases) to distinguish them from class D  $\beta$ -lactamases that are devoid of carbapenemase activity.<sup>10</sup> Recent studies have documented, however, that some OXA-type enzymes that behave as noncarbapenemases in various bacteria are capable of producing clinically significant levels of resistance to carbapenem antibiotics when expressed in *A. baumannii*.<sup>11</sup>

Six *A. baumannii* CHDLs, OXA-23, -24/40, -51, -58, -143, and -235 are responsible for the majority of the infections caused by carbapenem-resistant *Acinetobacter* in clinics around the world.<sup>12–15</sup> The genes for the OXA-23, -24/40, -58, -143, and -235 enzymes are located on various plasmids, which could have contributed to their rapid spread in *A. baumannii*.<sup>16–19</sup> Unlike all other clinically important CHDLs, the OXA-51-like  $\beta$ -lactamases are encoded by genes located on the chromosome of the vast majority of *A. baumannii* isolates and thus are native to this species.<sup>20,21</sup> Although OXA-51 is regarded as a weak carbapenemase, it often produces resistance to carbapenems in *A. baumannii* when its gene acquires a strong promoter or/and changes its location from the chromosome to a plasmid, which is expected to result in the increase in the gene copy number

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Table 1. MICs ( $\mu\text{g/mL}$ ) of Carbapenem Antibiotics against *A. baumannii* ATCC 17978 Producing Various CHDLs

antibiotic	enzyme					
	control <sup>a</sup>	OXA-51	OXA-51 M <sup>b</sup>	OXA-23 <sup>c</sup>	OXA-24/40 <sup>c</sup>	OXA-58 <sup>c</sup>
imipenem	0.125	2	16	32	64	8
meropenem	0.25	8	64	64	128	4
ertapenem	2	32	256	256	>256	32
doripenem	0.125	8	64	32	128	4

<sup>a</sup>Parental *A. baumannii* ATCC 17978 strain with the plasmid pNT221 without a  $\beta$ -lactamase gene. <sup>b</sup>OXA-51 M denotes the OXA-51 Trp222Met mutant enzyme. <sup>c</sup>The MICs of OXA-23, OXA-24/40, and OXA-58 for carbapenems have been previously reported.<sup>11</sup>

Table 2. Kinetics Parameters for Hydrolysis of Carbapenems by OXA-51 and the OXA-51 Trp222Met Mutant

enzyme	parameter	antibiotic			
		imipenem	meropenem	ertapenem	doripenem
OXA-51	$k_{\text{cat}}$ ( $\text{s}^{-1}$ )	$0.41 \pm 0.01$	$0.113 \pm 0.001$	$0.044 \pm 0.001$	$0.052 \pm 0.001$
	$K_{\text{m}}$ ( $\mu\text{M}$ )	$89 \pm 7$	$7.1 \pm 0.4$	$6.0 \pm 0.6$	$5.5 \pm 0.6$
	$k_{\text{cat}}/K_{\text{m}}$ ( $\text{s}^{-1} \text{M}^{-1}$ )	$(4.6 \pm 0.4) \times 10^3$	$(1.6 \pm 0.1) \times 10^4$	$(7.3 \pm 0.8) \times 10^3$	$(9.5 \pm 1.0) \times 10^3$
OXA-51 M <sup>a</sup>	$k_{\text{cat}}$ ( $\text{s}^{-1}$ )	$0.28 \pm 0.01$	$0.089 \pm 0.001$	$0.039 \pm 0.001$	$0.053 \pm 0.001$
	$K_{\text{m}}$ ( $\mu\text{M}$ )	$4.7 \pm 0.3$	$0.67 \pm 0.08$	$0.51 \pm 0.07$	$0.52 \pm 0.09$
	$k_{\text{cat}}/K_{\text{m}}$ ( $\text{s}^{-1} \text{M}^{-1}$ )	$(6.0 \pm 0.4) \times 10^4$	$(1.3 \pm 0.2) \times 10^5$	$(7.7 \pm 1.1) \times 10^4$	$(1.0 \pm 0.2) \times 10^5$

<sup>a</sup>OXA-51 M denotes the OXA-51 Trp222Met mutant enzyme.

and the amount of the enzyme produced.<sup>22</sup> Mutant OXA-51  $\beta$ -lactamases have also been reported in the carbapenem resistant clinical isolates of *A. baumannii*, but their role in enhanced resistance has not been studied.<sup>23,24</sup> The OXA-51-like  $\beta$ -lactamases have thus a potential to become the most important and deleterious CHDLs of *Acinetobacter*, as their presence in every *A. baumannii* isolate and the rapid evolution of the enzyme toward more robust carbapenemase activity could render every *A. baumannii* isolate resistant to carbapenem antibiotics. While OXA-51-type enzymes constitute the largest family of *A. baumannii* CHDLs that includes more than 100 closely related enzymes, their role in carbapenem resistance of this important pathogen is currently the subject of debate. To gain insight into the kinetic and structural basis for the carbapenemase activity of OXA-51, we performed kinetic analyses and determined the crystal structure of this ubiquitous *A. baumannii*  $\beta$ -lactamase. We have further demonstrated how a single amino acid substitution in OXA-51 significantly elevates its catalytic efficiency and transforms the enzyme into a robust carbapenemase.

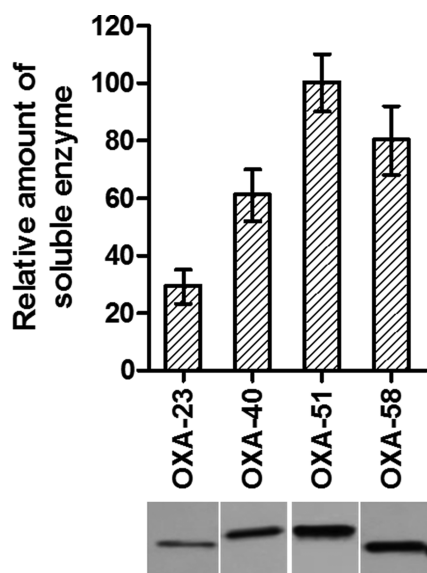
We first determined the MICs of carbapenem antibiotics conferred by OXA-51 and compared them to the MICs produced by other CHDLs, OXA-23, -24/40, and -58. All of these genes were cloned into the pNT221 shuttle vector<sup>25</sup> to express them under the same promoter in *A. baumannii* ATCC 17978. MICs were determined as previously described.<sup>11</sup> In the presence of OXA-51 the MICs of imipenem, meropenem, ertapenem, and doripenem increased 16-, 32-, 16-, and 64-fold when compared to the background control strain. The MICs of the carbapenems produced by OXA-51 were similar to those conferred by OXA-58, but well below the levels produced by two other *A. baumannii* CHDLs, OXA-23 and OXA-24/40 (Table 1).

Next we evaluated the steady-state kinetic parameters for the turnover of the carbapenems by purified OXA-51 (Table 2). The enzyme turned over these carbapenem substrates rather inefficiently ( $k_{\text{cat}}$  values from 0.11 to 0.41  $\text{s}^{-1}$ ). The  $k_{\text{cat}}$  values for OXA-51 with meropenem, ertapenem, and doripenem were overall similar to those reported for the CHDLs OXA-23 and OXA-24/40 and were 4- to 5-fold higher than those for OXA-

58.<sup>11</sup> With imipenem the  $k_{\text{cat}}$  value for OXA-51 was identical to that for OXA-23 and was 4- to 4.5-fold below that for OXA-58 and OXA-24/40.<sup>11</sup> The  $K_{\text{m}}$  value of OXA-51 for imipenem was 89  $\mu\text{M}$  (Table 2), well above ( $\geq 50$ -fold) the values determined by us for the OXA-23, OXA-24/40, and OXA-58  $\beta$ -lactamases.<sup>11</sup> For the three other carbapenems,  $K_{\text{m}}$  values were in the 5.5–7.1  $\mu\text{M}$  range, also well above the reported  $K_{\text{m}}$  values for the other three CHDLs.<sup>11</sup>

The amount of soluble  $\beta$ -lactamase produced by the bacterial cell has a significant impact on the produced level of antibiotic resistance. To evaluate the relative amount of OXA-51 produced by *A. baumannii* ATCC 17978 versus the amount of the three other CHDLs (OXA-23, OXA-24/40, and OXA-58) produced by the same host strain, the hexa-His tag was introduced to the C-termini of these enzymes. The amount of produced  $\beta$ -lactamases were subsequently evaluated as described by us recently.<sup>11</sup> This analysis demonstrated that while *A. baumannii* ATCC 17978 produced similar amounts of each of the CHDLs, the amount of soluble  $\beta$ -lactamases differed. Production of soluble OXA-51 was the highest (100%), followed by OXA-58, OXA-24/40, and OXA-23 (80%, 60%, and 30% of the OXA-51 amount, respectively; Figure 1). These data combined with our kinetic measurements demonstrate that it is not the amount of enzyme produced but rather its lower relative affinity for carbapenems that is responsible for lower levels of resistance to these antibiotics conferred by OXA-51 when compared to the three other CHDLs.

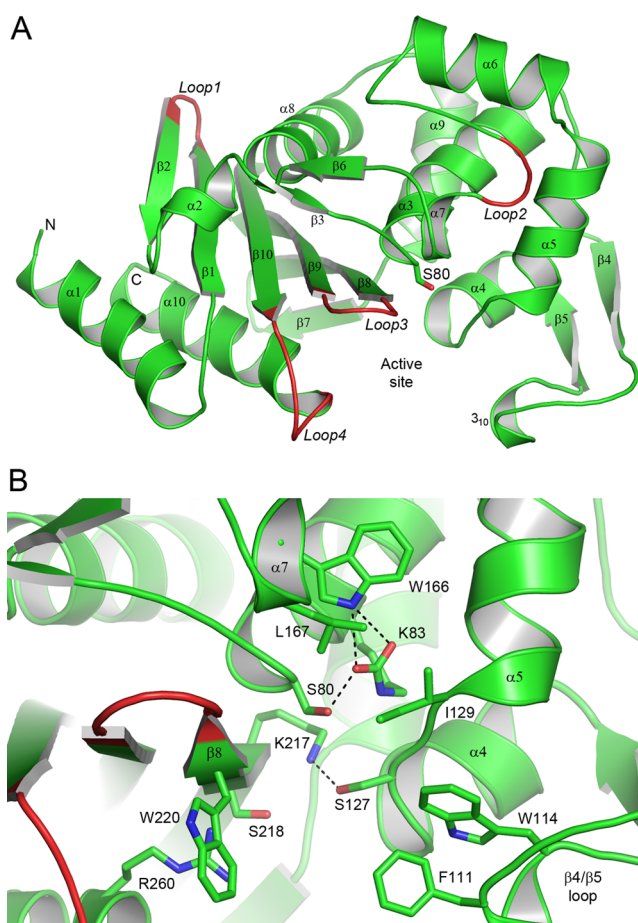
The OXA-51 carbapenemase was purified to homogeneity and crystallized prior to diffraction data collection at SSRL beamline BL12-2. The final refined OXA-51 model comprises 243 amino acids (residues Ser38 to Leu280), folded into two structural domains (Figure 2A). Some representative electron density is shown in Figure S1. The overall structure of OXA-51 resembles that of other class D  $\beta$ -lactamases whose structures have been determined, including OXA-1,<sup>26,27</sup> OXA-2 (unpublished, PDB code 1K38), OXA-10,<sup>28,29</sup> OXA-13,<sup>30</sup> OXA-23,<sup>25,31</sup> OXA-24/40,<sup>32,33</sup> OXA-45 (unpublished, PDB code 4GN2), OXA-46,<sup>34</sup> OXA-48,<sup>35</sup> OXA-58,<sup>36</sup> and OXA-146.<sup>31</sup> Superposition of OXA-51 onto these enzymes gives rmsds for all



**Figure 1.** Expression levels of OXA carbapenemases. (Bottom) Immunoblot of the soluble OXA-23, -40, -51, and -58 produced by *A. baumannii* ATCC 17978. (Top) Relative amounts of enzymes produced are normalized to that of OXA-51.

matching  $C_{\alpha}$  atoms ranging from 0.6 Å for OXA-23 and OXA-24/40, and up to 1.8 Å for OXA-45 (Table S1). Not surprisingly, OXA-51 shows high structural agreement with the four other OXA enzymes from *A. baumannii* (OXA-23, -24, -58, and -146), consistent with a significant degree of sequence identity (54% to 67%) between these five enzymes. The largest structural deviations among the OXA enzymes occur in the loops connecting strands  $\beta 1$  and  $\beta 2$  (Loop1), helices  $\alpha 6$  and  $\alpha 7$  (Loop2), strands  $\beta 8$  and  $\beta 9$  (Loop3), and in the final turn between strand  $\beta 10$  and helix  $\alpha 10$  (Loop4). The two latter loops are near the opening of the active site (Figure 2A).

The active site is located in a cleft at the interface of the two structural domains (Figure 2A), which is exposed to the external solvent and filled with a number of water molecules. A number of universally conserved residues line the active site including the catalytic Ser80 and the neighboring residues Lys83, Ser127, Trp166, Lys217, and Arg260 (Figure 2B). Ser127 from the loop between helices  $\alpha 4$  and  $\alpha 5$  forms a hydrogen bond with the side chain of Lys217, and this is the only direct connection between the two sides of the cleft. The Trp166 residue on helix  $\alpha 7$  helps to anchor the carboxylated Lys83 via a hydrogen bonding interaction (Figure 2B). The Arg260 residue has been shown to be responsible for anchoring the carboxylate moiety, a common feature on the ring immediately adjacent to the  $\beta$ -lactam ring of penicillins, cephalosporins, and carbapenems, in known OXA enzyme complex structures.<sup>25,26,30,33</sup> There are also several partially conserved amino acids that have been suggested to play a role in either substrate specificity or/and the reaction mechanism. These include two aromatic residues at the end of the long  $\beta 4/\beta 5$  loop, Phe111 and Trp114 in OXA-51 (motif 2, Figure S2), small hydrophobic residues on the  $\alpha 4/\alpha 5$  loop (Ile129, motif 3) and helix  $\alpha 7$  on the  $\Omega$ -loop (Leu167, motif 5), and a hydroxyl (Ser218) and an aromatic residue (Trp220) on strand  $\beta 8$  (motif 7) (Figure 2B). Table S2 indicates the most common amino acids at these positions in 156 OXA enzymes. The two aromatic residues (Phe111 and Trp114 in OXA-51) on the  $\beta 4/\beta 5$  loop appear to make hydrophobic interactions with

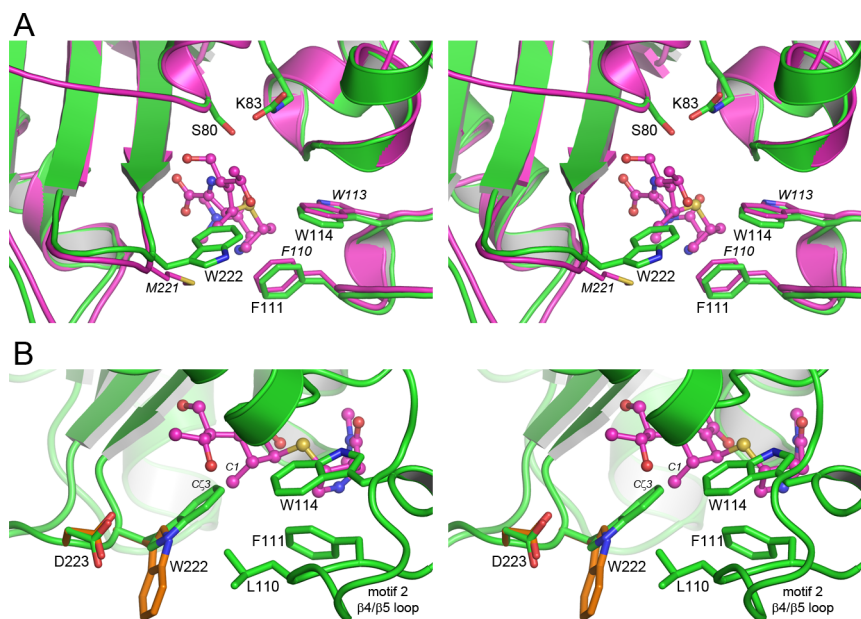


**Figure 2.** OXA-51 structure. (A) Ribbon representation of the *A. baumannii* OXA-51  $\beta$ -lactamase showing the secondary structure labeling used. The location of the active site is indicated by the side chain of the catalytic serine, Ser80. Four loops (loops 1–4), which show the largest structural variation between OXA-51 and the other OXA enzymes, whose structures are known, are also indicated. (B) Close-up view of the OXA-51 active site showing some of the conserved and partially conserved residues, which are important for substrate binding and/or the enzyme mechanism.

substrates in some OXA enzyme–substrate complexes.<sup>25,33</sup> Another role for the phenylalanine residue in some OXA enzymes seems to be the formation of a hydrophobic “bridge” or “tunnel” across the mouth of the active site. In these enzymes, the phenylalanine interacts with a partially conserved methionine residue from motif 7 at the C-terminal end of strand  $\beta 8$  (Figure 3A). This hydrophobic interaction was first observed in the structure of OXA-24/40<sup>33</sup> and subsequently in OXA-23,<sup>25</sup> and it was suggested that the carbapenemase activity of these enzymes might be dependent upon the formation of this bridge, whereby it can direct the orientation of the carbapenem substrate and make it more susceptible to deacylation. The recent structure of the carbapenemase OXA-58 shows that this hydrophobic bridge does not exist in this enzyme despite the presence of the same residues (Phe114 and Met225, OXA-58 numbering), which would normally form the hydrophobic interaction.<sup>36</sup> In OXA-51 and almost all of the known OXA-51-like variants, the residue at the equivalent position to this methionine is a tryptophan (Trp222) (Figure 3A).

In OXA-51 the side chain of Trp222 projects across the opening of the active site (Figure 3B) and is anchored in this





**Figure 3.** Trp222 side chain in OXA-51 would occlude the active site. (A) Stereoview of the active site of OXA-51 (green) superimposed on OXA-23 (magenta). The location of meropenem in the OXA-23 structure is shown as magenta ball-and-stick. The position of Ser80 in OXA-51 shows the location of the site of acylation. (B) Stereoview of the outer end of the OXA-51 active site, showing the location of Trp222 and the residues from the  $\beta 4/\beta 5$  (Leu110, Phe111, and Trp114) loop, which make hydrophobic contacts with Trp222. Meropenem from OXA-23 is shown as magenta ball-and-stick. The critical clash between the indole ring of Trp222 and the C1 methyl atom of the substrates is indicated. The alternate rotamer configuration of Trp222 from energy minimization calculations is shown as orange sticks, and the small rotation of the Asp223 side chain is also indicated.

conformation by several aromatic and hydrophobic interactions with the side chains of Leu110, Phe111, and Trp114 with the edge-to-face interaction with Phe111, in particular, being moderately strong.<sup>37</sup> Moreover, the Leu110/Trp222 pairing, albeit on opposite sides of the active site cleft, is a characteristic sequence motif for the OXA-51-like family of class D enzymes. This leucine residue precedes the partially conserved phenylalanine of motif 2 (Phe111 in OXA-51) and is universally conserved in all of the OXA-51-like enzymes, whereas in 156 non-OXA-51 enzymes, the residue at this position is highly variable.

Superposition of the apo OXA-51 structure with the published structures of the OXA-1-doripenem,<sup>26</sup> OXA-13-meropenem,<sup>30</sup> OXA-23-meropenem,<sup>25</sup> and OXA-24/40-doripenem<sup>33</sup> acyl enzyme complexes show that the presence of Trp222 in OXA-51 would severely hinder the binding of carbapenem substrates (Figure 3B). The indole ring of Trp222 is directed toward the pyrrolidine ring of the carbapenem, such that the indole C $\zeta$ 3 atom and the C1 methyl group of meropenem and doripenem almost overlap. There are also additional potential clashes between Ile129 and Leu167 of the enzyme and the  $\alpha$ -hydroxyethyl group of the carbapenem, but those can be readily alleviated by small rotations of the isoleucine and leucine side chains. Occlusion of the active site of OXA-51 by the Trp222 side chain is further exacerbated by the extensive hydrophobic interactions it makes with the  $\beta 4/\beta 5$  loop, which may present an additional energy barrier to efficient carbapenem binding. According to our kinetic data, OXA-51 is an active enzyme with modest carbapenemase activity, an indication that the enzyme is capable of the binding and hydrolysis of carbapenem antibiotics. The only way carbapenems can productively bind to the active site of OXA-51 is by displacing the side chain of Trp222 from the position it occupies in the current apo-OXA-51 structure.

A total of seven rotamers of the Trp222 side chain, with probabilities of greater than 1%, are plausible based on the statistical analysis of side chain rotamer preferences in proteins.<sup>38</sup> Analysis of these rotamers with the graphics program COOT<sup>39</sup> indicates that five of the rotamer variants are sterically disfavored (Figure S3). The only sterically viable rotamer configuration is a 180° rotation about the C $\beta$ –C $\gamma$  bond of the tryptophan side chain, pointing the indole ring in the opposite direction to that observed in the OXA-51 structure (Figure 3B). In this alternate configuration, although the indole ring would still be stabilized by the extensive hydrophobic interactions with the side chains of Leu110 and Phe111, it would potentially clash with the side chain of Asp223. To confirm whether this alternate rotamer configuration is accessible in the context of the OXA-51 structure, optimization of the five residue  $\beta 8/\beta 9$  loop (with sequence WGWDV), centered on Trp222, with a meropenem fixed in the OXA-51 active site based upon a superposition of the OXA-23-meropenem structure<sup>25</sup> was performed. The minimization resulted in a side chain configuration for Trp222 closely resembling the predicted viable rotamer variant, with the plane of the indole rings system within 10° of the indole of the rotamer variant. Moreover the neighboring Asp223 side chain also showed a small concerted movement in order to avoid the potential clash with the newly positioned Trp222 indole ring. Such transitioning from the observed tryptophan rotamer to the alternate configuration would require an energy barrier to be overcome, which may explain the higher  $K_m$  for OXA-51 with the carbapenem substrates (Table 2) when compared to other class D carbapenemases.<sup>11</sup> It should be noted that there is no evidence of this alternate conformation in the apo-OXA-51 electron density (Figure S1).

An alignment of the 117 reported OXA-51-like sequences shows that the residue at position 222 is almost invariably

tryptophan; only four enzymes, OXA-79 (glycine) and OXAs-172, -173, and -200 (all leucine), have other amino acid residues at this position. While these mutant enzymes have not been isolated and characterized, it has been reported that clinical isolates carrying these OXA-51 variants have increased resistance to carbapenems.<sup>40</sup>

To evaluate whether the bulky Trp222 in OXA-51 is responsible for the lower apparent affinity of the enzyme for carbapenem antibiotics when compared to the OXA-23, -24/40, and -58 carbapenemases and, ultimately, attenuates its carbapenemase activity, we have substituted it with methionine, a residue that is found in this position in other clinically important CHDLs and their variants. When expressed from the same plasmid and promoter in the *A. baumannii* ATCC 17978 recipient, this mutant enzyme produced an 8-fold increase in MICs of all four carbapenems tested when compared to the parental enzyme (Table 1). The resulting MIC values of imipenem, meropenem, ertapenem, and doripenem were similar to those against *A. baumannii* ATCC 17978 producing the OXA-23 and OXA-24/40 CHDLs and are well above the MICs against the same strain producing the OXA-58 carbapenemase (Table 1). The Trp222Met substitution had little effect on the  $k_{\text{cat}}$  values of the enzyme but resulted in a significant increase in its affinity for carbapenem antibiotics as judged by lower  $K_m$  values. Overall, the catalytic efficiency of the Trp222Met mutant enzyme against carbapenems increased 10-fold when compared to the parental OXA-51  $\beta$ -lactamase. Of importance, several mutant derivatives of OXA-51 have recently been reported in carbapenem-resistant clinical *A. baumannii* isolates. DNA sequencing of the gene for OXA-51 has identified several amino acid substitutions, including mutations of Trp222.<sup>40</sup> While the contribution of the mutant OXA-51 enzymes to the decreased susceptibility of the clinical isolates has not been evaluated, our present study demonstrates that Trp222 indeed is crucial for the ability of the OXA-51-type  $\beta$ -lactamase to produce high, clinically important levels of resistance to carbapenem antibiotics.

**Conclusions.** The OXA-51-like enzymes comprise the largest family of *A. baumannii*  $\beta$ -lactamases. Our studies of OXA-51 have demonstrated that the bulky Trp222 residue transiently occludes the active site and is responsible for the weak carbapenemase activity of the enzyme. We have demonstrated that substitution of this tryptophan with a less bulky methionine converts this enzyme into a robust CHDL that produces high levels of resistance to these last resort antibiotics. As the gene for OXA-51 is ubiquitous in *A. baumannii*, its evolutionary plasticity may, in short time, transform the OXA-51-like family of enzymes into carbapenemases of major clinical importance.

## METHODS

See Supporting Information.

## ASSOCIATED CONTENT

### Supporting Information

Tables of crystallographic data and crystallographic refinement statistics. Figures of the final electron density in the active site, a structure-based sequence alignment of the OXA enzymes whose structures are known, and the alternate rotamer conformations for Trp222. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschembio.5b00090.

## Accession Codes

The atomic coordinates and the structure factors for OXA-51 were deposited to the Protein Data Bank (Berman, H. M.; *et al.* (2000) The Protein Data Bank. *Nucleic Acids Res.* 28(1), 235–242) with PDB code 4ZDX.

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### Notes

The authors declare no competing financial interest.

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## REFERENCES

- (1) Peleg, A. Y., Seifert, H., and Paterson, D. L. (2008) *Acinetobacter baumannii*: Emergence of a successful pathogen. *Clin. Microbiol. Rev.* 21, 538–582.
- (2) Perez, F., Hujer, A. M., Hujer, K. M., Decker, B. K., Rather, P. N., and Bonomo, R. A. (2007) Global challenge of multidrug-resistant *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 51, 3471–3484.
- (3) Roca, I., Espinal, P., Vila-Farres, X., and Vila, J. (2012) The *Acinetobacter baumannii* oxymoron: Commensal hospital dweller turned pan-drug-resistant menace. *Front. Microbiol.* 3, 1–30.
- (4) Boucher, H. W., Talbot, G. H., Bradley, J. S., Edwards, J. E., Gilbert, D., Rice, L. B., Scheld, M., Spellberg, B., and Bartlett, J. (2009) Bad bugs, no drugs: No ESCAPE! An update from the Infectious Diseases Society of America. *Clin. Infect. Dis.* 48, 1–12.
- (5) Davies, T. A., Queenan, A. M., Morrow, B. J., Shang, W., Amsler, K., He, W., Lynch, A. S., Pillar, C., and Flamm, R. K. (2011) Longitudinal survey of carbapenem resistance and resistance mechanisms in *Enterobacteriaceae* and non-fermenters from the USA in 2007–09. *J. Antimicrob. Chemother.* 66, 2298–2307.
- (6) Higgins, P. G., Dammhayn, C., Hackel, M., and Seifert, H. (2010) Global spread of carbapenem-resistant *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* 65, 233–238.
- (7) Kallen, A. J., Hidron, A. I., Patel, J., and Srinivasan, A. (2010) Multidrug resistance among gram-negative pathogens that caused healthcare-associated infections reported to the National Healthcare Safety Network, 2006–2008. *Infect. Control Hosp. Epidemiol.* 31, 528–531.
- (8) Rhomberg, P. R., and Jones, R. N. (2009) Summary trends for the Meropenem Yearly Susceptibility Test Information Collection Program: A 10-year experience in the United States (1999–2008). *Diagn. Microbiol. Infect. Dis.* 65, 414–426.
- (9) Rosenthal, V. D., Maki, D. G., Jamulitrat, S., Medeiros, E. A., Todi, S. K., Gomez, D. Y., Leblebicioglu, H., Abu Khader, I., Miranda Novales, M. G., Berba, R., Ramirez Wong, F. M., Barkat, A., Pino, O.

- R., Duenas, L., Mitrev, Z., Bijie, H., Gurskis, V., Kanj, S. S., Mapp, T., Hidalgo, R. F., Ben Jaballah, N., Raka, L., Gikas, A., Ahmed, A., Thu le, T. A., and Guzman Siritt, M. E. (2010) International Nosocomial Infection Control Consortium (INICC) report, data summary for 2003–2008, issued June 2009. *Am. J. Infect. Control* 38, 95–104.
- (10) Poirel, L., and Nordmann, P. (2006) Carbapenem resistance in *Acinetobacter baumannii*: Mechanisms and epidemiology. *Clin. Microbiol. Infect.* 12, 826–836.
- (11) Antunes, N. T., Lamoureaux, T. L., Toth, M., N.K. S., Frase, H., and Vakulenko, S. B. (2014) Class D  $\beta$ -lactamases: Are they all carbapenemases? *Antimicrob. Agents Chemother.* 58, 2119–2125.
- (12) Poirel, L., Naas, T., and Nordmann, P. (2010) Diversity, epidemiology, and genetics of class D  $\beta$ -lactamases. *Antimicrob. Agents Chemother.* 54, 24–38.
- (13) Queenan, A. M., and Bush, K. (2007) Carbapenemases: The versatile  $\beta$ -lactamases. *Clin. Microbiol. Rev.* 20, 440–458.
- (14) Zhao, W. H., and Z.Q. H. (2012) *Acinetobacter*: A potential reservoir and dispenser for  $\beta$ -lactamases. *Crit. Rev. Microbiol.* 38, 30–51.
- (15) Higgins, P. G., Perez-Llarena, F. J., Zander, E., Fernandez, A., Bou, G., and Seifert, H. (2013) OXA-235, a novel class D  $\beta$ -lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 57, 2121–2126.
- (16) Bou, G., Oliver, A., and Martinez-Beltran, J. (2000) OXA-24, a novel class D beta-lactamase with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. *Antimicrob. Agents Chemother.* 44, 1556–1561.
- (17) Higgins, P. G., Poirel, L., Lehmann, M., Nordmann, P., and Seifert, H. (2009) OXA-143, a novel carbapenem-hydrolyzing class D  $\beta$ -lactamase in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 53, 5035–5038.
- (18) Poirel, L., Marque, S., Heritier, C., Segonds, C., Chabanon, G., and Nordmann, P. (2005) OXA-58, a novel class D  $\beta$ -lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 49, 202–208.
- (19) Scaife, W., Young, H. K., Paton, R. H., and Amyes, S. G. (1995) Transferable imipenem-resistance in *Acinetobacter* species from a clinical source. *J. Antimicrob. Chemother.* 36, 585–586.
- (20) Brown, S., Young, H. K., and Amyes, S. G. B. (2005) Characterisation of OXA-51, a novel class D carbapenemase found in genetically unrelated clinical strains of *Acinetobacter baumannii* from Argentina. *Clin. Microbiol. Infect.* 11, 15–23.
- (21) Evans, B. A., and Amyes, S. G. (2014) OXA  $\beta$ -lactamases. *Clin. Microbiol. Rev.* 27, 241–263.
- (22) Lee, Y. T., Turtton, J. F., Chen, T. L., Wu, R. C., Chang, W. C., Fung, C. P., Chen, C. P., Cho, W. L., Huang, L. Y., and Siu, L. K. (2009) First identification of blaOXA-51-like in non-baumannii *Acinetobacter* spp. *J. Chemother.* 21, 514–520.
- (23) Chen, T. L., Lee, Y. T., Kuo, S. C., Hsueh, P. R., Chang, F. Y., Siu, L. K., Ko, W. C., and Fung, C. P. (2010) Emergence and distribution of plasmids bearing the blaOXA-51-like gene with an upstream ISAbal in carbapenem-resistant *Acinetobacter baumannii* isolates in Taiwan. *Antimicrob. Agents Chemother.* 54, 4575–4581.
- (24) Zander, E., Chmielarczyk, A., Heczko, P., Seifert, H., and Higgins, P. G. (2013) Conversion of OXA-66 into OXA-82 in clinical *Acinetobacter baumannii* isolates and association with altered carbapenem susceptibility. *J. Antimicrob. Chemother.* 68, 308–311.
- (25) Smith, C. A., Antunes, N. T., Stewart, N. K., Toth, M., Kumarasiri, M., Chang, M., Mobashery, S., and Vakulenko, S. B. (2013) Structural basis for carbapenemase activity of the OXA-23  $\beta$ -lactamase from *Acinetobacter baumannii*. *Chem. Biol.* 20, 1107–1115.
- (26) Schneider, K. D., Karpen, M. E., Bonomo, R. A., Leonard, D. A., and Powers, R. A. (2009) The 1.4 Å crystal structure of the class D  $\beta$ -lactamase OXA-1 complexed with doripenem. *Biochemistry* 48, 11840–11847.
- (27) Sun, T., Nukaga, M., Mayama, K., Braswell, E. H., and Knox, J. R. (2003) Comparison of  $\beta$ -lactamases of classes A and D: 1.5-Å crystallographic structure of the class D OXA-1 oxacillinase. *Protein Sci.* 12, 82–91.
- (28) Golemi, D., Maveyraud, L., Vakulenko, S., Tranier, S., Ishiwata, A., Kotra, L. P., Samama, J. P., and Mobashery, S. (2000) The first structural and mechanistic insights for class D  $\beta$ -lactamases: Evidence for a novel catalytic process for turnover of  $\beta$ -lactam antibiotics. *J. Am. Chem. Soc.* 122, 6132–6133.
- (29) Paetzel, M., Danel, F., de Castro, L., Mosimann, S. C., Page, M. G. P., and Strynadka, N. C. J. (2000) Crystal structure of the class D  $\beta$ -lactamase OXA-10. *Nat. Struct. Biol.* 7, 918–924.
- (30) Pernot, L., Frénois, F., Rybkine, T., L'Hermite, G., Petrella, S., Delettré, J., Jarlier, V., Collatz, E., and Sougakoff, W. (2001) Crystal structures of the class D beta-lactamase OXA-13 in the native form and in complex with Meropenem. *J. Mol. Biol.* 310, 859–874.
- (31) Kaitany, K. C., Klinger, N. V., June, C. M., Ramey, M. E., Bonomo, R. A., Powers, R. A., and Leonard, D. A. (2014) Structures of the class D carbapenemases OXA-23 and OXA-146: Mechanistic basis of activity against carbapenems, extended-spectrum cephalosporins and aztreonam. *Antimicrob. Agents Chemother.* 58, 4848–4855.
- (32) Santillana, E., Beceiro, A., Bou, G., and Romero, A. (2007) Crystal structure of the carbapenemase OXA-24 reveals insights into the mechanism of carbapenem hydrolysis. *Proc. Natl. Acad. Sci. U. S. A.* 104, 5354–5359.
- (33) Schneider, K. D., Ortega, C. J., Renck, N. A., Bonomo, R. A., Powers, R. A., and Leonard, D. A. (2011) Structures of the class D carbapenemase OXA-24 from *Acinetobacter baumannii* in complex with doripenem. *J. Mol. Biol.* 406, 583–594.
- (34) Docquier, J. D., Benvenuti, M., Calderone, V., Giuliani, F., Kapetis, D., De Luca, F., Rossolini, G. M., and Mangani, S. (2010) Crystal structure of the narrow-spectrum OXA-46 class D beta-lactamase: relationship between active-site lysine carbamylation and inhibition by polycarboxylates. *Antimicrob. Agents Chemother.* 54, 2167–2174.
- (35) Docquier, J. D., Calderone, V., De Luca, F., Benvenuti, M., Giuliani, F., Bellucci, L., Tafi, A., Nordmann, P., Botta, M., Rossolini, G. M., and Mangani, S. (2009) Crystal structure of the OXA-48  $\beta$ -lactamase reveals mechanistic diversity among class D carbapenemases. *Chem. Biol.* 16, 540–547.
- (36) Smith, C. A., Antunes, N. T., Toth, M., and Vakulenko, S. B. (2014) Crystal Structure of Carbapenemase OXA-58 from *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 58, 2135–2143.
- (37) Hunter, C. A., Singh, J., and Thornton, J. M. (1991) Pi-pi interactions: The geometry and energetics of phenylalanine-phenylalanine interactions in proteins. *J. Mol. Biol.* 218, 837–846.
- (38) Dunbrack, R. L., and Cohen, F. E. (1997) Bayesian statistical analysis of protein sidechain rotamer preferences. *Protein Sci.* 6, 1661–1681.
- (39) Emsley, P., and Cowtan, K. (2004) Coot: Model-building tools for molecular graphics. *Acta Crystallogr. D* 60, 2126–2132.
- (40) Zander, E., Nemec, A., Seifert, H., and Higgins, P. G. (2012) Association between  $\beta$ -lactamase-encoding bla(OXA-51) variants and DiversiLab rep-PCR-based typing of *Acinetobacter baumannii* isolates. *J. Clin. Microbiol.* 50, 1900–1904.