Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2019.0502.47**

STUDY OF METHICILLIN BETA LACTAM ANTIBIOTIC WITH PENICILLIN BINDING PROTEIN 2A

B. Padmavathi Bai

Department of Botany, S.N.S.R Degree college, Velgode, Kurnool, India.

ABSTRACT: The *mecA* gene is a gene found in bacterial cells. The *mecA* gene allows a bacterium to be resistant to antibiotics such as methicillin, penicillin and other penicillin-like antibiotics. The most commonly known carrier of the *mecA* gene is the bacterium known as MRSA. Apart from *Staphylococcus aureus* and other *Staphylococcus* species, it can also be found in *Streptococcus pneumoniae* strains resistant to penicillin-like antibiotics. In *Staphylococcus* species, *mecA* is spread on the SCC*mec* genetic element. The *mecA* gene does not allow the ring like structure of penicillin-like antibiotics to bind to the enzymes that help form the cell wall of the bacterium (transpeptidases), and hence the bacteria is able to replicate as normal. The gene encodes the protein PBP2A (penicillin binding protein 2A). PBP2A has a low affinity for beta-lactam antibiotics such as methicillin and penicillin. This enables transpeptidase activity in the presence of beta-lactams, preventing them from inhibiting cell wall synthesis.

KEYWORDS: Methicillin, Mec A gene, *Staphylococcus aureus*, PBP2A, Beta lactam.

Corresponding Author: Dr. B. Padmavathi Bai* Ph.D.

Department of Botany, S.N.S.R Degree college, Velgode, Kurnool, India.

1.INTRODUCTION

The multiple antibiotic resistances of methicillin-resistant strains of *Staphylococcus aureus* (MRSA) has become a major clinical problem worldwide. Rates of MRSA infection are increasing [1]. In some centers, MRSA is becoming less susceptible to vancomycin, and these strains have been associated with worse clinical outcomes [2]. Intermediate or fully resistant vancomycin strains of MRSA have emerged clinically, whereas community acquired MRSA has become epidemic. Since the major difference between methicillin sensitive *Staphylococcus aureus* (MSSA) and MRSA is gene mecA, studies to find novel inhibitors of PBP2a Protein are being carried out in order to counter

the resistance of MRSA against β-lactam antibiotics [3, 4]. According to recent findings, Linezolid, daptomycin and tigecycline have been approved during the last decade to treat infections due to MRSA. Although these agents are extremely valuable in the fight against MRSA, each one has limitations because of their side effects. New lipoglycopeptides (telavancin, dalbavancin and oritavancin) are in advanced phase of clinical development. One of the new approaches for the discovery of compounds with anti MRSA activity is to search for molecules from a wide array of photochemicals from plants. Review of literature indicated that the active substances present in many plants could be used as therapeutic alternatives for MRSA infection. Screening of phytochemicals from plant extracts have shown that higher plants and their secondary metabolites provide diverse bioactive compounds with antibacterial activity [5-8]. Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are discovered on a rational basis, using computational methods to simulate drug – receptor interactions insilico saving a lot of time, effort and expenditure in the search for lead compounds. The present study is an attempt to find such compounds using Bioinformatic tools on selected plant products for their binding and inhibiting capacity of the target Protein PBP2a, responsible for resistance of MRSA [9-11].

2. MATERIALS AND METHODS

Primer design

For designing the primer, DNA template sequence is required that can be taken from any of the available sequence databases, e.g., RefSeq database. The in silico validation can be carried out using BLAST tool and Gene Runner software, which check their efficiency and specificity. Thereafter, the primers designed in silico can be validated in the wet lab. After that, these validated primers can be synthesized for use in the amplification of concerned gene/DNA fragment [12-14].

Retrieval Of Target Sequence

The protein sequence of the protein PBP2a had been retrieved from UniProt, and saved in fasta format that gives the specific information regarding the number of amino acids in the sequence and other sequence related information.

Performing Template Search

The protein sequence of the protein PBP2a had been retrieved from UniProt and the search for the template had been done using *BLAST* algorit.

Homology Modeling

Homology modeling, also known as **comparative modeling** of protein refers to constructing an atomic-resolution model of the "target" protein from its amino acid sequence and an experimental three-dimensional structure of a related homologous protein. Homology modeling relies on the identification of one or more known protein structures likely to resemble the structure of the query sequence, and on the production of an alignment that maps residues in the query sequence to residues in the template sequence. The sequence alignment and template structure are then used to produce

a structural model of the target. Because protein structures are more conserved than DNA sequences, detectable levels of sequence similarity usually imply significant structural similarity [15-18]. The quality of the homology model is dependent on the quality of the sequence alignment and template structure. The approach can be complicated by the presence of alignment gaps (commonly called indels) that indicate a structural region present in the target but not in the template, and by structure gaps in the template that arise from poor resolution in the experimental procedure (usually X-ray crystallography) used to solve the structure.

Homology modeling can produce high-quality structural models when the target and template are closely related, which has inspired the formation of a structural genomics.

- Steps in model production

The homology modeling procedure can be broken down into four sequential steps: template selection, target-template alignment, model construction, and model assessment.[1] The first two steps are often essentially performed together, as the most common methods of identifying templates rely on the production of sequence alignments

1. Template selection and sequence alignment

The critical first step in homology modeling is the identification of the best template structure. The simplest method of template identification relies on serial pairwise sequence alignments aided by database search techniques such as FASTA and BLAST. More sensitive methods based on multiple sequence alignment. This family of methods has been shown to produce a larger number of potential templates and to identify better templates for sequences that have only distant relationships to any solved structure. Protein threading, also known as fold recognition or 3D-1D alignment, can also be used as a search technique for identifying templates to be used in traditional homology modeling method [19].

2. Model generation

Given a template and an alignment, the information contained therein must be used to generate a three-dimensional structural model of the target, represented as a set of Cartesian coordinates for each atom in the protein. Three major classes of model generation methods have been proposed.

- Fragment assembly

The original method of homology modeling relied on the assembly of a complete model from conserved structural fragments identified in closely related solved structures. Thus unsolved proteins could be modeled by first constructing the conserved core and then substituting variable regions from other proteins in the set of solved structures.

- Segment matching

The segment-matching method divides the target into a series of short segments, each of which is matched to its own template fitted from the Protein Data Bank. Thus, sequence alignment is done over segments rather than over the entire protein.

- Satisfaction of spatial restraints

The most common current homology modeling method takes its inspiration from calculations required to construct a three-dimensional structure from data generated by NMR spectroscopy.

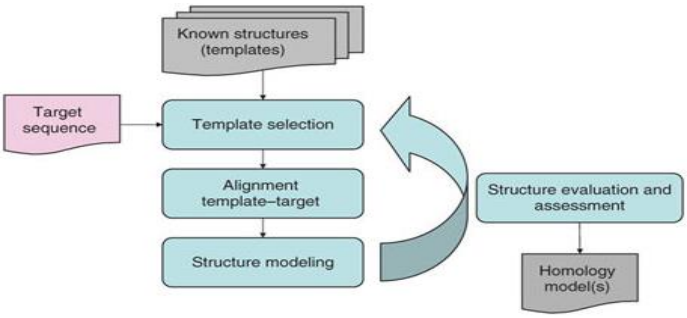
3. Loop modeling

Regions of the target sequence that are not aligned to a template are modeled by loop modeling; they are the most susceptible to major modeling errors and occur with higher frequency when the target and template have low sequence identity.

4. Model assessment

Assessment of homology models without reference to the true target structure is usually performed with two methods: statistical potentials or physics-based energy calculations. Both methods produce an estimate of the energy (or an energy-like analog) for the model or models being assessed; independent criteria are needed to determine acceptable cutoffs. Neither of the two methods correlates exceptionally well with true structural accuracy, especially on protein types underrepresented in the PDB, such as membrane proteins. Physics-based energy calculations aim to capture the interatomic interactions that are physically responsible for protein stability in solution, especially van der Waals and electrostatic interactions. These calculations are performed using a molecular mechanics force field; proteins are normally too large even for semi-empirical quantum mechanics-based calculations [20-22].

Flow Chart Of Homology Modeling



Validation Of Modeled Protein Using Rapper

As the loop of the structure is built up and the terminals are removed, the modeled structure is now given for validation through RAPPER. The structure is given in .pdb form to it to analyze the structure and to generate the RAMACHANDRAN plot of the structure to find the validity of the structure. This is again also carried out after minimization of the structure is done

Pocket Determination Using CASTP

Now that we have the final modeled structure the active pockets of the protein are found by uploading the .pdb file of modeled protein to castP. The residues in the pockets are noted.

Ligands For Docking Studies

Antibiotics shorten the course of the disease and reduce the severity of the symptoms; however, oral rehydration therapy remains the principal treatment. Tetracycline is typically used as the primary antibiotic, although some strains of V. cholerae have shown resistance. Other antibiotics that have been proven effective against V. cholerae include cotrimoxazole, erythromycin, doxycycline, chloramphenicol, and furazolidone, Fluoroquinolones such as norfloxacin also may be used,. Ligands are extracted from various sources such as PUBCHEM, DRUGBANK,

Molecular Modeling

Molecular modelling is a collective term that refers to theoretical methods and computational techniques to model or mimic the behaviour of molecules. The techniques are used in the fields of computational chemistry, computational biology and materials science for studying molecular systems ranging from small chemical systems to large biological molecules and material assemblies. The simplest calculations can be performed by hand, but inevitably computers are required to perform molecular modelling of any reasonably sized system. The common feature of molecular modelling techniques is the atomistic level description of the molecular systems; the lowest level of information is individual atoms (or a small group of atoms). This is in contrast to quantum chemistry (also known as electronic structure calculations) where electrons are considered explicitly. The benefit of molecular modelling is that it reduces the complexity of the system, allowing many more particles (atoms) to be considered during simulations.

Designing of Methicillin

Methicillin Antibiotic structure was designed for docking studies. The structure of this antibiotic was constructed and optimized using “Chemsketch” software.

Active site Identification

Active site of PBP2a was identified using CASTp server. A new program, CASTp, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CASTp identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings.

Docking Method

Molecular docking was performed using the Gold version 3.0.1 (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA), to study the binding orientation of compounds into the PBP2a structure. This method allows partial flexibility of protein and full flexibility of compounds. The designed Methicillin was docked to the active site of the PBP2a. The binding site identification of PBP2a structure was carried out using CastP server. CastP identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the

atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings. The interaction of methicillin with the active site residues are studied using molecular mechanics calculations. The parameters used for GA were population size (100), selection pressure (1.1), number of operations (10,000), number of island (1) and niche size (2). Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. Default cutoff values of 3.0 A° (dH-X) for hydrogen bonds and 6.0 A° for vanderwaals were employed. During docking, the default algorithm speed was selected and the binding site in the PBP2a was defined within a 10 A° radius with the centroid as CE atom of GLN207. The number of poses for each inhibitor was set 100, and early termination was allowed if the top three bound conformations of a compound were within 1.5A° RMSD. After docking, the individual binding poses of each compound were observed and their interactions with the protein were studied. The best and most energetically favorable conformation of each compound was selected [23-26].

Gold Score Fitness Function

Gold Score performs a force field based scoring function and is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond); 2. Protein-ligand vander Waals energy (external vdw); 3.Ligand internal vander Waals energy (internal vdw); 4.Ligand intramolecular hydrogen bond energy (internal- H- bond). The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

$$\text{GoldScore} = S(\text{hb\_ext}) + S(\text{vdw\_ext}) + S(\text{hb\_int}) + S(\text{vdw\_int})$$

Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intramolecular strain in the ligand.

3. RESULTS AND DISCUSSION

Primer Design

The primers for mec A gene were identified using Primer3 software. The *Staphylococcus aureus* genome was submitted to primer3 and the forward and reverse primers which amplify the mecA gene were predicted. The primers were 20 nucleotide in length with high GC content. These primers were synthesized and used to amplify the mec A gene of MRSA (Table 1).

Table 1: Primer sequences for mecA gene

OLIGO	start	len	tm	gc%	any	3' seq
LEFT PRIMER	4591	20	60.02	45.00	4.00	2.00 AAGAAGCGTTGTCGCTGAAT
RIGHT PRIMER	4820	20	60.04	50.00	7.00	2.00 GGACAAAGCCGTTGTACGTT

Homology Modeling of PBP2a

All molecular simulations were performed on AMD 64 bits dual processing hi-end Linux desktop. The amino acid sequence of PBP2a from MRSA was obtained from the protein sequence databank in the Swiss-prot or Uniprot_KB at the site www.expasy.org (Fig 1).

Primary sequence of PBP2a Protein in MRSA

>tr|Q7DHH4|Q7DHH4_STAAU MecA OS=*Staphylococcus aureus* GN=mecA PE=4 SV=1

MKKIKIVPLILIVVVVGFGIYFYASKDKEINNTIDAIEDKNFKQVYKDSSYISKSDNGEV
EMTERPIKIYNSLGVKDINIQDRKIKKVSKNKKRVDAQYKIKTNYGNIDRNVQFNFVKED
GMWKLDWDHSVIIPGMQKDQSIHIENLKSERGKILDRNNVELANTGTAYEIGIVPKNVSK
KDYKAIAKELSISEDYIKQQMDQNWVQDDTFVPLKTVKKMDEYLSDFAKKFHLTTNETES
RNYPLGKATSHLLGYVGPINSEELKQKEYKGYKDDAVIGKKGLEKLYDKKLQHEDGYRVT
IVDDNSNTIAHTLIEKKKKDGKDIQLTIDAKVQKSIYNNMKNDYGSGTAIHPQTGELLAL
VSTPSYDVYPFMYGMSNEEYNKLTEDKKEPLLNKFQITTSPGSTQKILTAMIGLNNKTLD
DKTSYKIDGKGWQKDKSWGGYNVTRYEVVNGNIDLKQAIESSDNIFFARVALELGSKKFE
KGMKKLGVGEDIPSDYPFYNAQISNKNLDNEILLADSGYGQGEILINPVQILSIYSALEN
NGNINAPHLLKDTKNKVWKKNIISKENINLLTDGMQQVVNKTHKEDIYRSYANLIGKSGT
AELKMKQGETGRQIGWFISYDKDNPNMMMAINVKDVQDKGMASYNAKISGKVYDELYENG
NKKYDIDE

Primary sequence analysis of MRSA PBP2a protein:

Amino Acid		Number	Mol%
Ala	A	13	6.40
Cys	C	4	1.97
Asp	D	15	7.39
Glu	E	13	6.40
Phe	F	11	5.42
Gly	G	6	2.96
His	H	5	2.46
Ile	I	10	4.93
Lys	K	12	5.91
Leu	L	23	11.33
Met	M	9	4.43
Asn	N	11	5.42
Pro	P	4	1.97
Gln	Q	8	3.94
Arg	R	13	6.40
Ser	S	10	4.93
Thr	T	10	4.93
Val	V	16	7.88
Trp	W	4	1.97
Tyr	Y	6	2.96

Figure 1: Represents the amino acid composition and molecular weight of the PBP2a protein, in our results shows Leu is highly present compared to other residues.

Graphical Representation of amino acid composition:

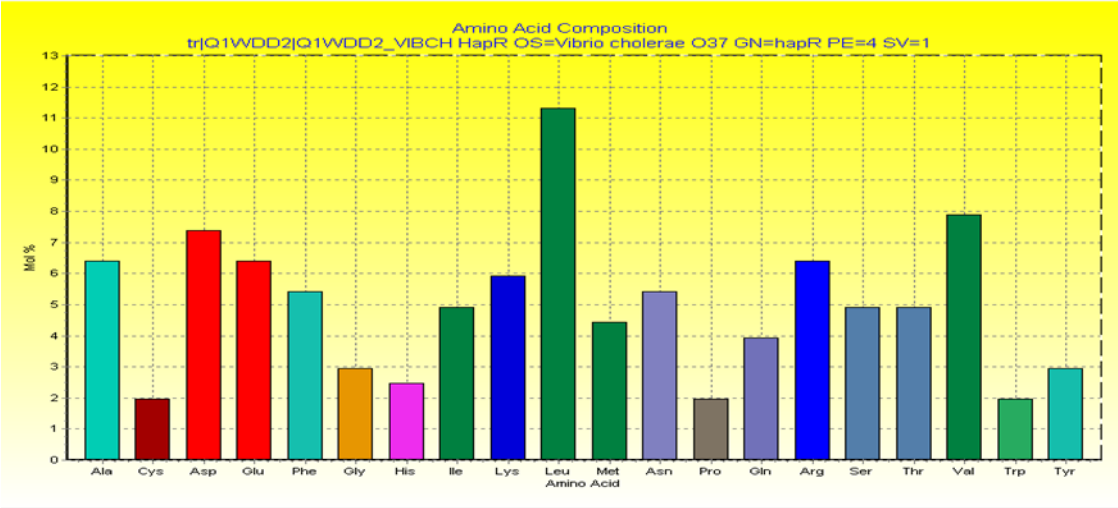


Figure 2: Hydrophobic profile of PBP2a protein sequence

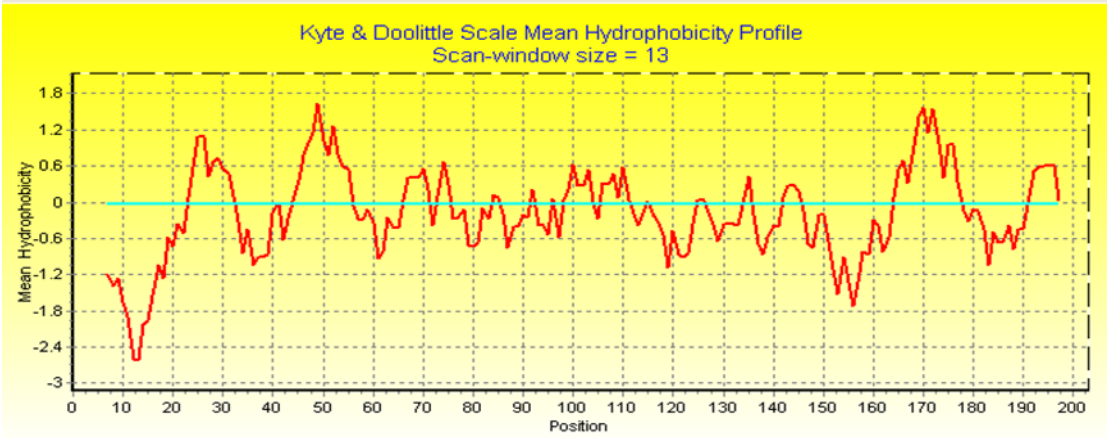


Figure 3: Diagram shows hydrophobicity profile of PBP2a ,which was developed by Kyte &doolite program ,it was representing hydrophobic regions 22-32,42-58,162-180

Parker HPLC –Hydrophilicity Profile:

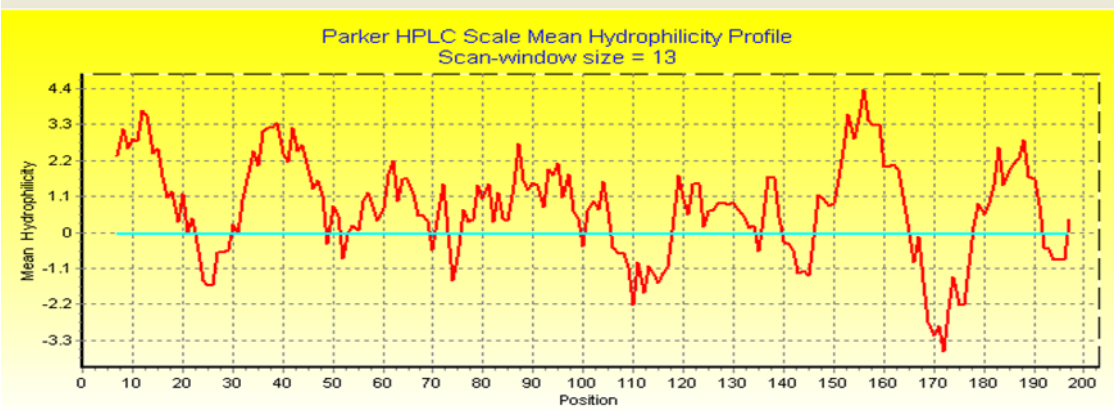


Figure 4: diagram shows hydrophilicity profile of PBP2a ,which was developed by parker HPLC program ,it was representing hydrophiliic regions 31-50,148-165 etc

Helical-wheel diagram

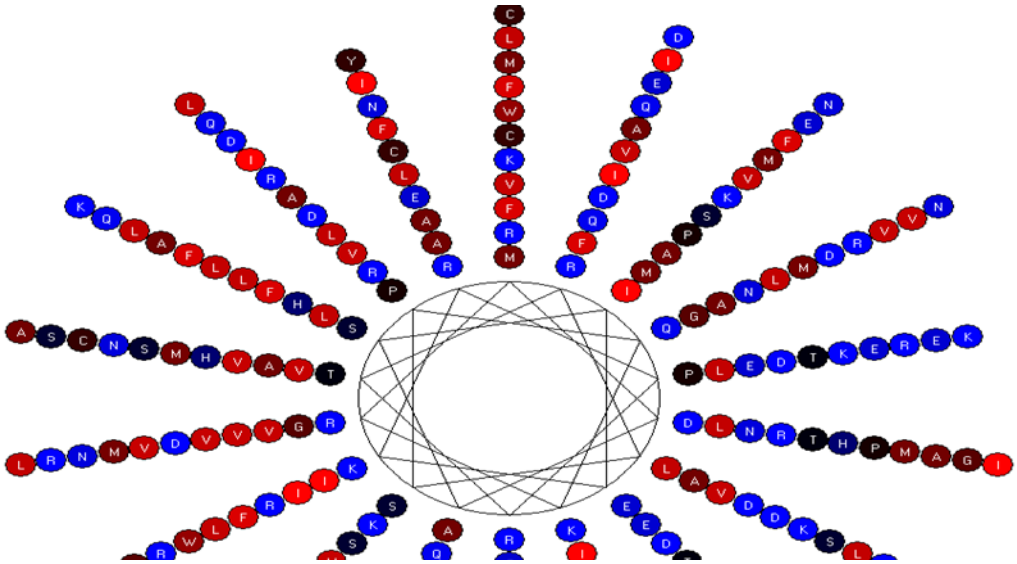
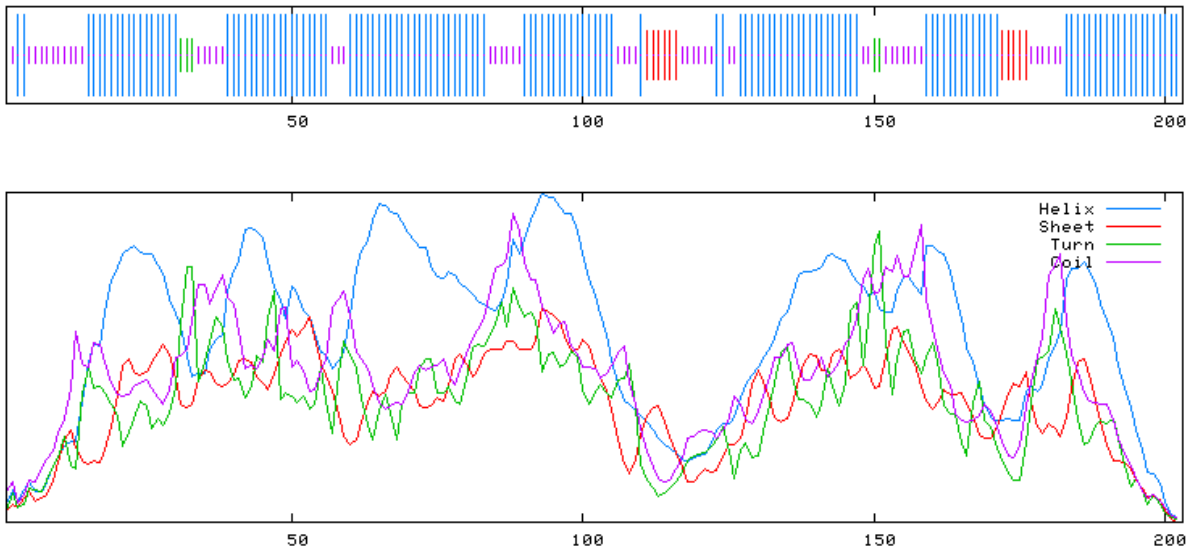


Figure 5: shows pattern of amino acid representation by helical wheel diagram ,central region is the hydrophobic and similar color residues represent they are belongs to same branch of amino acids

Secondary structure analysis of PBP2a Protein




```
Parameters :
   Window width         :  17
   Similarity threshold :   8
   Number of states     :   4
```

Figure 6: Shows secondary structure analysis of PBP2a protein sequence

Template selection is a process of identifying a suitable protein which shares nearly the same structure of the query protein which doesn't possess the 3D structure. Template selection is very important in comparative protein modeling. Templates can be chosen by various tools such as BLAST, FASTA, Swiss-model, etc. In the case of Blast and Fasta the sequence of protein in fasta format can be uploaded and the templates can be manually selected by considering the score value

and the E value. In the case of Swiss-Model server, it automatically chooses the template and models the protein structure (Fig 2-6).

BLAST Search

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. In the results of BLAST search against PDB, only one-reference protein 2PBX has a high level of sequence identity and the identity of the reference protein with the Tau-protein domain are 80% (Fig 7).

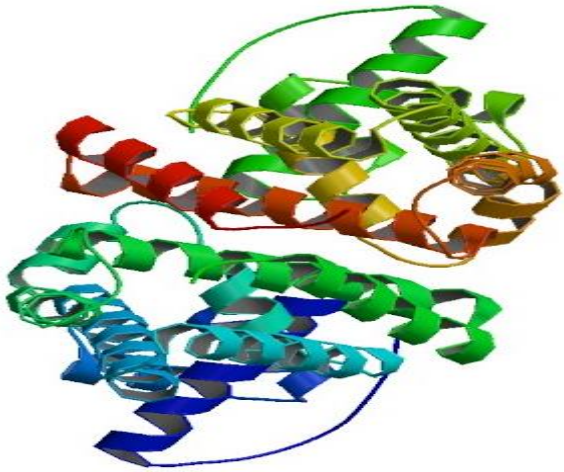


Figure 7: Template structure: 2PBX

Sequence Alignment

In the following study, we have chosen 2PBX as a reference structure for modeling PBP2a domain (Fig 8, 9). Coordinates from the reference protein (2PBX) to the SCRs, structurally variable regions (SVRs), N-termini and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints. Sequence of the reference structures were extracted from the respective structure files and aligned with the target sequence using the default parameters in ClustalW.

Theoretical model of PBP2a Protein

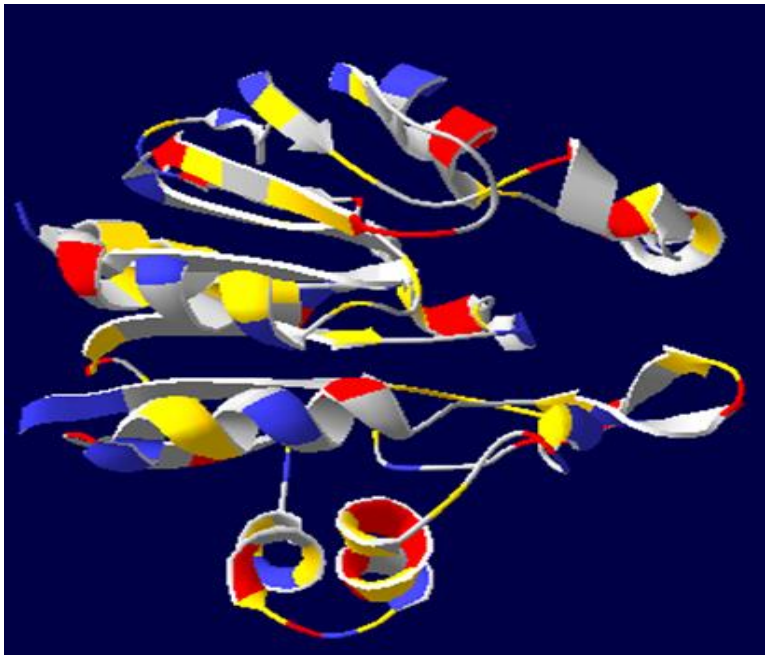
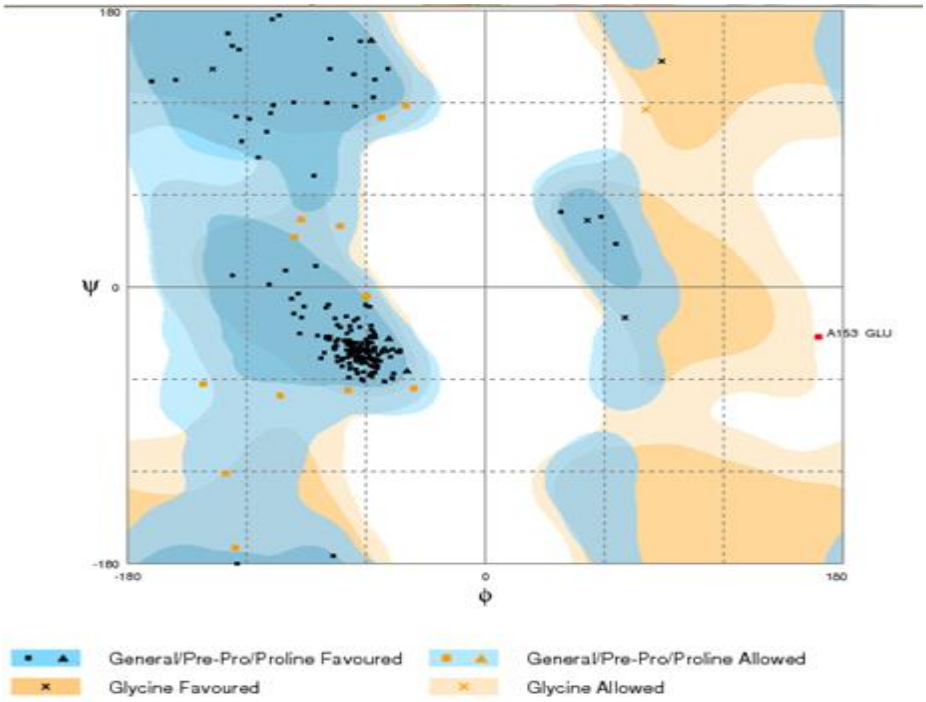


Figure 8: PBP2a structure

Model verification by Rapper



Number of residues in favoured region	(~98.0% expected)	:	182 (92.4%)
Number of residues in allowed region	(~2.0% expected)	:	14 (7.1%)
Number of residues in outlier region		:	1 (0.5%)

Figure 9: Ramachandranplot analysis

Active site Identification

The selected docked conformations of the PBP2a binding site are shown in Figure 10. The docked conformations revealed that methicillin located in the hydrophobic binding pocket surrounding the binuclear copper active site. In this study, docked methicillin was found to have some interaction between an oxygen atom of the methicillin and PBP2A. Moreover, these docked conformations also formed an H-bonding interaction with in the active site. In the binding pocket, common H-bonding interactions were formed between all docked compounds and GLY 135, GLN 137, GLN 140, HIS 143, GLU 145, GLN 145, GLN 207, ASP 209, HIS 232, THR 300, and HIS 311.

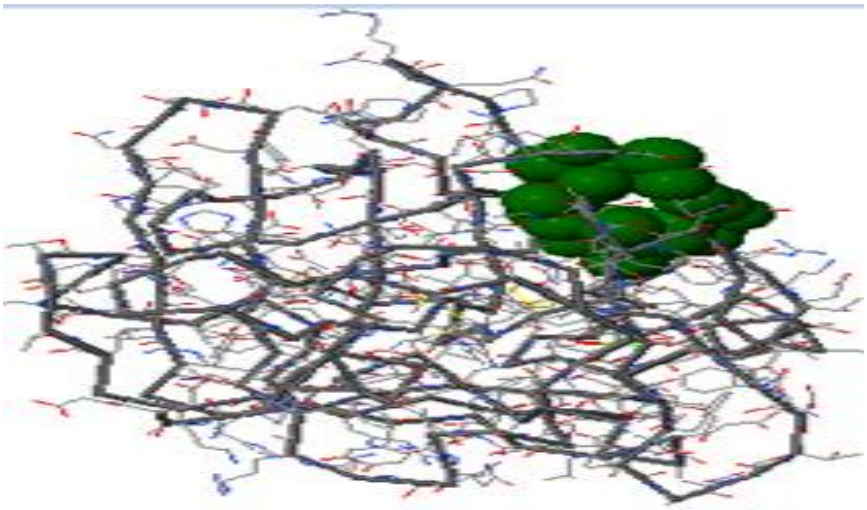


Figure 10: active site of PBP2a

After constructing the methicillin and optimizing them using "Chemsketch" software ,the crystal model and the possible binding sites of PBP2a were searched with CASTP server. From the binding site analysis of PBP2a we identified that, the binding pockets are identical in all chains and the largest binding pocket was taken for further docking studies. The crystal structures of PBP2a are similar and we have therefore taken 1VQQ (chain A) as representative structure for docking studies. The docking of compounds into the active site of PBP2a was performed using the GOLD software and the docking evaluations were made on the basis of GoldScore fitness functions. We preferred Gold fitness score than Chemscore fitness as Gold fitness score is marginally better than Chemscore fitness function (Fig 11).

In order to explain the binding of methicillin, the H-bonding interactions with the other surrounding residues in the hydrophobic binding pocket were also investigated. The docking results showed that methicillin has affinity towards PBP2a [27-31].

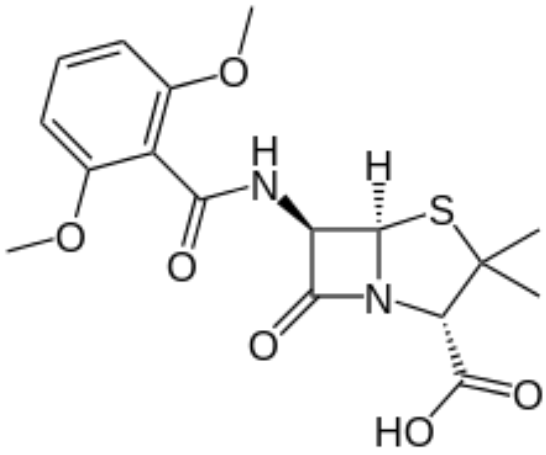


Figure 11: Methicillin structure

Docking of the methicillin with PBP2a was performed using GOLD 3.0.1, which is based on genetic algorithimn. This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the binding pocket and then passes each molecule against a negative image of the binding site. Poses clashing with this 'bump map' are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. We defined the binding pocket using the ligand-free protein structure and a box enclosing the binding site. This box was defined by extending the size of a cocrystalized ligand by 4A. This dimension was considered here appropriate to allow, for instance, compounds larger than the cocrystallized ones to fit into the binding site. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used for docking was converted in 3D with SILVER. To this set, the substrate corresponding to the modeled protein were added. Docking of best inhibitor with the active site of protein showed the activity of the molecule on protein function (Fig 12).

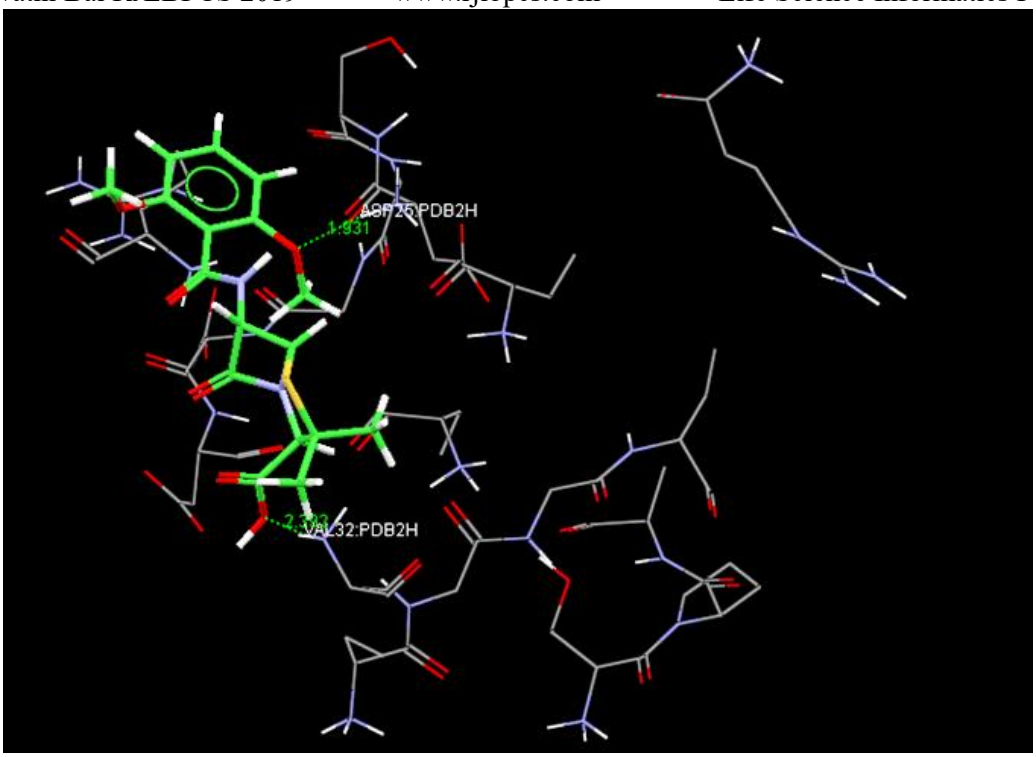


Figure 12: Docking studies of Methicillin with PBP2a

Table 2: docking score

Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)	Compound
31.62	0.00	40.06	0.00	-23.45	Methicillin

4. CONCLUSION

The docking results showed that Methicillin showed docking fitness and this revealed the binding orientation in the PBP2a binding pocket surrounding the active site, which resulted that PBP2A has affinity for beta-lactam antibiotics such as methicillin. This enables transpeptidase activity in the presence of beta-lactams, preventing them from inhibiting cell wall synthesis. From the results we can conclude that PBP2a is competitor protein to Methicillin to bind to other proteins.

ACKNOWLEDGEMENT

Authors are thankful to Dr.Jayasimha Rayalu Daddam for helping in preparation of manuscript.

CONFLICT OF INTEREST

Authors declare that they don't have any conflict of interest

REFERENCES

1. Appelbaum, P.C. The emergence of vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus*. Clinical Microbiology and Infection. 2006; 12(1): 16-23.
2. Chambers HF. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin Microbiol Rev 1997; 10(4):781-791
3. Katayama Y, Ito T and Hiramatsu K . A new class of genetic element, Staphylococcus cassette chromosome mec, encodes methicillin resistance in *Staphylococcus aureus*. Antimicrob Agents chemother 2000; 44(6): 1549-1555

4. Khan SA, Kumar S, Maqsood A. M . Virtual Screening of Molecular Properties and Bioactivity Score of Boswellic Acid Derivatives in Search of Potent Anti-Inflammatory Lead Molecule. International Journal of Interdisciplinary and Multidisciplinary Studies,2013,Vol 1,No.1,8-12.
5. Kirby WMM . Extraction of a highly potent penicillin inactivator from penicillin resistant staphylococci. Science. 1944; Jun 2; 99:452-3.
6. Krishnakumar KL, Paul M . Synthesis, Characterization of some heterocyclic curcumin analogues and their copper complexes as antitubercular and antimicrobial agents. International J of recent scientific research.2013; Vol 4, Issue no. 2:122- 127
7. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Delivery Rev. 1997;23: 4-25
8. Masroor H, Parvateesam M , Daddam JR , Naidu NV. Molecular Docking Studies of some Selected Plant Products as Inhibitors of Pencillin Binding Protein 2a (PBP2a) Proteins of Methicillin Resistant *Staphylococcus aureus.* 2015. Online Journal of Bioinformatics**.**Volume 16 (1):125-136.
9. Moghadamtousi SZ Kadir HA , Hassandarvish P Tajik H Abubakar S Zandi K . A review on antibacterial, antiviral, and antifungal activity of curcumin**..** Biomed Res Int. 2014
10. Othman A. Hamed, Noha Mehdawi, Adham Abu Taha, Emad M. Hamed, Mohammed A. Al-Nuri, and Ayman S. Hussein. Synthesis and Antibacterial Activity of Novel Curcumin Derivatives Containing Heterocyclic Moiety.Iranian journal of pharmaceutical research. 2013;12(1): 47-56.
11. Engemann JJ, Carmeli Y, Cosgrove SE, Fowler VG, Bronstein MZ, Trivette SL, et al. Adverse clinical and economic outcomes attributable to methicillin resistance among patients with *Staphylococcus aureus* surgical site infection. Clin Infect Dis. 2003;36(5):592-8.
12. Huang MT, Wang ZY, Georgiadis CA, Laskin JD, Conney AH.Inhibitory effect of curcumin on tumor initiation by benzo[a] pyrene and 7,12-dimethylbenz[a]anthracene. Carcinogenesis 1992.13: 2183-2186.
13. Jones G, Willett P, Glen RC Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation. J Mol Biol 1995.245: 43–53.
14. Jones G, Willett P, Glen RC, Leach AR, Taylor R Development and validation of a genetic algorithm for fl exible docking. J Mol Biol 1997. 267: 727–748.
15. Katyama, Y. Ito, T. and Hiramatsu, K. A new class of genetic element *Staphylococcus cassete* chromosome mec together encodes methicillin resistance in *Staphylococcus aureus.* Antimicrobial agents chemother . 2000. Vol 44 (6) : 1549-15 55.
16. Klevens, R.N., Morrison, M.A., Nadle, J. Invasive Methicillin Resistant *Staphylococcus aureus* Strains in US JAMA, 2007. VOL , 298 ; 1763-1771.

17. Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharm 1990.58: 237-240.
18. Lin JK, Lin-Shiau SY.Mechanisms of Cancer Chemoprevention by Curcumin. Proc Natl Sci Counc Repub China B 2001.25: 59-66.
19. Liu JY, Lin SJ, Lin JK.Inhibitory effects of curcumin on protein kinase C activity induced by 12-O-tetradecanoyl-phorbol-13-acetate in NIH 3T3 cells. Carcinogenesis 1993.14: 857-861.
20. Masroor, H. Parvateesam, M. Daddam, J.R.Naidu, N.V. Molecular Docking Studies of some Selected Plant Products as Inhibitors of Penicillin Binding Protein 2a(PBP2a)Proteins of Methicillin Resistant *Staphylococcus aureus*. Online Jjournal of Bioinformatics.Vol 2015.16(1):125-136.
21. Moghadamtousi , S. Z . Kadir , H.A. Hassandarvish, P. Tajik, H. Abubakar, S. Zandhi, K.A. Review On Antibacterial, Aniviral and Antifungal Activity Of Curcumin. 2014.Biomed Res Int.
22. Nimmo G.R., Coombs G.W., Pearson J.C., O'Brien F.G. and Christiansen K.J. Methicilloin Resistant *Staphylococcus aureus* in the Austrilian Community; an involving epidemic . Med. J. Aust. 2006.Vol 184 : 384-388.
23. Anand, P. Thomas, S.G, Kunnumakkara, A.B. Sundaram, C. Harikumar, K.B. Sung, B. Tharakan, S.T. Misra, K. Priyadarshini, I.K.Rajasekharan, K.N. Aggarwal, B.B. Biological Activities of Curcumin and its Analogues made by Man and mother nature.Biochem Pharmacol. 2008. Vol 76(11):1590-611.
24. Conney AH, Lysz T, Ferraro T, Abidi TF, Manchand PS, et al. Inhibitory effect of curcumin and some related dietary compounds on tumor promotionand arachidonic acid metabolism in mouse skin. Adv Enzyme Regul 1991. 31: 385-396.
25. Melter O, Santos Sanches I, Schindler J, Aires de Sousa M, Mato R, Kovarova V, et al. Methicillin-resistant *Staphylococcus aureus* clonal types in the Czech Republic. J Clin Microbiol. 1999;37:2798–803.
26. Muto CA, Jernigan JA, Ostrowsky BE, Richet HM, Jarvis WR, Boyce JM, et al. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and enterococcus. Infect Control Hosp Epidemiol. 2003; 24:362–86.
27. Govindarajan VS Turmeric--chemistry, technology, and quality. Crit RevFood Sci Nutr 1980. 12: 199-301.
28. Halliwell B, Gutteridge John MC. Oxygen radicals and the nervoussystem. Trends Neurosci 1985. 8: 22-26.
29. Huang MT, Lou YR, Ma W, Newmark HL, Reuhl KR, et al. Inhibitoryeffects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. Cancer Res 1994. 54: 5841-5847.
30. Huang MT, Ma W, Lu YP, Chang RL, Fischer C, et al. Effects of curcumin, demethoxycurcumin,

bisdemethoxycurcumin and tetrahydrocurcumin on 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion. Carcinogenesis 1995. 16: 2493-2497.

31. Huang MT, Ma W, Yen P, Xie JG, Han J, et al. Inhibitory effects of topical application of low doses of curcumin on 12-O-tetradecanoylphorbol-13- acetate-induced tumor promotion and oxidized DNA bases in mouse epidermis. Carcinogenesis 1997. 18: 83-88.