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A review on the mechanistic details of OXA enzymes of ESKAPE pathogens

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ABSTRACT

The production of β-lactamases is a prevalent mechanism that poses serious pressure on the control of bacterial resistance. Furthermore, the unavoidable and alarming increase in the transmission of bacteria producing extended-spectrum β-lactamases complicates treatment alternatives with existing drugs and/or approaches. Class D β-lactamases, designated as OXA enzymes, are characterized by their activity specifically towards oxacillins. They are widely distributed among the ESKAPE bugs that are associated with antibiotic resistance and life-threatening hospital infections. The inadequacy of current β-lactamase inhibitors for conventional treatments of 'OXA' mediated infections confirms the necessity of new approaches. Here, the focus is on the mechanistic details of OXA-10, OXA-23, and OXA-48, commonly found in highly virulent and antibiotic-resistant pathogens *Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Enterobacter spp*. to describe their similarities and differences. Furthermore, this review contains a specific emphasis on structural and computational perspectives, which will be valuable to guide efforts in the design/discovery of a common single-molecule drug against ESKAPE pathogens.

KEYWORDS

B-lactamase; OXA-10; OXA-23; OXA-48; ESKAPE bugs

Introduction

β-lactam antibiotics are life-saving drugs that kill bacterial cells by irreversibly preventing the proper formation of the peptidoglycan polymers in their cell walls through the inactivation of the peptidoglycan transpeptidases. Unfortunately, bacteria evolve different resistance mechanisms to counteract the action of these wonder drugs [1–3]. The production of β-lactamases is one of the most prevalent resistance mechanisms encountered in clinical settings. Just like the peptidoglycan transpeptidases, β-lactamases form an intermediate complex with β-lactams and then render these drugs ineffective by cleaving their β-lactam rings, the chemical moiety with the amidic function [4,5]. The use of β-lactamase inhibitors in combinatorial treatments is one major strategy to restore the efficacy of β-lactam antibiotics to prevent the antibiotic from being hydrolyzed by the enzyme [3].

There exist two different schemes for the classification of β-lactamases. Bush-Jacoby-Medeiros classification divides the enzymes into groups based on substrate and inhibitor profiles [6]. The more widely used Ambler classification divides the enzymes based on the similarities of the catalytic site residues and the motifs in their primary sequences [7,8]. Originally, Ambler specified only the A and B classes. For catalysis, while class A enzymes had a serine residue in the active site, class B enzymes required the metal zinc in the active site. Later, two more classes with active-site serine residues but with little sequence similarity to class A enzymes [9] were discovered; 'Amp C' β-lactamases, designated as the class C [10], and 'OXA' β-lactamases, designated as the class D [11,12].

Most OXA-enzymes have the ability to hydrolyze the so-called 'last resort' β-lactams, such as carbapenems and oxyimino-substituted agents as ceftazidime and cefotaxime [13]. For the management of antibiotic resistance, in-depth knowledge of mechanistic details of these enzymes, especially those with carbapenemase activity, is crucial. Hence, in order to guide drug and inhibitor development efforts, this review will focus on three OXA enzymes widely distributed in highly virulent and antibiotic-resistant bacterial pathogens associated with life-threatening hospital infections. There will be specific emphasis on the OXA enzymes of ESKAPE bugs [14], *Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Enterobacter spp*., from structural and computational perspectives to guide efforts in the design/discovery of a single-molecule targeting all four pathogens.

Class D β-lactamases of 'ESKAPE' bugs

Class D β-lactamases are commonly known as oxacillinases based on their strong hydrolytic activity against the semisynthetic penicillin oxacillin. Thus, they are conventionally named using the 'OXA' nomenclature [15]. Later studies have confirmed their activities also against cephalosporins and carbapenems. Within β-

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lactamases, this class has a unique mechanism for catalysis, which is featured by a carboxylated lysine [16].

Most genes encoding OXA-type β-lactamases are located on large, transferable plasmids in Gram-negative bacteria, constituting a danger for their wide dispersal. More recent studies report that they can be encoded by both chromosomal and plasmid-mediated genes in Gram-negative and Gram-positive bacteria [17,18]. Currently, there are more than 750 types of OXA-enzymes displaying heterogeneous substrate profiles; consequently, they are categorized as narrow- or extended-spectrum enzymes [13,19]. Generally, OXA-enzymes resist inhibition by common β-lactam inhibitors (i.e. clavulanate, sulbactam, and tazobactam) and confer resistance to the amino-, carboxy-, and ureidopenicillins [20]. Not surprisingly, these enzymes are widely distributed among the clinically challenging ESKAPE bugs [21]. Hence, OXA-enzymes deserve special attention under the topic of β-lactomics, the term introduced by Khan [22]. Here the focus will be on the OXA-10, OXA-23, and OXA-48 β-lactamases, commonly found in highly virulent and antibiotic-resistant pathogens *A. baumannii, K. pneumoniae, P. aeruginosa*, and *Enterobacter spp.*

P. aeruginosa primarily produces the class D β-lactamases of the OXA-10 group [23,24]. The bla_{OXA-10} gene was first characterized from a *P. aeruginosa* plasmid and its product has been initially named as PSE-2 [25]. Later, it was transferred to the OXA group and renamed as OXA-10 [26]. Due to horizontal gene transfer, they are also encountered in different bacterial species [27–29]. Many enzymes within this group are generally narrow-spectrum with weak carbapenemase activity; however, natural variants with point mutations on OXA-10 such as OXA-11, OXA-14, OXA-16, OXA-17, and OXA-35 have been shown to be extended-spectrum class D β-lactamases [13,30]. This demonstrates how these enzymes have the potential to evolve into clinically important variants under selective pressure [31].

The general drug resistance associated with *A. baumannii* is through the production of OXA-type-β-lactamases with carbapenem hydrolyzing activities. These enzymes could be intrinsic like the chromosomal OXA-51 group enzymes or acquired (e.g. OXA-23 group, OXA-40 group, OXA-58 group, etc.) [13]. Among the plasmid-borne enzymes, the OXA-23 group is the most prevalent element [32]. All around the world, outbreaks of *A. baumannii* synthesizing OXA-23 have been reported [33–39]. The bla_{OXA-23} gene was first characterized from an *A. baumannii* strain resistant to imipenem, penicillins, and all classes of cephalosporins. Its product has been initially named as ARI-1 [40] and later renamed as OXA-23 [41]. This was the first carbapenem-hydrolyzing class D β-lactamase (CHDL) on a self-transferable plasmid [42].

Different *K. pneumoniae* strains are capable of synthesizing β-lactamases of all classes from A to D; however, drug resistance to multiple antibiotics is mostly associated with the synthesis of extended-spectrum β-lactamases of the Ambler class A (NMCA, IMI, SME, GES, and KPC), of extended-spectrum metallo β-lactamases (IMP, VIM), and of extended-spectrum oxacillinase, OXA-48 [43–50]. The first bla_{OXA-48} gene was identified in a *K. pneumoniae* strain [50], but now surveillance studies identify the OXA-48 group enzymes as the 2nd or 3rd most common carbapenemases among global *Enterobacteriaceae* [20].

Structural and catalytic features of OXA-10, OXA-23, and OXA-48

Despite having varying amino acid sequences, class D β-lactamases assume very similar secondary structures [51–53]. In Figure 1, the aligned OXA-10, OXA-23, and OXA-48 enzymes are presented to provide an insight into their sequential and structural similarities. The limited sequence identity of 33.4–45.6 % (Figure 1 -a-c) yielded Cα RMSD values between 0.782 and 1.092 Å, which indicates quite similar secondary structures (Figure 1 -b-d). Similar folding patterns in these OXA structures might be associated with the motifs present in their primary sequences. In particular, all three OXA enzymes have the major conserved motifs of class D enzymes, as well as the Ser-x-x-Lys motif, which is the minimal requirement for serine acylation [51,54–58]. Multiple sequence alignment of OXA-10, OXA-23, and OXA-48 further revealed additional shared motifs, which are summarized in Table 1.

Class D β-lactamases are commonly found in monomeric and/or dimeric forms. In accordance with this, OXA-23 primarily exists as a monomer, while OXA-10 and OXA-48 usually exist as homodimers [52]. Their dimerization is stabilized via cation-binding sites, chloride ions, and hydrogen bonding, as cations are hydrogen-bonded to water molecules in their vicinity. For OXA-10, an additional β-strand has been shown to mediate the dimer formation [58]. Size-wise, the dimerization surface area of OXA-48 is narrower compared to OXA-10. When this dimeric structure is disrupted, a decrease in enzyme activity against antibiotics such as oxacillin and penicillin is observed in OXA-10, whereas this is not the case for OXA-48 [9,53,56,60].

OXA-10, OXA-23, and OXA-48 fold as two-domain proteins, like most other β-lactamases (Figure 2). The first domain (D1) is a mixed α/β domain containing the β5-β6 carbapenemase loop, the Lys[SerThr]Gly motif, and N- and C-termini; whereas the second domain (D2) is a helix-only domain, containing the active site serine acylation motif, the Ω-loop, and other active site stabilizing elements [51,55–58]. The active sites of these OXA enzymes are positioned in the cleft between the two domains like a transition zone, and the conserved

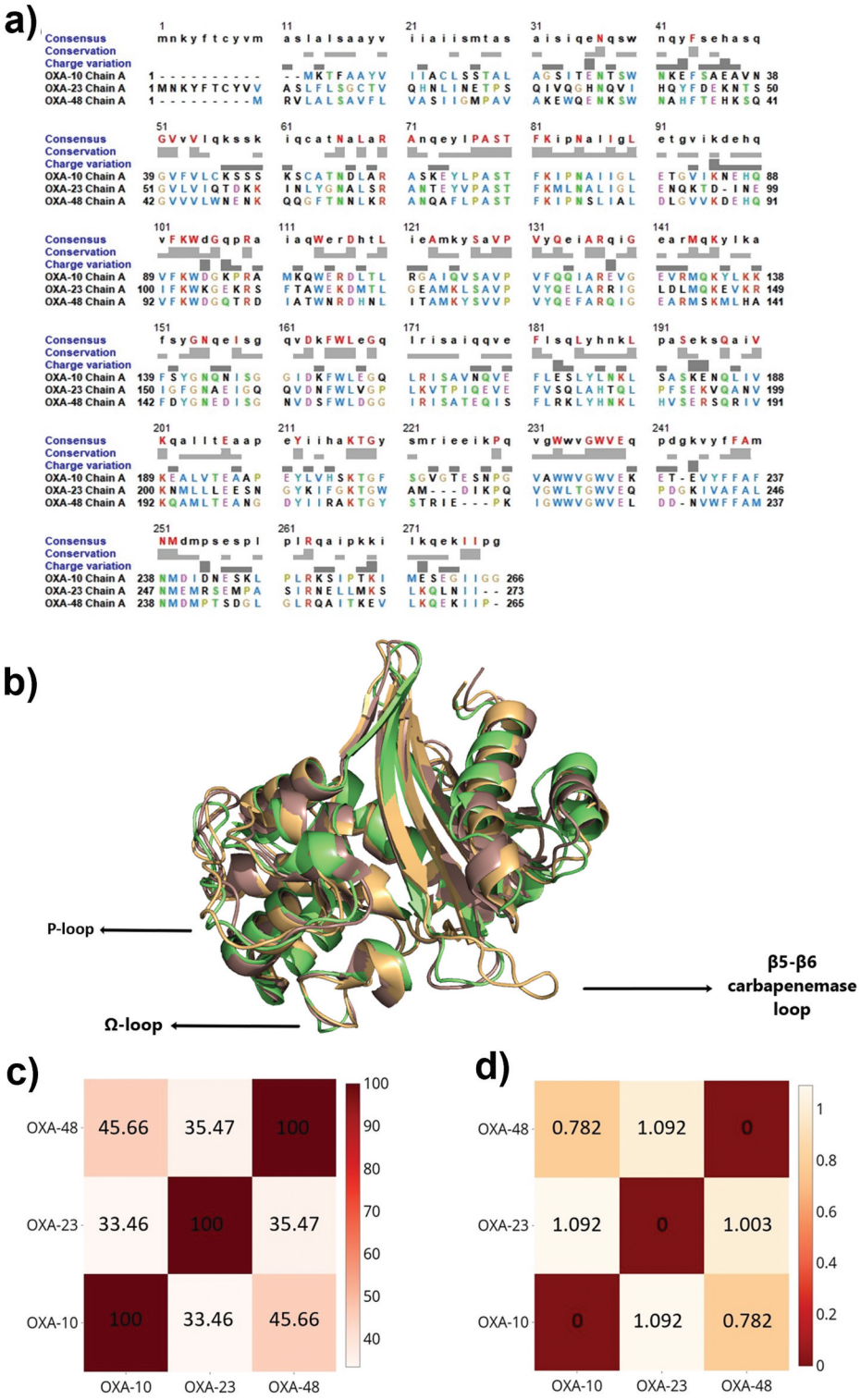

Figure 1. a) Multiple sequence alignment of OXA-10 (PDB ID: 1FOF), OXA-23 (PDB ID: 4K0X), and OXA-48 (PDB ID: 3HBR). Consensus residues are colored and indicated in the upper case at the top row. The degree of conservation is presented in light grey. b) Aligned 3D structures of OXA-10 (light Orange), OXA-23 (pale green), and OXA-48 (dark salmon) highlighting the differences occurring especially on important loops surrounding active site cleft. c) Heatmap of sequential identities on a scale of 0 to 100, where 100 denotes the highest similarity. d) Heatmap of RMSD differences, where 0 denotes the highest similarity.

motifs (Table 1) present in this region are important for activity. Active site residues of OXA-10 [58,60–63], OXA-23 [57,64–66], and OXA-48 [51,61,62,67–69] are summarized in Table 2 and presented in Figure 3. Overall, the total number of active site residues (marked in bold in Table 2) is highest in OXA-10, while the least number of active site residues is in OXA-48. For the most part, many of the residues are conserved in the primary sequences and on the superimposed structures. The strictly conserved residues Ser70, Lys73, Ser118, Lys216, and Gly218 (given in DBL numbering) [70] are overlaid when superimposed,

Table 1. Conserved motifs of OXA-10, OXA-23, and OXA-48 enzymes.

	OXA-10	OXA-23	OXA-48
Conserved motifs with DBL numbering [59]			
SER^{70}-x-x-LYS	Ser^{67}-Thr-Phe-Lys	Ser^{79}-Thr-Phe-Lys	Ser^{70}-Thr-Phe-Lys
SER^{118}-x-VAL	Ser^{115}-Ala-Val	Ser^{126}-Ala-Val	Ser^{118}-Val-Val
LYS^{216}-THR-GLY	Lys^{205}-Thr-Gly	Lys^{216}-Thr-Gly	Lys^{208}-Thr-Gly
Additional common motifs with DBL numbering [59]			
GLY^{40}-VAL-x-VAL	Gly^{39}-Val-x-Val	Gly^{51}-Val-x-Val	Gly^{42}-Val-x-Val
PHE^{87}-LYS-TRP-x-GLY	Phe^{90}-Lys-Trp-x-Gly	Phe^{101}-Lys-Trp-x-Gly	Phe^{93}-Lys-Trp-x-Gly
PHE^{163}-TRP-LEU **GLY^{235}-TRP-VAL-GLU**	Phe^{153}-Trp-Leu Gly^{224}-Trp-Val-Glu	Phe^{164}-Trp-Leu Gly^{232}-Trp-Val-Glu	Phe^{156}-Trp-Leu Gly^{224}-Trp-Val-Glu

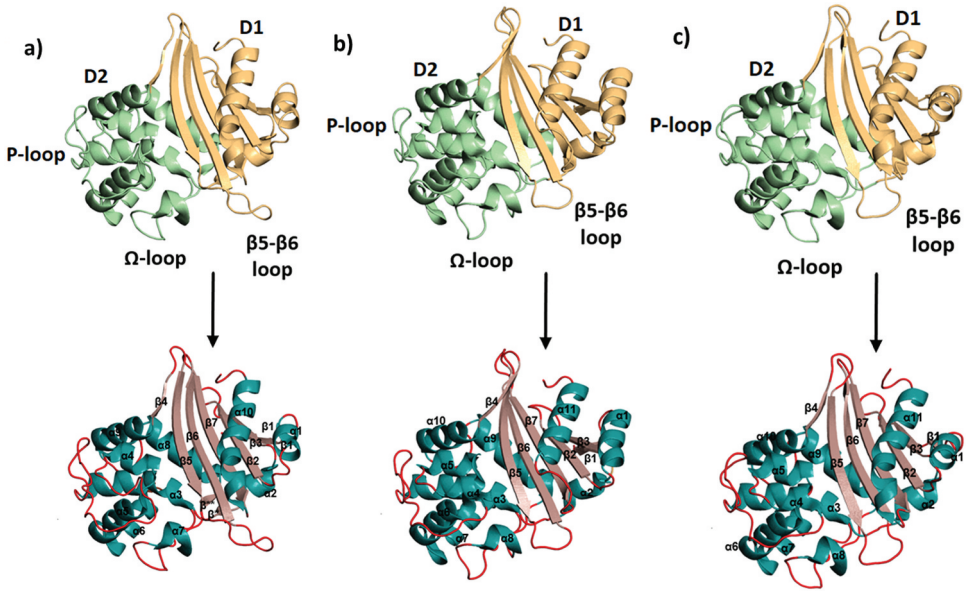

Figure 2. Domains (top row) and secondary structural elements (bottom row) of OXA-10, OXA-23, and OXA-48. First domain (D1) is shown in yellow, the second domain (D2) is shown in lime green, α-helices are shown in dark green, β-strands are shown in pink and the loops are shown in red. a) OXA-10 (PDB ID: 1FOF) α1 (28–34), α2 (55–60), α3 (65–80), α4 (107–114), α5 (116–128), α6 (127–139), α7 (152–157), α8 (163–175), α9 (181–192), α10 (243–261): β1 (21–24), β2 (39–45), β3 (50–53), β* (62–63), β**(162–163), β4 (193–196), β5 (200–208), β6 (218–228), β7 (231–241). b) OXA-23 (PDB ID: 4K0X),: α1 (37–47), α2 (67–73), α3 (77–92), α4 (110–114), α5 (118–126), α6 (127–139), α7 (139–151), α8 (163–168), α9 (173–186), α10 (192–203) α11 (257–270); β1 (32–34), β2 (51–56), β3 (61–65), β4 (204–208), β5, (211–220), β6 (226–235), β7 (241–250). **c)** OXA-48 (PDB ID: 3HBR) α1 (31–37), α2 (58–63), α3 (68–83), α4 (102–106), α5 (110–117), α6 (119–131), α7 (131–143), α8 (155–160), α9 (166–178), α10 (184–195), α11 (243–261); β1 (26–27), β2 (42–48), β3 (53–56), β* (65–66), β** (164–165), β4 (196–199), β5 (204–212), β6 (219–227), β7 (232–240) (β* and β** not shown).

though the last three are present in the active site of OXA-23 only, none of these three residues contribute to the active site in OXA-48. Found within these residues are the active-site Ser70 and the carboxylated Lys73 [16]. This carboxylysine residue acts as the general base in the hydrolysis mechanism, playing a particularly essential role in the deacylation step of catalysis [71,72]. The residues 102, 105, 120, 165, 219, and 260 (DBL numbering) [59] confer hydrophobic character to the active site region. The residues 120 and 165 (DBL numbering) are key elements for carbapenem binding [51–53,57,73,74]. These conserved residues interact with the antibiotics through hydrophobic interactions, thus, contribute to the adoption of different tautomerizations [52,53].

In the active sites of the enzymes, there is an 'oxyanion hole' (Figure 4), which is responsible for the tetrahedral oxyanion intermediate stabilization (Table 3). Of the residues stabilizing the oxyanion hole, the first one is the conserved active-site serine residue while the second one is an aromatic amino acid. The identity of the aromatic amino acid is different in all three OXA enzymes. These aromatic residues are found as a part of a nonpolar patch important for binding substrate side-chains [15]. A water molecule resides in the oxyanion hole of all three enzymes, OXA-10 [16], OXA-23 [75], and OXA-48 [51]:, in their apo structures.

Table 2. Structural alignment of active site residues of OXA-10, OXA-23, and OXA-48 [51,52,56,57].

DBL numbering [59]	OXA-10	OXA-23	OXA-48
69	**Ala66**	**Ala78**	**Ala69**
70	**Ser67**	**Ser79**	**Ser70**
73	**Lys70^{CX}**	**Lys82^{CX}**	**Lys73^{CX}**
76	**Asn73**	Asn85	Asn76
102	**: Met99**	**: Phe110**	**: Ile102**
105	**Trp102**	Trp113	**Trp105**
118	**Ser115**	**Ser126**	**Ser118**
120	**Val117**	**Val128**	**Val120**
123	**: Phe120**	: Tyr131	: Tyr123
124	**Gln121**	Gln132	Gln124
164	*Trp154*	*Trp165*	*Trp157*
165	**Leu155**	**Leu166**	**Leu158**
216	**Lys205**	**Lys216**	Lys208
217	**Thr206**	**Thr217**	Thr209
218	Gly207	**Gly218**	Gly210
219	: PHE208	: TRP219	: TYR211
220	**Ser209**	: Ala220	**Ser212**
221	**Gly210**	**Met221**	**Thr213**
225	Glu214	Asp222	**Arg214**
257	**Glu244**	. Glu253	. Ser242
260	**Leu247**	Ala256	Leu247
263	**Arg250**	**Arg259**	**Arg250**

* Amino acids in bold are active site residues. Residues stabilizing the oxyanion cleft of the active region are capitalized, residues connecting the conserved active Ser and Lys residues are italicized, and carboxylated Lys residues are labeled with 'CX'.

The active sites of the enzymes are surrounded mainly by the two characteristic loops, the Ω-loop, residing in D2, and the carbapenemase loop, connecting β5 and β6 in D1 (β5-β6 loop) (Figure 3). Variations in these loops are responsible for the differences in observed activities and substrate profiles. The Ω-loop, which has a variable length, forms one side of the active site [15]. It adopts similar conformations in all three enzymes. While in OXA-10 this loop connects α6 and α7; in OXA-23 and OXA-48, it connects α7 and α8. It is also shorter in size and has a more compact conformation in OXA-10 [58]. The absence of this loop is found to affect not only antibiotic recognition profiles but also acylation by third-generation cephalosporins [76]. The β5-β6 loop residing close to the active site connects two β-strands and delimits one side of the active site in OXA-23 and OXA-48, while folds in the opposite direction in OXA-10, pointing outwards to the active site [51,56,58]. Insertions and deletions within this loop have been shown to affect functional properties [52] and the substrate profiles, particularly for carbapenems [62]. In OXA-23 and OXA-48, this loop has similar folds and extends towards the outer portion of the active site crevice, changing its charge and narrowing its width. In OXA-10, it adopts an open conformation easing the passage of water molecules, which can be related to the weaker carbapenemase activity but stronger activity against bulky chain antibiotics, such as penicillin due to the larger entrance region [1,2]. An additional study showed that OXA-10 adopted carbapenemase activity when its β5-β6 loop has been replaced with the β5-β6 loop of OXA-48 or OXA-24. However, oxacillinase, ampicillinase, and cephalotinase activities were not altered in the same variants, pointing out their independence from this loop [62].

OXA-23 displays carbapenemase activity as a result of mutations and mechanisms it has evolved [57,66]. A conformational change in OXA-23 characterized by turning of the Ω-loop element, Leu166, away from the Val128 side chain upon substrate binding, plays a key role in providing access for the hydrolytic water molecule to the N-carboxylated Lys for efficient deacylation of the bound substrate. In OXA-23, there is a tunnel-like entrance to the active site forming a hydrophobic barrier to give access only to certain substrates [52,57]. The hydrophobic bridge that forms between Phe110 and Met221 of OXA-23 narrows its active site significantly. Hydrophobic-bridge deficient OXA-23 mutants exhibit lower carbapenemase activity [57]. This bridge was initially hypothesized to be responsible for carbapenemase activity in class D β-lactamases but later it was found not to be a universal feature for activity [70,77], e.g. it is not found in OXA-10 and OXA-48 [53]. Due to the absence of the hydrophobic bridge, OXA-48 has a more open active site [78]. OXA-48 has enhanced hydrolytic activity against carbapenems like OXA-23, yet its functional mechanism is almost completely different [53]. The carboxylate group of the Lys73 (DBL numbering) is responsible for activation of the water molecule which leads to deacylation and it is protected by a hydrophobic patch formed by juxtaposed aliphatic residues, Val120 and Leu165 (DBL numbering) that form the 'deacylating water

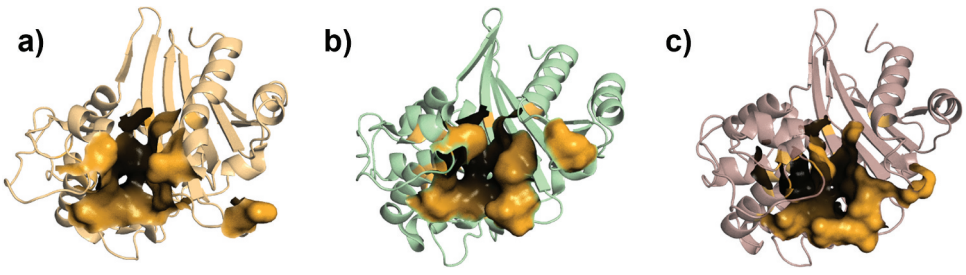

Figure 3. Active site regions of a) OXA-10, b) OXA-23, and c) OXA-48 are shown on the 3D structures.

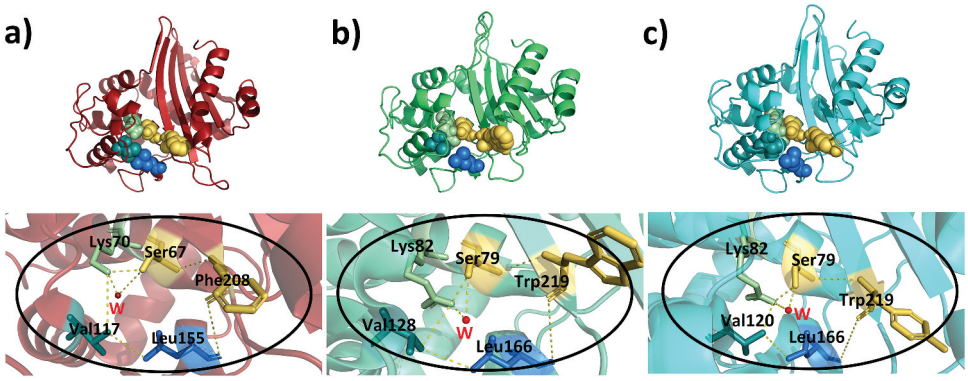

Figure 4. Oxyanion hole of a) OXA-10, b) OXA-23, and c) OXA-48. In the above panels, residues surrounding and stabilizing the oxyanion holes are represented with spheres. In the below panels, the zoomed views of the oxyanion holes with stick representation of the stabilizing residues which form the crevice are given. The water molecules are shown in red and the stabilizing residues are shown in yellow-orange spheres.

Table 3. Residues correlated with the oxyanion hole.

	Residues around the oxyanion hole	Residues stabilizing the oxyanion hole
OXA-10	Ser67, Lys70, Val117, Leu155	Ser67, Phe208
OXA-23	Ser79, Lys82, Val128, Leu166	Ser79, Trp219
OXA-48	Ser70, Lys73, Val120, Leu158	Ser70, Tyr211

channel'. In OXA-23, the surface is fully closed over the lysine and this channel opens via the movement of residues of the hydrophobic patch upon substrate binding, while in OXA-48 there is a preexisting channel between the hydrophobic patch and the catalytic serine, which slightly widens upon substrate binding [57,77]. On the other hand, OXA-48 has lower activity against the antibiotics with bulkier side-chain substituents [52] compared to OXA-10, which is consistent with its higher carbapenemase activity and narrower active site [78,79].

Substrate and inhibitor profiles of OXA-10, OXA-23, and OXA-48

Substrate profiles of OXA-10, OXA-23, and OXA-48 can be determined by measuring the kinetic parameters such as k_{cat} and K_m. As a general rule, the k_{cat}/K_m ratio provides an idea about the catalytic efficiency of the enzymes, e.g. a high k_{cat}/K_m value would indicate high catalytic efficiency despite low k_{cat} and K_m values. Thus, the actual catalytic efficiency of a β-lactamase against a β-lactam antibiotic is commonly determined by evaluating k_{cat} and k_{cat}/K_m values together [80]. Following this, the kinetic values presented in Tables 4, 5, and 6 clearly show that OXA-10, OXA-23, and OXA-48 display diverse substrate specificities. However, overall, they hydrolyze penicillins more efficiently when compared to other classes of β-lactams. Despite their higher affinity for carbapenems (in the nanomolar range for selected antibiotics), their hydrolysis is very slow. This demonstrates that hydrolysis of cephalosporins and monobactams is not as efficient as penicillins.

In literature, reports on the kinetic parameters for different class D β-lactamases are available. However, there exist variations in the reported values for OXA-10, OXA-23, and OXA-48, even with the same substrate. The major reason for this discrepancy is whether or not a CO_2 source (such as $NaHCO_3$) is present during kinetic measurements. Class D β-lactamases require a CO_2 source for the N-carboxylation of the catalytic lysine [16]. In the absence of a CO_2 source, the enzyme may be inactive, which in turn significantly alters kinetic measurements. Some other studies also explain this discrepancy among the reports by the difference in the enzyme formulations employed, which could be crude extracts, partially purified enzymes, or enzymes that lose part of their activity upon purification [30].

Following the discovery of β-lactamase inhibitors, which can be coadministered with β-lactam antibiotics to restore drug efficacies, much research was directed towards finding new inhibitor molecules to be used in combinatorial therapies. The evaluation of the efficiency of an inhibitor molecule requires the measurement of additional kinetic parameters, IC_{50} and/or K_i. In Table 7 presented are the kinetic parameters of different inhibitors tested against OXA-10, OXA-23, and OXA-48.

First-generation inhibitors (clavulanic acid, sulbactam, and tazobactam) are effective against class A serine β-lactamases, while they are proven to be futile against class C and class D serine β-lactamases. The limited spectrum of first-generation inhibitors necessitated the development of novel β-lactamase inhibitors [81]. Currently, as new generation inhibitors, different inhibitor scaffolds are available; such as diazabicyclooctane (DBO) derivatives, boronic acid

Table 4. Kinetic values of OXA-10 β-lactamase against β-lactam antibiotics.

	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($mM^{-1}.s^{-1}$)
Carbapenems			
Imipenem	<0.1 [62]	0.04 ± 0.007 [62]	ND [62]
	0.041 ± 0.001 [30]	2.0 ± 0.1 [30]	21 ± 3 [30]
	0.047 ± 0.001 [31]	>2 [31]	>23 [31]
Meropenem	0.039 ± 0.001 [30]	5.6 ± 0.8[30]	7 ± 0.2 [30]
	0.023 ± 0.001[31]	>2 [31]	>12 [31]
Ertapenem	0.022 ± 0.001 [30]	4.1 ± 0.5 [30]	5.4 ± 0.7 [30]
Doripenem	0.037 ± 0.001 [30]	4.8 ± 0.8 [30]	8 ± 1 [30]
Penicillins			
Benxylpenicillin	89 ± 10 [60]	63 ± 6 [60]	$1.41x10^3$ [60]
(Penicillin G)	109 ± 3 [16]	23 ± 0.4 [16]	$(5 ± 1)x10^3$ [16]
	120 ± 10 [104]	20 ± 1 [104]	$(6.0 ± 0.6)x10^3$ [104]
	120 ± 5 [105]	20 ± 1 [105]	$(6 ± 0.4)x10^3$ [105]
	91 ± 1 [31]	14 ± 1 [31]	$6.7x10^3$ [31]
Oxacillin	608 ± 10 [60]	222 ± 16 [60]	$2.74x10^3$ [60]
	$(1.26 ± 0.03)x10^3$ [16]	29 ± 2 [16]	$(43 ± 3)x10^3$ [16]
	300 ± 10 [104]	100 ± 20 [104]	$(3 ± 0.6)x10^3$ [104]
	300 ± 4 [105]	100 ± 20 [105]	$(3 ± 0.6)x10^3$ [105]
	660 [62]	96 [62]	$6.9x10^3$ [62]
	530 ± 10 [30]	87 ± 5 [30]	$(6.1 ± 0.3)x10^3$ [30]
	346 ± 3 [31]	65 ± 5 [31]	$5.3x10^3$ [31]
Ampicillin	$(5.59 ± 0.03)x10^3$ [60]	235 ± 30 [60]	$2.5x10^3$ [60]
	143 ± 7 [16]	34 ± 4 [16]	$(4.2 ± 0.6)x10^3$ [16]
	220 ± 20 [104]	35 ± 5 [104]	$(6.02 ± 0.1)x10^3$ [104]
	220 ± 10 [105]	35 ± 5 [105]	$(6.3 ± 0.9)x10^3$ [105]
	530 [62]	77 [62]	$6.9x10^3$ [62]
Carbenicillin	31 ± 1 [60]	195 ± 13 [60]	159 [60]
	112 ± 14 [16]	92 ± 16 [16]	$(1.2 ± 0.3)x10^3$ [16]
Cloxacillin	530 ± 36 [60]	$(2.64 ± 0.3)x10^3$ [60]	196 [60]
	$(1.53 ± 0.02)x10^3$ [16]	114 ± 22 [16]	$(13 ± 3)x10^3$ [16]
	120 ± 10 [104]	110 ± 10 [104]	$(1 ± 0.1)x10^3$ [104]
Cephalosporins			
Cephalothin	6 ± 0.1 [60]	38 ± 2 [60]	158 [60]
	8.3 ± 0.1 [16]	32 ± 2 [16]	260 ± 10 [16]
	2.5 ± 0.1 [104]	7 ± 1 [104]	380 ± 50 [104]
	10 ± 1 [105]	35 ± 2 [105]	300 ± 30 [105]
	2.9 ± 0.1 [62]	11 ± 1.4 [62]	260 [62]
	1.07 ± 0.03 [31]	4.4 ± 1.4 [31]	250 [31]
Cephaloridine	79 ± 8 [60]	$(2.34 ± 0.3)x10^3$ [60]	33 [60]
	57 ± 14 [16]	374 ± 94 [16]	150 ± 50 [16]
	70 ± 20 [104]	400 ± 100 [104]	180 ± 60 [104]
Cefoxitin	>0.07 [31]	>200 [31]	0.38 ± 0.012 [31]
Cefotaxime	9 ± 0.2 [60]	346 ± 19 [60]	26 [60]
	2.06 ± 0.19 [31]	104 ± 17 [31]	19 [31]
Ceftriaxone	3 ± 0.3 [60]	55 ± 2 [60]	54 [60]
Ceftazidime	ND [60]	ND [60]	ND [60]

ND: Hydrolysis not detected.

derivatives, and β-lactam derivatives. Unlike first-generation inhibitors, DBO and boronic acid derivatives have non-β-lactam scaffolds that also target the active site of the enzyme [81]. The DBO inhibitors display different activities against OXA-10, OXA-23, and OXA-48. For example; OXA-48 is more susceptible to avibactam with IC_{50} values in the nanomolar range and with a k_2/K value of $(1.4 ± 0.1)x10^3$ M^{-1} s^{-1}, while OXA-10 is not susceptible at all. On the other hand, the same DBO moderately inhibits OXA-23. Other DBO-derived inhibitors, durlobactam, ETX1317, and WCK4234 are potent inhibitors for all three enzymes. Among the boronic acid inhibitors; taniborbactam shows inhibitory effect against OXA-10 and OXA-48. QPX7728 shows a high affinity for OXA-23 and OXA-48 with very low K_i values. IC_{50} values of this inhibitor are very low, which indicates its great potential for clinical use. LN-1-255 is a β-lactam inhibitor among these new generation inhibitors with higher or broader activity than the first-generation inhibitors. Kinetic values show that this inhibitor has a significant inhibitory effect on OXA-23 and OXA-48 enzymes (Table 7). Other novel inhibitors such as ANT2681 (Antabio), J-110,441, and J-111,225 have displayed potency against other classes of β-lactamases but information on their activities against OXA-10, OXA-23, and OXA-48 is not available.

Computational studies on OXA-10, OXA-23, and OXA-48

Computational studies gain increasing attention since they not only shed light on the mechanistic details at the structural level but also support experimental findings. To this end, several approaches, e.g. molecular

Table 5. Kinetic values of OXA-23 β-lactamase against β-lactam antibiotics.

	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($mM^{-1}.s^{-1}$)
Carbapenems			
Imipenem	0.35 ± 0.01 [57]	4.8 ± 0.3 [57]	74 ± 4 [57]
	2.8 [65]	6.26 [65]	450 [65]
	0.490 ± 0.01 [66]	0.204 ± 0.023 [66]	(2.4 ± 0.3)x10^3 [66]
	0.35 ± 0.01 [30]	≤2.0 [30]	≥180 [30]
	0.5 [106]	80 [106]	6 [106]
Meropenem	0.068 ± 0.001 [57]	≤1 [57]	≤68 [57]
	0.7 [65]	17 [65]	40 [65]
	0.068 ± 0.001 [30]	≤2.0 [30]	≥34 [30]
Ertapenem	0.021 ± 0.001 [57]	0.50 ± 0.10 [57]	42 ± 8 [57]
	0.021 ± 0.001 [30]	≤2.0 [30]	≥ 11 [30]
Doripenem	0.036 ± 0.001 [57]	0.70 ± 0.09 [57]	52 ± 7 [57]
	0.028 ± 0.003 [66]	0.018 ± 0.002 [66]	(1.5 ± 0.3)x10^3 [66]
	0.036 ± 0.001 [30]	≤2.0 [30]	≥18 [30]
Penicillins			
Benzylpenicillin	78 [65]	188 [65]	400 [65]
(Penicillin G)	40 [106]	60 [106]	670 [106]
Oxacillin	320 ± 10 [30]	110 ± 10 [30]	(3.1 ± 0.2)x10^3 [30]
Ampicillin	460 ± 10 [66]	82 ± 9 [66]	(5.7 ± 0.6)x10^3 [66]
	31 [65]	161 [65]	200 [65]
	<0.1 [106]	ND* [106]	ND* [106]
Ticarcillin	7 [106]	70 [106]	100 [106]
Piperacillin	47 [65]	302 [65]	150 [65]
Cephalosporins			
Cefotaxime	5.5 ± 0.1 [66]	340 ± 30 [66]	16 ± 2 [66]
Ceftriaxone	0.016 ± 0.001 [66]	3.7 ± 0.5 [66]	4.4 ± 0.7 [66]
Ceftazidime	<0.01 [66]		
Cefepime	3 [65]	173 [65]	18 [65]
	20 ± 1 [73]	(1.1 ± 0.2)x10^3 [73]	18 ± 3 [73]
Cefiderocol	<0.1 [106]	ND [106]	ND [106]
Monobactams			
Aztreonam	0.24 ± 0.01 [66]	(2.4 ± 0.14)x10^3 [66]	0.10 ± 0.01 [66]

ND*: not determined due to instability of the hydrolysis complex.
ND: Hydrolysis not detected.

docking and molecular dynamics (MD), are commonly employed to study enzymes (Figure 5). The primary goal in such studies is usually to elucidate catalytic mechanisms, to find potential inhibitors, and to understand inhibition mechanisms at the molecular level. Computational docking and MD simulation studies of OXA enzymes with a specific emphasis on OXA-10, OXA-23, and OXA-48 structures and their selected variants are briefly summarized below.

In one of the earlier studies of OXA-48, the crystallized structure of the protein (PDB ID: 3HBR) was used for computations to get an insight into the mechanism of carbapenem hydrolysis [51]. Molecular docking followed by 10 ns MD simulations were conducted with the inhibitor (meropenem) bound structure to identify critical residues for carbapenem hydrolysis. The structural basis for different functional properties of the OXA enzymes toward carbapenems and oxacillins was investigated and a new catalytic mechanism, which relied on the nature and conformation of the β5-β6 loop residues was proposed. A comparative docking study by Stojanoski et al. (2015) on OXA-48 and OXA-163 structures undertook an effort to understand the underlying mechanism in their different substrate specificities [107]. Different than OXA-48 by four amino acid deletions (214-Arg-Ile-Glu-Pro -217) in the loop region connecting β5 and β6 strands and one substitution on the β5-strand (Ser212Asp), OXA-163 displays increased cephalosporinase and decreased carbapenemase activities due to an enlargement in the active site. A later study by Pina Vaz et al. (2016) tried to understand the affinity of different drugs to different types of enzymes produced by *Enterobacteriaceae* using experimental methods complemented with computational approaches [82]. Their results showed that among ertapenem, doripenem, meropenem, and imipenem; ertapenem had the highest affinity, while imipenem had the lowest. A similar combined approach of experimental and molecular docking techniques to investigate OXA enzymes was by Fröhlich et al. (2019), which focused on the effects of ceftazidime (CAZ) and CAZ-AVI (Ceftazidime-Avibactam) on OXA-48 and the epidemic OXA-48 plasmid in *Escherichia coli* [79]. Based on their experiments with both wild-type and mutated OXA-48 structures (OXA-48: Pro68Ala, Tyr211Ser), they concluded that CAZ hydrolysis is mechanistically infeasible in OXA-48 and that Pro68Ala mutation results in increased activity towards CAZ, whereas Pro68Ala and Tyr211Ser mutations together lead to decreased activity. Since crystallization of the structure with

Table 6. Kinetic values of OXA-48 β-lactamase against β-lactam antibiotics.

	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($mM^{-1}.s^{-1}$)
Carbapenems			
Imipenem	2 [50]	14 [50]	145 [50]
	4.8 [51]	13 [51]	370 [51]
	22.48 [69]	28.3 [69]	794 [69]
	6.7 ± 0.2 [30]	5.3 ± 0.6 [30]	(1.3 ± 0.2)x10^3 [30]
	2.7 ± 0.2 [107]	3.7 ± 0.7 [107]	900 [107]
	4.5 ± 0.8 [108]	7.9 ± 0.1 [108]	570 [108]
	1.5 ± 0.1 [109]	60.3 ± 12.4 [109]	25 [109]
	1 [106]	15 [106]	70 [106]
	4.8 ± 0.2 [79]	13 ± 2 [79]	365 ± 71 [79]
	11.4 ± 0.5 [110]	57.7 ± 9.3 [110]	200 ± 30 [110]
	5 ± 0.3 [111]	2.3 ± 0.07 [111]	(2.0 ± 0.2)x10^3 [111]
Meropenem	0.1 [50]	200 [50]	0.5 [50]
	0.07 [51]	11 [51]	6.2 [51]
	0.112 [69]	5.5 [69]	20.4 [69]
	0.16 ± 0.01 [30]	≤2.0 [30]	≥80 [30]
	0.11 ± 0.01 [107]	6.0 ± 1.2 [107]	17 [107]
	0.098 ± 0.005 [108]	1.0 ± 0.2 [108]	98 [108]
	0.71 ± 0.02 [79]	4 ± 1 [79]	177 ± 50 [79]
	0.087 ± 0.01 [110]	<1.9 [110]	<45 [110]
	0.12 ± 0.001 [111]	0.06 ± 0.002 [111]	(1.9 ± 0.1)x10^3 [111]
Ertapenem	0.13 [51]	100 [51]	1.3 [51]
	0.112 [69]	2.4 [69]	46.7 [69]
	0.067 ± 0.001 [30]	≤2.0 [30]	≥34 [30]
	0.3 ± 0.02 [109]	123.7 ± 36.2 [109]	2 [109]
	0.03 ± 0.002 [111]	0.2 ± 0.003 [111]	200 ± 30 [111]
Doripenem	ND [69]	≤2.0 [30]	≥70 [30]
	0.14 ± 0.01 [30]	4.1 ± 0.6 [107]	16 [107]
	0.066 ± 0.002 [107]		
Panipenem	1.4 [51]	14 [51]	100 [51]
Faropenem	0.038 [51]	13 [51]	2.9 [51]
Penicillins			
Benzylpenicillin	245 [50]	40 [50]	6.1x10^3 [50]
(Penicillin G)	446 [112]	79 [112]	5.6x10^3 [112]
	(8.0 ± 1.1)x10^3 [108]	700 ± 300 [108]	1.1x10^4 [108]
	1750 [106]	200 [106]	8.75x10^3 [106]
Oxacillin	25 [50]	30 [50]	850 [50]
	130 [51]	95 [51]	1.4x10^3 [51]
	160 ± 10 [30]	≤30 [30]	≥6x10^3 [30]
Ampicillin	340 [50]	5200 [50]	65 [50]
	955 [51]	395 [51]	2.4x10^3 [51]
	1349 [69]	572.7 [69]	2.36x10^3 [69]
	560 ± 30 [108]	150 ± 30 [108]	3.7x10^3 [108]
	370 [106]	1.1x10^3 [106]	340 [106]
	608 ± 53 [79]	370 ± 70 [79]	(1.64 ± 0.46)x10^3 [79]
Ticarcillin	45 [50]	55 [50]	820 [50]
	70 [106]	90 [106]	780 [106]
Piperacillin	75 [50]	410 [50]	180 [50]
	3.9 ± 0.5 [79]	898 ± 155 [79]	4 ± 1 [79]
Temocillin	0.3 [51]	45 [51]	6.6 [51]
Cephalosporins			
Cephalothin	3 [50]	20 [50]	150 [50]
	44 [51]	195 [51]	230 [51]
	2.8 ± 0.1 [107]	140 ± 10 [107]	20 [107]
Cephaloridine	2 [50]	27 [50]	75 [50]
Cefoxitin	>0.05 [51]	>200 [51]	0.26 [51]
Cefotaxime	10 [50]	190 [50]	60 [50]
	>9 [51]	>900 [51]	10 [51]
	ND [107]	>1.0x10^3 [107]	4.7 [107]
	<0.001 [111]	174 ± 14 [111]	<0.006 [111]
Ceftazidime	4 [50]	5.1x10^3[50]	1 [50]
	ND [69]	ND [107]	ND [107]
	ND [107]	300 ± 150 [79]	10 ± 8 [79]
	3.0 ± 0.8 [79]	(9.9 ± 0.74)x10^3 [111]	ND [111]
	ND [111]		
	ND [51]		
Cefepime	1 [50]	160 [50]	6 [50]
	>0.6 [51]	>550 [51]	1.1 [51]
	7.87 [69]	2514.5 [69]	3.13 [69]
	9 ± 2 [108]	300 ± 110 [108]	30 [108]
	1.7 ± 0.6 [79]	(1.68 ± 0.7)x10^3 [79]	1 ± 0.8 [79]
Cefpirome	8 [50]	390 [50]	20 [50]
Cefiderocol	<0.1 [106]	ND [106]	ND [106]
Monobactams			
Aztreonam	ND [50]	ND [50]	ND [50]

ND: Hydrolysis not detected.

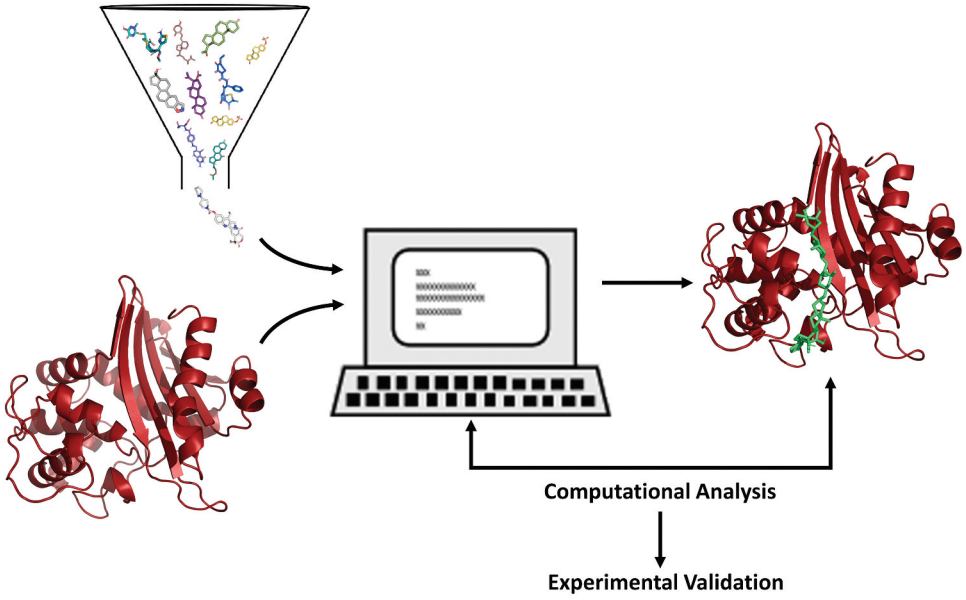

Figure 5. Schematic representation of computational workflow for drug discovery.

these two mutations (OXA-48: Pro68Ala, Tyr211Ser) was unsuccessful, computational modeling was helpful to get a deeper understanding of the underlying mechanism. More recently, Hirvonen et al. (2020) [78] used QM/MM and MD simulations to understand cephalosporin breakdown by OXA-48 and OXA-163 variants. They reported that in OXA-163, extra water molecules were able to enter the active site accelerating ceftazidime breakdown, which suggested that the differences in the variants can be related to changes in solvation. In another study, molecular docking calculations were performed with imipenem and temocillin to shed light on the observation of different hydrolytic parameters [83]. They suggested alternative binding modes and conformations. Together with their experimental observations, which were consistent with computational results, their mutational analysis showed that residue 214 in OXA-48-like β-lactamases is critical for carbapenemase activity. More recently, Pestana-Nobles et al. (2022) [84] conducted a computational study based on molecular docking and MD simulations aiming to find new inhibitors from microalgal metabolites against six β-lactamase enzymes including OXA-48. Their computational studies revealed that metabolites belonging to the same structural families, (phenylacridine (4-Ph), quercetin (Qn), and cryptophycin (Cryp)), exhibit better performance with β-lactamase than the existing commercial inhibitors (clavulanic acid, sulbactam, and tazobactam). To this end, they suggested the usage of these metabolites as novel inhibitors as well as possible structural templates to be used in further studies.

One of the first modeling studies with OXA-10 was conducted by Johnson et al. (2010) [85]. As they re-evaluated the cyclobutanone analogs as potential inhibitor compounds, they modeled enzyme-inhibitor complexes to gain insight into specific active site interactions and provide support for the inhibition observed with the synthesized molecules. Though their inhibition was modest, with molecular modeling studies they were able to suggest modifications on the inhibitor compounds to increase their affinity with the enzymes. Malathi et al. (2016) conducted molecular docking and MD simulations with imipenem and imipenem-like drugs retrieved from the ZINC database [86] not only to propose new non-hydrolyzing inhibitors for *P. aeruginosa* OXA-10 (PDB: 1FOF) but also to understand the resistance mechanism at a structural level [87]. They came up with the molecule ZINC44672480 possessing ideal characteristics to be a potential inhibitor. In another study, Singh et al. (2017) [88] focused on an OXA-10 from an *A. baumannii* strain. They first constructed the 3D structure of OXA-10 from *A. baumannii* by homology modeling and then carried out molecular docking with different protease inhibitor compounds of cyanobacterial origin. They suggested kempopeptin as a potential inhibitor to be tested for its *in vivo* inhibitory activities. In the studies by Kotsakis et al. (2019) and Leiros et al. (2020), the focus was on the differences among OXA-10, OXA-656, and OXA-655.

Table 7. Kinetic parameters of different inhibitors tested against OXA-10, OXA-23, and OXA-48.

		OXA-10	OXA-23	OXA-48
FIRST GENERATION INHIBITORS				
Clavulanic acid	IC50 (nM)	810 [113]	$2.1x10^8$ [65]	$1.6x10^4$ [114] $2.85x10^4$ [115]
	K_i (nM)	$(1.04 \pm 0.02)x10^5$ [116]		
Sulbactam	IC50 (nM)	$3.7x10^4$ [113]	$3.3x10^9$ [65]	$5x10^4$ [114]
	K_i (nM)		$1.3x10^5$ [117]	
	k_{inact}/K_i (M^{-1} s^{-1})	22 [117]		4 [117]
Tazobactam	IC50 (nM)	$9.4x10^2$ [113]	$(1.5 \pm 0.2)x10^3$ [118] $(2.13 \pm 0.80)x10^3$ [90] $3.1x10^7$ [65]	550 [119] $(1.5 \pm 0.5)x10^3$ [109] $1.7x10^3$ [114] $2.0x10^4$ [115]
	K_i (nM)	$(1.7 \pm 0.17)x10^5$ [116]	$(1.14 \pm 0.25)x10^4$ [90]	$(3 \pm 0.3)x10^4$ [109]
	k_{inact}/K_i (M^{-1} s^{-1})		$(1.18 \pm 0.078)x10^3$ [90]	$(3 \pm 0.5)x10^3$ [109]
	k_2/K (M^{-1} s^{-1})		193.53 ± 39.10 [90]	
NEW GENERATION INHIBITORS				
Diazabicyclooctane derivatives				
Avibactam (NXL104)	IC50 (nM)	445 [61]	$1.78x10^3$ [61] $(3.1 \pm 0.6)x10^3$ [120] $(8.93 \pm 0.99)x10^3$ [90]	180 ± 50 [120] 550 [119] 593 [61] 880 [121] $(1.7 \pm 0.2)x10^3$ [118]
	K_i (nM)		$(1.7 \pm 0.4)x10^3$ [122] $>1.0x10^5$ [123]	27 ± 15 [122] 260 ± 5 [124] $(3 \pm 0.3)x10^4$ [123]
	k_{inact}/K_i (M^{-1} s^{-1})	70 [125]	100 [125] 286.60 ± 61.78 [90]	$5x10^3$ [125]
	k_2/K (M^{-1} s^{-1})	11 ± 1 [126]	300 ± 20 [127]	$(1.4 \pm 0.1)x10^3$ [126]
Relebactam (MK-7655)	IC50 (nM)			$(9 \pm 0.3)x10^4$ [120]
	K_i (nM)		$>1.0x10^5$ [123]	$>1.0x10^5$ [123]
	k_{inact}/K_i (M^{-1} s^{-1})	44 [128]	6 [128]	39 [128]
Zidebactam (WCK-5107)	IC50 (nM)			
	K_i (nM)		$>1.0x10^5$ [123]	$>1.0x10^5$ [123]
Durlobactam (EXT2514)	IC50 (nM)			
	K_i (nM)			
	k_{inact}/K_i (M^{-1} s^{-1})	$(9 \pm 2)\times10^3$ [125]	$(5.1 \pm 0.2)x10^3$ [125]	$(8 \pm 2)x10^5$ [125]
ETX1317	IC50 (nM)			77 [121]
	K_i (nM)			
	k_{inact}/K_i (M^{-1} s^{-1})	680 ± 30 [125] $8.6x10^3$ [128]	$(1.54 \pm 0.06)x10^3$ [125] $5.1x10^3$ [128]	$(5.3 \pm 0.2)x10^4$ [125] $8.3x10^5$ [128]
WCK4234	IC50 (nM)			
	K_i (nM)		$8.0x10^3$ [123]	290 [123]
	k_{inact}/K_i (M^{-1} s^{-1})	$9.5x10^3$ [128]	$1.4x10^4$ [128]	$6x10^5$ [128]
Boronic acid derivatives				
Vaborbactam (RPX7009)	IC50 (nM)	$>4x10^5$ [129]	$(1.2 \pm 0.2)x10^5$ [120]	$(6.9 \pm 2.3)x10^3$ [120] $3.2x10^4$ [129] $3.88x10^4$ [119]
	K_i (nM)		$> 4.0 \times 10^4$ [122]	$(1.4 \pm 0.5)x10^4$ [122] 350 ± 7 [124]
Taniborbactam (VNRX-5133)	IC50 (nM)	$234^-/645^+$ [130]		$537^-/2390^+$ [130] 420 [119]
	K_i (nM)			350 ± 7 [124]
QPX7728	IC50 (nM)		1.2 ± 0.4 [120]	1.1 ± 0.4 [120]
	K_i (nM)		0.74 [122]	0.28 [122]
	k_2/K (M^{-1} s^{-1})		$(9.9 \pm 0.6)\times10^5$ [120]	$(2.75 \pm 0.09)x10^6$ [120]
β-lactam derivatives				
Enmetazobactam (AAI101)	IC50 (nM)			$(11 \pm 1)x10^3$ [118]
	K_i (nM)			
LN-1-255	IC50 (nM)		12±8 [90]	3 ± 0.3 [109]
	K_i (nM)		88 ± 6 [90]	170 ± 10 [109]
	k_{inact}/K_i (M^{-1} s^{-1})		$(1.39 \pm 0.29)x10^5$ [90]	
	k_2/K (M^{-1} s^{-1})		$(2.90 \pm 0.03)x10^4$ [90]	$(10 \pm 1)\times10^4$ [109]

$^{-/+}$: in the absence/presence of 100 mM aqueous sodium bicarbonate.

An experimental study was complemented with 10 ns MD simulations to elucidate interaction profiles on a residue-based level [31,89]. Simulations revealed Val117Leu to affect internal interactions, which in turn has a role in carbapenem alignment through the structural shifts in the active site.

As for OXA-23, computational studies are very limited. In a study by Smith et al. (2013), MD simulations were conducted to complement experimental observations, which included the crystallization of OXA-23 structures of wild type strains, under varying pH conditions and in complex with meropenem (PDB ID: 4JF4,

4JF5, and 4JF6) [57]. The molecular mechanism of carbapenemase activity was investigated in detail and it was observed that the binding of the carbapenem substrate causes a substantial movement in the conserved sites, which is stabilized by the special fold of the β6/β7 loop allowing a water molecule to enter the enzyme's active site. In another study, the inhibitory activity of LN-1-255 was characterized and compared with avibactam and tazobactam against class D carbapenemases in *A. baumannii* (OXA-23, OXA-24/40, OXA-58, OXA-143, OXA-235, and OXA-51) [90]. Experimental studies followed by computational docking studies showed that LN-1-255 was effective and is a potential new inhibitor that might have a significant role against infections. A recent computational biology study aimed to find novel inhibitors for OXA enzymes (OXA-1, OXA-10, OXA-23, OXA-24/40, OXA-48, OXA-51, and OXA-58) [91] using virtual screening followed by 50 ns MD simulations. Two known structures (cilastatin and meropenem) and two newly identified potential inhibitors were docked and binding energies and bond formations were examined in detail. As a result, two new novel inhibitors were proposed (M1593 and M2680) for OXA variants that can be used as reversible competitive inhibitors.

Future prospects in novel inhibitor search

The alarming increase in the transmission of multi-drug-resistant (MDR) pathogenic bacteria, especially those expressing extended-spectrum β-lactamases, makes it very difficult to cope with such bacteria using existing drugs and approaches. Furthermore, the most widely used β-lactamase inhibitors of combinatorial treatments in the clinic are commonly ineffective against class B, C, and D enzymes. Prudent support for the development of new antibiotics and alternative solutions are required to avoid antibacterial agents exhibiting cross-resistance [92]. In this context, the focus is turned to nature, which serves as a rich source for untapped novel bioactive compounds. Indeed, clavulanic acid, the first β-lactamase inhibitor introduced into clinical medicine, is a natural product [3]. These compounds can be administered alone or in combinatorial therapies to fight against increased incidences of antibiotic resistance. Thus, natural products obtained from different sources, which are not limited to microbial and plant cells, play key roles in the discovery of new molecules with distinct antimicrobial mechanisms and multi-target properties [93].

Several experimental studies report the potential of natural compounds to inhibit the different classes of β-lactamases, also providing limited mechanistic details [94–99]. However, these studies primarily focus on enzyme classes other than D or work with a collection of strains that may synthesize OXA types along with other enzyme classes. Therefore, the specificity of the inhibitor molecule under question remains vague. Among the available experimental studies specific to OXAs, Somboro et al. (2019) [100] and Nishimura et al. (2021) [101] have reported natural product based inhibitors for OXA-48, tannic acid (a polyphenolic biomolecule) and JBIR-155 (a molecule from a *Streptomyces polymachus* strain). A few recent studies report the antibacterial and synergistic activities of natural products against OXA-48 producing *K. pneumoniae* [102] and OXA-23 producing *A. baumannii* [103], but no details on their mechanisms are available; thus it is not clear if these molecules can function as inhibitors.

Present findings strongly suggest the importance of molecules of natural origin for the identification of new compounds and medicinal leads in β-lactamase inhibition. Despite this potential, studies with natural products targeting OXA enzymes of ESKAPE bugs are very limited. The increase in the availability of structural and mechanistic details of these enzymes, combined with the development of novel computational tools, is valuable to push this rate in the discovery of new inhibitor molecules. The implementation of the power of computers and bioinformatic tools not only to find ligands targeting active sites but also to find ligands targeting allosteric sites will further increase the pace of the fight against this worldwide health crisis. Here, a challenging approach would be to design/discover a candidate molecule to target multiple pathogens.

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Disclosure statement

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