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ORIGNAL ARTICLE

Comparative In-Silico Evaluation of Novel Peptide Analogues and Ceftaroline Against Penicillin-Binding Protein 2A in MRSA

Ahmad Raza[1], Warda Ahmed[1], Muhammad Rashid Mehmood[1], Faqiha Tayyeb[1], Syeda Maria Ali[1], Syeda Haniya Jamal[1], Ruqia Khan[1]

[1] Informatix BioLab, Lahore, Pakistan

Correspondence
Ahmad Raza
Email: ahmadraza009293@gmail.com

Conflict of Interest
All the authors have no conflict of interest

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Abstract
Staphylococcus aureus that is resistant to β -lactam is a major health problem in the world and is likely to be a cause of various complications around the globe. This resistance mainly occurs through penicillin-binding protein 2a (PBP2a) which has low affinity to the conventional antibiotics. Even though ceftaroline was found to be effective against MRSA, its usefulness is confined by the emergence of PBP2a mutations. The objective of this research was to examine the thesis of whether HH2 and HG4 novel analog peptides exhibit an increased binding affinity to PBP 2a relative to ceftaroline. The PBP2a protein sequence was retrieved and sequence alignment was carried out against four clusters of PBP2 to determine the conserved regions. To achieve the correct modeling, physiochemical characterization, secondary and tertiary structure prediction of PBP2a were carried out. Molecular docking studies were then performed to evaluate and compare the binding of ceftaroline, HH2 and HG4 to the PBP2a active site. The obtained results of molecular docking demonstrated that ceftaroline showed higher affinity with the PBP2a active site and formed more stable interactions compared to the analog peptides HH2 and HG4. Interactions between the binding energies and essential hydrogen bonding proved that ceftaroline is high in stability, in the binding pocket. Ceftaroline had a greater binding affinity and molecular interactions with PBP2a than HH2 and HG4. These results indicate that, although there are still resistance problems, ceftaroline is a better therapeutic choice in MRSA infection compared to the novel analog peptides trying to be tested. The new research must be directed at structural optimization of peptide analogs in order to increase their binding capacity and counteract new resistance.
***Keywords**: Staphylococcus aureus, MRSA, PBP2a, Ceftaroline, Antimicrobial peptides, Molecular docking*

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant threat to worldwide public health because it has wide-ranging resistance to many different antimicrobials, leading to high levels of morbidity, mortality, and high healthcare costs. Antibiotic resistance is characterized as bacteria's ability to survive and proliferate while being exposed to drugs that should destroy them or slow down their growth. It is a serious crisis in the field of worldwide healthcare. MRSA, a strain of Staphylococcus aureus that is resistant to methicillin and other β-lactam antimicrobials, has emerged as a highly virulent pathogen that has caused a category of serious infections ranging from soft tissue and skin infections to potentially lethal infections like pneumonia and bacteremia. Current epidemiologic reports show that MRSA is a significant percentage of healthcare facility infections, with rates of resistance and corresponding mortality still at alarming levels (CDC, 2014).

The resistance mechanism in Methicillin-resistant *Staphylococcus aureus* (MRSA) is because the bacteria have acquired the mecA gene, that encodes penicillin-binding protein 2a (PBP2a). PBP2a is an altered transpeptidase enzyme with a greatly reduced affinity for β-lactam antibiotics (Kelly et al., 2015). Penicillin-binding proteins (PBPs) are integral bacterial enzymes that facilitate the peptidoglycan crosslinking in its cell wall, a critical activity for bacterial structural integrity and viability. In normal strains of *Staphylococcus aureus*, β-lactam antibiotics

act by inhibiting PBPs, thus readily blocking cell wall construction with resultant bacterial cell lysis. In contrast, PBP2a confers resistance by retaining cell wall crosslinking activity in beta-lactam presence, thereby enabling MRSA strains to survive otherwise lethal antibiotic exposure. The underlying molecular mechanism involves PBP2a allosteric control and its changed binding site that limits antibiotic association and supports an inhibitory result.

Ceftaroline is one of the essential fifth-generation cephalosporin antibiotics because it has the characteristics of high-affinity binding of PBP2a, to restore the activity of β-lactam for MRSA infections. In contrast to earlier cephalosporins, ceftaroline shows strong bactericidal activity. It suppresses both native PBPs and resistant PBP2a and is an efficient therapeutic choice to treat complex skin and tissue infections as well as community-acquired pneumonia induced by MRSA. Ceftaroline exhibits broad-spectrum activity towards both MRSA and multidrug-resistant gram-positive organisms, indicating its potential to control resistant infections.

Its quite disappointing that despite ceftaroline possesses the therapeutic potential, the appearance of resistance through PBP2a mutations is still commonly occurring. This necessitates the need for in silico computational approaches to help with the drug design. From literature, it was observed that in silico analysis and docking of PBP2a with ceftaroline along with novel peptide analogs like HH2 and HG4 could be potentially explored.

Therefore, this study aims to provide in silico docking and evaluation of PBP2a with ceftaroline and novel peptide ligands HH2 and HG4. We will explore them as potential therapeutic agents against MRSA. By integration of different molecular docking approaches, this research work seeks to advance understanding of candidate drug-PBP2a interactions and identify promising candidates for overcoming antibiotic resistance in MRSA infections.

2. Material and methods

Sequence Retrieval

Protein sequence (FASTA format) of *PBP2* protein from *Staphylococcus aureus* with accession number of ALJ10987.1 with 668 amino acid was obtained from National Center for Biotechnology Information Center(NCBI) **(**Available at https://www.ncbi.nlm.nih.gov/snp/). The same FASTA sequence was further used as an input sequence for computational analysis

Sequence Alignment

Sequence alignment was done by using BLAST tool (Available at https://blast.ncbi.nlm.nih.gov/Blast.cgi). there are different variations of BLAST, but we used BLASTp for alignment purpose. We aligned four clusters of *PBP2* protein to analyze the sequence similarity and and find conserved region.

Primary Structure Analysis

Expasy (https://web.expasy.org/translate/) an online tool was used to translate the nucleotide sequence to protein sequence. A nucleotide sequence was given to Expasy and the results were given in the form of Open Reading Frames. The nucleotide sequence was translated to amino-acid sequence and the frames were highlighted red to visualize the protein-coding region.

Physiochemical Analysis

Protparam (https://web.expasy.org/protparam/) was used for the physiochemical properties' analysis of the given protein sequence. The physiochemical properties are given in the form of molecular weight, amino acid composition, theoretical pI, extinction cofficient etc. (Khan et al., 2016).

Secondary Structure Prediction

Psipred (https://bioinf.cs.ucl.ac.uk/psipred/&uuid=a7b8d360-84cc-11f0-94ce-00163e100466) was used to predict the secondary structure of protein. The secodary structure is predicted by providing the FASTA sequence of protein, the predicted structure is shown in the form of helices, coils and beta sheets marked with different colors.

Tertiary Structure Prediction

I-TASSER (https://zhanggroup.org/I-TASSER/) and trRosseta (https://yanglab.qd.sdu.edu.cn/trRosetta/) were used to predict the 3D structures of protein,which provides complete details of the molecular basis of protein function. TrRosetta utilize advanced DL technique to predict protein structure with accuracy while I-TASSER rely on homology modeling for 3D structure building (Kumar & Kim, 2024).

Structural Visualization

PyMOL (https://www.pymol.org/).used to visualize the structure of protein and macromolecules. PyMOL gives the detailed structure of proteins, ligands, and other molecules in the form of lines, cartoon, ribbon, cells, dots and many other forms (DeLano, 2002).

Structural Validation

PROCHECK was used to validate the protein structure. The results are given in the form of Ramachandran plot. The plots has the phi (φ) and psi

(ψ) backbone dihedral angles of every amino acid residue (Sharma & Maheshwari, 2021). This plot assists in determining if residues are within favored, was edited and the Ramachandran plot was optimized by using Discovery studio.

Ligand Selection

Pubchem (https://pubchem.ncbi.nlm.nih.gov) is the largest freely available database, which contains the complete information of different biological molecules. It gives us the complete details of a molecule including its name, molecular formula, compound ID and its date of creation. It also provides the 2D structure of the ligand or molecule being used in research. The ligands we used in research were Ceftaroline (Compound CID 9852981), HH2(Compound CID 135440058), HG4(Compound CID 132000066).

Molecular Docking

Autodock vina is a fast molecular docking tool. It has different versions including AutoDock Vina 1.2.0 and AutoDock 4.2, both of the versions support along scoring function, multiple ligand binding, batch processing and new Python binding to facilitate scripting and workflow development (Eberhardt et al., 2021). Autodock Vina is available at https://github.com/ccsb-scripps/AutoDock-Vina.

allowed, or disallowed areas, and it is an important measure of model quality. The structure of protein

CB Dock is a protein-ligand docking tool that streamlines the docking process by automatically detecting potential binding sites, which calculate their center and size and customize the box size according to the ligand.

Protein–Ligand Interaction Analysis

Protein–Ligand Interaction Profiler (PLIP) (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index) was used for non-covalent interaction between biological macromolecules and ligands. This tool identifies the interactions including hydrogen bonds, hydrophobic contracts and salt bridges. (Salentin et al., 2015)

3. Results

Secondary structure prediction

The secondary structure of protein pbp2a was predicted using PSIPRED. The sequence-based prediction revealed a mixed composition of α-helices, β-strands, and coils (as shown in Fig.1) which are consistent with structural characteristics of penicillin-binding proteins. Further, the distribution and organization of these secondary elements within the protein sequence is illustrated by corresponding cartoon representation (Figure 1.A).

(A) (B)

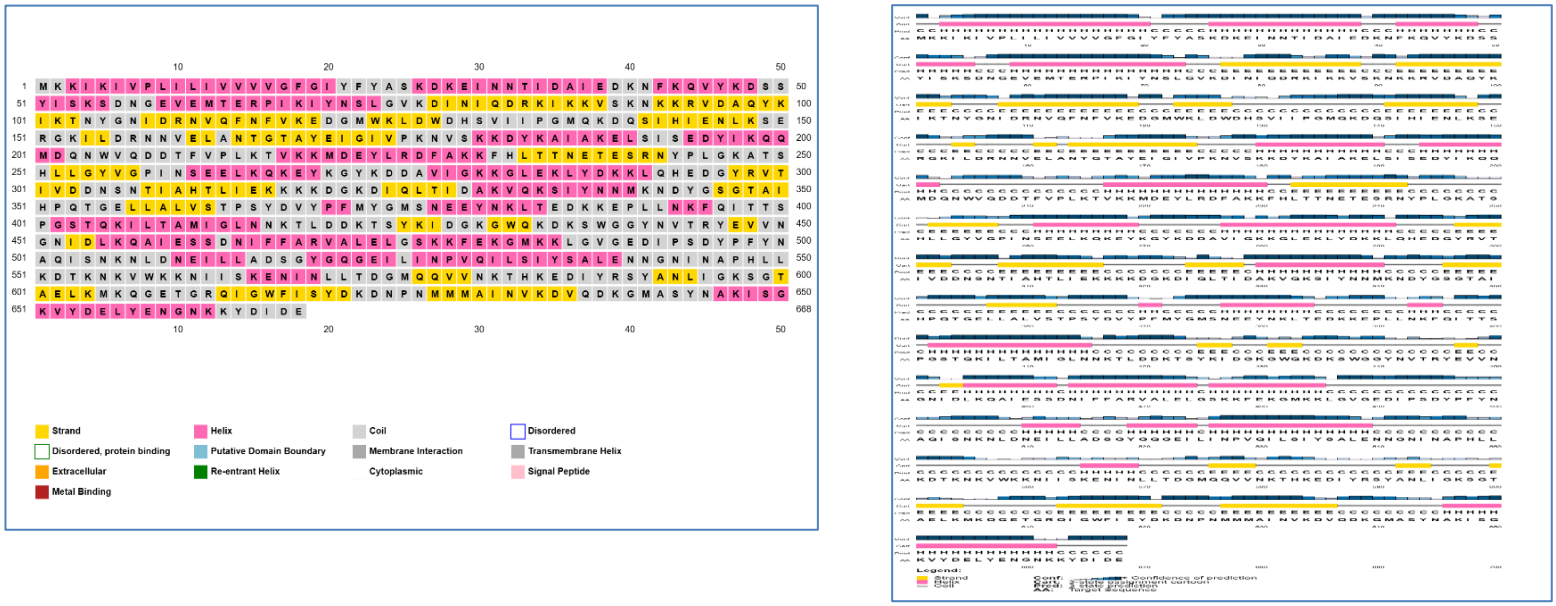


Figure 1. (A) PSIPRED secondary structure prediction sequence plot. **(B)** PSIPRED cartoon plot showing helices, strands and coils

Tertiary structure prediction

The 3D structure of the pbp2a protein was generated using trRosetta. Among the available models, model 1 was selected due to its superior confidence scores. The unrefined model (Fig.2) was further optimized through GalaxyRefine giving a refined structural model with better stereochemical quality (Fig.2).

Both the models were used for docking analysis to assess the influence of refinement on protein-ligand interaction.

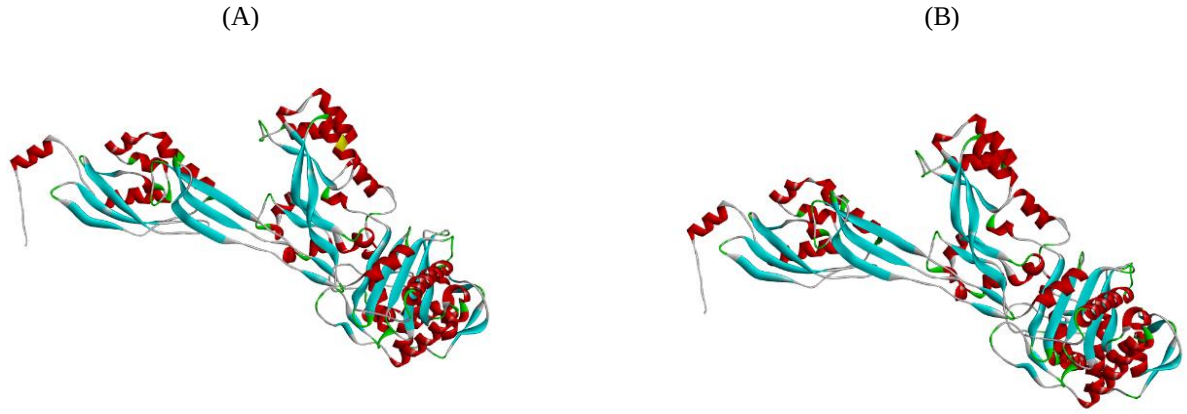


Figure 2. (A) Predicted trRosetta model (unrefined). **(B)** Refined trRosetta model obtained through GalaxyRefine).

Ligand retrieval

Three ligands were selected for docking studies: ceftaroline, HH2 and HG4. The ligands were retrieved from PubChem in SDF format and converted to PDB format for compatibility with AutoDock Vina. The PubChem IDs and structure of these ligands are summarized in Table 1.

Physiochemical properties of protein

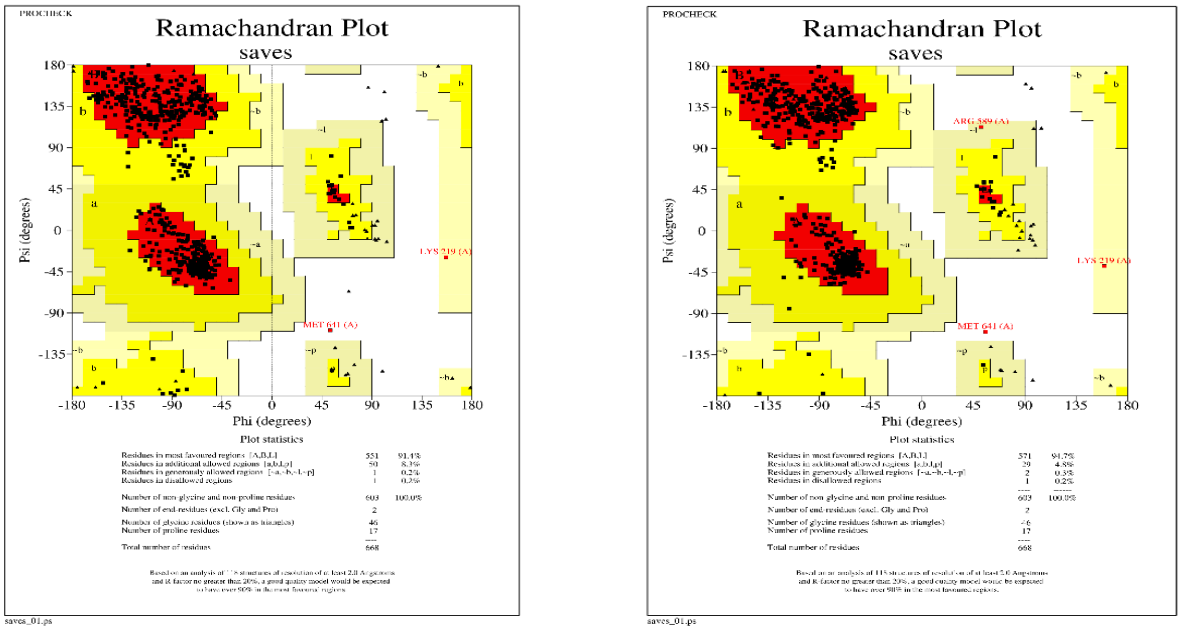
The physiochemical characteristics of protein were computed using ProtParam tool available on the Expasy server(Gasteiger, et al., 2005). The protein has 668 amino acids, with theoretical molecular weight of 76.17 kDa and an isoelectric point of 8.77, which suggests slightly basic nature. The overall instability index (31.03) classifies protein as stable, while the aliphatic index (82.43) indicates thermostability. The GRAVY score (-0.714) reflects a hydrophilic character.

The protein contains 94 acidic residues (Asp+Glu) and 102 basic residues (Arg+Lys). It's computed molecular formula is $C_{3396}H_{5395}N_{903}O_{1047}S_{17}$. The predicted extinction coefficient is 93,630 $M^{-1}cm^{-1}$, with an absorbance value of 1.229 for a 0.1% solution at 280nm. The estimated half life is 30 hours in mammalian reticulocytes (in vitro), over 20 hours in yeast (in vivo), and more than 10 hours in *Escherichia coli* (in vivo)

Structural validation

The quality of the predicted 3D structure was evaluated using Ramachandran plot obtained from PROCHECK website. For the unrefined model 91.4% of the residues were found in the most favoured regions, which indicates acceptable model but with room for improvement. The refined model showed an increase to 94.7% residues in favoured regions. This indicates that

the refinement process improved the stereochemical quality of the structure by increasing the proportion of residues and energetically favourable conformations.



(A) (B)

Figure 3: Ramachandran Plot of (A) unrefined trRosetta model (B) refined trRosetta model.

Docking analysis

The evaluation of binding affinities of ceftaroline, HH2, and HG4 against target protein pbp2a from *Staphylococcus MRSA strain*was done through molecular docking performed using AutoDock Vina. Two structural models of protein were used for this purpose; the trRosetta-predicted model and the refined trRosetta model.

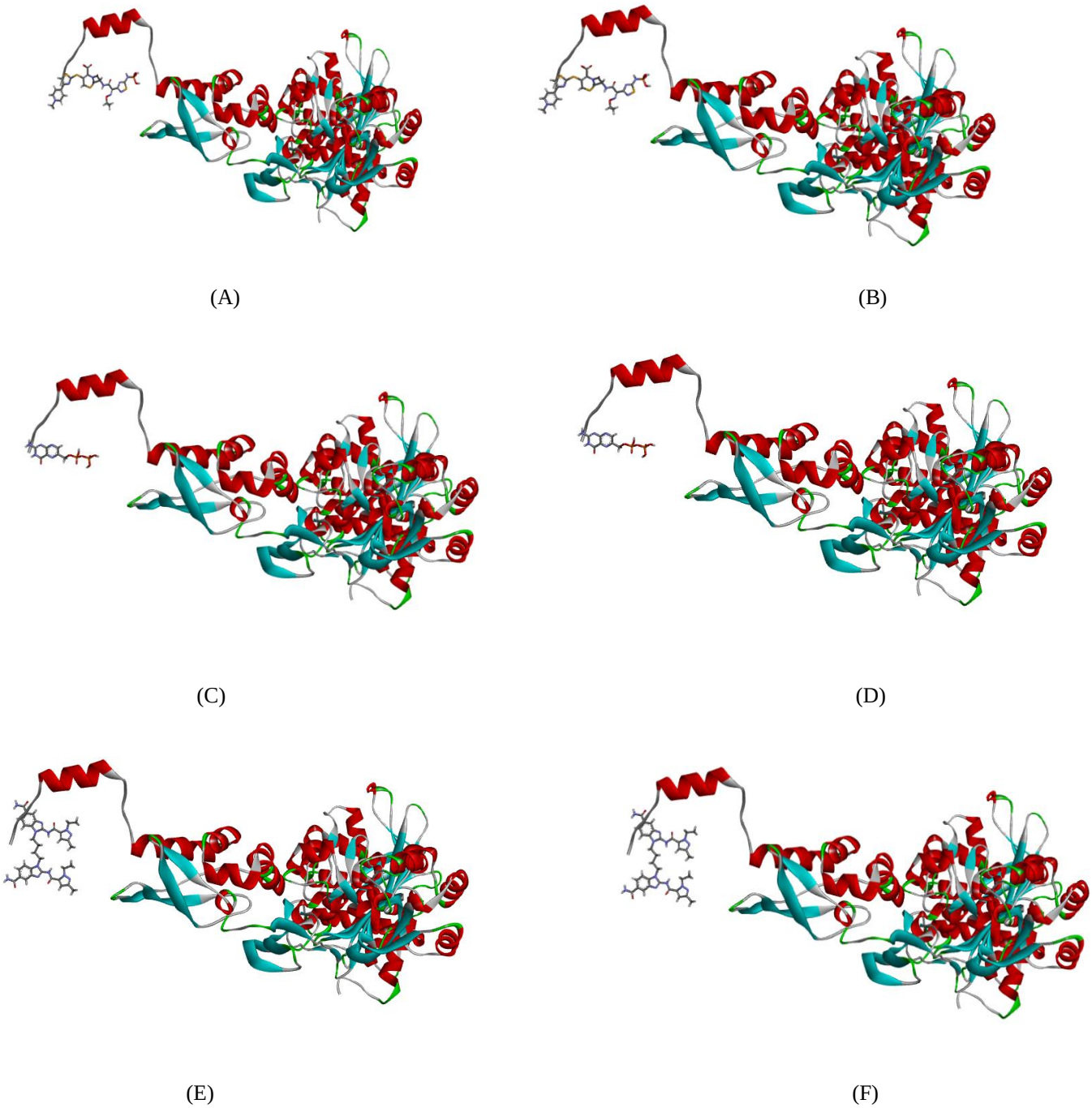
The docking results (shown in Table 2) demonstrated that across both the models ceftaroline consistently exhibited strongest bonding affinity, with binding energies around -7.3 kcal/mol (unrefined trRosetta model) and -7.1 kcal/mol (refined trRosetta model), favourable interactions were shown by HG4 while HH2 displayed weaker binding affinities comparatively.

Binding-site analysis

CB-Dock2 was used for the analysis of binding site. It revealed that all three ligands preferentially occupied C1 cavity in trRosetta model with a cavity volume of 1526Å³ and grid center coordinates at (63, -18, -28). According to ligand size the pocket dimensions varied with ceftaroline docked at 30×30×30Å³, HH2 at 23×23×23Å³, and HG4 at 27×27×27Å³.

In case of purified trRosetta model ceftaroline and HH2 also bounded to C1 cavity with same cavity volume and grid center coordinates as that of unrefined trRosetta's, whereas HG4 occupied distinct C4 cavity, with cavity volume of 460Å³ and grid center coordinates at (84, -10, -29). Indicating possible shift in the preference of HG4 with structural refinement of the protein model.

Consistent with this, visual inspection of docking poses (Figure 4) showed ceftaroline and HH2 in nearly identical orientations in both models while HG4 occupied a slightly elevated position compared to other ligands, reflecting its altered cavity assignment in refined structure.



(A) (B)

(C) (D)

(E) (F)

Figure 4: Docked binding poses of ligands in unrefined and refined trRosetta models of *S.aureus* pbp2a.

(A, B) Ceftaroline in unrefined **(A)** and refined **(B)** models, showing similar orientation.

(C, D) HH2 in unrefined **(C)** and refined **(D)** models, also maintaing consistent position.

(E, F) HG4 in unrefined **(E)** and refined **(F)** models, showing slightly different position

Drug-ligand Interaction profile
Using PLIP, protein-ligand interaction analysis was performed to identify hydrogen bond, hydrophobic interactions and other interactions stabilizing ligand binding.

Ceftaroline: In both the models, ceftaroline formed three hydrophobic interactions (with MET1A AND LYS2A) and four hydrogen bonds (with LYS2A, LYS3A, TYR23A). The recurrence of these interactions across both models highlights the reproducibility of ceftaroline's binding mode.
HH2: HH2 demonstrated weaker binding, in both the models HH2 formed single hydrophobic interaction (MET1A) and four hydrogen bonds primarily involving MET1A, LYS2A, LYS3A.
HG4: Formed only one hydrophobic interaction and no hydrogen bonds in either model.

4. Discussion
In this study, molecular docking revealed that ceftaroline exhibited strong binding affinity with PBP2a, comparable to or even higher than the standard peptide ligands HH2 and HG4. The PBP2a is a major contributor towards the MRSA resistance of *Staphylococcus aureus*. This result is important because PBP2a plays a central role in methicillin-resistant *S. aureus* (MRSA) resistance by reducing the effectiveness of β-lactam antibiotics. The ability of ceftaroline to form stable interactions with the active site residues of PBP2a suggests that it may effectively disrupt this resistance mechanism and restore antibacterial activity of the β-lactam antibiotics (Otero et al., 2013). This reflects that the ceftaroline is an ideal candidate to break the adaptiveness of *S. aureus* towards the cell wall synthesis by binding and inhibiting the PBP2a and hence, inhibition of the growth of the bacteria. This hypothesis also suggests that ceftaroline overcome the steric hindrance associated with the β-lactam resistance in MRSA. It also enables the effective inhibition in even the highly resistant PBP2a variant strains.
Otero et al., (2013) demonstrated that ceftaroline exhibits unique mechanism of binding to allosteric sites of PBP2a among β-lactams, where it can induce conformational changes and sufficiently convert the catalytic site to inhibition site (Otero et al., 2013). Otero et al. (2013) also showed that ceftaroline binding at an allosteric site ~60 Å away triggers structural rearrangements that open the active site for subsequent acylation. This type of allosteric hindrance is of particular importance because as it represents the importance of conformational plasticity rather than relying only on the direct active-site inhibition. More recently, Jiao et al. (2023) confirmed that ceftaroline stabilizes the allosteric domain while enhancing the catalytic region through molecular dynamics simulations. Through this enhancement it is clear that ceftaroline could effectively captures the active-site opening process. These findings by Jiao et al., (2023) are consistent with the docking results performed in this study where strong interactions of ceftaroline with key residues of the catalytic site make it an effective antibacterial agent. The findings by Moisan et al., (2010) also support the results of our analysis. The affinity predictions based conformational changes through docking and molecular dynamic simulations not only strengthens our reliance on computational approaches but also suggests that ceftaroline-PBP2a interactions are energetically favored (Moisan et al., 2010). The study conducted by Acebrón (2015) and colleagues further emphasized that targeting both the allosteric and active sites of PBP2a represents a novel example in combating MRSA resistance. This dual-targeting mechanism of ceftaroline supports its classification under the next generation antibiotics.
The novelty of our work also lies in the comparative docking analysis with standard ligands HH2 and HG4, which allowed us to benchmark ceftaroline's binding affinity. This comparative framework has not been extensively reported before and provides an additional dimension to evaluating ceftaroline's inhibitory potential. We have employed peptide ligands as reference standards which represent more quantitative and biologically relevant context for interpretation of docking scores. These discoveries contribute to the broader understanding of β-lactam–PBP2a interactions and would aid in the rational design of next-generation antibiotics. Nevertheless, this study has certain limitations. Docking simulations are only predictive and do not fully consider the protein flexibility, solvent effects, or the dynamic behavior of ligand–protein complexes under real physiological conditions. Therefore, while our results strongly suggest the inhibitory potential of ceftaroline, the experimental validation through molecular dynamics simulations, enzyme inhibition assays, and microbiological testing is essential to confirm its activity against MRSA.
Furthermore, it is advisable for forthcoming investigations to integrate long timescale simulations with free-energy estimates. It is also suggested to enhance the accuracy of binding affinity estimates and augment the translational relevance. In conclusion, our data substantiate the efficacy of ceftaroline as a significant inhibitor of PBP2a and affirm its therapeutic importance in addressing MRSA resistance. This study enhances the current

understanding by providing comparative structural stresses the need to combine computer methods with experimental research in the search for antibiotics. Overall, the integrative method may speed up the search for new and effective drugs to fight bacteria that are resistant to multiple drugs and the rise of antimicrobial resistance around the world.

5. Author Contributions:

Ahmad Raza[1] contributed to the conceptualization of the study, experimental design, and overall supervision of the research work.
Warda Ahmed[2] was responsible for data collection, bioinformatics analysis, and interpretation of results.
Muhammad Rashid Mehmood[3] performed molecular docking and structure prediction analyses.
Faqiha Tayyeb[4] assisted in literature review, data curation, and preparation of figures and tables.
Syeda Maria Ali[5] contributed to manuscript writing, editing, and formatting for journal submission.
Syeda Haniya Jamal[6] participated in methodology validation, data visualization, and statistical evaluation.
Ruqia Khan[7] assisted in proofreading, referencing, and critical revision of the manuscript for intellectual content.

6. References

1. Acebrón I, Chang M, Mobashery S, Hermoso JA. The allosteric site for the nascent cell wall in penicillin-binding protein 2a: An Achilles' heel of methicillin-resistant Staphylococcus aureus. *Curr Opin Microbiol*. 2015;27:41–7.
2. DeLano, W. L. PyMOL: An open-source molecular graphics tool. *DeLano Scientific.* 2002. https://pymol.org
3. Eberhardt, J., Santos-Martins, D., Tillack, A. F., & Forli, S. AutoDock Vina 1.2. 0: new docking methods, expanded force field, and python bindings. *Journal of chemical information and modeling*, 2021; *61*(8), 3891-3898.
4. Jiao F, Bao Y, Li M, Zhang Y, Zhang F, Wang P, et al. Unraveling the mechanism of ceftaroline-induced allosteric regulation in penicillin-binding protein 2a: insights for novel antibiotic development against MRSA. *Antimicrob Agents Chemother.* 2023; 67(12):e00895-23. Available from:https://pubmed.ncbi.nlm.nih.gov/37971241
5. Kelley, W.L., Jousselin, A., Barras, C., Lelong, E. and Renzoni, A. Missense mutations in PBP2A Affecting ceftaroline susceptibility detected in epidemic hospital-acquired methicillin-resistant *Staphylococcus aureus* clonotypes ST228 and ST247 in Western Switzerland archived since 1998. *Antimicrobial Agents and Chemotherapy*, 2015; *59*(4), pp.1922-1930.
6. Khan, A., Hossain, A., Jahan, N., Ali, S. R., Amin, A., & Morshed, M. N. An in silico Approach for Structural and Functional Annotation of Salmonella enterica serovar typhimurium Hypothetical Protein R_27. *International Journal Bioautomation*, 2016; 20(1): 31-42.
7. Kumar, H., & Kim, P. Artificial intelligence in fusion protein three-dimensional structure prediction: Review and perspective. *Clinical and Translational Medicine*, 2024; *14*(8), e1789om. https://pubmed.ncbi.nlm.nih.gov/25760091
8. Moisan H, Pruneau M, Malouin F. Binding of ceftaroline to penicillin-binding proteins of Staphylococcus aureus and Streptococcus pneumoniae. *Journal of antimicrobial chemotherapy,* 2010; 65(4):713-6.
9. Otero LH, Rojas-Altuve A, Llarrull LI, Carrasco-López C, Kumarasiri M, Lastochkin E, et al. How allosteric control of *Staphylococcus aureus* penicillin binding protein 2a enables methicillin resistance and physiological function. Proc Natl Acad Sci U S A. 2013;110(40):16808–13. https://pubmed.ncbi.nlm.nih.gov/24085846
10. Salentin, S., Schreiber, S., Haupt, V. J., Adasme, M. F., & Schroeder, M. PLIP: fully automated protein–ligand interaction profiler. *Nucleic acids research*, 2015. *43*(W1), 443-447.
11. Sharma, S., & Maheshwari, S. Protein Structure and Function Prediction of SARS-CoV 2: Prospective Antivirus Active Drug Binding Sites. *Materials Science and Engineering Conference Series,* 2021; 1170(1).