ORIGINAL ARTICLE

Mechanism of Antibiotic Resistance in Multidrug-Resistant *Klebsiella* spp.: Protein and Drug Interactions

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Abstract

Background: Multi-drug-resistant *Klebsiella pneumoniae* poses a significant global health threat due to its resistance mechanisms against multiple antibiotic classes. The SHV-1 β-lactamase enzyme, a type of serine-based β-lactamase, plays a crucial role in hydrolyzing β-lactam antibiotics, contributing to treatment failures. **Objective:** This study investigates the molecular interactions between tazobactam, a β-lactamase inhibitor, through computational approaches. **Materials and Methods:** The PyMOL 2.5.2 tool was used for *in silico* analysis, and the three-dimensional structure of SHV-1 β-lactamase was retrieved from the Protein Data Bank. Hydrogen atoms were incorporated after water molecules by the AutoDockTools package. GROMACS 2025.1 with the AMBER99SB-ILDN force field was used for the MD simulation, and ADME analysis was performed using the SwissADME web server. **Results:** Molecular docking using AutoDock 4.2 revealed strong binding affinity (-9.46 kcal/mol) between tazobactam and SHV-1 β-lactamase, with key interactions involving hydrogen bonds with ARG-205, MET-A186, THR-A71, and LYS-A234 residues. Molecular dynamics simulations over 100 nanoseconds using GROMACS 2025.1 demonstrated complex stability with RMSD fluctuations ranging from 0.15-0.2 nm after initial equilibration. RMSF analysis identified flexible regions (0.1-0.2 nm) important for drug binding dynamics. The computational analysis was complemented by ADME profiling to assess drug-like properties. Results indicate that tazobactam exhibits promising binding affinity and structural stability against SHV-1 β-lactamase, supporting its therapeutic potential in combination therapy. **Conclusion:** This study provides molecular-level insights into the mechanism of β-lactamase inhibition and contributes to understanding structure-activity relationships essential for developing effective treatments against multidrug-resistant *Klebsiella* infections.

Key words: Antibiotic resistance, *Klebsiella pneumoniae*, molecular docking, sulfhydryl variable β-lactamase enzymes-1 β-lactamase, tazobactam

INTRODUCTION

Antimicrobial drug resistance and multidrug resistance (MDR) are becoming global threats nowadays.[1] Particularly, *Klebsiella pneumoniae*, which belongs to the Enterobacteriaceae family, has recently emerged as a non-stoppable contagious organism during clinical practice. It uses several mechanisms to restrict the entry of antibiotics, like surface porin proteins (OmpK35, and OmpK36, LamB, OmpK26, PhoE, and KpnO); using an active efflux system (AcrAB-TolC); biofilm formation. All these proteins were controlled by genes such as blaSHV, blaTEM, kpnEF, PhoPQ, etc.[2,3] This organism produces nosocomial infections and is predominantly responsible for catheter-associated urinary tract infections, pneumonia, and blood-borne infections[4] in the intensive care patients.[5] As this is the opportunistic pathogen which has drug resistance potential and increases the treatment cost,[6] the World Health Organisation recently announced it as a critical priority

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pathogen.[7] It indicates MDR *K. pneumoniae* infections need quick diagnostic protocols and novel treatment methods.[8]

Latest technologies have unlocked its capacity to produce sulfhydryl variable β-lactamase enzymes (SHV-1) to breakdown the β-lactam ring of next-generation cephalosporins and penicillin antibiotics.[9] This is due to the establishment of a covalent bond with the β-lactam antibiotic using the active site serine residue containing 238- and 240 -positioned amino acids.[10] Hence, new and combined β-lactamase inhibitor antibiotic therapy has been proposed to break the β-lactamase-mediated resistance.[11,12] SHV-1 β-lactamase is a type of β-lactamase enzyme Class A, which is enriched with an α-helices arrangement and encircled by a five-stranded β-sheet. The active site Ser70 (serine) is present in the conserved SXXK motif with the 240s loop and the Ω-loop.[13]

Tazobactam, a type of β-lactamase inhibitor, can make the acylation of the serine active site by initial reversible binding, which helps to make a covalent bond with serine β-lactamase. Hence, the acylation of the active site's serine is caused by the initial reversible binding. The combination of tazobactam and piperacillin efficiently produced synergistic activity to suppress the activity of β-lactamases.[14] However, identifying a similar active site is quite a cumbersome process in drug development.

Studying the protein-drug interactions at the molecular level may help to prescribe the precision medicine. It can be possible to excel at molecular docking (MD) and molecular dynamics simulations (MDS) to understand conformational changes, structural stability, and binding mechanisms of protein-drug interactions[15] and protein-ligand interactions by high-resolution molecular dynamics.[16] Furthermore, thermodynamics, binding kinetics, and conformational dynamics are essential to learning the drug mechanisms.[17] Hence, the current study was carried out to determine the interaction between tazobactam and SHV-1 β-lactamase with the computational biological tools.

MATERIALS AND METHODS

Protein structure preparation

Using PyMOL 2.5.2, the three-dimensional structure of SHV-1 β-lactamase was extracted from Protein Data Bank Id: 1SHV.[18,19] Hydrogen atoms were incorporated after water molecules by the AutoDockTools package. Heteroatoms were removed.[20]

Ligand preparation

Tazobactum (PubChem database [CID: 123630]) was optimized with the help of Gaussian 15 software and the B3LYP/6-31G(d,p). Energy minimization was carried out to identify the most stable conformer.[21]

MD

AutoDock 4.2.6 software was utilized to perform MD.[22]

The binding site was identified based on known catalytic residues with a grid box centered on Ser70. The Lamarckian genetic algorithm was deployed with 100 independent runs, a population size of 150, and maximum evaluations of 2.5×10^6.

MDS

GROMACS 2025.1 with the AMBER99SB-ILDN force field was used for the MDS study.[23] The TIP3P water model within the dodecahedral box was used to solvate the protein-ligand complex. The system was buffered with Na+ and Cl- ions at 0.15 M concentration. Energy minimization was done using the steepest descent algorithm, followed by NVT and NPT equilibration for 100 ps each. Production runs were done for 100 ns with a 2 fs time step[24] (https://manual.gromacs.org/2025.1/).

Absorption, distribution, metabolism, and excretion (ADME) analysis

ADME characters were examined using the SwissADME web server[25] and the pkCSM platform.[26] Lipinski's Rule of Five and Veber's criteria were used to evaluate drug-likeness.

Data analysis

GROMACS analysis tools were used to calculate binding energies, root mean square deviation (RMSD), and root mean square fluctuation (RMSF). VMD 1.9.4. Statistical analysis was performed in R software version 4.3.2 to analyse hydrogen bonds.[27]

RESULTS

MD analysis

The strong binding affinity between tazobactam and SHV-1 β-lactamase, has the binding energy of −9.46 kcal/mol, was evaluated during the MD study. Similar binding energy was already proved for tazobactam with class A β-lactamases.[28] The docking pose showcased the optimal positioning of tazobactam within the active site, developing a crucial attachment with key catalytic residues. Hydrogen bond analysis determined four primary interactions: MET-A186 (2.3 Å), THR-A71 (1.9 Å), and LYS-A234 (2.2 Å) [Figure 1a] and ARG-205 (2.1 Å) [Figure 1b]. These binding affinities explore strong electrostatic and polar contacts that stabilise the inhibitor-enzyme complex. This binding energy proves that tazobactam makes a thermodynamically stable complex with SHV-1 β-lactamase, which is essential for potential inhibition.

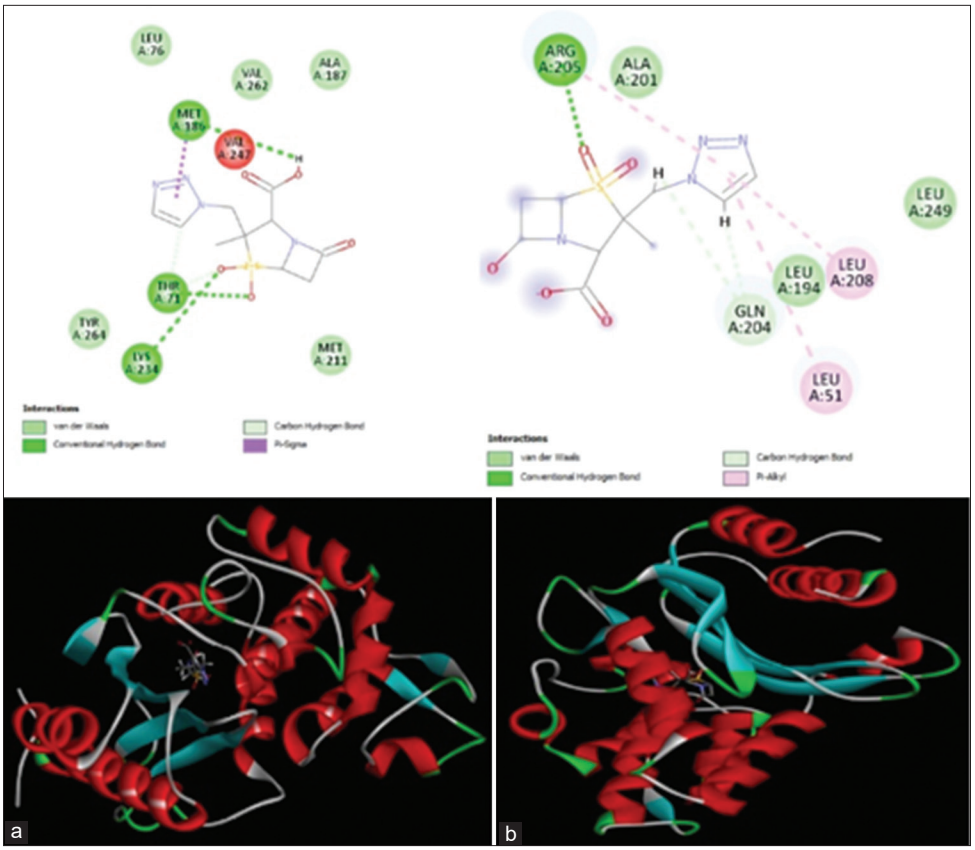

Figure 1: Binding affinity of the tazobactam with sulfhydryl variable β-lactamase enzymes-1 β-lactamase (a) interaction with MET-A186 (2.3 Å), THR-A71 (1.9 Å), and LYS-A234 (2.2 Å); (b) ARG-205 (2.1 Å)

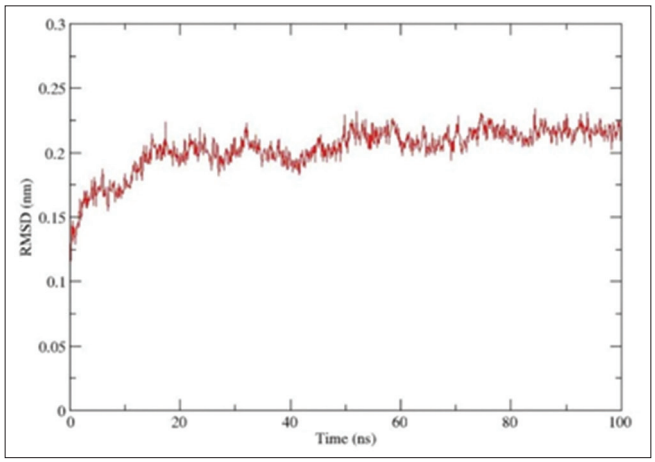

Figure 2: Tazobactam – sulfhydryl variable β-lactamase enzymes-1 complex determination in root mean square deviation during molecular dynamic simulation analysis

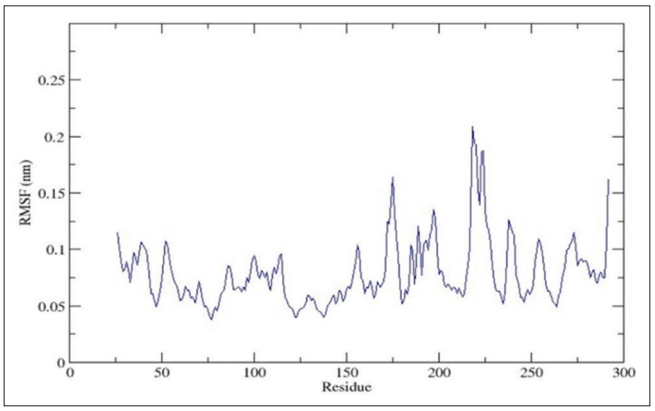

Figure 3: Flexibility pattern analysis in root mean square fluctuation analysis during molecular dynamic simulation analysis

In the present study, the main affinity with ARG-205 is very crucial due to substrate binding and catalysis in class A β-lactamases.[29] This proves the importance of the hydrogen bonding pattern with certain residue interactions to make the stable inhibitor-enzyme complex. The binding of tazobactam with THR-A71 at the catalytic serine (Ser70) indicates that this could stop the nucleophilic attack, which is necessary for β-lactam hydrolysis.[13]

The molecular-level interaction of tazobactam with SHV-1 β-lactamase yields significant insights. Fragmentation of tazobactam causes inactivation of SHV-1 β-lactamase. Pagan-Rodríguez *et al*. have demonstrated that fragmentation of tazobactam happens when it attached to the active site Ser70 in this enzyme and revealed that the Ser→Gly substitution at amino acid position 130 is not crucial for enzyme inactivation.[30] Further, this study was in agreement with the research of Bellini *et al*.,[31] who demonstrated tazobactam's interaction with β-lactamase OKP-B-6 of *K. pneumoniae*.

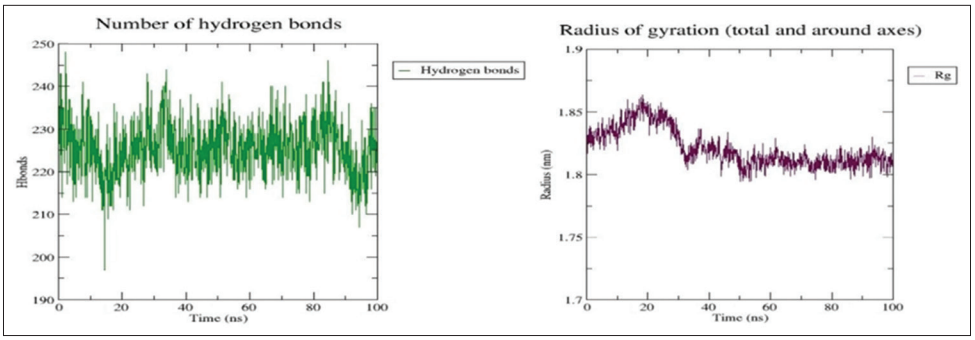

Figure 4: Hydrogen bonds between tazobactam and sulfhydryl variable β-lactamase enzymes-1 β-lactamase

MDS

The 100-ns MDS offered insights into the dynamic activity of the tazobactam-SHV-1 complex. RMSD analysis proved the initial fluctuations during the first 10 ns, followed by stabilisation with values ranging from 0.15 to 0.2 nm [Figure 2].

During RMSD analysis, stabilisation after the first equilibration confirms the stable formations of inhibitor-enzyme formation. In this study, the MDS result confirms the stability of tazobactam-SHVI complex for over longer period of time. This supports the hypothesis that β-lactamase inhibitors bind with antibiotic in irreversible mode.[32] Furthermore, the RMSD values of 0.15–2 nm are relatively low indicates that tazobactam binding does not induce major alteration in protein structure. The protein backbone RMSD demonstrated excellent stability throughout the simulation, indicating that tazobactam binding does not impact confirmational changes overall protein structure. This makes the inhibitor more effective in SHV-I.

In RMSF analysis, most regions showing fluctuations between 0.1 and 0.2 nm, confirms residue specific flexibility patterns. Notable flexibility at the Ω-loop (residues 164–179) and the 240s loop is known for substrate binding and catalysis [Figure 3]. The above result was consistent the work done by Egorov *et al*.[33] It is known that these loop regions change shape when they bind to a substrate and when they catalyze a reaction.

It further confirms that tazobactam does not induce protein to get rigid.[34] The active site residues showed minimal fluctuations, suggesting stable binding of the inhibitor.

Hydrogen bond dynamics

In the entire simulation study, the number of hydrogen bonds between tazobactam and SHV-1 β-lactamase fluctuated between 2 and 5, with an average of 3.2 bonds per frame. The most persistent interactions involved ARG-205 and THR-A71, maintaining contact for >80% of the simulation time [Figure 4].

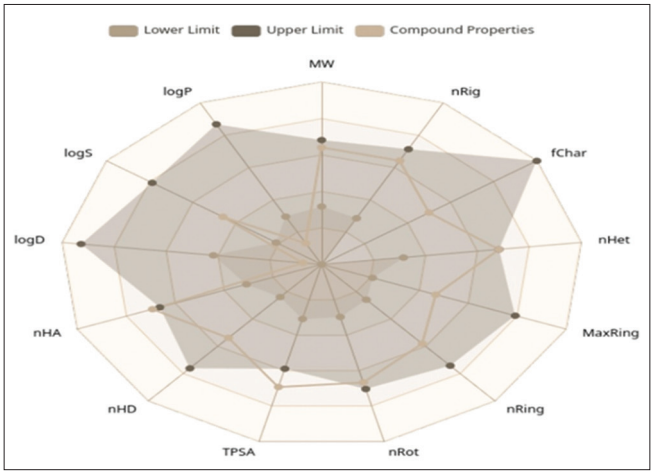

Figure 5: Pharmacokinetic analysis

The binding kinetics of tazobactam were studied via the H2 bond dynamics. The interactions with the residues ARG-205 and THR-A71 are most essential for the recognition and binding of the inhibitor. This is the best perfect character of the effective inhibitor binding.[35] The change in the H2 bond counts (2–5) shows that the dynamic nature of protein-ligand interactions is always changing and that unique binding conformations affect the overall binding affinity.[36]

ADME properties

ADME properties help to understand a compound's drug-likeness property. In this study, a molecular weight of 300.29 Dawith the LogP (−1.23) and polar surface area value of 112.73 Ų was observed for tazobactam during ADME analysis [Figure 5]. The above characters were fulfilling the criteria of Lipinski's principle. This predicts the molecule's water solubility and moderate permeability, for which it can be used in an intravenous solution. As per ADME analysis, tazobactam reached the drug-likeness standards, indicating the human body can easily absorb this molecule with enhanced stability.[37] Further, the moderate permeability predictions confirm that the tazobactam can be distributed equally in tissue compartments.[38] Hence, it is a more suitable candidate for clinical application as a β-lactamase inhibitor.

Crucial binding affinity areas will be helpful to understand mutational areas to clearly identify the resistance mechanisms.[39-41] In this way, the current study offered a detailed key finding on structural insights that paved the way to comprehend the resistance mechanisms to formulate the potential and novel β-lactamase inhibitors.[42] The current study suggests that future drug development requires cross-checking the computation predictions and experimental validation of participating binding modes and drug kinetic parameters.

CONCLUSION

The efficacy of tazobactam's role as a β-lactamase inhibitor was demonstrated in this study. The compound's binding affinity (−9.45 kcal/mol) and stability with ARG-205, MET-A186, THR-A71, and LYS-A234 residues were confirmed. RMSD values in MD were recorded as 0.15–0.2 over 100 ns. The H2 dynamic pattern was elucidated for ARG-205 and THR-A71, and tazobactam's ADME properties were also explored. The above screened characters confirm that tazobactam can be the potential β-lactamase inhibitor against multidrug-resistant *K. pneumoniae*. In addition, the present study findings offered a novel route for the development of generation β-lactamase inhibitors to combat the emanating resistance mechanisms.

DATA AVAILABILITY STATEMENT

Not applicable.

ETHICS STATEMENT

Not applicable.

AUTHORS' CONTRIBUTIONS

B. Arihanth Kumaran, M. Nivaedhitha– conceived the idea and experimentations; T. Selvankumar R. Jeya Chitra – conceived the idea, drafting, editing and proofing.

GENERATIVE AI STATEMENT

No AI tool used.

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