Identification of Molecular Basis and Catalytic Specificity of TEM and CTX-M Extended-Spectrum β-Lactamase Resistance: *An in silico* study

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Abstract: *Antibiotic resistance is at its peak to numerous antibiotics and has indeed necessitated exploring new potential targets and effective antibiotics. The prevalence of TEM and CTX-M type ESBLs poses a great threat to the clinical use of antibiotics for the treatment of severe infections. In this study, we have explored the catalytic binding speficity of antibiotics against TEM and CTX-M proteins that impose ESBL resistance. The TEM and CTX-15 proteins from E.coli, K.pneumoniae and P.aeruginosa were subjected to BLAST-P and Clustal W to identify the potential templates for homology modelling and models were built by using Modeller 9v9. The stereo chemical quality of the modeled protein has been validated by the PROCHECK analysis program using SAVS (Structure Analysis Verification Server). The docking analysis was carried out by using the Autodock tools (ADT) v1.5.4 and Auto dock v4.2 program. The docking analysis revealed the key amino acid residues within the catalytic sites of TEM & CTX-M-15 protein that has favored the interactions with specific antibiotics. Among the key residues that favored interactions, the amino acid residues SER, TYR and THR were found to be crucial in favoring the interactions with all the antibiotics. Thus the study provides the molecular insight of key amino acids from ESBL resistant strains which provides the path to design the novel inhibitors to overcome these long challenging ESBL resistances.*

Keywords: **Homology modelling, Molecular docking, ESBL, antibiotics, TEM and CTXM**

1.Introduction

Antibiotic resistance in Gram negative bacteria is a major health concern. This resistance is due to the emergence of beta lactamase producers conferring resistance against antibiotics [1]. High proportion of drug resistance in bacterial pathogens indicated loss of efficacy of conventional antibiotics as only one third of the diseases could be cured by currently available drugs [2,3]. The emerging resistance of bacterial pathogens to some synthetic antimicrobial agents makes it necessary to continue the exploration for new antimicrobial agents [4].Several reports of higher level of antibiotic resistance and ESBL production in India are available but in this region of South Tamilnadu in India, no such study has been done to analyse the type of ESBL responsible for the development of antibiotic resistance in gram negative bacteria. No data have been documented regarding the prevalence of genes responsible for the beta lactam resistance in our region. The present study was initiated to determine the prevalence of ESBL genes in our geographical region and also provides molecular insight into the types of ESBL circulating among the selected gram negative bacteria *E.coli, K.pneumoniae* and *P.aeruginosa.* This study also attempted to identify the amino acid residues crucial to the interaction between TEM and CTX-M-15.This information might be useful for the scientists involved in drug-designing in their search for more potent and versatile beta lactamase inhibitors.

The process of drug discovery is very complex and requires an interdisciplinary effort to design effective and commercially feasible drugs. The objective of drug design is to find a chemical compound that can fit to a specific cavity on a protein target both geometrically and chemically. After passing the animal tests and human clinical trials, this compound becomes a drug available to patients. The conventional drug design methods include random screening of chemicals found in nature or synthesized in laboratories. The problems with this method are long design cycle and high cost. Modern approach including structure-based drug design with the help of informatics technologies and computational methods has speeded up the drug discovery process in an efficient manner. Remarkable progress has been made during the past five years in almost all the areas concerned with drug design and discovery. An improved generation of software with easy operation and superior computational tools to generate chemically stable and worthy compounds with refinement capability has been developed. These tools can tap into cheminformation to shorten the cycle of drug discovery, and thus make drug discovery more cost-effective. Over 50 compounds have entered into the clinical trial through computer aided drug design method. Among the 50 compounds some of them are approved by FDA (Jorgensen, 2004).Molecular docking is one of the major fields in computer aided drug design methods. This docking method involves a combined posing and scoring process, in which many different protein-ligand conformations are sampled and a scoring function is used to rank the estimated interaction energies of each conformation [5].

Trying to predict if a given compound interacts with a protein target of interest, using only bioinformatics tools, is not an easy task. The docking tools must find the optimum binding orientation for the

compound in the active site of the protein. This means that it must predict the correct ligand conformation and orientation, usually term the POSE. In addition the *in silico* method must also try to calculate the relative affinity of the compound. This quantitative value is usually referred as the SCORE. Many docking methods and programs have been developed and tested as docking applications. Docking POSE accuracy is usually evaluated by the ability to reproduce the experimentally determined binding mode of a ligand. The best docking programs correctly dock around 70–80% of the docked ligands, when tested on large sets of protein–ligand complexes, although these percentages are highly dependent of protein structures available and the accuracy of given software. It is widely accepted that different docking software, because they use different POSE search algorithms, performed better for different protein structures, so it is always a sound methodology to use and test more than one docking software in a drug discovery project [6].

The docking SCORE accuracy is usually evaluated by predicting the binding energy (ΔG) or the constant inhibition (Ki) values for a number of known inhibitors of the protein target studied, and comparing them to known experimental values. A good correlation between predicted and experimental values will demonstrate a good performance of given docking software in predicting POSE and SCORE of other tested compounds [7,8].Finding out the best POSE for each compound into the binding site of the protein structure, and evaluating and comparing the SCORES of each docked compound are thus the main object to determine the potential of the studied compounds as inhibitors of a given protein target of interest [9]. There is a number of docking software, either commercial or free for academic use. Among the latter, one of the most used software is Auto Dock 4 (AD4). As all docking software and structural based drug design (SBDD) methodologies in general, the knowledge of the 3D experimental structures of the protein target of interest is essential. AD4 require the knowledge of the 3D structure that must include the binding site of the target protein.

AD4 is maintained by the Molecular Graphics Laboratory, Scripps Research Institute, La Jolla. Auto Dock 4 uses a Lamarckian Genetic algorithm to get fast predictions of the POSE and the SCORE as free energy of binding. This type of algorithm simulates the genetic selection that occurs in nature. A number of conformations of the ligand are generated (population) and evaluated, and the ligand structure with the best binding energy are selected and used to generate the next population. This process is performed millions of times till eventually the docked pose of the ligand with the best SCORE and POSE is obtained. In order to search efficiently the selected 3D conformational space and to speed up the interaction energy calculation, AD4 prepare grid map for each possible atom in the ligand or protein structure. AD4 is one of the first software to be developed and is one of the more widely used as there are a large number of studies that use AD4 [10]. So in the present study we used AD4 for docking studies of selected antibiotics with the target protein TEM & CTX-M-15.

2 Material and methods

2.1 In silico analysis of TEM & CTX-M-15 protein sequence analysis

BLAST-P search and ClustalW was used to find suitable templates and align for homology modelling of TEM and CTX-M-15 protein. Based on the high percentage of sequence identity $bla_{TEM\ 1}$ and $bla_{CTX\text{-}M\text{-}15}$ from *E.coli* and *K.pneumoniae* respectively with 99% similarity was selected as a template for homology modeling. The Clustal W results in a color alignment with a histogram showing the degree of similarity between all sequences [11].

In the present study, homology modelling of the TEM & CTX-M-15 protein was performed based on the crystal structures of 1ZG4 and 5T66. This was used as templates to build the three-dimensional structure of TEM and CTX-M-15 protein. The coordinate file of the templates was retrieved from the protein databank. The 3D model was built using Modeller 9v9.19 software based on a given sequence alignment and template [12].The modeled structure was visualized through PYMOL and were ranked based on the internal scoring function (DOPE score), and those with the least internal score were identified and utilized for further model validation process.Quality of the models was assessed with respect to their energy and stereo chemical geometry [13]. The stereo chemical quality of the modeled protein has been validated by the inspection of Phi/Psi distributions of Ramachandran Plot from PROCHECK analysis program using SAVS (Structure Analysis Verification Server) [14].

2.2 Active Site Prediction and Docking

The 3D structure of 19 selected anti-bacterial agents such as Beta lactamse – Ampicillin (6249), Aminoglycoside - (Amikacin (37768), Carbapenems - (Imipenem (104838), Meropenem (441130), Cephems - (Cefoxitin (441199), Cefexime (5362065), Cefalothin (6024), Ceftazidime (5481173), Ceftriaxone (5479530), Cefepime(5479537), Cefotaxime(5280980)), Monobactam - (Aztreonam (5742832)), Nitrofurans - (Nitrofurantoin (6604200), β-lactam inhibitors (Amoxicillin (33613), Clavulanic acid (5080980), Piperacillin (43672), Tazobactam (123630), Polypeptide – Colistin (44144393) and Fluroquinolones-Ciprofloxacin (2764) were retrieved as SD File format from PubChem Database [15]. To prepare the structure of homology modeled structure of TEM and CTX-M-15 protein for docking studies, the ligand and all water molecules were removed. Charges and non-polar hydrogen atoms were added using the prepare_receptor4.py script from MGLTools. The structures of 19 compounds were downloaded from pubchem database. **A**ll structures were energy minimized by the MM2 method and converted to. pdb extension file which is readable at the ADT interface.

2.3 Protein-Ligand Interaction using Autodock

The docking analysis was carried out by using the Autodock tools [16] (ADT) v1.5.4 and Autodock v4.2 program. In order to run the docking, we used a searching grid extended of selected target proteins and polar hydrogen was added to the ligand moieties. Kollman charges were assigned and atomic salvation parameters were added. Polar hydrogen charges of the Gasteiger-type were assigned and the non-polar hydrogen was merged with the carbons and the internal degrees of freedom and torsions were set. Selected Compounds were docked in to the TEM and CTX-M-15 protein with the molecule considered as a rigid body and the ligand being flexible. The search was extended over the whole receptor protein used as blind docking. Affinity maps for all the atom types present, as well as an electrostatic map, were computed with a grid spacing of 0.375 A°. The search was carried out with the Lamarckian Genetic Algorithm; populations of 150 individuals with a mutation rate of 0.02 were evolved for 10 generations. Evaluation of the results was done by sorting the different complexes with respect to the predicted binding energy. A cluster analysis based on root mean square deviation values (RMSD values), with reference to the starting geometry, was subsequently performed and the lowest energy conformation of the more populated cluster was considered as the most trustable solution.

3 Result

3.1 Sequence Alignment between Template and Target

Sequence Alignment between Template and Target is given in Figure 1 and Figure 2.To perform homology modeling, the main criteria are template selection and sequence alignment between the target and template. To select the template sequence, PSI – BLAST was performed. BLAST results showed that the sequence identity between the target sequence of TEM & CTX-M-15 protein and the templates (PDBI D1ZG4 and 5T66.) is 99% and 99% respectively. Using Clustal W, alignment was performed between the protein sequences of target TEM & CTX-M-15 protein and templates (PDB ID:1ZG4 and 5T66). The alignments which included the residues that were conserved in both the template and query sequences were shown in Figure 1 & 2. The identical residues between the query and the template sequences are shown with the same color.

Figure1. Alignment of target protein [TEM] with template [1ZG4]

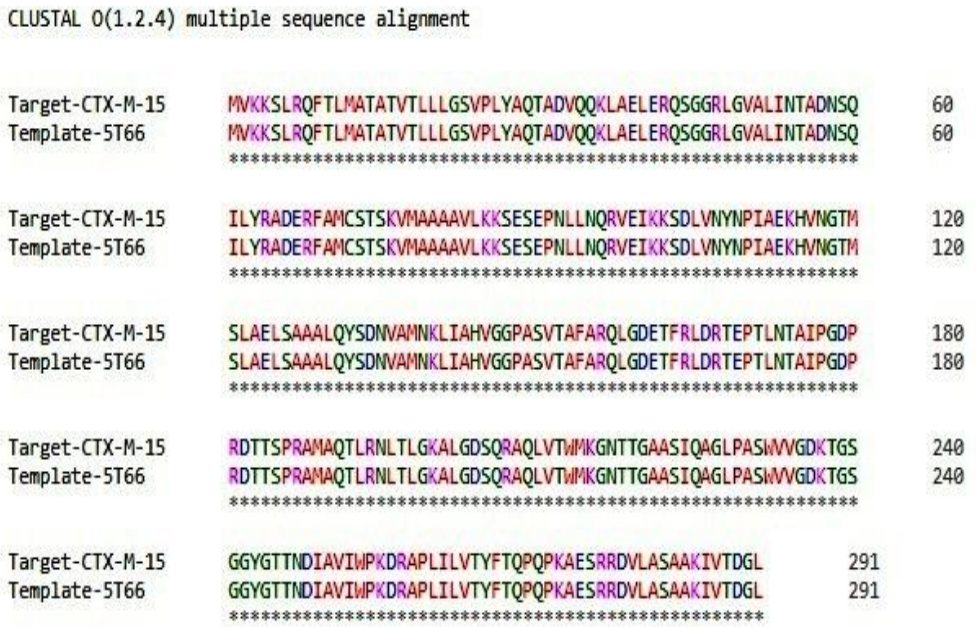


Figure 2. Alignment of target protein [CTX-M-15] with template [5T66]

3.2 Homology Modeling of TEM & CTX-M-15 protein

Homology modeling gives a clear relationship of homology between the target protein sequence and the protein sequence whose structure has been solved. The three dimensional structure of the protein provides a valuable insight into its molecular function and helps to analyze their interactions with suitable substrates or inhibitors. As a result of homology modeling, totally five models were generated using MODELLER 9.19 software for each protein. Dope scores were calculated for all the generated models using the model-single.py

command. The model TvLDH.B99990001.pdb for TEM and TvLDH.B99990004.pdb for CTX-M-15 having the minimal dope score value was considered as the best model. The dope score of the modeled structures are given in Table 1 and Table 2. These two modeled structure has similar structural features to the template protein. The best modeled structure is shown in Figure 3 and Figure 4. The N-terminal and C-terminal domains were recognized which provides valuable insight into molecular function and also enables the protein-ligand interaction to be analyzed.

3.3 Model Validation

The modeled structures were then subjected to Model validation to confirm the stereo chemical quality of the modeled structure. Validation of the modeled structure was carried out by using SAVS (Structure Alignment Verification Server). Ramachandran plot calculations are computed with PROCHECK program. The Phi/Psi distributions of the Ramachandran plot for TvLDH.B99990001.pdb (TEM) have shown 85.5 % of residues in the most favoured regions, 10.6 % residues in the allowed regions and 2..8% residues in the generously allowed regions and 1.1% in the disallowed regions (Figure 5) and TvLDH.B9999000.pdb (CTX-M-15) have shown 87.8% of residues in the most favored regions, 9.9% residues in the allowed regions and 1.0% in the generously allowed regions and 1.3% in the disallowed regions (Figure 6). The root mean square deviation value obtained as a result of superimposition of target and template protein using Chimera was 0.097 Å & 0.10 respectively which indicates that the generated model is quite similar to the template.

Table 1. Dope score of modeled structure of TEM

S.No	File Name	Dope Score
1	**TvLTH.B99990001.pdb**	**-37375.90234**
2	TvLTH.B99990002.pdb	-37072.73828
3	TvLTH.B99990003.pdb	-37112.60156
4	TvLTH.B99990004.pdb	-37062.25391
5	TvLTH.B99990005.pdb	-36358.01953

Table 2.Dope score of modeled structure of CTX-M-15

S.No	File Name	Dope Score
1	TvLDH.B99990001.pdb	-29056.332031
2	TvLDH.B99990002.pdb	-28726.529297
3	TvLDH.B99990003.pdb	-29003.167969
4	**TvLDH.B99990004.pdb**	**-29378.070313**
5	TvLDH.B99990005.pdb	-29234.333984

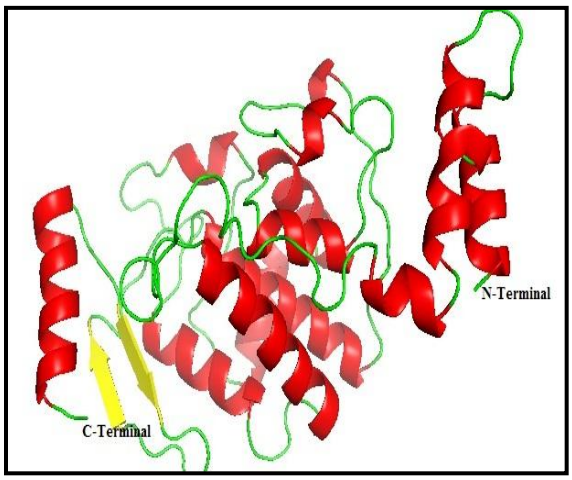


Figure 3. The best modeled structure of the TEM protein by using modeler 9.19. The red color indicates the alpha-helix, green color indicates loops and yellow color indicates alpha sheets.

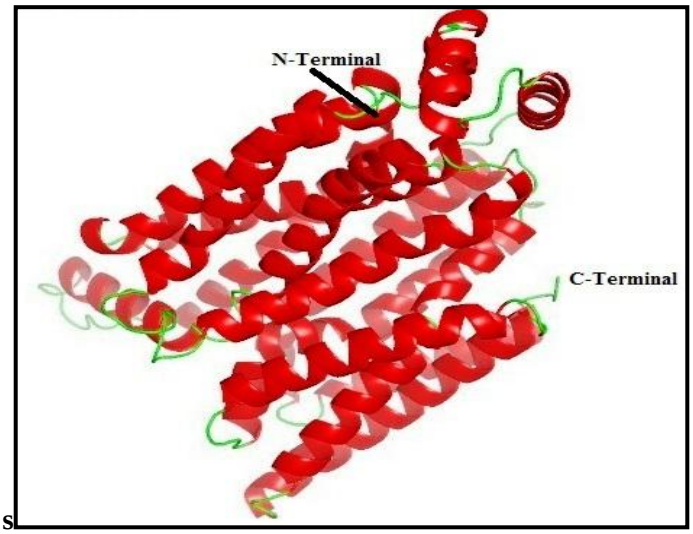


Figure 4. The best modeled structure of the CTX-M-15 protein by using modeler 9.19.The red color indicates alpha-helix and green color indicates loops.

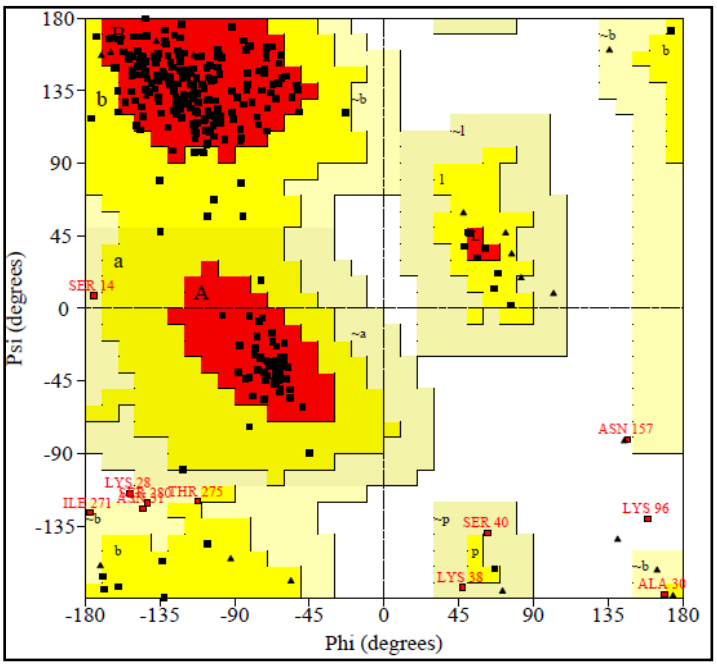


Figure 5. Ramachandran plot of the developed homology model of TEM protein. The most favored regions are colored red; additional allowed, generously allowed and disallowed regions are shown as yellow, light yellow and white fields, respectively.

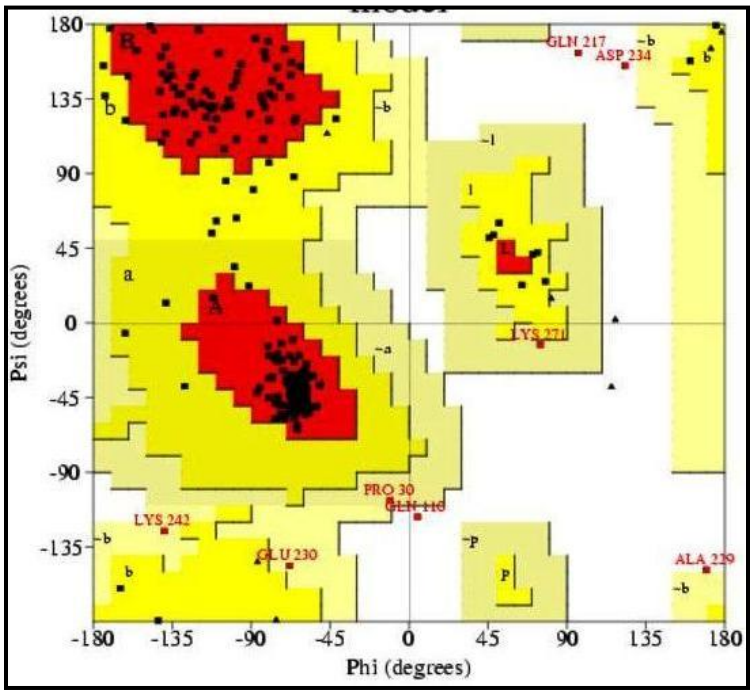


Figure 6. Ramachandran plot of the developed homology model of CTX-M-15 protein. The most favored regions are colored red; additional allowed, generously allowed and disallowed regions are shown as yellow, light yellow and white fields respectively.

3.4 Active Site Prediction

Active Site analysis using CAST p module reveals that the compounds were bound in the cavity of the protein containing the following residues GLU166, ASN170, SER235, ARG244, ASN 132, SER130, GLU166, SER70, VAL216, ALA237, ARG244 were involved in making hydrogen bonds with TEM protein with selected antibiotics and amino acids. SER237, THR235, THR216, TYR219, ASN245, SER220, ARG276, SER130, SER70 residues of CTX-M-15 protein with selected antibiotics form an interaction through hydrogen (H) bonds. Hence these amino acids in both proteins play a major role in the binding affinity with the ligand molecule.

3.5 Molecular Docking interactions of various antibiotics used in the study

Results of docking study clearly showed that the beta lactam (Ampicillin) interacted with both proteins in active way in terms of binding energy and hydrogen bond interaction. The binding energy and hydrogen bond interaction are shown in Figure 7 -12 and Table 3 & 4: The amino acids favoring the docking interactions within the active site of TEM and CTXM-15 modeled proteins, binding affinities (kcal/mol), Inhibition Constant (nM) and ligand efficiency for all the antibiotic compounds used in the study were reported in Table 3 and 4 respectively. The binding interactions of all the compounds docked within the predicted binding sites of TEM and CTXM-15 are presented (Fig 7-12). From the docking results, it is evident that all compounds exhibited better binding energy against TEM and CTXM-15.

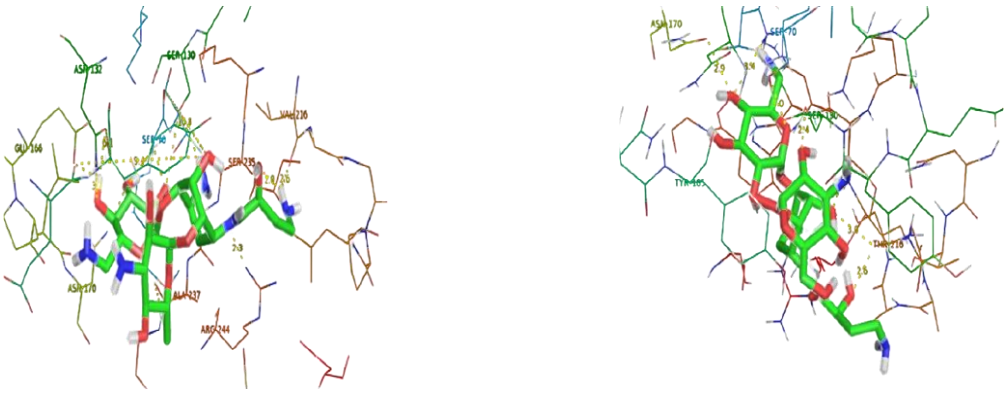
Table 3. Docked amino acid residues of TEM protein and their interactions with selected compounds

S.No	Antibiotics	No of Hydrogen Bonds	Amino Acids Residues of Target Protein involved in Hydrogen Bonding	Binding affinity (kcal/mol)	Inhibition Constant (nM)	Ligand Efficiency
1.	Ampicillin	4	Glu166, Asn170, Ser235,Arg244	-7.09	6.38	-0.3
2.	Amikacin	10	Asn132,Ser130,Glu166,Ser70,Val216,Ser235, Ala237,Arg244,Asn170,Glu166	-15.49	11.89	0.39
3.	Imipenem	5	Asn132,Ser70,Ser235,Ala237,Arg244	-6.04	37.34	-0.3
4.	Meropenem	4	Ser130,Tyr105,Ser70,Ala237	-5.84	1.34	-0.15
5.	Cefoxitin	4	Ala237,Ser70,Ser130, Asn132	-6.12	32.45	-0.17
6.	Cefexime	3	Asn132,Lys73,Ser130	-6.77	8.02	-0.23
7.	Cefalothin	3	Ser235,Ser130,Ser70	-6.57	10.85	-0.21
8.	Ceftazidime	3	Ala23,Ser70,Asn170	-4.89	7.65	-0.08
9.	Ceftriaxone	4	Tyr105,Gly236,Pro167,Asn170	-4.97	227.56	-0.18
10.	Cefepime	5	Asn170,Asn132,Ser70,Ser130,Ser235	-6.95	10.89	-0.26
11.	Cefotaxime	3	Asn132,Ser70,Asn170	-5.69	67.78	-0.19
12.	Aztreonam	6	Lys234,Ser130,Ser235,Arg244,Ser70,Asn132	-8.13	10.12	-0.29
13.	Nitrofurantoin	4	Ser130,Ser70,Ala237,Asn170	-5.50	402.74	-0.27
14.	Amoxicillin	4	Glu240,Pro167,Asn170,Ser70	-4.46	539.69	-0.18
15.	Clavulanic acid	3	Ser130,Lys234,Tyr105	-3.68	24.0	-0.26
16.	Piperacillin	3	Ser235,Ala237,Ser70	-5.91	426.74	1.55
17.	Tazobactam	4	Ser130,Ser235,Ser70,Glu104	-4.61	420.46	-0.23
18.	Colistin	-	Not Docked	---	17.65	---
19.	Ciprofloxacin	2	Ser130,Ser 70	-2.97	27.56	-0.18

Table 4. Docked amino acid residues of CTX-M-15 protein and their interactions with selected compounds

S.No	Antibiotics	No of Hydrogen Bonds	Amino Acids Residues of Target Protein involved in Hydrogen Bonding	Binding Affinity (kcal/mol)	Inhibition Constant (nM)	Ligand Efficiency
1.	Ampicillin	4	Ser237,Thr235,Thr216,Tyr219	-5.77	1.72	-0.15
2.	Amikacin	5	Asn170,Ser70,Ser130,Tyr105,Thr216)	-8.04	3.89	5.98
3.	Imipenem	4	Tyr129,Ser237,Ser70,Tyr105	-5.92	177.3	-0.26
4.	Meropenem	3	Ser237,Gly236,Lys234	-5.0	217.84	-0.19
5.	Cefoxitin	5	Arg276,Ser237.Lys234,Ser130,Tyr129	-4.41	581.95	-0.16
6.	Cefexime	5	Tyr129,Thr216,Thr235,Ser130,Arg276	-6.51	16.95	-0.22
7.	Cefalothin	3	Thr235,Thr216,Gly217	-3.13	5.08	-0.1
8.	Ceftazidime	3	Tyr129,Ser237,Thr215	-4.69	1.97	-0.1
9.	Ceftriaxone	6	Ser237,Asn132,Ser130,Thr216,Lys73,Ala219	6.56	16.05	0.46
10.	Cefepime	5	Tyr105,Asn132,Ser70,Ser130,Thr235	-6.74	26.55	-0.24
11.	Cefotaxime	3	Asn132,Ser70,Asn170	-3.56	2.37	0.12
12.	Aztreonam	4	Ser130,Ser70,Ala237,Asn170	-5.11	180.71	-0.18
13.	Nitrofurantoin	7	Asn245,Ser220,Arg276,Thr216,Ser130,Ser237,Ser70	-6.63	93.47	-0.32
14.	Amoxicillin	4	Ser237,Thr235,Thr216,Tyr219	-4.77	1.72	-0.15
15.	Clavulanic acid	4	Ser237,Ser130,Thr235,Thr216	-4.61	421.17	-0.33
16.	Piperacillin	5	Asn132 Ser130,Ser237,Thr244,Thr215	-6.35	58.27	1.68
17.	Tazobactam	5	Thr235,Gly236,Ser130,Ser70,Asn132	-6.09	34.1	-0.3
18.	Colistin	5	Thr71,Val74,Thr189,Ser182,Tyr264	-5.60	17.65	27.41
19.	Ciprofloxacin	3	Thr216,Ala219,Ser237	-4.97	3.28	-0.14

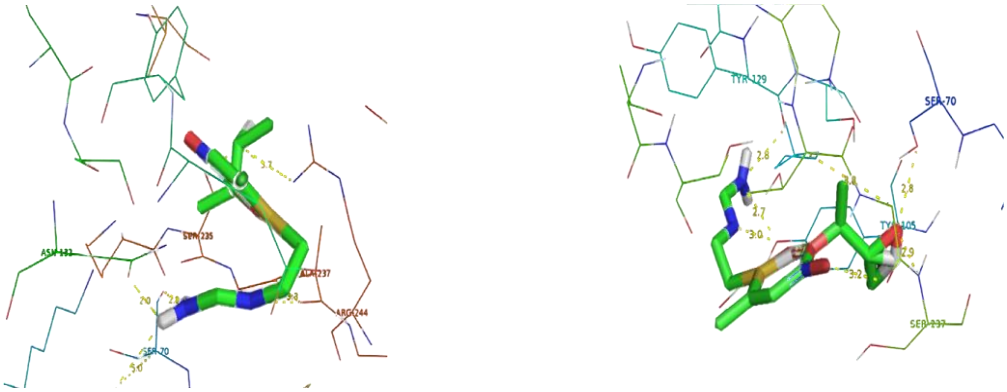
The docking interactions of Amikacin (CID 37768) are favored by H-bond interactions with TEM binding site residues: Asn132, Ser130, Glu166, Ser70, Val216, Ser235, Ala237, Arg244, Asn170, and Glu166 with binding affinity of -15.49 kcal/mol. While it has shown the binding affinity of -8.04 kcal/mol with CTXM15 protein while forming the favorable interactions with binding site residues: Asn170, Ser70, Ser130, Tyr105, Thr216. The best possible binding affinities of the Amikacin at two targeted protein's active sites are displayed in Figure (7a) & (7b) and their corresponding energy values were listed in Table 3 & 4 respectively.



TEM **CTX-M-15**

Figure 7a & 7b.Docking orientation and interactions of Amikacin with TEM and CTX-M-15

Imipenem showed better interaction with both the target protein TEM and CTX-M-15 when compared to Meropenem. The docked pose of TEM and CTX-M-15 with Imipenem and Meropenem is shown in Figure 8a & 8b and Table 3 & 4.

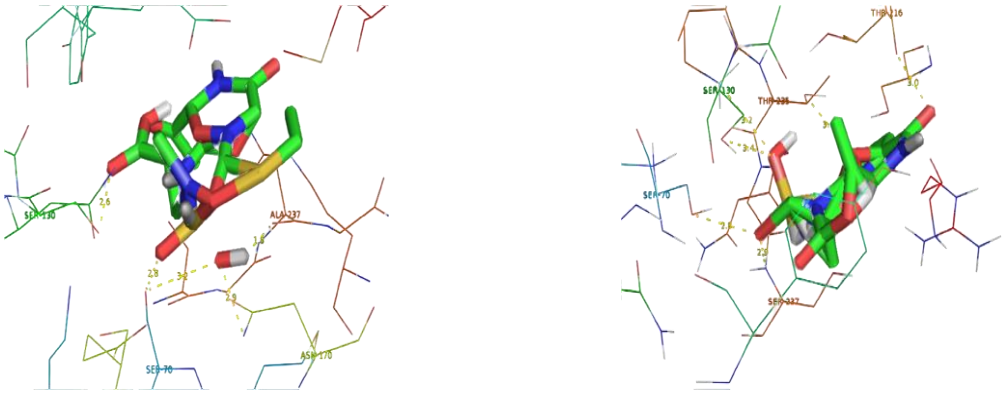


TEM **CTX-M-15**

Figure 8a & 8b. Docking orientation and interactions of Imipenem with TEM and CTX-M-15

The docked pose of TEM with Cefoxitin, Cefexime, Cefalothin, Ceftazidime, Ceftriaxone, Cefepime, Cefotaxime reveals good binding affinity, number of hydrogen bond and amino acids involved in hydrogen bonding details are given in Table 3.are shown in Figure to respectively. Analysis of these results clearly demonstrated the binding positions of the ligands with the protein. The docked pose of CTX-M-15 with Cefoxitin, Cefexime, Cefalothin, Ceftazidime, Ceftriaxone, Cefepime, Cefotaxime reveals good binding affinity, number of hydrogen bond and amino acids involved in hydrogen bonding details are given in Table 4. Comparative analysis of all compounds in Cephems revealed that Cefepime had very good interaction with both TEM & CTX-M-15 protein because it has the good binding energy and worthy number of hydrogen bond interaction than other compounds in Cephems.

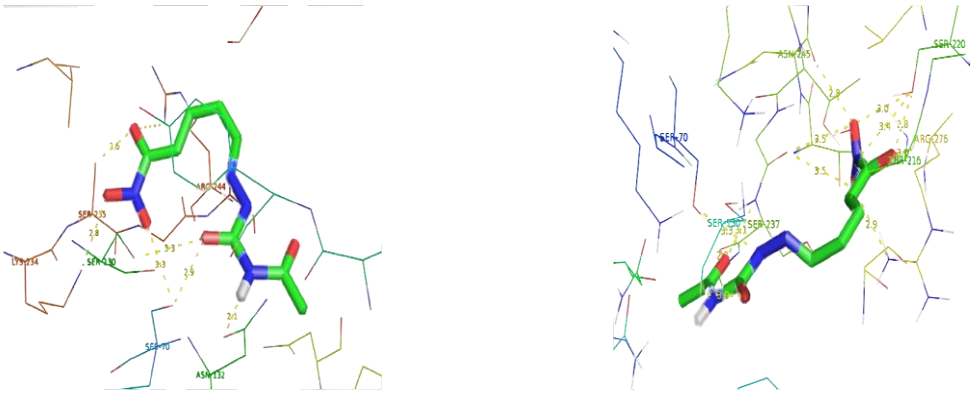
Docking results showed that Aztreonam is one of the high affinity compounds with TEM and CTX-M-15 protein. It had strong interaction and exactly bound to the active site cavity of TEM and CTX-M-15 protein. The drug–receptor interaction details were given in Table 3 & 4 and their docking pose shown in Figure 9a & 9b.



TEM **CTX-M-15**

Figure 9a & 9b. Docking orientation and interactions of Aztreonam with TEM and CTX-M-15

Nitrofurantoin had better interaction with CTX-M-15 than TEM protein. The best possible binding affinities of the Nitrofurantoin at two targeted protein's active sites are displayed in Figure 10a & 10b and their corresponding energy values are listed in Table 3 & 4.

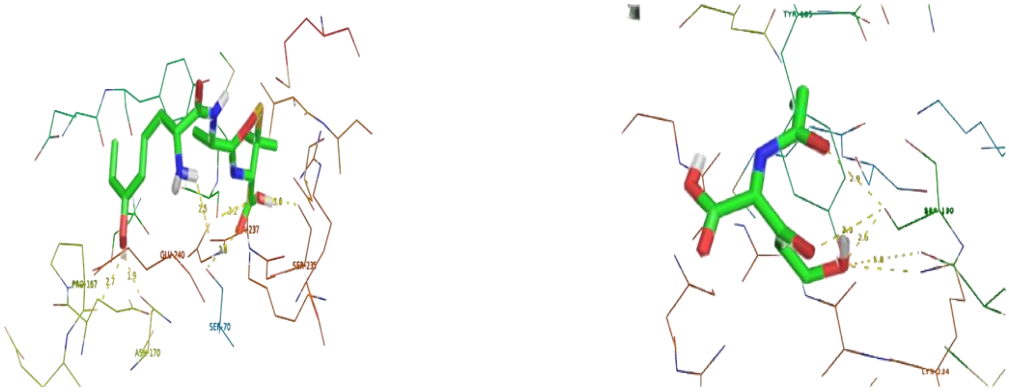


TEM **CTX-M-15**

Figure 10a & 10b. Docking orientation and interactions of Nitrofurantoin with TEM and CTX-M-15

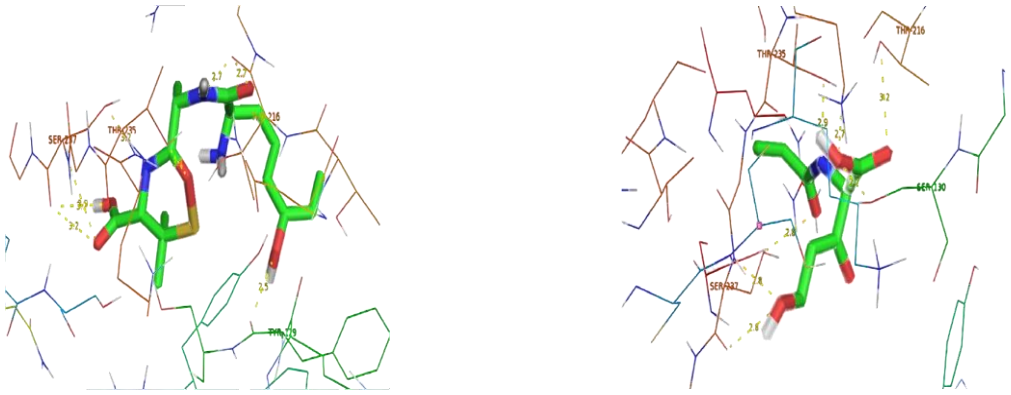
The docked pose of TEM protein with Amoxicillin is shown in Figure 11a and Clavulanic acid and their binding affinities with corresponding energy values are shown in Table 3 and Figure 11b.Amoxicillin showed high binding energy with TEM when compared to Clavulanic acid. The docked pose of CTX-M-15 protein with Amoxicillin and Clavulanic acid is shown in Figure 12 a and 12 b clearly demonstrated the

binding positions of the ligand with the protein. The comparison of these results indicates that, the Amoxicillin showed the better interaction with both target protein than Clavulanic acid.



Amoxyciliin **Clavulanic acid**

Figure 11a & 11b. Docking orientation and interactions of Amoxyciliin and Clavulanic acid with TEM



TEM **CTX-M-15**

Figure 12a & 12b. Docking orientation and interactions of Amoxyciliin and Clavulanic acid with CTX-M-15

The results clearly showed that Piperacillin, Tazobactam and Ciprofloxacin had strong interaction with CTX-M.15 than TEM.The analysis of the results points out that Colistin had good binding affinity with both TEM than CTX-M-15 protein.

4. Discussion

The treatment of bacterial infections with antibiotics is one of the major fields of human medicine. However, the effectiveness of antibiotics has become limited owing to an increase in bacterial antibiotic resistance, which represents a global health problem with a strong social and economic impact (Bonnet, Rice). The resistance to commonly used antibiotics, including penicillins and cephalosporins, which are amongst the

most widely used class of antibiotics, is a serious problem and needs an immediate attention. Hence in the present study we used Insilco analysis to identify the amino acids residues which is responsible for the resistance of antibiotic.

In-silico analysis is the initial step in the drug discovery research before entering into laborious experimental part, which always reduces the cost and time. The computational method is used to predict the mode of interaction between particular compounds with the biological macromolecules. It also assists in finding the desirable chemical properties of the compound. In microbiology research field there is a wealth of reports signifying the successful application of Computer Aided Drug Design (CADD). The results of the *in-silico* analysis of selected antibiotics are discussed in the following sections.

Molecular interactions between protein and ligand play important roles in many biological processes such as signal transduction, cell regulation, and other macromolecular assemblies. Therefore, determination of the binding mode and affinity between the constituent molecules in molecular recognition is crucial to understanding the interaction mechanisms and to designing therapeutic interventions. Due to the difficulties and economic cost of the experimental methods for determining the structures of complexes, computational methods such as molecular docking are desired for predicting putative binding modes and affinities. Molecular docking is a widely-used computational tool for the study of molecular recognition, which aims to predict the binding mode and binding affinity of a complex formed by two or more constituent molecules with known structures [17].

Numerous successes of designed drugs were reported, including Dorzolamide for the treatment of cystoid macular edema [18], Zanamivir for therapeutic or prophylactic treatment of influenza infection [19], Sildenafil for the treatment of male erectile dysfunction [20] and Amprenavir for the treatment of HIV infection [21, 22]. With increasing evidences of success in translating docking analysis, as an efficient filtering system in the drug development process, it is encouraging to test this approach in the present research study.

In the present study molecular docking was carried out with 19 antibiotics. Docking analysis was performed to identify the best compound. Docking analysis typically starts with the identification of suitable targets. Such targets are biomolecules that are usually proteins. For the present analysis the proteins chosen are TEM and CTX-M-15. Results of this docking clearly showed that most of the compounds showed very good interaction with both TEM and CTX-M-15 in terms of docking energy and number of hydrogen bond interaction.

The drugs (Ampicillin, Amoxycillin, Amikacin, Imipenem, Meropenem, Cephalothin, Cefoxitin, Ceftazidime, Cefexime, Ceftriazone, cefepime, cefotaxime, Aztreonam, Nitrofurantoin, Colistin, Ciprofloxacin as well as inhibitors Clavulanic acid, Piperacillin and Tazobactam) were docked into modeled structure of TEM and CTX-M-15 protein. Pymol analysis of the docked structures revealed that the amino acid residues ASN170, GLU 166 ASN132, SER70, ALA237, SER235, SER70, SER130, LYS234, TYR105, ASN170 of TEM protein interacted with most of the antibiotics used in the present

study.Similarly,Ser237,Thr235,Thr216,Tyr219,Asn170,Ser70,Ser130,Tyr105,Thr21,Tyr129,Lys234,Arg276, Thr235, Thr244,Thr215 amino acid residues of CTX-M-15 found to be a common interactive site for all the compounds. So these amino acids residues are responsible for the function of that particular protein. These information might be useful for the future development of a TEM & CTX-M resistant antibiotics.

5. Conclusion

This is the first study to the best of our knowledge in South Tamilnadu, India to report the docking of drugs as well as inhibitors (Clavulanate and Tazobactam) with TEM and CTX-M-15 for the identification of amino acid residues crucial to the enzyme-drug and enzyme-inhibitor interaction. Pymol analysis of the docked structures revealed that the amino acid residues Asn170, Glu 166 Asn132, Ser70, Ala237, Ser235, Ser70, Ser130, Lys234, Tyr105, Asn170 of TEM protein interacted with most of the antibiotics used in the present study. Similarly, Ser237, Thr235, Thr216, Tyr219, Asn170, Ser70, Ser130, Tyr105, Thr216, Tyr129, Lys234, Arg276, Thr235, Thr244, Thr215 amino acid residues of CTX-M-15 found to be a common interactive site for all the compounds. So these amino acids residues are responsible for the function of that particular protein. This information might be useful for the future development of a versatile TEM & CTX-M-15 resistant antibiotics.

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