ISSN 2063-5346

IN SILICO QUANTUM COMPUTATION ASSESSMENT OF VARIOUS DRUG COMPOUNDS AGAINST PENICILLIN BINDING PROTEIN 2A (PBP2A) OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

Amit Ram[1], *Ashish Saraf[2] and Vishwaprakash Roy[3]

[1,2,3]School of Sciences, MATs University, Raipur, C.G.India

Article History: Received: 15.04.2023 **Revised:** 10.05.2023 **Accepted:** 25.05.2023

Abstract

Bacterial resistance to β-lactam antibiotics pose a serious threat to human health. Penicillin binding proteins (PBPs) and β-lactamases are involved in both antibacterial activity and mediation of β-lactam antibiotic resistance. The two major reasons for resistance to β-lactams include: (i) pathogenic bacteria expressing drug insensitive PBPs rendering β-lactam antibiotics ineffective and (ii) production of β-lactamases along with alteration of their specificities. Thus, there is an urgent need to develop newer β-lactams to overcome the challenge of bacterial resistance. Therefore, the present study aims to identify the binding affinity of β-lactam antibiotics with different types of PBPs and β-lactamases. In this study, Cephalosporins and Carbapenems are docked in to PBP2a of Staphylococcus aureusand SHV-1β-lactamase of Escherichia coli. The results reveal that Ceftobiprole can efficiently bind to PBP2a and not strongly with SHV-1 β-lactamase. Furthermore, molecular dynamics (MD) simulations are performed to refine the binding mode of docked complex structure and to observe the differences in the stability of free PBP2a. MD simulation supports greater stability of Ceftobiprole-PBP2a complex compared to free PBP2a. This work demonstrates that potential β-lactam antibiotics can efficiently bind to different types of PBPs for circumventing β-lactam resistance and opens avenues for the development of newer antibiotics that can target bacterial pathogens.

Key words: MRSA, molecular docking, PBP2a, Cephalosporins, Carbapenems, β-lactamase.

Introduction

Integration of computational approaches for drug designing is one of the promising tools to find lead molecules. In continuation of the recent development of drug discovery approaches, computational methods such as molecular modelling, molecular docking/dynamics, and QSAR provide the better resolution to understand the underlying mechanism of molecular action at the atomic level and in-depth understanding to rationalize the structure-activity relationship (SAR) of molecules for further lead optimization (Mishra and Pathak, 2018). At the end of the lead optimization step, only those derivatives with the very best, experimentally confirmed, binding affinity will be retained and find their way into the next step.

Antibiotics originally were defined as metabolites of small microbes that inhibit the growth or kill other microorganisms (Romano, 1953; Mlynarczyk et al., 1979). Now they refer more generally to drugs used for the treatment of bacterial infections. The first antibiotic, penicillin (Patelski and Hobby, 1952; Fletcher, 1984), was discovered in 1927 by

Alexander Fleming (Hughes, 1952; Lachowicz, 1960) and first used for medical treatment in 1940s (Steinman, 1962;Sabath et al., 1977). Penicillin changed medical care fundamentally, reduced disease and death, and in some cases eliminated the bacterial disease as a cause of death. Although the first antibiotics were natural compounds produced by other microorganisms, these were followed by some semi-synthetic antibiotics such as roxithromycin and clindamycin that derived produced by chemical modification of natural antibiotics (Kayser et al., 1970; Whipp, 1987). Now over 10,000 antibiotics have been characterized and synthesized and grouped into several different classes, and the most frequently used is the β-lactam class (Sieradzki et al., 2003; Kareiviene et al., 2006)

β-lactams (Penicillins, Cephalosporins and Carbapenems) are potent inhibitors and have been used effectively over several decades against different types of bacterial infections, due to their higher effectiveness, low cost, ease of use, and minimal side effects (Frere and Joris, 1985, Matagne*et al.,* 1999). β-lactam antibiotics form stable acyl enzyme complex with penicillin binding proteins (PBPs) in the bacterial cell membrane, thus inhibiting the final stages of peptidoglycan biosynthesis (Macheboeuf*et al.,* 2006, Chain, 1948a, Florey, 1948b). However, Gramnegative bacteria have developed resistance to β-lactams through three different strategies: (i) structural modification in PBP targets, (ii) production of β-lactamase (Williams and Moosdeen, 1986, Reid, 1987, Jorgensen, 1982) and (iii) active expulsion of β-lactam antibiotics via efflux pumps (Wilke *et al.,* 1948). Penicillin binding proteins (PBPs) are enzymes that catalyze the steps involved in bacterial cell wall biosynthesis and are the target enzymes of βlactam antibiotics (Fischer *et al.,* 2005, Macheboufet *al.,* 2006) PBPs have been classified into two types, high molecular weight (hmv) PBPs act as transpeptidases and low molecular weight (lmv) PBPs generally act as D-alanyl-Dalanine-carboxypeptidases (DD-carboxypeptidases) (Macdonough *et al.,* 2002) The β-lactam antibiotics inhibit both transpeptidase and DD-carboxypeptidase activities by acylating the active-site serine of PBPs (Hakenbeck, 1998). Alterations of the PBPs reduce their binding affinity for β-lactam antibiotics, resulting in drug resistance. Another common mechanism of bacterial resistance to the β-lactam antibiotics is the production of β-lactamase that inactivates β-lactams by hydrolyzing the amide group of the β-lactam ring (Matagne*et al.,* 1990, Matagne and Freare, 1995) Therefore there is an urgent need to tackle this bacterial resistance with the help of a newer antibiotics.

Cephalosporins have a broad spectrum of activity against Gram negative and Gram-positive organisms such as *Streptococcus pneumoniae, Haemophilus influenzae, Streptococcus pyogenes, Klebsiella pneumoniae* and *Staphylococcus aureus*. (Clark *et al.,* 2002, Fenoll*et al.,* 2007, Lee *et al.,* 2006, Shimizu *et al*, 2007, Soriano *et al.,* 2003, Soriano *et al.,* 2004)*S. aureus* and *S. pneumoniae* are leading causes of hospital and community acquired bacterial infection and they are global health threat (Chambers, 1997, Enright *et al.,*2007). However, many community-based infections are becoming more difficult to treat owing to the emergence of resistant organism such as multidrug-resistant *S. pneumoniae* (MDRSP) and methicillin-resistant *S. aureus* (MRSA) (Bambeke*et al.,* 2007, Satnkovic*et al.,* 2007). These two organisms are developing resistance to many of the β-lactam antibiotics (Chiu *et al.,*2007, Niederman and Chest, 2007).*S. pneumoniae* contains six PBPs, PBP1a, PBP1b, PBP2a, PBP2b, PBP2x and PBP3. β-lactam antibiotics resistance in *S. pneumoniae* is caused by alterations in the penicillin-binding domains of one or more of these six PBPs (Hakenbeck*et al.,* 1998, Hakenbeck, 1999, Laible*et al.,*1991).

MRSA acquires resistance to such antibiotics due to altered PBP2a that have low affinity for β-lactam antibiotics.Several studies revealed the mechanism of resistance of S. pneumoniae and MRSA to β-lactams using only a few cephalosporins and Carbapenems. This prompted us to investigate in detail using a wide spectrum of β-lactam antibiotics (both Cephalosporins and Carbapenems). Our study mainly focused on PBP2a of MRSA. Molecular docking studies are performed to investigate possible binding mechanism of Cephalosporins and Carbapenems with PBP2a of MRSA. Furthermore, molecular dynamics (MD) simulations are carried out to refine the binding conformation of docked complex structure and to investigate the structural stability PBP2a in the absence and presence of ligand.The present study also compares Cephalosporins and Carbapenem to find out which one exhibits the lowest binding affinity with SHV-1 β-lactamase.

Materials and Methods

Data set

3-Dimensional (3D) structures of the PBPs and β-lactamase were obtained from Protein Data Bank (PDB) (Berman *et al.,* 2000). Co-crystallized ligands were identified and removed from the target proteins and then crystallographic water molecules were eliminated from the 3D coordinate file. Missing side chains were reconstructed to the target protein structures and minimizations were performed using SwissPDBviewer (Guex and Peitsch, 1997) The structures of β-lactam antibiotics were obtained from NCBI PubChem Compound database (Cheng *et al.,* 2010) and the structures were drawn using Chemsketch (Li *et al.,* 2004). Hydrogen atoms were added to all the structures and gasteiger atomic partial charges were computed. A geometry optimization of all the compounds was performed by using chimera (Pettersen *et al.,* 2004) for flexible conformations of the compounds during the docking. The PDB ID, source and detail of PDB structures employed for the study are listed in Table 1.

Table 1 Details of structures selected for Molecular Docking Studies

PDB id	Detail	Source	Reference
4CJN	Crystal structure of PBP2a of MRSA in complex with quinazolinone ligand	Methicillin resistant *Staphylococcus aureus*	Lim and Strynadka, 2002
1IYS	Crystal structure of class A β-lactamase toho-1 (chain A)	Escherichia coli	Ibuka *et al*., 2003

Molecular Docking studies

Molecular docking is a very much investigated technique for recognizing the potent compound without putting excessively exertion and investment in research. AutoDock 1.5.6 software is used by us to investigate the activity in terms of binding affinity (Kcal/mol), and there after the outcomes are compared in binding affinity score for best-docked conformation. All the ligand structures were optimized by energy minimization using MM2 method and converted to readable format at the ADT interface. The outcomes of results were analyzed by AutoDock Vina (ADT) result which reveals close contact, hydrogen bond, hydrophilic, and hydrophobic interactions.First, the validation of protein (4CJN) was done by extraction of ligand and after extraction of ligand from the protein; it was prepared for the docking study by adding the polar hydrogen, detecting root, and converting it to pdbqt extension file. For docking studies, after extraction of ligand, the protein is prepared by removing water molecules, repairing missing atoms, adding polar hydrogen

only, and subsequently adding the Kollman charges. Further, the grid box was generated keeping the ligand as a centre. From grid output file the configuration file "conf.txt" was prepared and command prompt was used for ADT molecular docking by giving command "program files\the scripps research institute\autodock\autodock4. exe - config conf.txt - log log.txt" It generated the output file with the docking score or binding affinity (Kcal/mol), similarly, all the designed molecules were studied.

Molecular dynamics simulation

Ceftobiprole has least docking score when compared to other compounds. MD simulation was carried out for PBP2a-Ceftobiprole docked complex. GROMACS 2018.1 package was used to run and analyze 100 ns MD simulation. GROMOS96 43a1(force field) in single point charge (SPC) water models was used to generate protease and ligand force field and parameter files for the protein and PRODRG server for ligand respectively. The system was then solvated with water molecules in separated cubic boxes with 10 Å distance from the edge of the box. The system was neutralized by adding 4 NA^+ counter ions. The energy minimization the system was performed through running the steepest descent minimization algorithm with 50000 steps to achieve stable system with maximum force < 1000 kJ mol^{-1} nm^{-1}. Prior to the running of real dynamics, the solvent and ions of the system was equilibrated by NVT (constant under number of particles, volume, and temperature) and NPT (constant number of particles, pressure, and temperature) ensemble. After the completion of the MD simulation run, the trajectories were used for various dynamics analysis such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), number of hydrogen bonds, etc.The trajectories were analyzed using the tools from GROMACS distribution. All the graphs were generated using QTgrace tool.62

Results and discussion

Ligand conformation

Evaluation of binding affinity of β-lactam antibiotics with PBP2aand SHV-1 βlactamase are performed using AutoDock. The binding poses for each ligand molecule into the PBPs and SHV-1 β-lactamase are determined and different poses are generated based on the total score (Dock score). The total score is an indicator of the binding affinity of a ligand-receptor complex. The docking scores (-logKd) for fourth and fifth generation Cephalosporins and Carbapenems are depicted in Table 2. The binding conformation for each ligand molecule into the PBP2aand SHV-1 β-lactamase target proteins are determined and the one having highest docking score with PBPs and lower docking score with SHV-1 β-lactamase aregenerated. The higher docking scores represent better protein-ligand binding affinity compared to lower docking score values. Among the 13 ligands, the fifth-generation cephalosporin, Ceftobiprole has high docking score value with PBPs (Dock score for PBP2a=-5.2) and low docking score for SHV-1 β-lactamase (Dock score SHV-1=-3.2). From the docking results, the variation is observed in the binding affinity of Ceftobiprole with PBP2a and SHV-1 β-lactamase. Ceftobiprole shows high binding affinity with PBPs especially it has highest binding affinity with PBP2a where-as for SHV-1 β-lactamase binding affinity of Ceftobiprole is lower. We further analyzed the docked conformation for finding the binding mode of Ceftobiprole into PBP2aand SHV-1 β-lactamase target proteins to validate the position obtained likely to represent reasonable binding conformations.

Docking of Ceftobiprole into Penicillin binding proteins PBP2a

Binding affinity of Ceftobiprole towards PBP2a is investigated in detail. The number of H-bonds and binding residues of PBP2a with Ceftobiprole complexes are shown in Table 3-6. From the post docking analysis, it is found that the Ceftobiprole shows high binding affinity with PBP2a. On analysis of the interaction of Ceftobiprole in the active site of PBP2a, it is observed the residues Gln292, Asp295 and His293 in PBP2a are participating in the H-bond interaction (Fig. 1A). From the Fig 1A, it is found that His293 and Asp295 contributed the greatest number of H-bond interaction with Ceftobiprole. Moreover, these two residues are present in buried region of protein structure.

Docking of Ceftobiprole in to SHV-1 β-lactamase

Docking analysis of β-lactam antibiotics with SHV-1 β-lactamase is carried out to identify the drug which is having lowest binding affinity with SHV-1 β-lactamase. Among the 13 β-lactam antibiotics selected for this study, Ceftobiprole shows the dock score value of -4.5 (Table. 2). It is lower when compared to other β-lactam antibiotics. It indicates Ceftobiprole has lower binding affinity with SHV-1 β-lactamase. From the interaction residues analysis, it is observed that only three amino acid residues Gln292, Asp295 and His293 are involved in the interaction with Ceftobiprole and formed less number of hydrogen bonds (Table 2). Among these interacting residues, Asp295 is present in buried region of protein structure. The possible binding mode of Ceftobiprole into the binding site of SHV-1 β-lactamase and corresponding 2D interaction models, number of hydrogen bonds and bond distance are shown in Fig 1B.

Hydrophobic interaction

Hydrophobic interactions are also a crucial element of binding for Ceftobiprole. Hydrophobic interactions should play an important role in the ligand-protein interaction. The residues of PBP2aand SHV-1 β-lactamase involved in the hydrophobic interaction with Ceftobiprole are analysed using Ligplot tool (Fig. 2). In PBP2a, Tyr297, Asn146, Lys273, Asp295, Glu294 and Val277 are in hydrophobic contact with Ceftobiprole (Fig. 2A). In SHV-1 β-lactamase, only four residues Tyr241, Asp240, Gly238 and Tyr105 are hydrophobic contact with Ceftobiprole (Fig. 2D). These observations are significant and might be the probable cause for higher affinity of Ceftobiprole to PBPs and lower binding affinity to SHV-1 β-lactamase.

Molecular dynamics simulation

MD simulations are conducted for the protein-ligand complex as well as for the free enzyme. This provided a better picture of the overall stability of the PBP2a and PBP2a complex with Ceftobiprole within nanosecond time scale. Ceftobiprole-PBP2a complex is selected because Ceftobiprole has high binding affinity with PBP2a. The complex model and the free enzyme are subjected to 10 ns MD simulations in order to find the stability of the PBP2a in the presence of the Ceftobiprole. Root-mean-square-deviation (RMSD), Root mean square fluctuation (RMSF), Radius of gyration (Rg) and H-bonds are used to check the stability of the model system.

Root mean square deviation

The RMSD, a crucial parameter to analyse the equilibration of MD trajectories. RMSD of the protein backbone atoms are plotted as a function of time to check the stability of each system throughout the simulation. The RMSD values of the PBP2a backbone with and without Ceftobiprole are calculated against the simulation time scale (0-10000 ps) and results are shown in Fig 3. It can be noted that two trajectories have RMSD values within 0.1- 0.6 Å during 10000 ps simulation. From the Fig 3, it is seen that

for free PBP2a system, The RMSD values is 0.35 nm at 6000 ps. After this, the RMSD value is increased up to 0.6 nm. For PBP2a-Ceftobiprole complex, the RMSD values steadily increase till 3000 ps followed by a slow increase up to 4500 ps. After this there is no further increment of RMSD values and the complex systems reached equilibrium. After 6000 ps, PBP2a-Ceftobiprole complex system shows lower RMSD value than the free PBP2x system. The decrease in RMSD value of the complex from that of the free PBP2a indicates increased rigidity and stability of the PBP2x upon binding with Ceftobiprole.

Root mean square fluctuation

The RMSF with respect to the average MD simulation conformation is used as a mean describing flexibility differences among residues. The RMSF of the backbone atoms of each residue in the PBP2a-Ceftobiprole and in free PBP2a is calculated to reveal the flexibility of the backbone structure. The high RMSF value shows more flexible where as low RMSF value shows limited movements during simulation in relation to its average position. The RMSF of the residues are shown in Fig 4, clearly depicting different flexibility in the PBP2a in the absence and presence of Ceftobiprole. In Fig 4, it is found that the residues (100-156 and 558-566) in PBP2a without Ceftobiprole show more fluctuation than the PBP2a-Ceftobiprole complex. The residues in the PBP2a that bind with the Ceftobiprole shows a small degree of flexibility with RMSF of less than 4.00 nm when compared with the free PBP2a, reveals that the residues of PBP2a in the presence of Ceftobiprole seem to be more rigid as a result of binding to Ceftobiprole.

Radius of gyration and H-bond network

We also performed Rg to understand the level of compaction in the structure of PBP2a in the absence and presence of Ceftobiprole. The Rg is defined as the mass weighted root mean square distance of a collection of atoms from their common center of mass. Hence this analysis gives us the overall dimensions of the protein. The calculated Rg values over the simulation time scale for the PBP2a and the PBP2a-Ceftobiprole complex are shown in Fig 5. Rg value of PBP2a-Ceftobiprole and free PBP2a varies between 2.55 nm to 2.75 nm. As shown in Fig 5, it is observed that for PBP2a, the Rg values fluctuate near 2.76 nm and then decrease to a minimum value of 2.68 nm and for PBP2a-Ceftobiprole complex the Rg value initially fluctuate near 2.60 nm after 200 ps the Rg value is decreased up to 2.50 nm. From the Rg plot, the PBP2a and PBP2a-Ceftobiprole complex curve differs significantly and PBP2a-Ceftobiprole complex shows lower Rg value than the PBP2a. During simulation the change of Rg value from PBP2a to PBP2a-Ceftobiprole over simulation time reveals stabilization and little conformational changes in PBP2a when bound to the Ceftobiprole. The intermolecular hydrogen bonding between the protein and ligand plays an essential role in stabilizing the protein-ligand complexes. The stability of hydrogen bond network formed between Ceftobiprole and PBP2a is calculated throughout the simulation for the ligated system. Total number of H-bonds in Ceftobiprole-PBP2a complex versus time at 300K is shown in Fig 6. Ceftobiprole-PBP2a complex exhibited seven H-bonds throughout the simulation time period. It indicates that the Ceftobiprole shows stable and strong H-bonds with PBP2a.

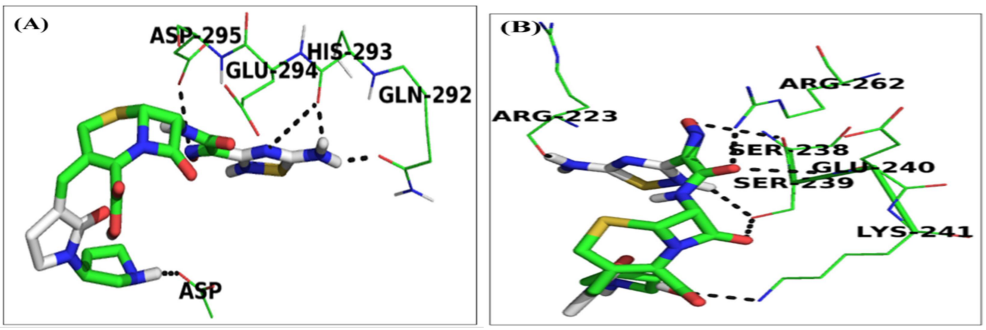

Fig.1 Docking results of Ceftobiprole in to PBP2a, PBP2b, PBP2x and SHV-1. (A) Binding mode of Ceftobiprole in PBP2a. (B) Binding mode of Ceftobiprole with SHV1. Ligand atoms are coloured by its type. The interacted amino acids residues, hydrogen bond networks in the binding pocket are all shown.

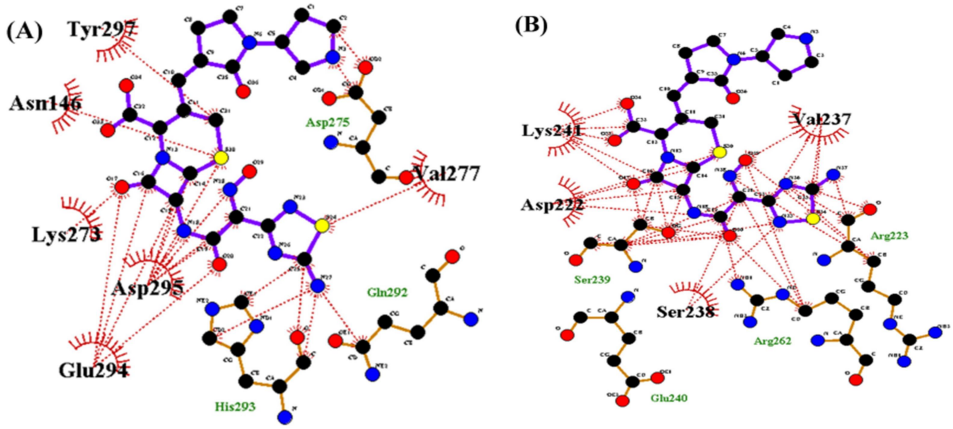

Fig. 2 Schematic representation of the hydrophobic interaction between Ceftobiprole with A) PBP2a (B) SHV-1 produced using the LIGPLOT program [43]. Hydrophobic contacts are indicated by an arc with spokes radiating towards the ligand atoms they contact. The interacted atoms are spokes radiating back.

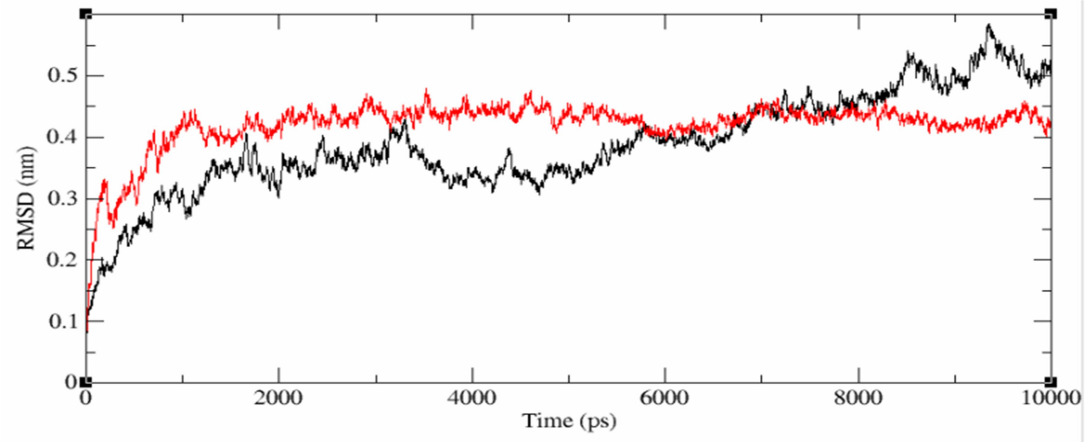

Fig. 3 Backbone RMSDs are shown for PBP2a in the absence and presence of Ceftobiprole at 300K. Black color indicates PBP2a in the absence of Ceftobiprole, PBP2x-Ceftobiprole complex shown in red.

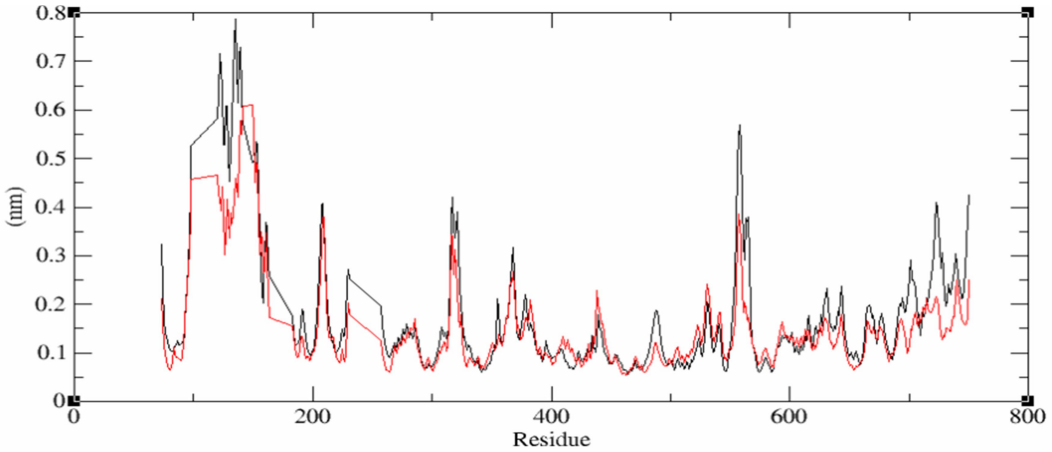

Fig. 4 RMSF of the backbone atoms of PBP2a in the presence and absence of Ceftobiprole at 300K. Black color indicates PBP2a in the absence of Ceftobiprole, PBP2a-Ceftobiprole complex shown in red.

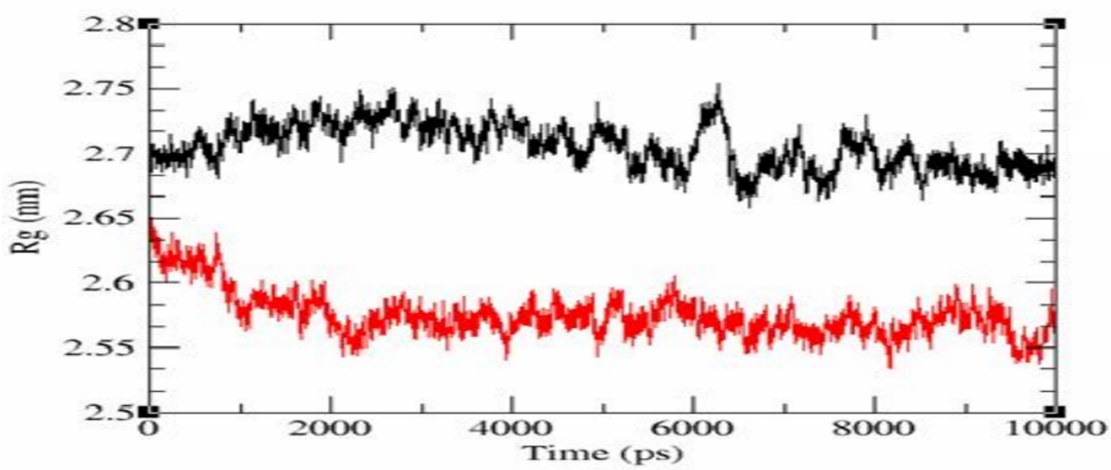

Fig. 5 Radius of gyration of Cα atoms of PBP2a in the presence and absence of Ceftobiprole. Black color indicates PBP2x in the absence of Ceftobiprole, PBP2a-Ceftobiprole complex shown in red.

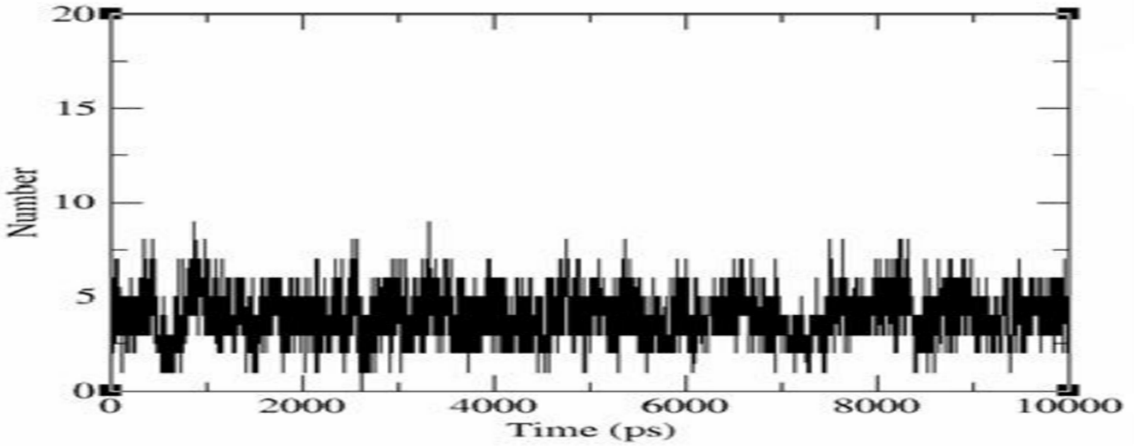

Fig. 6 Total number of H-bond between Ceftobiprole-PBP2a complex versus time at 300K.

Table 2 Docking results of β-lactam antibiotics with Penicillin binding proteins (PBP2A) and SHV-1 β-lactamase

S.No	Ligands	PBP-2a	SHV-1	Interacting residues	H-Bonds
1.	Cefepime	-3.3	-2.4	His293, Asp295, Glu239	3
2.	Cefozopran	-4.2	-2.1	His293, Asp295, Lys148	3
3.	Cefpirome	-3.4	-2.3	Lys273	1
4.	Cefquinome	-3.2	-3.1	Asp295	1
5.	Ceftaroline	-4.0	-2.9	Tyr297	2
6.	Ceftobiprole	-5.2	-4.5	Gln292, Asp295, His293	5
7.	Doripenem	-4.2	-3.8	His293, Asp295, Glu239	5
8.	Ertapenem	-4.3	-3.4	Asp295, Ser149, Lys148, Thr165	4
9.	Faropenem	-3.7	-3.0	Ser149, Lys48	2
10.	Imipenem	-3.9	-3.2	Asp295, Val277, Gln292	5
11.	Meropenem	-4.8	-4.5	Asp295, Lys148, Glu239	5
12.	Tebipenem	-4.2	-3.6	His293, Asn146	2
13.	Thienamycin	-4.3	-2.1	Lys273, Val277, His293	3

Conclusion

In the present study, the molecular docking and MD simulations are performed to investigate the reasonable binding conformation of β-lactam antibiotics with PBP2a of *S. aureus*. The best docked conformation is selected based on binding energy scores, hydrogen bonding and hydrophobic interaction. Cephalosporins show higher affinity with PBPs than Carbapenems. Especially the fifth generation Cephalosporin, Ceftobiprole shows best results with PBP2a. The conclusion drawn from this docking is that Ceftobiprole has the highest binding affinity with PBP2a of S. aureus. Several Class-A β-lactamase enzymes have the potential to hydrolyze Cephalosporins. Therefore, the present study also investigated the binding affinity of β-lactams (Cehaplospoirns and Carbapenems) with SHV-1 β-lactamase and solvent accessible surface area of amino acid residues involved in both H-bond and hydrophobic interactions with Ceftobiprole are identified. Our observations on amino acid residues in the active site suggest, that they are buried in PBPs and there are more hydrophobic and H-bond interactions with Ceftobiprole. However, the residues in the active site of SHV-1 β-lactamase are exposed and there are only a few H-bond and hydrophobic interactions with Ceftobiprole. Thus, Ceftobiprole may not be hydrolyzed by SHV-1 β-lactamase while it binds strongly to PBPs. Furthermore, MD simulation is performed to check the stability of the Ceftobiprole-PBP2a complex. RMSD, RMSF, Rg, H-bond and PCA results indicates that Ceftobiprole-PBP2a complex is highly stable compared to free PBP2x. Over all, from the results of the present study, it is strongly suggested that Ceftobiprole is a potent inhibitor of PBP2a,which can be further modified and explored as a potential next generation β-lactam antibiotic for S. aureus and S. pneumoniae infections.

References:

1. Mishra, V. and Pathak, C. Structural insights into pharmacophore-assisted in silico identification of protein-protein interaction inhibitors for inhibition of human toll-like receptor 4 - myeloid differentiation factor-2 (hTLR4-MD-2) complex. Journal of biomolecular structure & dynamics 2018:1-24.
2. Romano, A. (1953). Strain of Staphylococcus aureus of high resistance to antibiotics. *Revista de la sanidadmilitarargentina*, *52*(1), 107-108.
3. Młynarczyk, A., Młynarczyk, G., &Osowiecki, H. (1979). Mechanisms of resistance to antibiotics in Staphylococcus aureus. *Polski TygodnikLekarski (Warsaw, Poland: 1960)*, *34*(39), 1537-1540.
4. Patelski, R., & Hobby, G. L. (1952). The first decade of antibiotics; 1941-51; penicillin to terramycin. *Medicine illustrated*, *6*(4), 155-166.
5. Fletcher, C. (1984). First clinical use of penicillin. *British Medical Journal (Clinical research ed.)*, *289*(6460), 1721.
6. Hughes, J. D. (1952). Antibiotic and chemotherapeutic agents in diseases of the gastrointestinal tract. *Journal of the American Medical Association*, *150*(15), 1456-1459.
7. STEINMAN, H., & BRANDRISS, M. (1962). Current developments in penicillin therapy and hypersensitivity. *The Medical annals of the District of Columbia*, *31*, 689-698.
8. Sabath, L. D., Laverdiere, M., Wheeler, N., Blazevic, D., & Wilkinson, B. (1977). A new type of penicillin resistance of Staphylococcus aureus. *The Lancet*, *309*(8009), 443-447.
9. Kayser, F. H., Benner, E. J., &Hoeprich, P. D. (1970). Acquired and native resistance of Staphylococcus aureus to cephalexin and other β-lactam antibiotics. *Applied microbiology*, *20*(1), 1-5.
10. Whipp, P. (1987). Staph aureus: resistance to antibiotics. *Professional nurse (London, England)*, *2*(9), 282-284.
11. Sieradzki, K., Leski, T., Dick, J., Borio, L., & Tomasz, A. (2003). Evolution of a vancomycin-intermediate Staphylococcus aureus strain in vivo: multiple changes in the antibiotic resistance phenotypes of a single lineage of methicillin-resistant S. aureus under the impact of antibiotics administered for chemotherapy. *Journal of clinical microbiology*, *41*(4), 1687-1693.
12. Kareivienė, V., Pavilonis, A., Sinkutė, G., Liegiūtė, S., &Gailienė, G. (2006). Staphylococcus aureus resistance to antibiotics and spread of phage types. *Medicina*, *42*(4), 332-339.
13. Frère, J. M., Joris, B., &Shockman, G. D. (1984). Penicillin-sensitive enzymes in peptidoglycan biosynthesis. *CRC Critical reviews in microbiology*, *11*(4), 299-396.
14. P. Macheboeuf, C. Contreras-Martel, V. Job, O. Dideberg and A. Dessen, FEMS Microbiol. Rev., 2006, 30, 673-691.
15. Chain, E. Á. (1958). Chemistry and biochemistry of antibiotics. *Annual Review of Biochemistry*, *27*(1), 167-222.
16. FLOREY, H. W. (1955). Antibiotic products of a versatile fungus. *Annals of internal medicine*, *43*(3), 480-490.
17. Williams, J. D., &Moosdeen, F. (1986). Antibiotic resistance in

Haemophilus influenzae: epidemiology, mechanisms, and therapeutic possibilities. *Reviews of Infectious Diseases*, *8*(Supplement_5), S555-S561.

18. Jorgensen, J. H. (1992). Update on mechanisms and prevalence of antimicrobial resistance in Haemophilus influenzae. *Clinical infectious diseases*, *14*(5), 1119-1123.
19. Fisher, J. F., Meroueh, S. O., &Mobashery, S. (2005). Bacterial resistance to β-lactam antibiotics: compelling opportunism, compelling opportunity. *Chemical reviews*, *105*(2), 395-424.
20. Macheboeuf, P., Contreras-Martel, C., Job, V., Dideberg, O., &Dessen, A. (2006). Penicillin binding proteins: key players in bacterial cell cycle and drug resistance processes. *FEMS microbiology reviews*, *30*(5), 673-691.
21. McDonough, M. A., Anderson, J. W., Silvaggi, N. R., Pratt, R. F., Knox, J. R., & Kelly, J. A. (2002). Structures of two kinetic intermediates reveal species specificity of penicillin-binding proteins. *Journal of molecular biology*, *322*(1), 111-122.
22. Hakenbeck, R. (1998). Mosaic genes and their role in penicillin-resistant Streptococcus pneumoniae (minireview). *Electrophoresis*, *19*(4), 597-601.
23. Matagne, A., Lamotte-Brasseur, J., & Frère, J. M. (1998). Catalytic properties of class A β-lactamases: efficiency and diversity. *Biochemical Journal*, *330*(2), 581-598.
24. Matagne, A., & Frère, J. M. (1995). Contribution of mutant analysis to the understanding of enzyme catalysis: the case of class A β-lactamases. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, *1246*(2), 109-127.
25. Clark, C. L., Nagai, K., Dewasse, B. E., Pankuch, G. A., Ednie, L. M., Jacobs, M. R., & Appelbaum, P. C. (2002). Activity of cefditoren against respiratory pathogens. *Journal of Antimicrobial Chemotherapy*, *50*(1), 33-41.
26. Fenoll, A., Giménez, M. J., Robledo, O., Coronel, P., Gimeno, M., Casal, J., & Aguilar, L. (2007). Activity of cefditoren against clinical isolates of Streptococcus pneumoniae showing non-susceptibility to penicillins, cephalosporins, macrolides, ketolides or quinolones. *International Journal of Antimicrobial Agents*, *29*(2), 224-226.
27. Lee, M. Y., Ko, K. S., Oh, W. S., Park, S., Lee, J. Y., Baek, J. Y., ... & Song, J. H. (2006). In vitro activity of cefditoren: antimicrobial efficacy against major respiratory pathogens from Asian countries. *International journal of antimicrobial agents*, *28*(1), 14-18.
28. Shimizu, A., Maebashi, K., Niida, M., Mikuniya, T., Hikida, M., &Ubukata, K. (2007). In vitro activities of oral cephem and telithromycin against clinical isolates of major respiratory pathogens in Japan. *Journal of Korean medical science*, *22*(1), 20-25.
29. Soriano, F., Granizo, J. J., Fenoll, A., Gracia, M., Fernandez-Roblas, R., Esteban, J., ... & Santos, F. (2003). Antimicrobial resistance among clinical isolates of Streptococcus pneumoniae isolated in four southern European countries (ARISE project) from adult patients: results from the cefditoren surveillance

program. *Journal of chemotherapy*, *15*(2), 107-112.
30. Soriano, F., Granizo, J. J., Coronel, P., Gimeno, M., Ródenas, E., Gracia, M., ... &Gadea, I. (2004). Antimicrobial susceptibility of Haemophilus influenzae, Haemophilusparainfluenzae and Moraxella catarrhalis isolated from adult patients with respiratory tract infections in four southern European countries: the ARISE project. *International journal of antimicrobial agents*, *23*(3), 295-298.
31. Chambers, H. F. (1997). Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clinical microbiology reviews*, *10*(4), 781-791.
32. Enright, M. C., Robinson, D. A., Randle, G., Feil, E. J., Grundmann, H., & Spratt, B. G. (2002). The evolutionary history of methicillin-resistant Staphylococcus aureus (MRSA). *Proceedings of the National Academy of Sciences*, *99*(11), 7687-7692.
33. Van Bambeke, F., Reinert, R. R., Appelbaum, P. C., Tulkens, P. M., &Peetermans, W. E. (2007). Multidrug-resistant Streptococcus pneumoniae infections: current and future therapeutic options. *Drugs*, *67*, 2355-2382.
34. Stankovic, C., Mahajan, P. V., &Asmar, B. I. (2007). Methicillin-resistant Staphylococcus aureus as a cause of community-acquired pneumonia. *Current Infectious Disease Reports*, *9*, 223-227.
35. Chiu, C. H., Su, L. H., Huang, Y. C., Lai, J. C., Chen, H. L., Wu, T. L., & Lin, T. Y. (2007). Increasing ceftriaxone resistance and multiple alterations of penicillin-binding proteins among penicillin-resistant Streptococcus pneumoniae isolates in Taiwan. *Antimicrobial agents and chemotherapy*, *51*(9), 3404-3406.
36. Niederman, M. S. (2007). Recent advances in community-acquired pneumonia: inpatient and outpatient. *Chest*, *131*(4), 1205-1215.
37. Hakenbeck, R., König, A., Kern, I., van der Linden, M., Keck, W., Billot-Klein, D., ... & Gutmann, L. (1998). Acquisition of Five High-M rPenicillin-Binding Protein Variants during Transfer of High-Level β-Lactam Resistance from Streptococcus mitis to Streptococcus pneumoniae. *Journal of bacteriology*, *180*(7), 1831-1840.
38. Hakenbeck, R. (1999). β-Lactam-resistant Streptococcus pneumoniae: epidemiology and evolutionary mechanism. *Chemotherapy*, *45*(2), 83-94.
39. Laible, G., Spratt, B. G., &Hakenbeck, R. (1991). Interspecies recombinational events during the evolution of altered PBP 2x genes in penicillin-resistant clinical isolates of Streptococcus pneumoniae. *Molecular microbiology*, *5*(8), 1993-2002.
40. Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., ... &Bourne, P. E. (2000). The protein data bank. *Nucleic acids research*, *28*(1), 235-242.
41. Guex, N., &Peitsch, M. C. (1997). SWISS-MODEL and the Swiss-Pdb Viewer: an environment for comparative protein modeling. *electrophoresis*, *18*(15), 2714-2723.
42. Li, Q., Cheng, T., Wang, Y., & Bryant, S. H. (2010). PubChem as a public resource for drug discovery. *Drug discovery today*, *15*(23-24), 1052-1057.

43. Z. Li, H. Wan, Y. Shi, and P. Ouyang, Personel experience with four kinds of chemical structure drawing softwares: review on ChemDraw, Chemwindow, ISIS/Draw and ChemSketch, *Journal of Chemical Information and Computer Sciences*, 2004, 44, 1886-90.
44. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry*, *25*(13), 1605-1612.