ORIGINAL ARTICLE

In Silico Study of Ceftazidime and Piperacillin Against Penicillin-Binding Protein 2, Beta-Lactamase (OXA-1 and SHV-28) of *Klebsiella pneumoniae* U25

Pavan Heggadadevanakote Kendaganna[1], Govindaraju Shruthi[2], Prashantha Karunakar[3], Sharanagouda Patil[4], Shiva Prasad Kollur[5], Chandrashekar Srinivasa[6], Chandan Shivamallu[1]

[1]Department of Biotechnology and Bioinformatics, School of Life Sciences, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India, [2]Research and Development, Glimetomics Bioresolve Pvt. Ltd., Mysuru, Karnataka, India, [3]Department of Biotechnology, PES University, Bengaluru, Karnataka, India, [4]Department of Virology, Indian Council of Agricultural Research-National Institute of Veterinary Epidemiology and Disease Informatics, Yelahanka, Bengaluru, India, [5]Department of Sciences, Amrita School of Arts and Sciences, Amrita Vishwa Vidyapeetham, Mysuru, Karnataka, India, [6]Department of Biotechnology, Davangere University, Davangere, Karnataka, India

Abstract

Background: To combat the action of beta-lactams, *Klebsiella pneumoniae* launches the enzymatic action by producing beta-lactamases to destruct the activity of ceftazidime and piperacillin. **Objective:** In our research, we want to know the action of ceftazidime and piperacillin on penicillin-binding protein2 (PBP2) and also does it have any interactions with beta-lactamases (OXA1 and SHV-28). Our idea is to prevent the action of beta-lactamases on ceftazidime and piperacillin. Hence, we have modified the beta-lactam core structures of ceftazidime and piperacillin and done the comparative docking interaction studies. **Materials and Methods:** *K. pneumoniae* U25 has been selected for the comparative docking analysis study with ceftazidime and piperacillin antibiotics by modifying its structures and targeting them against PBP2 and beta-lactamases (OXA-1 and SHV-28). **Results:** Our docking analysis revealed that ceftazidime and modified ceftazidime are forming hydrogen bonds, but piperacillin and modified piperacillin are showing hydrophobic interactions with an active site serine residue (Ser316) of PBP2 responsible for the transpeptidase activity in *K. pneumoniae* U25. Protective action by beta-lactamases (OXA-1 and SHV-28) to *K. pneumoniae* U25 against beta-lactam antibiotics is also revealed through our study by docking interactions of Ser71 of OXA-1 and Ser66 of SHV-28 with the ceftazidime, modified ceftazidime, piperacillin, and modified piperacillin.

Key words: Beta-lactamases, Ceftazidime, Docking interactions, *Klebsiella pneumoniae* U25, Penicillin-Binding Protein 2, Piperacillin

INTRODUCTION

Klebsiella pneumoniae U25 (CP012043.1) is a biofilm-forming, rod-shaped bacteria, with multidrug resistance, isolated from a urine sample in Chennai.[1] It has two cephalosporin resistance (*blaOXA-1* and *blaSHV-28*) genes in its genome.[1]

Penicillin-binding proteins (PBP) are the enzymes involved in cross-linking the peptidoglycans in the bacterial cell wall synthesis. Serine reactive PBPs have serine and lysine separated by two amino acids (*S--K*) in their active site,[2,3] conserved similar pattern residues observed in almost all the PBPs. PBP2 of *K. pneumoniae* U25 also contains the Ser316, Lys319 separated by Thr317 and Val318 residues at their active site. Beta-lactam antibiotics mainly target and inactivate PBPs by

Address for correspondence:
Chandan Shivamallu, Department of Biotechnology and Bioinformatics, School of Life Sciences, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India. E-mail: chandans@jssuni.edu.in

Received: 21-09-2020
Revised: 28-10-2020
Accepted: 05-11-2020

binding to their active sites.[4] Beta-lactam antibiotics have a 4-membered beta-lactam ring as their core structure; the beta-lactam core structure imitates the D-Ala-D-Ala peptide as the substrate for transpeptidase activity for bacterial cell wall synthesis.[5,6] Thus, binding of these antibiotics to the active site of the PBPs prevents the cross-linking activity, which intern causes the loosing of the bacterial cell wall resulting in the death of the bacteria.

Ceftazidime {1-{[(6R,7R)-7-[(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-[(1-carboxy-1-methylethoxy) imino] acetamido]-2-carboxylato-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl] methyl}pyridin-1-ium} is having the chemical formula $C_{22}H_{22}N_6O_7S_2$with the molecular weight 546.58 g/mol.

Ceftazidime is also the semisynthetic antibacterial agent with the core beta-lactam ring in its structure [Figure 2]. It is the third generation cephalosporin, active against both Gram-positive and Gram-negative bacteria such as *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Clostridium perfringens*, *Staphylococcus aureus*, and *Streptococcus pyogenes* and most of the *Enterobacteriaceae* such as *K. pneumoniae*, *Escherichia coli*, *Salmonella* species, and *Shigella* species.[7]

Ceftazidime is used as a treatment for urinary tract infections, lower respiratory tract infections, septicemia.[8] Daily in split doses, about 1–6 g of ceftazidime is administered intravenously or intramuscularly and for the children, about 30–100 mg/kg of the dose is administered.[7]

The penicillin analog piperacillin {(2S, 5R, 6R)-6-[(2R)-2-[(4-ethyl-2, 3-dioxopiperazine-1-carbonyl) amino]-2-phenylacetamido]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid} is having the chemical formula $C_{23}H_{27}N_5O_7S$ with the molecular weight 517.5 g/mol. Piperacillin is the semisynthetic antibacterial agent with the core beta-lactam ring in its structure [Figure 1]. It is the fourth generation antibiotic made by the attachment of piperazine and ampicillin derivative[9] to aminobenzylpenicillin.[10]

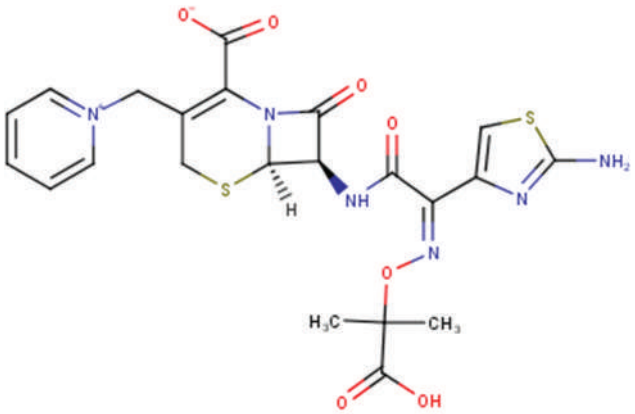

Figure 1: Structure of ceftazidime

Piperacillin antibiotic is used against *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and other groups of *Enterobacteriaceae*. Piperacillin sodium ($C_{23}H_{27}N_5NAO_7S$) is not absorbed in the gastrointestinal tract, so it is administered intramuscularly and intravenously. For the treatment of severe infections in the lower respiratory tract, urinary tract, and intra-abdominal region, about 3–4 g of piperacillin sodium are administrated intravenously every 4–6 h with the maximum dose of 24 g per day. For less severe infections, a maximum of 2 g can be given intramuscularly for one injection site. 1 g of piperacillin is soluble in 1.4 ml of water (pH 5.5–7.0).[11] Adverse effects by the usage of piperacillin include reactions specific to local, allergic, affect the liver, kidney, and also on blood cells.[12]

To combat the action of beta-lactams, *K. pneumoniae* launches the enzymatic action by producing beta-lactamases (OXA1 and SHV-28) to destruct the activity of ceftazidime and piperacillin. In our research, we want to know the action of ceftazidime and piperacillin on PBP2 and also does it have any interactions with beta-lactamases (OXA1 and SHV-28). Our idea is to prevent the action of beta-lactamases on ceftazidime and piperacillin. Hence, to enable protection, we have modified the beta-lactam core structures of ceftazidime and piperacillin and done the comparative docking interaction studies.

MATERIALS AND METHODS

K. pneumoniae U25[1] strain was selected for our research since its complete genome sequence was known and also it has been isolated in the southern part of India from the urine sample of the patient.[1] The complete genome was studied in search of potential targets for beta-lactam antibiotics. The beta-lactam antibiotics target PBP2 and the antibiotics threat beta-lactamases (OXA-1 and SHV-28) were selected and their proteins sequence in FASTA format was downloaded from the NCBI database of *K. pneumoniae* U25 genome.

(https://www.ncbi.nlm.nih.gov/genome/815?genome_assembly_id=259514).

FASTA sequences of the PBP2, Beta-lactamase (OXA-1and SHV-28) proteins were subjected to 3D structure building

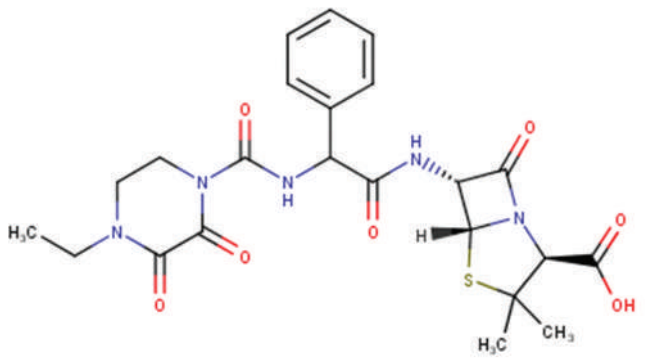

Figure 2: Structure of piperacillin

using the SWISS-MODEL[13] workspace tool. The output of this software was saved in PDB format. These proteins PDB files were uploaded to RAMPAGE[14] software for the angle of rotation (torsion angles) of amino acids, the number of residues in the favorable region (~98.0% expected), if the residues in the favorable region are less, the protein structure is uploaded to Galaxy Refine[15] for protein structure refinement. The quality of the refined protein structure was checked based on the Z-score using the ProSA-web[16] server. The resultant protein .pdb file is used for the molecular docking studies through PyRx software. The protein .pdb files along with the ligands .pdb file were uploaded to COACH-D[17,18] server for binding site prediction.

To prevent the action of beta-lactamase on beta-lactam antibiotics, we modified the ceftazidime and piperacillin antibiotics by introducing one extra carbon atom in the beta-lactam ring [Figure 3]. In our research, we want to know the action of modified ceftazidime and modified piperacillin in comparison with the ceftazidime and piperacillin. Hence, the ligand structures of ceftazidime, modified ceftazidime, piperacillin, and modified piperacillin were drawn in Marvin's sketch tool and saved in .sdf file format. Energy minimization of the ligands was done by loading .sdf files of the ligands to the open babel tool in the PyRx software and saved as .pdb file.

Docking studies were conducted using AutoDock Vina[19] through the PyRx tool. Ligands were then added to the AutoDock Vina of the PyRx software for docking with PBP2, OXA-1, and SHV-28. The grid box was generated for predicted binding site residues for PBP2 and beta-lactamase (OXA-1 and SHV-28) proteins against ceftazidime, modified ceftazidime, piperacillin, and modified piperacillin ligand structures.

Interaction analysis was carried out using PyMOL software, different orientations of the ligand with proteins were saved in. pdb format and analyzed using LigPlot+[20] tool. Each interaction of the ligand with protein is saved in a postscript file format. Postscript files are opened in the ghost viewer and saved into JPEG format for interaction analysis.

RESULTS AND DISCUSSION

K. pneumoniae U25 genome was searched for PBPs and beta-lactamases. The search resulted in PBP2 with 621 amino acids, beta-lactamases OXA-1 with 276 amino acids, and SHV-28 with 286 amino acids. The FASTA sequence of PBP2, OXA-1, and SHV-28 was downloaded from the NCBI *K. pneumoniae* U25 genome and subjected to homology modeling through SWISS-MODEL[13] workspace.

The homology model was developed using 6G9F has a template for PBP2 that had a 61.94% sequence similarity for Peptidoglycan D,D-transpeptidase MrdA. For OXA-1, the homology model was developed using 1M6K which had a 100% sequence similarity with beta-lactamase OXA-1. An SHV-28 homology model was built using 6NFD as a template, which had 98.50% similarity with beta-lactamase SHV-11.

The modeled proteins PBP2, OXA-1, and SHV-28 were subjected to structure refinement through the GalaxyWEB refinement tool. The refined models of proteins were then

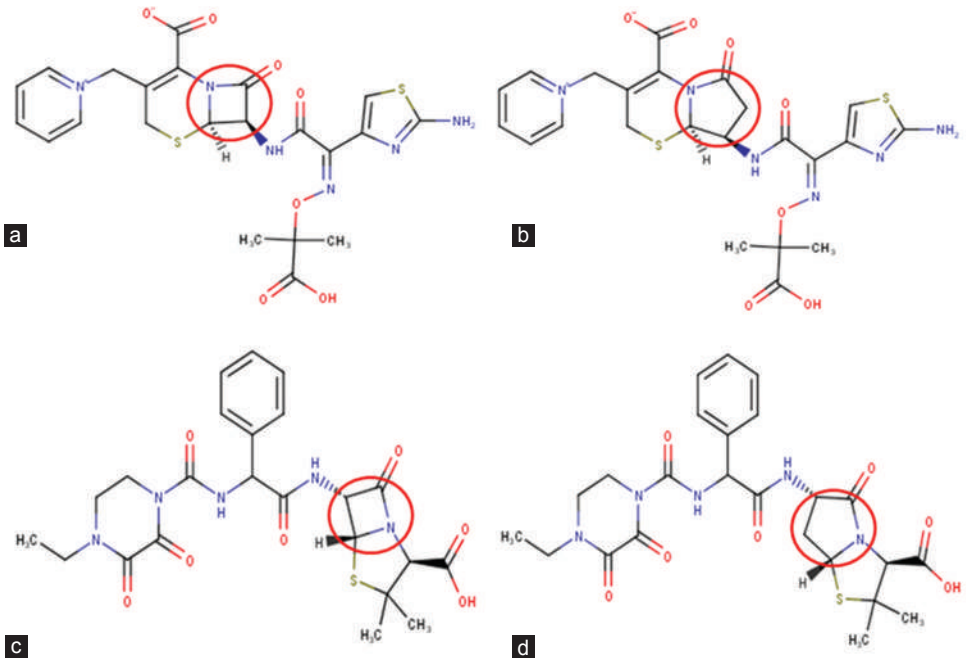

Figure 3: (a) Structure of ceftazidime, (b) modified structure of ceftazidime, (c) structure of piperacillin, (d) modified structure of piperacillin

loaded to ProSA-web[16,21] for detecting any errors of experimental and theoretical models in the protein structures. Model quality is determined by the Z-score and for refined protein modeled structures of PBP2, OXA-1, and SHV-28 Z-score was −10.27, −7.8, and −7.17 [Figures 4-6].

The refined protein structures were downloaded in .pdb format and subjected to the Ramachandra plot prediction through RAMPAGE. For PBP2, OXA-1, and SHV-28 modeled proteins, 98.4%, 98.8%, and 98.5% of residues were in the favored region [Figures 7-9]. Hence, the refined models were loaded to the AutoDock Vina of PyRx software for docking.

Docking Interaction Analysis

Beta-lactam antibiotics (ligands) exhibit their action by interacting with the active site serine residues of PBP2. To counter the antibiotics, beta-lactamases (OXA-1 and SHV-28) also exert their action through serine residues. PBP2 is having active site serine at 316th position, OXA-1 at 71st, and SHV-28 at 66th positions.

PBP2 Docking Results Interpretation

The binding site residues predicted through the COACH-D[17,18] server for PBP2 was ALA315, SER316, ARG354, TRP356, SER373, ASP375, SER436, ILE439, GLN441, THR516, SER532, GLY533, THR534, SER535, and GLN536 which was selected in the PBP2 protein loaded in AutoDock Vina of PyRx tool. Gridbox generated for these binding site residues resulted in the docking interactions, which are presented through LigPlot+ software [Figures 10 and 11].

Docking results of PBP2 with ceftazidime and modified ceftazidime revealed that ceftazidime is forming hydrogen bonds with Ser316 (3.19A^0), Asp375 (3.14A^0), Gln441 (3.28A^0), and Gln536 (2.92A^0 and 2.95A^0) at −7.1 Kcal/mol, whereas modified ceftazidime is also forming hydrogen bonds with Ser316 (2.93A^0), Asp375 (2.71A^0), and Gln441 (2.82A^0) at −7.8 Kcal/mol (Vina score).

Docking results of PBP2 with piperacillin and modified piperacillin revealed that piperacillin is forming hydrogen bonds

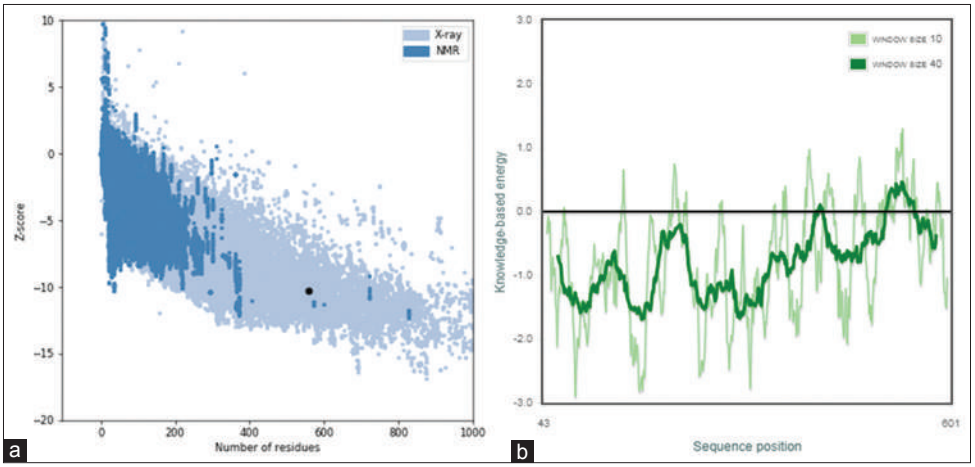

Figure 4: Plot exhibiting z-score of the PBP2 protein obtained through the ProSA-web server. (a) Plot exhibiting z-score (−10.27) of the model after refinement. (b) Energy plot of PBP2 amino acid sequence positions

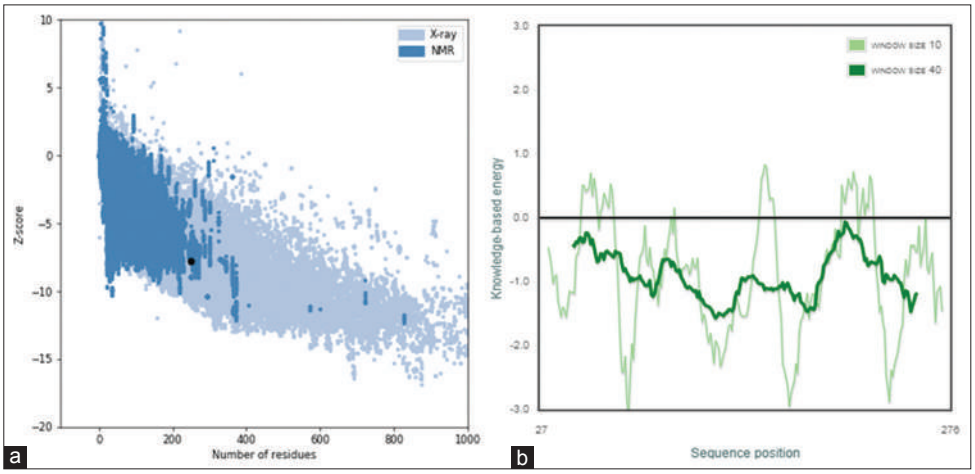

Figure 5: Plot exhibiting z-score of the OXA-1 protein obtained through the ProSA-webserver. (a) Plot exhibiting z-score (−7.8) of the model after refinement. (b) Energy plot of OXA-1 amino acid sequence positions

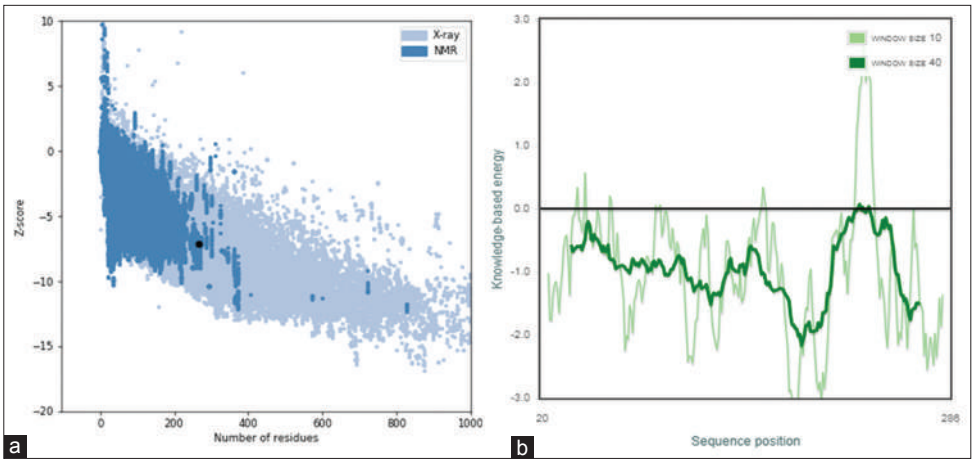

Figure 6: Plot exhibiting z-score of the SHV-28 protein obtained through the ProSA-web server. (a) Plot exhibiting z-score (–7.17) of the model after refinement. (b) Energy plot of SHV-28 amino acid sequence positions

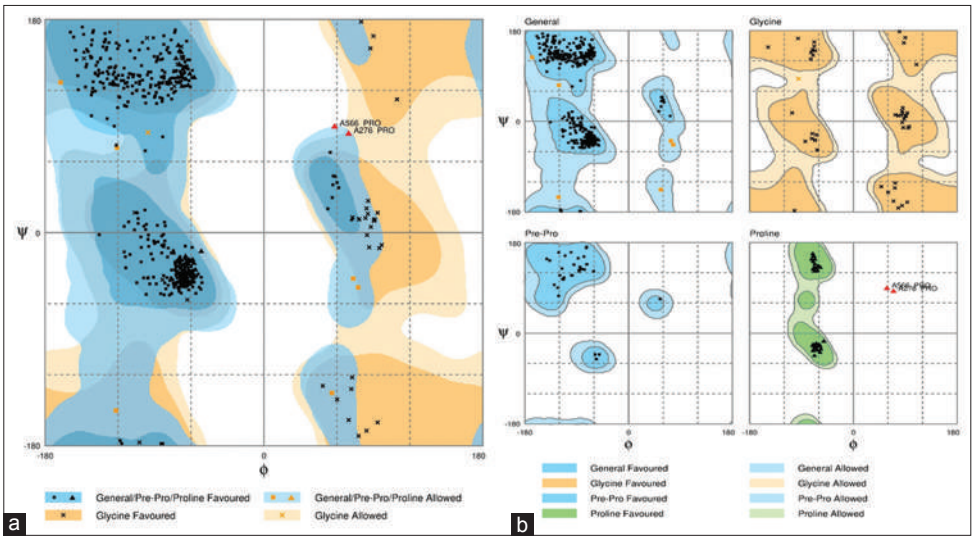

Figure 7: Ramachandran plot exhibiting residues of PBP2 refined model: (a) Generic plot exhibiting amino acid residues except for proline and glycine in favored and allowed region. (b) Plot depicting glycine, proline, and pre-pro residues in favored and allowed regions

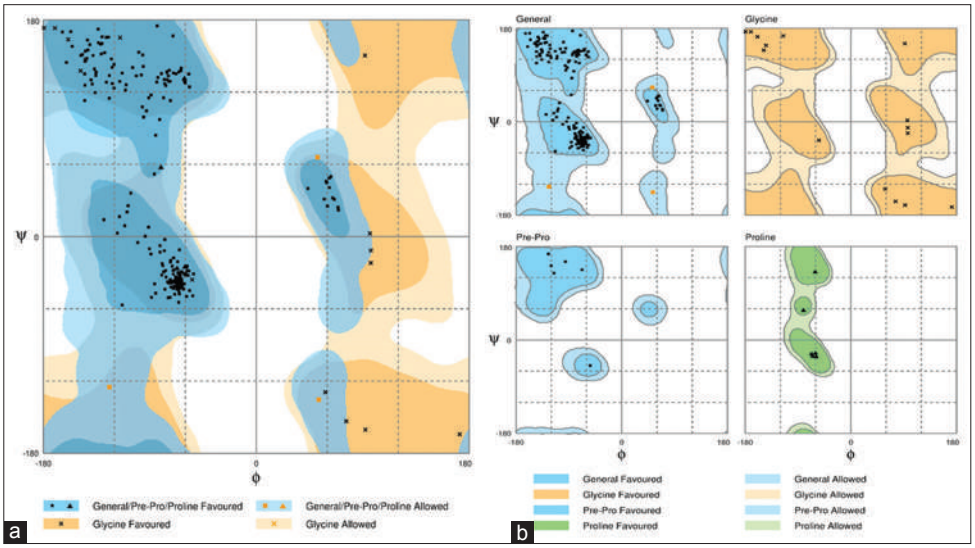

Figure 8: Ramachandran plot exhibiting residues of OXA1 refined model: (a) Generic plot exhibiting amino acid residues except for proline and glycine in favored and allowed region. (b) Plot depicting glycine, proline, and pre-pro residues in favored and allowed regions

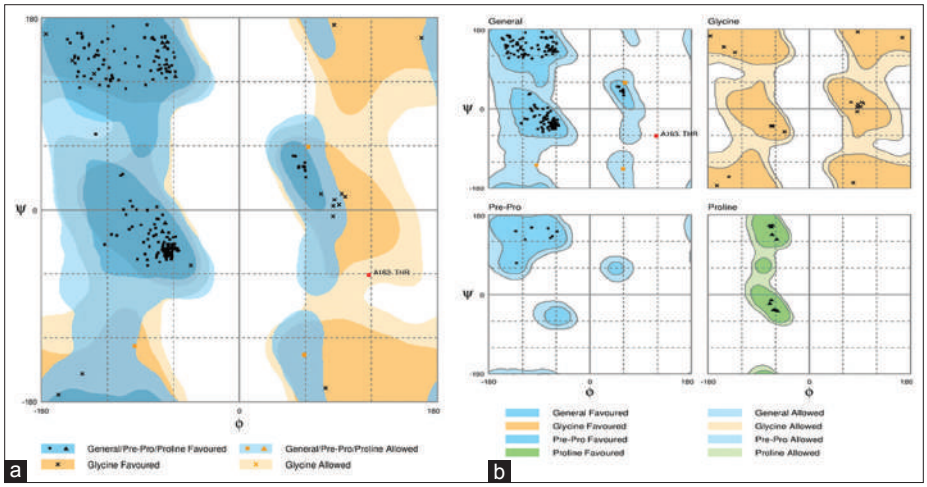

Figure 9: Ramachandran plot exhibiting residues of SHV28 refined model: (a) Generic plot exhibiting amino acid residues except for proline and glycine in favored and allowed region. (b) Plot depicting glycine, proline, and pre-pro residues in favored and allowed regions

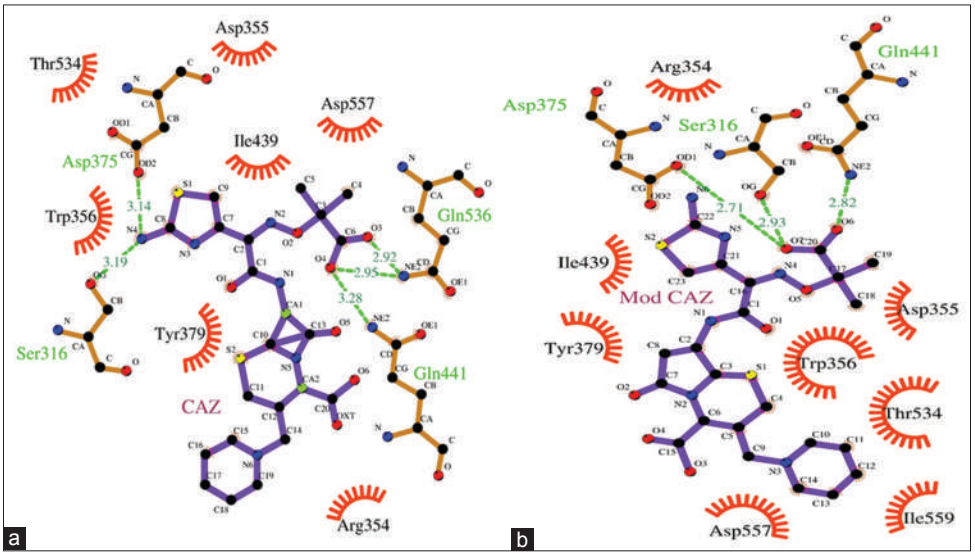

Figure 10: (a) Interaction of ceftazidime with PBP2; (b) interaction of modified ceftazidime with PBP2

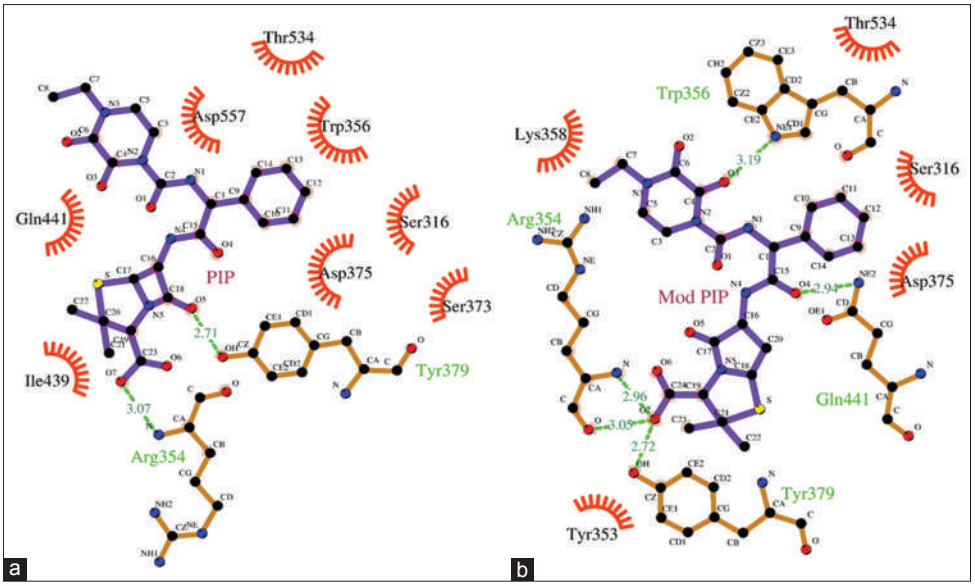

Figure 11: (a) Interaction of piperacillin with PBP2; (b) interaction of modified piperacillin with PBP2

with Arg354 (3.07A^0), Tyr379b (2.71A^0) at −7.5 Kcal/mol whereas modified piperacillin is also forming hydrogen bonds with Arg354 (2.96A^0 and 3.05A^0), Trp356 (3.19A^0), Tyr379 (2.72A^0), and Gln441 (2.94A^0) at −7.3 Kcal/mol (Vina score).

OXA-1 Docking Results Interpretation

The binding site residues predicted through the COACH-D[17,18] server for OXA-1 was ASP70, SER71, MET102, TRP105, SER118, VAL120, LEU164, GLU165, LYS215, THR 216, GLY217, ALA218, GLY219, and PHE220 which was docked through AutoDock Vina of the PyRx tool. Gridbox generated for these binding site residues resulted in the docking interactions, which are presented through LigPlot+ software [Figures 12 and 13].

Docking results of OXA-1 with ceftazidime and modified ceftazidime revealed that ceftazidime is forming hydrogen bonds with Ser71 (2.80A^0) and Asp70 (3.24A^0) at −6.2 Kcal/mol, whereas modified ceftazidime is also forming hydrogen bonds with Ser71 (3.05A^0) and Asp70 (3.06A^0) at −6.4 Kcal/mol (Vina score). Docking results of OXA-1 with piperacillin and modified piperacillin revealed that piperacillin is forming hydrogen bonds with Ser71 (3.06A^0), Thr216 (3.10A^0), Ala218 (2.98A^0), and Phe220 (2.91A^0) at −6.8 Kcal/mol, whereas modified piperacillin is forming hydrogen bonds with Ser118 (2.70A^0), Thr216 (2.84A^0), Asp218 (3.30A^0), and Ser260 (3.00A^0) at −8.5 Kcal/ mol (Vina score).

SHV-28 Docking Results Interpretation

The binding site residues predicted through the COACH-D[17,18] server for SHV-28 was MET65, SER 66, LYS69, ASP100, TYR101, SER126, ASN128, THR163, ASN166, VAL 212, LYS230, THR231, GLY 232, ALA 233,

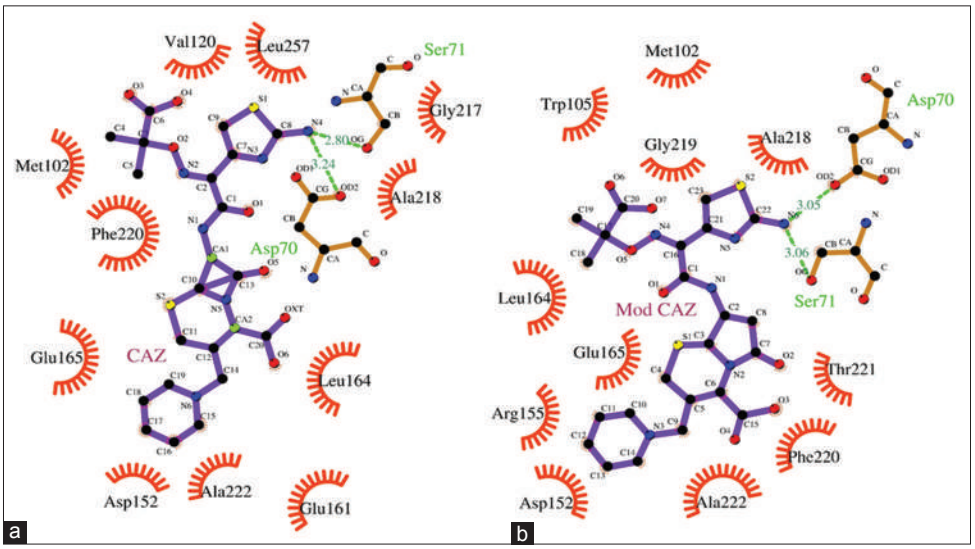

Figure 12: (a) Interaction of ceftazidime with OXA-1; (b) interaction of modified ceftazidime with OXA-1

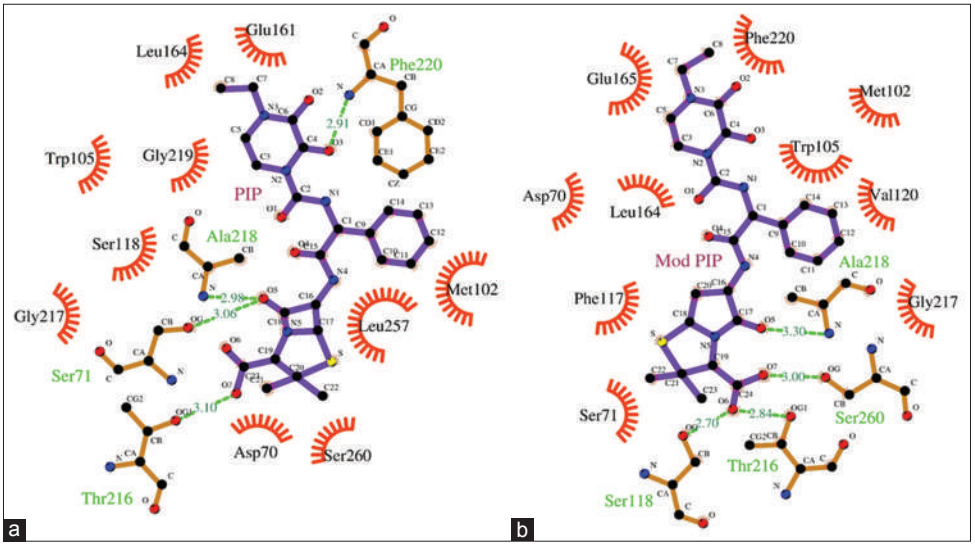

Figure 13: (a) Interaction of piperacillin with OXA-1; (b) interaction of modified piperacillin with OXA-1

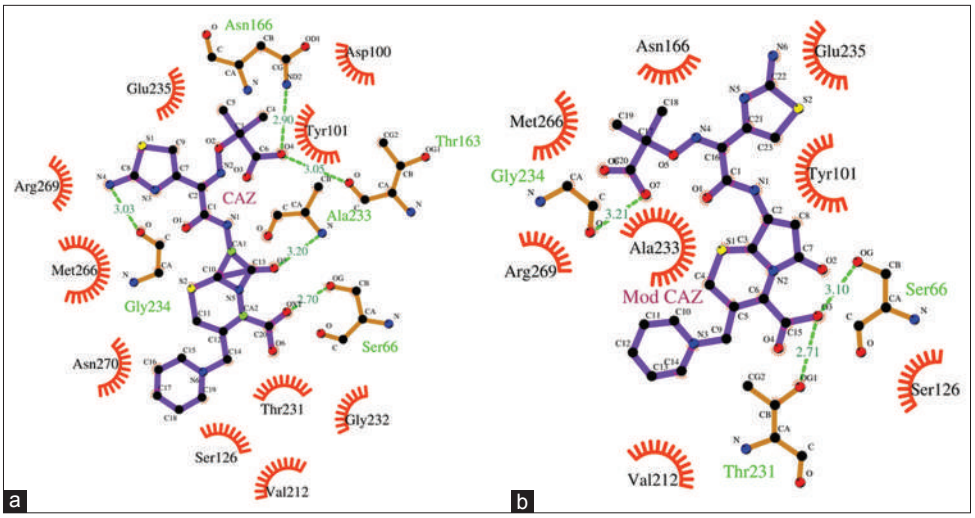

Figure 14: (a) Interaction of ceftazidime with SHV-28; (b) interaction of modified ceftazidime with SHV-28

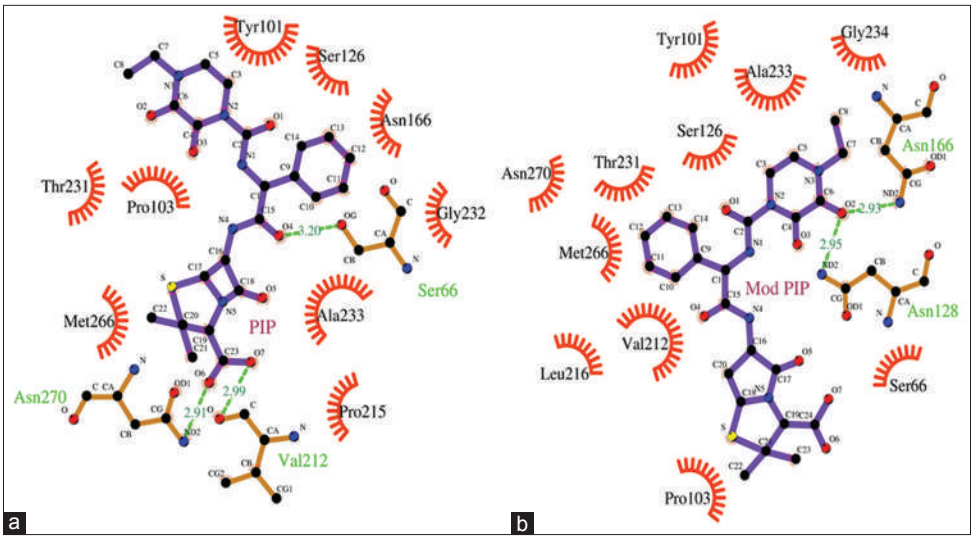

Figure 15: (a) Interaction of piperacillin with SHV-28; (b) interaction of modified piperacillin with SHV-28

GLY234, and GLU235 which was docked through AutoDock Vina of PyRx tool. Gridbox generated for these binding site residues resulted in the docking interactions, which are presented through LigPlot+ software [Figures 14 and 15]. Docking results of SHV-28 with ceftazidime and modified ceftazidime revealed that ceftazidime is forming hydrogen bonds with Ser66 (2.70A^0), Thr163 (3.05A^0), Asp166 (2.90A^0), and Gly234 (3.03A^0) at −7.2 Kcal/mol, whereas modified ceftazidime is also forming hydrogen bonds with Ser66 (3.10A^0), Thr231 (2.71A^0), and Gly234 (3.21A^0) at −7.0 Kcal/mol (Vina score).

Docking results of SHV-28 with piperacillin and modified piperacillin revealed that piperacillin is forming hydrogen bonds with Ser66 (3.20A^0), Val212 (2.99A^0), and Asn270 (2.91A^0) at −8.0 Kcal/mol, whereas modified piperacillin is also forming hydrogen bonds with Asn128 (2.95A^0) and Asn166 (2.93A^0) at −7.6Kcal/mol (Vina score).

CONCLUSION

Docking interaction analysis revealed that, in the case of PBP2, the ligands ceftazidime and modified ceftazidime are interacting (forming Hydrogen bond) with Ser316 of *K. pneumoniae* U25. However, piperacillin and modified piperacillin is showing hydrophobic interactions with Ser316 but not forming hydrogen bonds. Since ceftazidime and modified ceftazidime are interacting with the active site serine residues of PBP2, they can be used for the treatment of *K. pneumoniae* infections, whereas piperacillin and modified piperacillin are not interacting with active site serine. The action of preventing bacterial transpeptidase activity by binding to the active site serine is not achieved by piperacillin and modified piperacillin. Hence, piperacillin and modified piperacillin may not be suitable drugs to treat *K. pneumonia* U25 infections.

Beta-lactamases ensures protective action to the host against beta-lactam antibiotics. *K. pneumonia* U25 is having beta-lactamases (OXA-1 and SHV-28) in its genome. The same protective action of beta-lactamases against the beta-lactam antibiotics can be seen in our docking interaction analysis. In the case of beta-lactamase OXA-1, the ligands ceftazidime, modified ceftazidime, piperacillin, and modified piperacillin are interacting (forming hydrogen bond) with active site Ser71. Whereas in the case of SHV-28, the ligands ceftazidime and modified ceftazidime are forming a hydrogen bond with active site Ser66 but piperacillin and modified piperacillin showing hydrophobic interactions.

ACKNOWLEDGMENT

The author is thankful to Dr. Rama Vaidyanathan, Director R&D, Dr. A.P.J Abdul Kalam Centre for Innovation and Entrepreneurship, Dr. MGR Educational and Research Institute, Chennai-600095, India, for *K. pneumoniae* U25 strain support.

REFERENCES

1. Rafiq Z, Sam N, Vaidyanathan R. Whole genome sequence of *Klebsiella pneumoniae* U25, a hypermucoviscous, multidrug resistant, biofilm producing isolate from India. Mem Inst Oswaldo Cruz 2016;111:144-6.
2. Takasuga A, Adachi H, Ishino F, Matsuhashi M, Ohta T, Matsuzawa H. Identification of the penicillin-binding active site of penicillin-binding protein 2 of *Escherichia coli*. J Biochem 1988;104:822-6.
3. Powell AJ, Tomberg J, Deacon AM, Nicholas RA, Davies C. Crystal structures of penicillin-binding protein 2 from penicillin-susceptible and-resistant strains of *Neisseria gonorrhoeae* reveal an unexpectedly subtle mechanism for antibiotic resistance. J Biol Chem 2009;284:1202-12.
4. Sutaria DS, Moya B, Green KB, Kim TH, Tao X, Jiao Y, *et al*. First penicillin-binding protein occupancy patterns of beta-lactams and beta-lactamase inhibitors in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2018;62:1-12.
5. Tipper DJ, Strominger JL. Mechanism of action of penicillins: A proposal based on their structural similarity to acyl-D-alanyl-D-alanine. Proc Natl Acad Sci 1965;54:1133-41.
6. Egorov A, Rubtsova M, Grigorenko V, Uporov I, Veselovsky A. The role of the Ω-loop in regulation of the catalytic activity of TEM-type β-lactamases. Biomolecules 2019;9:1-16.
7. Richards DM, Brogden RN. Cefodizime: A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs 1985;29:105-61.
8. Gozzard DI, Geddes AM, Farrell ID. Ceftazidime-a new extended-spectrum cephalosporin. Lancet 1982;319:1152-6.
9. Pancoast S, Prince AS, Francke EL, Neu HC. Clinical evaluation of piperacillin therapy for infection. Arch Intern Med 1981;141:1447-50.
10. Dickinson GM, Droller DG, Greenman RL, Hoffman TA. Clinical evaluation of piperacillin with observations on penetrability into cerebrospinal fluid. Antimicrob Agents Chemother 1981;20:481-6.
11. Fortner CL, Finley RS, Schimpff SC. Piperacillin sodium : Antibacterial spectrum, pharmacokinetics, clinical efficacy, and adverse react ions. Pharmacotherapy 1982;2:287-96.
12. Holmes B, Richards DM, Brogden RN, Heel RC. Piperacillin a review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs 1984;28:375-425.
13. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, *et al*. SWISS-MODEL: Homology modelling of protein structures and complexes. Nucleic Acids Res 2018;46:W296-303.
14. Lovell SC, Davis IW, Arendall WB 3rd, de Bakker PI, Word JM, Prisant MG, *et al*. Structure validation by calpha geometry: Phi,psi and Cbeta deviation. Proteins 2003;50:437-50.
15. Heo L, Park H, Seok C. GalaxyRefine: Protein structure refinement driven by side-chain repacking. Nucleic Acids Res 2013;41:W384-8.
16. Wiederstein M, Sippl MJ. ProSA-web: Interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res 2007;35:407-10.
17. Wu Q, Peng Z, Zhang Y, Yang J. COACH-D: Improved protein-ligand binding sites prediction with refined ligand-binding poses through molecular docking. Nucleic Acids Res 2018;46:W438-42.
18. Yang J, Roy A, Zhang Y. Protein-ligand binding site recognition using complementary binding-specific substructure comparison and sequence profile alignment. Bioinformatics 2013;29:2588-95.
19. Trott O, Olson AJ. AutoDock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010;31:455-61.
20. Laskowski RA, Swindells MB. LigPlot+: Multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model 2011;51:2778-86.
21. Sippl MJ. Recognition of errors in three-dimensional structures of proteins. Proteins Struct Funct Genet 1993;17:355-62.

Source of Support: Nil. **Conflicts of Interest:** None declared.