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In silico mutations of TEM-1 β-lactamase show changes in structure and drug-enzyme affinity binding by molecular docking

Jose Arturo Molina-Mora[1], Sergio Jiménez Morgan[2]

[1]Faculty of Microbiology [2]School of Medicine
[1,2] University of Costa Rica, San José, Costa Rica

Abstract: **Bacterial resistance refers to bacteria capacity to evade antibiotic action, which constitutes a public health issue. This resistance is given by β-lactamase enzymes that break the drug rings and alter its function. To counteract this effect, some β-lactamase inhibitors, that have a higher affinity and irreversibly bond, have been used. However, as a consequence of selective pressure, some mutations have caused enzyme-drug affinity changes. TEM-1 is a serine- β-lactamase in which this process has been proved, giving particular interest for evaluating how these mutations affect drug-enzyme binding force. When making simulations with four mutations M182T, V184A, T160H and A224V and undertaking molecular docking, a change in the affinity pattern was observed, aiding enzyme-antibiotic bindging rather than enzyme-inhibitor bindging, which would explain lab results in which the use of β-lactamase inhibitors has not been effective. Besides, with the purpose of exploring inhibition alternatives in the enzyme, simulations with one BLIP (β-lactamase inhibitor protein) were carried out, showing that the bond between β-lactamase and BLIP alters drug access to an active site.**

Keywords: **TEM-1 β-lactamase, molecular docking, structure simulation, antibiotic resistance.**

I. INTRODUCTION

Bacterial resistance refers to bacteria capacity to evade antibiotic action, which constitutes a public health issue [1]. In the laboratory it is very common to describe susceptibility based on minimum inhibitory concentration (MIC); this is the minimum antibiotic concentration that inhibits in vitro bacterial growing under standard conditions and determines susceptibility categories for bacterial groups and antibiotics. Action mechanisms for attacking bacteria include cell membrane alterations, protein synthesis, modifications in DNA synthesis and replication, and metabolic pathways obstruction [2].

Bacteria have developed different resistance mechanisms which even make them resistant to one or several drug groups at the same time [3], out of which β-lactam resistance mechanism is the best described [2]. Resistance mechanisms include drug receptor alteration, a decrease in the drug that may bind to the receptor by altering its entrance and easing its exit, destruction or deactivation of the drug and provision of substitute metabolic pathways [4]. These processes are not excluding, on the contrary, their combination benefits resistance to multiple antibiotics [5]. More precisely, β-lactams (including penicillins, cephalosporins and carbapenems) act by interfering with the last stage of cellular wall synthesis (transpeptidation), which allows acetyl-glucosamine and acetyl-muramic chains remain unbound causing cellular lysis by osmotic stress [6]. They may also act through autolysins activations which similarly causes bacterial destruction [7].

Resistance to β-lactams is caused by β-lactamase enzymes that break the β-lactamic ring while nucleophilic indirect activation takes place in a serine waste, serine deprotonation, the β-lactam carbonyl ring is attacked with a subsequent acylation where the acyl-enzyme complex is formed and eventually breaks the bond [8].

As a response to the high β-lactams resistance rate via β-lactamase, some antibiotics with β-lactamase inhibitor have been developed which possess a higher affinity than β-lactams, bind to β-lactamase and inhibit it, allowing β-lactams act and cause drug susceptibility [7]. Some common β-lactamase inhibitors of clinical use are Clavulanic Acid, Tazobactam and Sulbactam, which can also be used in β-lactamases A, B, C, D and E classification process. Metal-dependent ones, called Metallo-β-lactamases belong to Classes B (specifically zinc-dependent) and E; metal-independent, with serine active sites, are called serin- β-lactamases and belong to Classes A, C and D [9].

One important characteristic to phenotypically distinguish them is that serin- β-lactamases are inactivated by Clavulanic Acid, Tazobactam and Sulbactam, which makes bacterium susceptible. Metallo-β-lactamases are resistant to classical β-lactamases inhibitors, though inactivated by EDTA while cation chelating [10]. In this last case, the bacterium under study that has this type of enzyme would be resistant to β-lactamics containing β-lactamase inhibitors [11]. Again, selection pressure has led bacteria to gain gene mutations that codify for β-lactamases and allow changing affinity for chemical inhibitors or antibiotics; this results in a new resistance category in which bacteria break the β-lactamic ring and diminish the inhibitor's effect. Alternatively, some proteins that function as β-lactamase inhibitors (BLIP, β-lactamase inhibitor protein) have been discovered which, due to steric hindrance, impede access to an active site.

However, its real function remains unknown. These BLIP are produced by *Streptomyces clavuligerus*, same organism from which the molecule to produce Clavulanic Acid is extracted [12].

In this context, the aim of the study is to model structurally the mutation effect occurring in the gene that codifies for the TEM-1 β-lactamase and study its effect in interaction with Amoxicillin (β-lactamic), Clavulanic Acid (β-lactactamase inhibitor), and a BLIP by molecular docking.

II. METHODOLOGY

A. Protein Structure Selection:

*PDB (*Protein Data Bank, http://www.rcsb.org/pdb/home/home.do) was used to select the TEM-1 β-lactamase to be studied. The molecule was downloaded in pdb format for its analysis with *Chimera software* (https://www.cgl.ucsf.edu /chimera/). Using the same conditions, a search and analysis of BLIP proteins was performed in order to make the molecular simulations.

B. Drug Structure Selection:

DB (DrugBank, http://www.drugbank.ca/) was used to select the molecules of Amoxicillin and Clavulanic Acid. The downloaded files were included in software *Marvin 2D y 3D* (https://www.chemaxon.com/products/marvin/) to create the tridimensional structure and afterwards they were included in *Chimera software* to carry out the molecular docking analysis.

C. In silico induction of mutated β-lactamase and comparison:

Using bibliography and the molecules in PDB data base, it was possible to specify the changes in amino acid sequence in TEM-1 enzyme, from a clinically important perspective, which may allow creating a mutated enzyme. The changes as well as the tridimensional-geographic alignment in TEM-1 and TEM-1 mutated to evidence the internal changes were carried out using Chimera software

D. Drug-Enzyme Molecular Docking:

In order to evaluate the changes in drug-enzyme interaction affinity, with both TEM-1 and the mutated enzyme, some molecular docking tests were conducted using Chimera software, determining the values of the resulting binding energy

E. Enzyme-BLIP-Drug Molecular Docking:

With the objective of determining the possible function of BLIPs in drug-enzyme interaction, two docking tests were carried out, a previous one enzyme- BLIP to form a complex and then another docking with the antibiotic and the complex.

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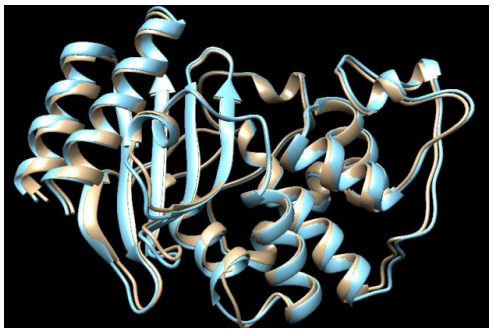
(A) TEM-1 β-lactamase enzyme

(B) Alignment of TEM-1 with TEM-1 mutated



(C) Amino acid composition of TEM-1 and TEM-1 mutated

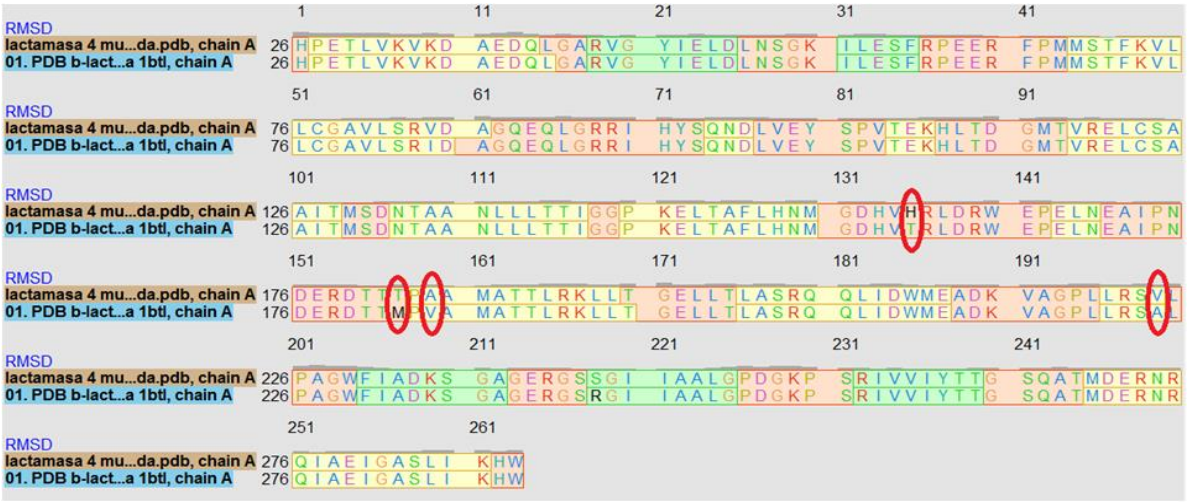


Fig. 1. Detailed Structure of TEM-1 β-lactamase y TEM-1 mutated

III. RESULTS AND DISCUSSION

Identification and characterization of resistance mechanisms can explain the changes in bacteria's drug susceptibility, a reason why it is so important to monitor the strains that are causing infections and could eventually evade the treatments used in clinics [4]. In most of the bacterial groups of clinical importance, strains from diverse geographic zones present genes that grant resistance to β-lactamics, tetracycline y clindamycin [13].

In the case of β-lactamics, the main resistance mechanisms are associated to β-lactamases that break the antibiotic. In Class A β-lactamases the most abundant enzyme among Gram-negative bacteria is TEM-1, a serine lactamase that hydrolyzes penicillins and cephalosporins. A general analysis of the primary enzyme structure using PDB database (ID "1btl") shows that TEM-1 has 263 residues and a disulfide bond between Cys77 and Cys123. As shown in Figure 1-A, TEM-1 also has several secondary structures corresponding to α-helices and β-pleated sheets, as well as a unique chain in its tridimensional conformation.

TEM-1 importance relies on that since the 1960's it has been responsible for more than 90% of the cases of *Escherichia coli* resistance to Ampicillin [14]. This is due in large part to indiscriminate and empirical use of antibiotics, and even agricultural misuse. These factors favor drug resistance through selective pressure mechanisms that vary between geographical zones as well as between medical centers from a same region, all of which is reflected in punctual mutations dynamics, gene conversion, genome rearrangements, and foreign DNA insertions and deletions [15].

At the phenotypic level, despite the existence of evidence to identify the types of β-lactamases, it is assumed that the serine-β-lactamases are susceptible to inhibitors such as Clavulanic Acid, omitting information of relevance with respect to changes in affinity enzyme-drug, which is plausible with point mutations in the genes encoding for the enzyme and leading to a different amino acid composition and three-dimensional conformation [16].

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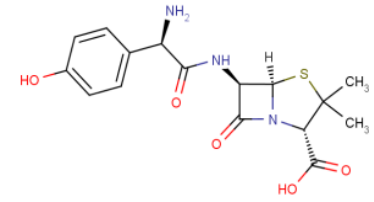
Particularly for TEM-1, different mutations have been reported that favor a low susceptibility to chemical inhibitors, including cases E104K, M182T and G238S as the most frequent in clinical isolates between 1994 and 2000 [16], and other cases as V184A, T160H and A224V in reports later [17].

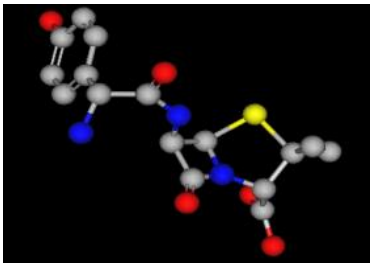
With the aim of evaluating the effect of mutations in this enzyme and its clinical importance, we selected the changes M182T, V184A, T160H and A224V to establish a mutated TEM-1 enzyme (synthetic). The comparison of the sequences of the two enzymes (TEM-1 and TEM-1-mutated) is shown in Figure 1-C. We also carried out an alignment in three-dimensional structures to evaluate the structural changes conferred by the mutations (see Figure 1-B), resulting in values of total RMSD of 0.655 Angstrom, which is considered as an optimal alignment (RMSD less than 1.0 Angstrom).

Of the mutations considered in the simulation, the first to be characterized included M182T and A224V, the first one being very frequent, causing reversion of destabilizing mutations, and that appears repeatedly in clinical isolates of β-lactamases of extended spectrum [16].

(A) 2D Structure



(B) 3D Structure



(B) General Information in *DrugBank*

Categories	• Anti-Bacterial Agents • Penicillins
CAS number	26787-78-0
Weight	Average: 365.404 Monoisotopic: 365.104541423
Chemical Formula	$C_{16}H_{19}N_3O_5S$
InChI Key	InChIKey=LSQZJLSUYDQPKJ-NJBDSQKTSA-N
InChI	InChI=1S/C16H19N3O5S/c1-16(2)11(15(23)24)19-13(22)10(14(19)25-16)18-12(21)9(17)7-3-5-8(20)6-4-7/h3-6,9-11,14,20H,17H2,1-2H3,(H,18,21)(H,23,24)/t9-,10-,11+,14-/m1/s1 Plain Text
IUPAC Name	(2S,5R,6R)-6-[(2R)-2-amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
SMILES	[H][C@]12SC(C)(C)[C@@H](N1C(=O)[C@H]2NC(=O)[C@H](N)C1=CC=C(O)C=C1)C(O)=O Plain Text
Mass Spec	Not Available

Fig. 2. Molecular composition of Amoxicillin (antibiotic)

Both mutations cause changes in the physico-chemical properties of the TEM-1, providing greater stability to urea and temperature [17]. Molecularly, M182T causes the hydroxyl of Thr182 to generate two new hydrogen bonds with the carbonyls of Glu63 and Glu64, which leads to an increase of the interaction between the two domains and possibly reduction of the conformational flexibility [16].

At the level of the phenotypic changes, the values of the MIC are clearly enhanced when there are mutations. For example with the antibiotic Cefotaxim, in comparison with TEM-1, the change G238S causes an increase of more than 8 times in the MIC, E104K increases more than 4 times, but a synergism can be observed when considering both mutations, given that the values of MIC increase by at least 267 times. In the case of M182T, the increase of MIC has been tested more than 100 times with respect to the non mutated enzyme [16].

With the objective of evaluating the changes in drug-enzyme affinity we selected drugs from the DrugBank: Amoxicillin as an antibiotic, and Clavulanic Acid as an inhibitor of the enzyme TEM-1. Figure 2 shows the detail of the Amoxicillin, including its dimensional conformation (2-A), three-dimensional (2-B), both structures were implemented in the software *Marvin*, and the details in the database (2-C). We performed the same procedure for the inhibitor of β-lactamase.

In order to investigate the effect of the enzyme mutations on the drug affinity, we conducted a multiple analysis undertaking molecular docking, a technique that calculates the potential energy of the molecules and the strength of interaction, both of attraction and repulsion, in order to obtain the three-dimensional configuration that leads to energy minimization and therefore their more stable form. In Figure 3 we show different cases of molecular docking, both for

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TEM-1 enzyme and Amoxicillin (3-A), as for mutated TEM-1 and Clavulanic Acid (3-B). Although not shown here, analyses were performed by docking other enzyme-drug combinations.

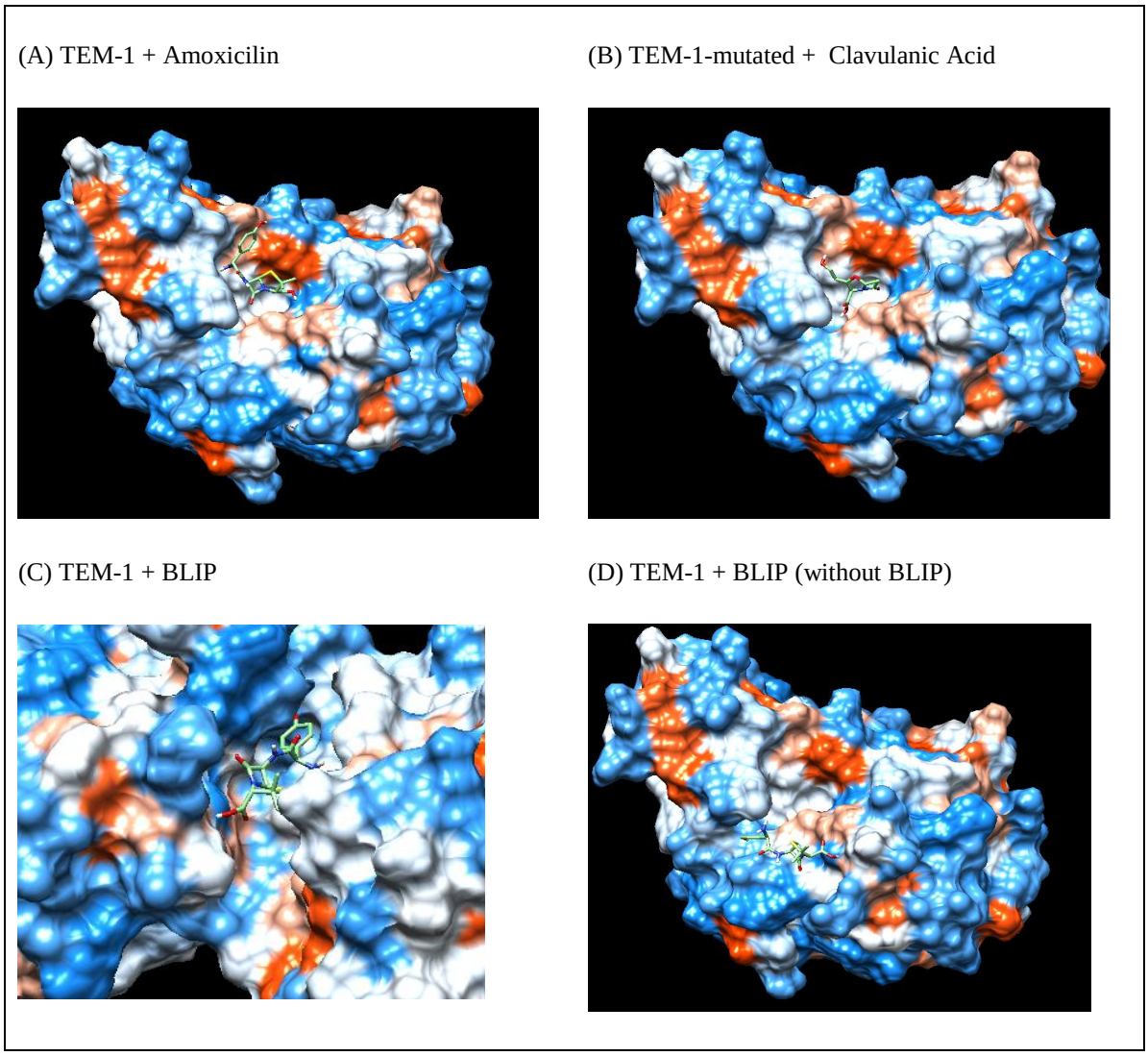

Fig. 3. Molecular Docking between β-lactamase enzymes, tested drugs and BLIP

TABLE 1. Comparison of binding energy molecular docking of β-lactamases and the evaluated drugs

Enzyme	Drug	Binding Energy
TEM 1	Amoxicillin	-5.9
	Clavulanic Acid	-7.2
TEM-1 mutated	Amoxicillin	-9.8
	Clavulanic Acid	-7.7

When comparing the results of the binding energy (shown in Table 1), it can be noticed that the cases with higher affinity are achieved with the mutated enzyme (lower binding energy means more stability). For TEM-1 the highest affinity is achieved with the Clavulanic Acid, which explains that the irreversible enzyme-Clavulanic Acid union is favored with respect to the enzyme-Amoxicillin union, making a blockade of the β-lactamase enzyme and allowing the antibiotic to have the possibility to act against the bacteria. On the other hand, in the case of the mutated TEM-1, the binding energy is lower for Amoxicillin, which is biologically consistent with the descriptions that associate those mutations to resistance, as bacteria are more likely to destroy the antibiotic instead of being inhibited by Clavulanic Acid.

Despite the fact that the alignment of the three-dimensional structures revealed no abrupt changes between TEM-1 and mutated TEM-1, the changes are sufficient to change the affinity for substrates such as drugs. These changes may affect the process of recognition in the active site, the proximity in certain regions and alter the electrostatic interactions that result in changes in the stability [2]. Structure modeling and molecular docking between the β-lactamase and the drug allows an assessment of the interaction with the enzyme. This strategy can be used in the decision making for the design of new molecules with a higher affinity in the binding site of both TEM-1 or TEM-1 with mutations, so as to effectively compete with the β-lactam and leave them available to attack the bacteria.

Another mechanism that has been discovered and which is available to alter the function of the β-lactamases is through BLIP proteins, which bind to these enzymes. In general, BLIP inhibit Class A β-lactamases including TEM-1, and are produced by *Streptomyces clavuligerus*. It has been verified that this protein is not essential for bacterial growth and its exact function remains unknown [12].

With the aim of simulating the effect of these BLIP proteins in the interaction drug-enzyme, we performed a prior molecular docking with the enzyme and the BLIP protein in order to form a complex, and then another docking with the complex and Amoxicillin. The selected BLIP protein (from the database PDB, ID "1jtd") is a single chain, 165 amino acids peptide. Despite its particular function is not known yet, its interaction with β-lactamases prevents inactivation by Clavulanic Acid or similar drugs. In fact, as shown in Figure 3-C, a steric hindrance occurs so that drugs cannot achieve the active site of the enzyme.

Figure 3-D shows the same docking but the protein BLIP has been removed of the complex to view the position of Amoxicillin and its inability to be placed in the active site (to be compared with Figure 3, where there was no impediment for the BLIP). This could represent both something positive and negative for the bacterium (as well as for treatment effects), as it could then not be inhibited by Clavulanic Acid but neither could break the β-lactam ring and be susceptible. Now, if the bacterium possesses alternative mechanisms of resistance as it might be to expel the antibiotic by efflux pumps, it would not be affected. In the absence of other mechanisms of resistance, BLIP could be used as an alternative to inhibit the enzyme regardless of affinity changes at the site where the inhibitors act, which would be highly important for cases where mutations have given advantage to bacteria.

In general, with this panorama of possible changes in the enzyme's affinity or the active site alteration, this resistance is unpredictable and responds to multiple biological processes, making it vital to constantly monitor the susceptibility characteristics of microorganisms at both genetic and phenotypic levels, as well as a structural and functional level. However, the concepts of sensitivity and resistance are definitely relative and depend on the location of the infection, the dose, route of administration and the bioavailability of the antibiotic [3]. Despite these conditions, the study of the susceptibility to antibiotics allows not only to have details of the biology, but to make decisions concerning the treatments given to patients.

IV. CONCLUSIONS

An analysis of a model of structures and simulation of a β-lactamase TEM-1 was presented, in which we evaluated its interaction with an antibiotic and a chemical inhibitor. In addition, we studied the effect of mutations of the enzyme in the interaction affinity and changes that occur when the active site is blocked with BLIP proteins. These results can be used to compare the phenotypic outcomes and make considerations regarding the clinical decisions that go beyond the simple classification of β-lactamases, in addition to giving a chemical-structural contribution that intends to explain the biological results of bacterial resistance in clinical isolates.

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