

# In Silico Modeling and Docking Analysis of CTX-M-5, Cefotaxime-Hydrolyzing $\beta$ -Lactamase from Human-Associated *Salmonella* Typhimurium

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## Abstract

**Objective:** To computationally model the CTX-M-5  $\beta$ -lactamase and establish its structure, which is exclusively present in human-associated *Salmonella*.

**Methods:** The CTX-M-5 amino acid sequence (Uniprot ID:O65975) of *Salmonella enterica* subsp. *enterica* serovar typhimurium was retrieved from UniProt database and subjected to homology modeling using MODELLER 9v7. The homology models were duly validated using RAMPAGE tool by generating Ramachandran plots, ERRAT graphs, and ProSA score. DoGSiteScorer server and ConSurf server were used to detect the cavities, pockets, and clefts to identify conserved amino acid sites in the predicted model. Subsequently, the modeled structure was docked using CLC Drug Discovery Workbench against proven drugs and known inhibitors.

**Results:** Obtained high-quality homology model with 91.7% of the residues in favorable regions in Ramachandran plot and qualified in other quality parameters. Docking studies resulted in a higher dock score for PNK (D-benzylpenicilloic acid) molecule when compared to other reported inhibitors.

**Conclusion:** This in silico study suggests that the compound PNK could be an efficient ligand for CTX-M-5  $\beta$ -lactamase and serve as a potent inhibitor of CTX-M-5.

## Keywords

Extended-spectrum  $\beta$ -lactamase (ESBL), *Salmonella typhimurium*, CTX-M-5, Homology modeling, Docking, PNK

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## Introduction

*Salmonella enterica* subsp. *enterica* serovar typhimurium (aka *S. typhimurium*), a member of the *Enterobacteriaceae* family, is a gram-negative, facultative anaerobe that causes self-limiting gastroenteritis in humans. On the other hand, infection is systemic in murine models that resemble typhoid fever in humans caused by *Salmonella enterica* subsp. *enterica* serovar typhi. *Salmonella* is one of the four major causes of diarrheal diseases and foodborne infections across the globe.<sup>1</sup> The World Health Organization reports 93,000,000 cases of enteric infections and 155,000 mortality cases annually.<sup>2</sup> Currently, drugs used in the treatment of gastroenteritis lead to the increasing emergence of antibiotic resistance and the most rampant antibiotic-resistant mechanism is the production of  $\beta$ -lactamases by

gram-negative bacteria.<sup>3</sup>  $\beta$ -lactamases inactivate  $\beta$ -lactam group of antibiotics by hydrolyzing the amide bond of a four-membered  $\beta$ -lactam ring. Based on sequence variation, about

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4300 different types of  $\beta$ -lactamases have been identified so far ([www.bldb.eu](http://www.bldb.eu)). According to the Ambler system of classification,  $\beta$ -lactamases are broadly categorized into four groups based on sequence similarity and hydrolytic mechanisms. They are active site serine  $\beta$ -lactamases, such as classes A, C, and D and metallo- $\beta$ -lactamases or zinc (Zn)-dependent class B  $\beta$ -lactamases.<sup>4</sup>

Extended-spectrum  $\beta$ -lactamases (ESBLs) are plasmid-mediated  $\beta$ -lactamases that provide resistance to the extended-spectrum group of cephalosporins and other closely related oxyiminocephalosporins namely ceftazidime, ceftriaxone, cefotaxime, and monobactam antibiotics such as aztreonam. However, they are susceptible to carbapenem antibiotics (imipenem, meropenem, and ertapenem), cephamycins, and  $\beta$ -lactamase inhibitors, such as clavulanic acid and ethylenediaminetetraacetic acid. Ambler's classification illustrated ESBL enzymes such as SHV, TEM, CTX-M, VEB, and GES as class A  $\beta$ -lactamases and OXA-ESBLs as class D  $\beta$ -lactamases.<sup>5</sup> Point mutations in the active site of known  $\beta$ -lactamases such as TEM, SHV, and OXA-10 led to the evolution of new  $\beta$ -lactamases called ESBLs. There were 193 SHV types and 223 TEM types of ESBLs as detailed in Beta-Lactamase Database (<http://bldb.eu/>). Apart from TEM- and SHV-type ESBLs, another different type of ESBL is CTX-M- $\beta$ -lactamases belonging to Ambler class A or Bush's group 2be.<sup>6</sup>

Although TEM and SHV ESBLs were more predominant during 1980s, in the recent past, CTX-Ms had completely supplanted them. At present, CTX-Ms have completely strewn the world referring as "CTX-M pandemic."<sup>7,8</sup> Being isolated from Munich and highly active on cefotaxime, they are named as CTX-Ms.<sup>9</sup> Currently, there are about 172 different varieties of CTX-M- $\beta$ -lactamases reported in the literature. According to Bush and Ambler's class A classification, CTX-Ms were clustered into five individual groups, such as CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25, named after the first member of each group.<sup>6</sup> Clustering of CTX-M-5 in CTX-M-2 group was first reported in *S. typhimurium*.<sup>10</sup> Later a widespread occurrence of CTX-M-5 across the world was observed.<sup>11–15</sup> CTX-M-5  $\beta$ -lactamase hydrolyses cefotaxime more actively than ceftazidimes, as methoxy iminogroup of cefotaxime provide better affinity to the enzyme than carboxy-isopropoximino group of ceftazidimes.<sup>9</sup>

CTX-M-2, CTX-M-3, CTX-M-9, CTX-M-14, and CTX-M-15 are found in both animal and human *Salmonella* isolates, whereas CTX-M-5 ESBLs are found only in human *Salmonella* isolates.<sup>10,16,17</sup> Members of CTX-M group such as CTX-M-9,<sup>18</sup> CTX-M-15, CTX-M-53, CTX-M-71, CTX-M-82, and CTX-M-89,<sup>19</sup> were docked with different drugs and inhibitors. However, the information about three-dimensional structure and functional analysis of the CTX-M-5 was not available so far. As CTX-M-5 is rapidly evolving in

*Salmonella* sps. across the world, there is an utmost need to model CTX-M-5 in silico and target its active site to combat its widespread. A 3D model will help to determine the active sites of protein for docking studies. Thus, modeling a structure using a computational biology approach is of enormous advantage as it consumes less time and is cost effective. Hence, the present study was carried out to predict a 3D structure for CTX-M-5  $\beta$ -lactamases protein, which is specific to human *Salmonella* isolates. Additionally, we focused on the evaluation of 3D structure and identification of conserved residue sites and ligand binding sites. Thus, the objectives of the present study are (a) homology modeling of CTX-M-5, (b) phylogenetic analysis of CTX-M-5, (c) evaluation of 3D structure, (d) assessment of functional sites in CTX-M-5, and (e) docking of CTX-M-5 with different drugs and inhibitors.

## Materials and Methods

### Data Retrieval and Template Selection

CTX-M-5  $\beta$ -lactamase amino acid sequence of *Salmonella enterica* subsp. *enterica* serovar typhimurium (Uniprot ID: O65975) was retrieved from UniProt database. To find out suitable templates for homology modeling, blaCTX-M-5 was subjected to BLAST-P search against Protein Data Bank (PDB).

### Phylogenetic Analysis

Phylogenetic analysis was carried out for a total of eight amino acid sequences using MEGA version 7.<sup>20</sup> Neighbor-joining method was employed to know the evolutionary history. The evolutionary distances were computed through the Poisson correction method. The bootstrap value of 1000 was used for the analysis of evolutionary history. Gaps and missing data were eliminated from positions.

### Homology Modeling and Validation

The three dimensional structures for CTX-M-5 were predicted using MODELLER 9v7. The predicted structures were evaluated using RAMPAGE (Ramachandran plot),<sup>21</sup> ERRAT graphs (Colovos and Yeates 1993)<sup>22</sup>, and ProSA analysis.<sup>23</sup>

### Functional Site Analysis

DoGSiteScorer server<sup>24</sup> was used to predict the cavities, pockets, and clefts. ConSurf server was used to determine the functional and conserved residue regions of the desired protein sequence with respect to other homologous sequences.<sup>25,26</sup> COACH, a meta server, was employed for determining ligand binding sites in the protein.<sup>27</sup>

## Molecular Docking

The predicted best structure of CTX-M-5 was docked with different drugs and inhibitors. The ligand molecules were retrieved from ZINC database (<https://zinc.docking.org/>). Docking was carried out using CLC Drug Discovery Workbench (CLC Inc., Aarhus, Denmark) with a maximum number of iterations of 200. The optimal binding mode of a molecule to the active site of macromolecule was interpreted to select more potent ligands as inhibitors.

## Results

### Data Retrieval and Template Selection

A complete amino acid sequence (Uniprot ID: O65975) of CTX-M-5  $\beta$ -lactamase (EC: 3.5.2.6) of *Salmonella enterica* subsp. *enterica* serovar typhimurium with 291 amino acid residues length was retrieved from the UniProt database (O65975). CTX-M-5  $\beta$ -lactamase protein sequence was subjected to PDB BLAST to identify structural homologs. The hit list consisted of chain A,  $\beta$ -lactamase Toho-1 of *Escherichia coli* (PDB: 4X69\_A; structure resolution: 1.42 Å, identity: 97.32%, coverage: 89%), chain A,  $\beta$ -lactamase Toho-1 of *E. coli* (PDB: 1IYS\_A; structure resolution: 1.65 Å, identity: 97.32%, coverage: 89%), chain A,  $\beta$ -lactamase Toho-1 of *E. coli* BL21 (PDB: 4BD0\_A, structure resolution: 1.2 Å, identity: 96.93%, coverage: 89%), and chain A, apo structure of class A  $\beta$ -lactamase Toho-1 of *E. coli* (PDB: 2ZQ8\_A, structure resolution: 1.03 Å, identity: 96.95%, coverage: 90 %). Amongst them, protein bearing PDBID: 2ZQ8\_A was selected as a template for homology modeling of the target protein as it has a better structural resolution (1.03 Å) compared to other proteins. Furthermore, the evolutionary analysis showed that 2ZQ8\_A, i.e., Toho-1 of *E. coli*, and CTX-M-5 of *S. typhimurium* clustered together in the phylogenetic tree, demonstrating their evolutionary relatedness and justifying the selection of 2ZQ8\_A as a template for the prediction of CTX-M-5 model. Phylogenetic

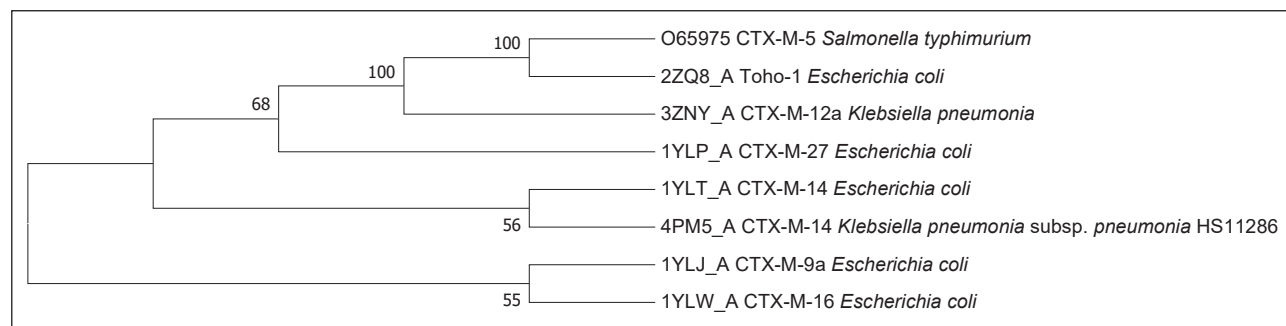
analysis (Figure 1) revealed the evolutionary relationship of CTX-Ms and reported the average rate of divergence as 0.02.

Phylogenetic analysis was performed in MEGA7, using the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.<sup>2</sup>

### Homology Modeling and Validation

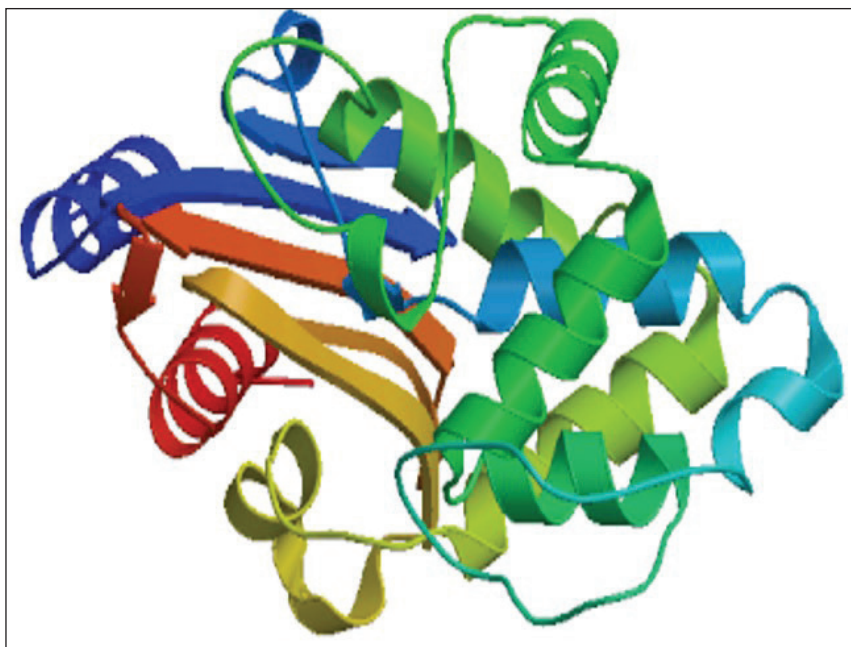
Homology modeling intends to build a 3D structure of a protein whose structure is experimentally unknown. It utilizes the structures of experimentally proven and verified structures of relevant protein family members as templates for prediction. MODELLER 9v7 was used to predict the 3D structure (Figure 2) for CTX-M-5 by using 2ZQ8\_A (PDB ID) as the template and the resulting structure was visualized using RasMol (Bernstein 2000).<sup>28</sup> A total of 100 3D structures were generated and the structure with the lowest discrete optimized protein energy score was chosen for further analysis. The molecule, (No: 62<sup>nd</sup>) with best DOPE score -30663.2 and GA341 score, 1.0 was selected for further studies. The total energy before and after energy minimization was -6143.1 kJ/mol and -13845 kJ/mol, respectively, and energy minimization was accomplished with GROMOS96 43B1 using Swiss-Pdb Viewer.

RAMPAGE analysis for the modeled CTXM-5 protein revealed that 91.7% of the residues in the allowed regions confirming the accuracy of the modeled structure (Figure 3). The Ramachandran plot displayed the statistical distribution of the combinations of the backbone dihedral angles  $\phi$  and  $\psi$  that are favorable, unfavorable, and disallowed. The permitted regions of the plot represent the values of  $\phi/\psi$  angles that are possible for an amino acid. Structure validation is based on the dispersion of  $\phi/\psi$  values in a protein structure. In the Ramachandran plot, dihedral angles observed in the forbidden regions denote low-quality homology models representing the structural problem. In the current model, all the favored and allowed regions resemble the conformations of the amino



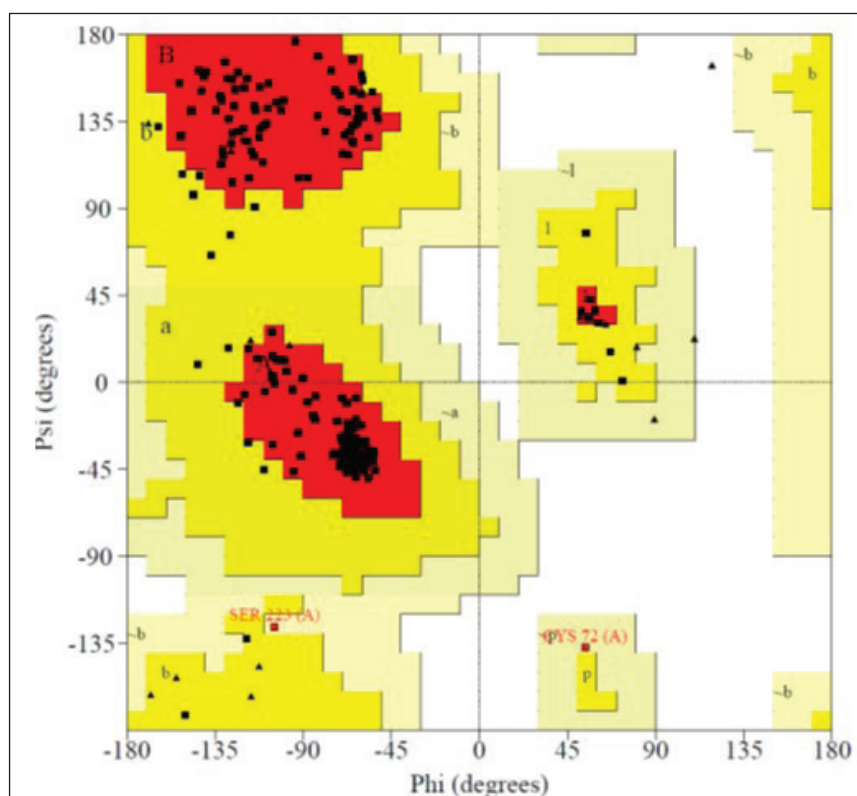
**Figure 1.** Evolutionary Relationships of Various  $\beta$ -Lactamases.

**Source:** Created by the Authors using MEGA7.



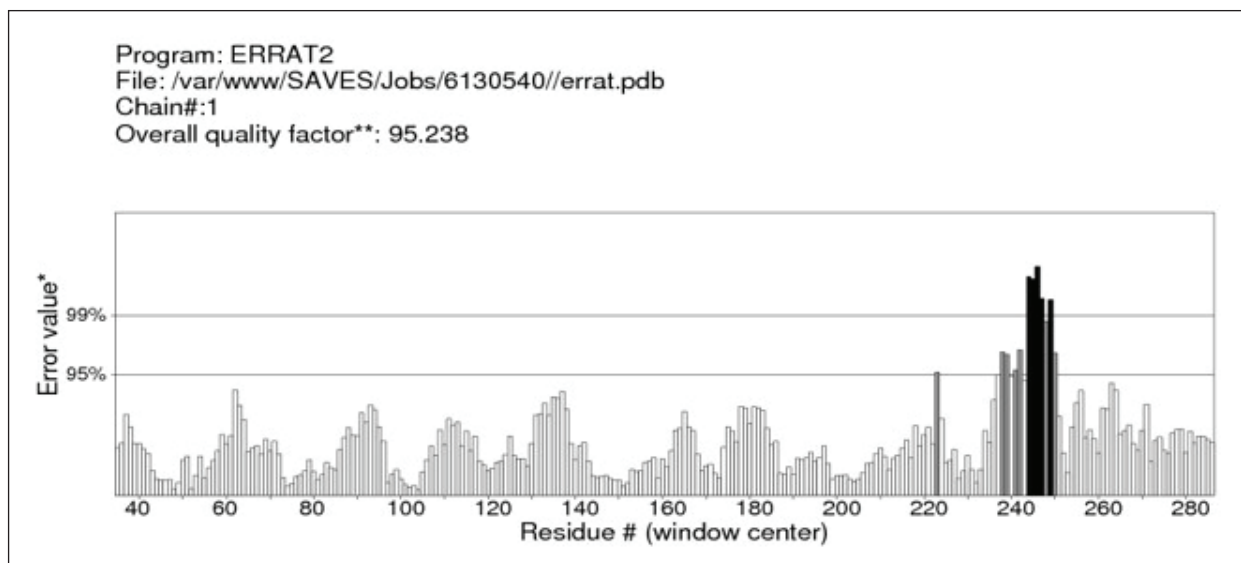
**Figure 2.** Predicted Model of CTX-M-5 Sequence of *Salmonella Enterica* Subsp. *Enterica* Serovar Typhimurium.

**Source:** Created by the Authors using MODELLER 9v7.



**Figure 3.** Ramachandran Plot (RAMPAGE: Residues in Most Favored Regions (A, B, L) = 91.7%, Residues in Additional and Generously Allowed Regions (a, b, l, p) = 8.3%, and Residues in Disallowed Regions = 0%. All Nonglycine and Proline Residues Are Shown as Filled Black Squares, Whereas Glycines (Nonend) Are Shown as Filled Black Triangles.

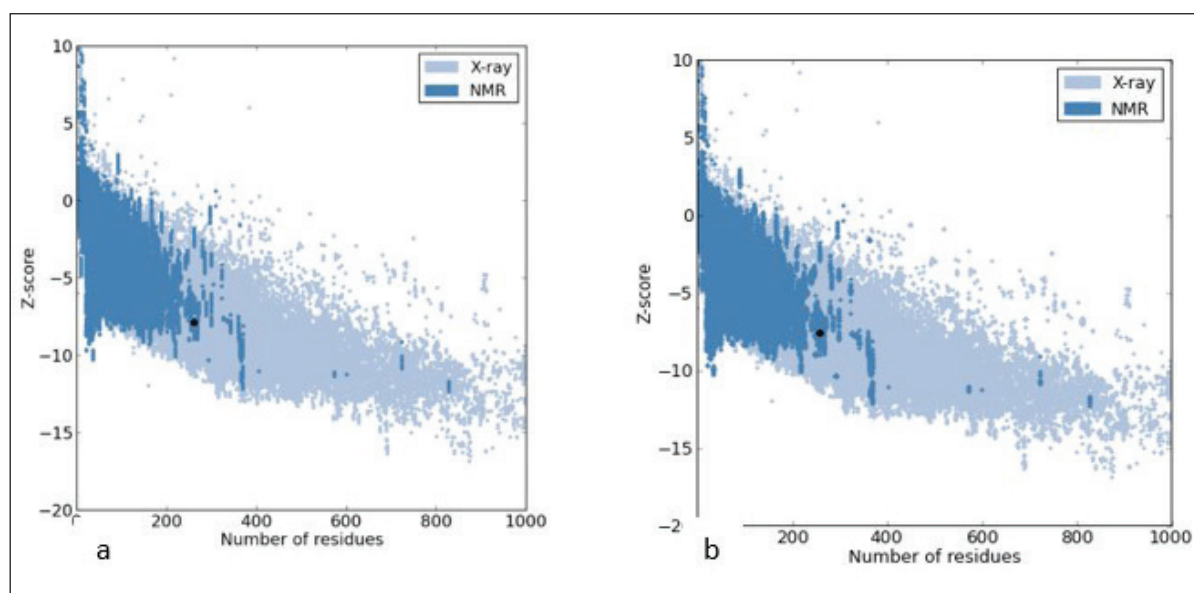
**Source:** Created by the Authors using RAMPAGE.



**Figure 4.** ERRAT Graph of the Predicted Structure.

**Source:** Created by the Authors using ERRAT.

**Note.** \*\*Expressed as the percentage of protein for which the calculated error value falls below the 95% rejection limit. Good high-resolution structures generally produce values around 95% or higher. \*On the error axis, two lines are drawn to indicate the confidence with which it is possible to reject regions that exceeded that error value.



**Figure 5.** ProSA-Web Z-Score of CTX-M-5 Protein Model.

**Source:** Created by Authors using ProSA.

acids and reported no steric hindrance. A good quality model would be expected to have a threshold value of over 90% in the most favored regions.

Verification of protein structure by ERRAT analysis exhibited an overall quality factor of 95.238 (Figure 4),

which depicts a good 3D structure of the predicted model. In addition to this, it also examines the statistics of nonbonded atomic interactions. It is the graph of the error value function plotted against the position of a nine-residue sliding window, calculated by comparing data from highly

refined structures with each other. Unrefined structures generally do not score well and the accepted range is  $>50$  for a high-quality model.

The ProSA Z-score indicates the overall model quality of the predicted 3D structure. Previous studies suggest that the Z-score of query protein should be within the range of our template protein in order to be considered a good quality score. The Z-score of the predicted protein ( $-7.63$ ) is within the range of our template protein ( $-7.88$ ) and hence the modeled structure is a valid one (Figure 5).

ProSA-web Z-scores of all protein chains in PDB are denoted by dots as determined by X-ray crystallography (blue dots) with respect to their length (number of residues). (a) The Z-score of proteins is highlighted (black dot). The Z-score of protein 2zq8 is  $-7.88$ . (b) The Z-score of the predicted structure is  $-7.63$ .

### Functional Site Analysis

DoGSiteScorer determines the potential active sites of a 3D protein structure of CTX-M-5. It uses a difference of Gaussian filter to detect potential pockets on the protein surface and splits them into subpockets, describing their size, shape, and chemical features in the given query protein structure. The higher the score, the more likely the pocket is to be druggable. Pocket P2 reported the higher score (Table 1) with a depth of about  $10.32 \text{ \AA}$  and the volume of about  $347.71 \text{ \AA}^3$ . It is predicted that pocket P2 contains the highest lipophilic surface when compared to other pockets, i.e., about  $479.31 \text{ \AA}^2$  comprising alanine and leucine as predominant amino acids in its pocket. Hence, P2 appears to be a potential active site for CTX-M-5.

### Conserved Sites Determination

The ConSurf server was used for identification of evolutionarily conserved amino acid residues in our predicted protein molecule. It uses the empirical Bayesian method for the calculation of conservation scores.<sup>29</sup> This method gives accurate conservation scores when compared to others' even when very small sequences are used. The conserved sites of CTX-M-5 were displayed both in sequence as well as in the 3D structure (Figure 6). Results were interpreted on a conservation scale of levels ranging from 1 to 9 represented with different colors. Levels 1 and 2 (blue) depict the variable region, level 5 (white) show average score, and levels 8 and 9

(bordeaux) show moderately conserved and highly conserved regions. According to the above analysis, CTX-M-5 protein has 65 highly conserved functional residues exposed and 14 highly conserved structural residues buried that contribute to the active sites of the protein. Thus, we suggest that this method of identification of conserved functional residues is merely helpful with respect to other methods in understanding the 3D structure of the protein.

### Protein–Ligand Binding Site Prediction

COACH server was used to determine the ligand-binding sites besides predicting an efficient ligand that can bind CTX-M-5 (Table 2). It generates complementary sites by searching against BioLip protein function database. C-score is the confidential score of the COACH server ranging from 0 to 1, where a PNK molecule with a 0.99 score stands as a reliable compound. The binding residues of CTX-M-5 from different bioinformatics tools like DoGSiteScorer, ConSurf, and COACH servers were compared (Table 3). From the analysis, Lys, Ala, Gly, Leu, and Thr were found to be common residues represented in bold font in Table 3 that could form hydrogen bond interactions with the drugs and inhibitors.

### Docking Analysis

Modeled CTX-M-5 was docked with different inhibitors (clavulanic acid, sulbactam, and tazobactam) and drugs (cefotaxime, cefipime, and cefixime) along with PNK molecule using CLC Drug discovery Workbench (Figure 7). The binding sites include a center binding pocket of  $138.75 \text{ \AA}^3$  and the number of iterations was set to 100. For each ligand, five docking outcomes were returned. When the protein and ligand interact, the CLC score simulates the possible energy change. A high negative value indicates a strong binding, whereas a lower negative or positive score indicates a weak binding (Table 4).

Docking result for the interaction of CTX-M-5 with ligands were shown in Table 4. The 3D diagrams depicting the interaction of CTX-M-5 with sulbactam, tazobactam, cefotaxime, cefipime, clavulanic acid, cefixime, and PNK (from left to right) were shown in Figure 7.

A  $\beta$ -lactam antibiotic when given in combination with a  $\beta$ -lactam inhibitor makes the antibiotic-resistant organism susceptible. As the inhibitor occupies the active site of the enzyme, the antibiotic is effective. The higher the negative

**Table 1.** Pockets and Descriptors Calculated for CTX-M-5 Using DoGSiteScorer.

| Name | Volume [ $\text{\AA}^3$ ] | Surface [ $\text{\AA}^2$ ] | Lipo Surface [ $\text{\AA}^2$ ] | Depth [ $\text{\AA}$ ] | Simple Score |
|------|---------------------------|----------------------------|---------------------------------|------------------------|--------------|
| P0   | 499.52                    | 766.70                     | 425.21                          | 24.83                  | 0.24         |
| P1   | 393.98                    | 605.82                     | 410.67                          | 21.22                  | 0.21         |
| P2   | 347.71                    | 702.29                     | 479.31                          | 10.32                  | 0.27         |
| P3   | 332.42                    | 501.77                     | 232.03                          | 11.41                  | 0.14         |

```

1   11   21   31   41
MNTQSRSSMLTVMATLPLL FSSATLHAQA NSVQQQLAL EKSSGGRLGV
e e e e e e e b b b b b b b b e e e e e e e e b e e e e b e e e e e e b b
f           f           f f s

51   61   71   81   91
ALINTADNSQILYLADERFA MCSTSKVMAA AAVLKQSE SD KHL LNQRVEI
b b b b e e e e e e e e e e b b b b e b b b b b b b e e e e e e e e e e
f           f f f s s f f           s s

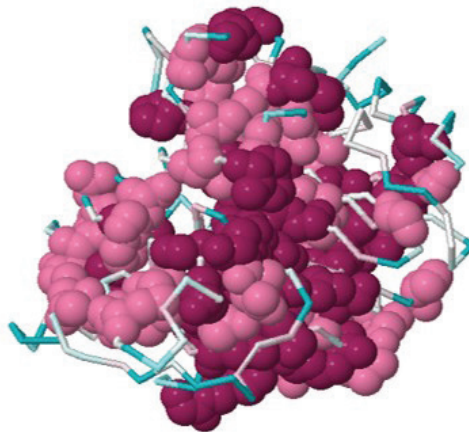
101  111  121  131  141
KASDLVNYNFI AEKHVNGT MTLAELGAGAL QYSDNTAMNK LIAHLGCPDK
e e e b b e e e e e e e e e e e e e e e e e e e e e e e e e e e e e e e
f f f           s f           s f f f f f f f f f f f f f f f f f f f f

151  161  171  181  191
VIAFARSLGDETERLDREPTLNSAPGDP RDTTIPAMIA QILKNL TLGK
b e e b b e e e e e e e e e e e e e e e e e e e e e e e e e e e e e e e
f f f f f f f f f f f f f f f f f f f f f f f f f f f f f f f f f f f

201  211  221  231  241
ALAEIQRAQLVTWLGKNTIG SASIRAGLPK CWVVGDKTGS GDVGTINDIA
b b e e e e e e e e e e e e e e e e e e e e e e e e e e e e e e e e e e
s f f           f f f f f f f f f f f f f f f f f f f f f f f f f f f f

251  261  271  281  291
VIWPE NHAQLVLVTYFTQPE QKAESRRDVL AAAAKIVTHG F
b b b b e e e e e e e e e e e e e e e e e e e e e e e e e e e e e e e e
f           f           f

```



The conservation scale:

1 2 3 4 5 6 7 8 9

Variable      Average      Conserved

e - An exposed residue according to the neural-network algorithm.

b - A buried residue according to the neural-network algorithm.

f - A predicted functional residue (highly conserved and exposed).

s - A predicted structural residue (highly conserved and buried).

X - Insufficient data - the calculation for this site was performed on less than 10% of the sequences.

**Figure 6.** ConSurf Representation of Conserved Sites in CTX-M-5.

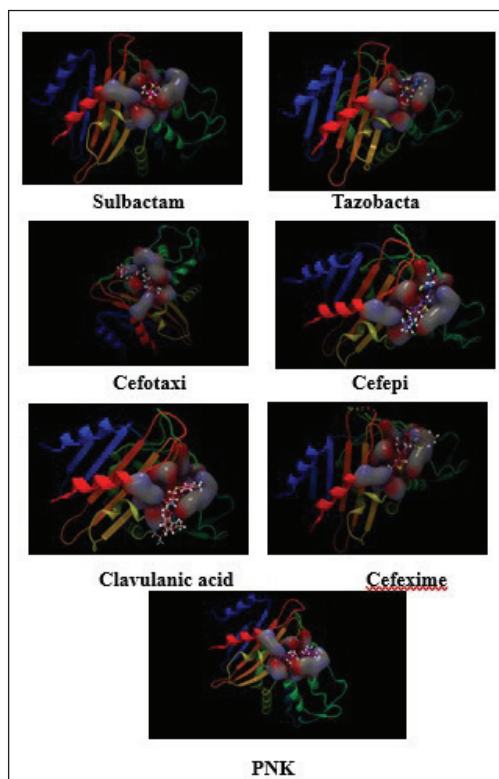
**Source:** Created by Authors using ConSurf server.

**Table 2.** Predicted Binding Residues Using COACH SERVER.

| C-Score | Ligand Name                                                                                    | Consensus Binding Residues                     |
|---------|------------------------------------------------------------------------------------------------|------------------------------------------------|
| 0.99    | PNK<br>4-carboxy-5,5-dimethyl- $\alpha$ -((phenylacetyl)amino)-2-thiazolidineacetic acid       | 42,43,46,77,78,103,105,143,208,209,210,211,212 |
| 0.44    | LN1<br>(3r)-4-[[[(3,4-dihydroxyphenyl)acetyl]oxy]-n-(2-formylindolizin-3-yl)-3-sulfinod-valine | 43,103,189,190,207,208,209,210,216,247         |
| 0.07    | MA4<br>cyclohexyl-hexyl-beta-d-maltoside                                                       | 199,204,218,220,222,232,234,250,251,255,258    |
| 0.06    | DN3<br>3-[(dimethylamino)methyl]-n-[3-(1h-tetrazol-5-yl)phenyl]benzamide                       | 52,55,56,59,115,120,124,127                    |
| 0.03    | CLS<br>Cephalothin                                                                             | 244,245,246,247,248                            |
| 0.02    | TEM<br>3-hydroxy-4-[[[(e)-3-oxoprop-1-enyl]amino]butanal                                       | 43,143,209,210,211,212                         |

**Table 3.** Comparison of Results from Different Tools Representing the Similarity of Active Site.

| S. No. | Name of the Tool | After Math Result                | Amino Acid Residues                                                                     |
|--------|------------------|----------------------------------|-----------------------------------------------------------------------------------------|
| 1      | DoGSiteScorer    | Pocket P2<br>(functional site)   | <b>LYS,ALA, GLY, LEU,THR,</b><br>ASN, PRO, TRP,<br>HIS, ILE, VAL                        |
| 2      | ConSurf          | Highly conserved regions         | <b>LYS,ALA, GLY, LEU,THR,</b><br>MET, GLU, SER, ASP<br>ASN, PRO, TRP,<br>ARG, GLN, PHE, |
| 3      | COACH            | PNK (ligand binding active site) | <b>LYS,ALA, GLY, LEU,THR,</b><br>MET, GLU, SER, ASP, VAL                                |

**Figure 7.** Docking Analysis.

**Source:** Created by Authors using CLC Drug discovery Workbench.

**Table 4.** CLC Score Toward the Interaction of Ligands with CTX-M-5.

| S. No | Substance                                                                                        | ZINC Database Number | CLC Score |
|-------|--------------------------------------------------------------------------------------------------|----------------------|-----------|
| 1     | Sulbactam                                                                                        | ZINC00897244         | -32.65    |
| 2     | Tazobactam                                                                                       | ZINC01483277         | -34.68    |
| 3     | Cefotaxime                                                                                       | ZINC03830437         | -42.81    |
| 4     | Cefepime                                                                                         | ZINC03871924         | -40.14    |
| 5     | Clavulanic acid                                                                                  | ZINC05280980         | -46.85    |
| 6     | Cefixime                                                                                         | ZINC13704177         | -41.49    |
| 7     | 2-((R)-carboxy[(phenylacetyl)amino]methyl)-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid (PNK) | ZINC56874249         | -52.59    |

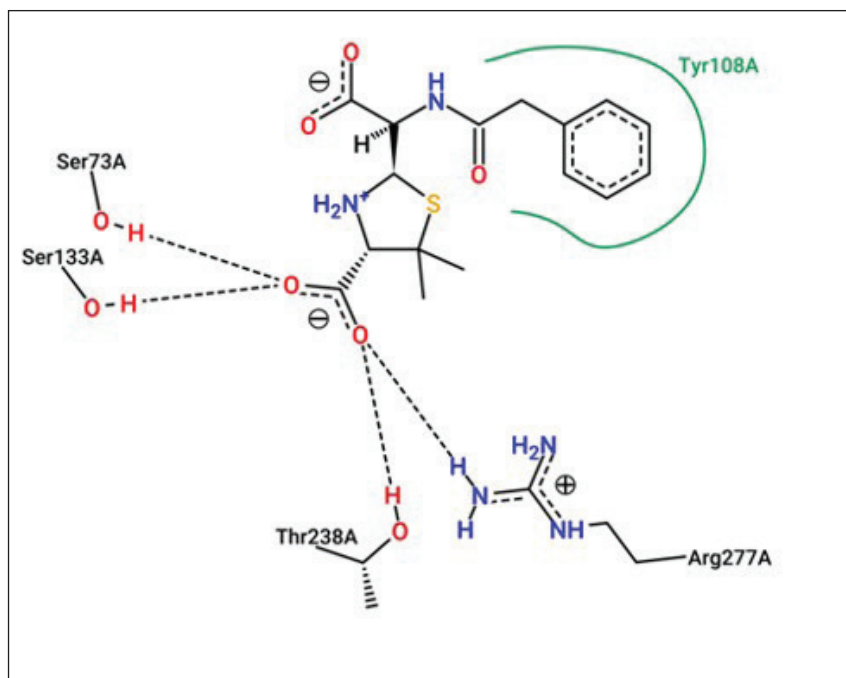
**Table 5.** Detailed Bond Interactions of Ligand with CTX-M-5.

| Hydrophobic Interactions |         |     |              |              |                                    |              |               |
|--------------------------|---------|-----|--------------|--------------|------------------------------------|--------------|---------------|
| Index                    | Residue | AA  | Distance     | Ligand Atom  |                                    | Protein Atom |               |
| 1                        | I07A    | ASN | 3.55         | 3941         |                                    | I164         |               |
| 2                        | I08A    | TYR | 3.69         | 3941         |                                    | I178         |               |
| Hydrogen Bonds           |         |     |              |              |                                    |              |               |
| Index                    | Residue | AA  | Distance H-A | Distance D-A | Donor Angle                        | Donor Atom   | Acceptor Atom |
| 1                        | 73A     | SER | 3.15         | 3.84         | 126.75                             | 3927 (N3)    | 639 (O3)      |
| 2                        | 73A     | SER | 1.82         | 2.55         | 132.64                             | 3935 (O.co2) | 639 (O3)      |
| 3                        | I33A    | SER | 2.26         | 2.63         | 102.63                             | 3948 (O.co2) | 1547 (O3)     |
| 4                        | I35A    | ASN | 2.62         | 3.23         | 117.94                             | 1572 (Nam)   | 3938 (O2)     |
| 5                        | 240A    | SER | 2.22         | 3.01         | 131.95                             | 3152 (O3)    | 3927 (N3)     |
| 6                        | 240A    | SER | 2.94         | 3.79         | 140.26                             | 3147 (Nam)   | 3927 (N3)     |
| TI-Stacking              |         |     |              |              |                                    |              |               |
| Index                    | Residue | AA  | Distance     | Angle        | Ligand Atoms                       |              |               |
| 1                        | I08A    | TYR | 5.07         | 76.67        | 3940, 3941, 3942, 3943, 3944, 3945 |              |               |
| Salt Bridges             |         |     |              |              |                                    |              |               |
| Index                    | Residue | AA  | Distance     | Ligand Group |                                    | Ligand Atoms |               |
| 1                        | 76A     | LYS | 5.21         | Carboxylate  |                                    | 3934, 3935   |               |
| 2                        | 237A    | LYS | 4.20         | Carboxylate  |                                    | 3947, 3948   |               |
| 3                        | 277A    | ARG | 5.15         | Carboxylate  |                                    | 3947, 3948   |               |

energy, higher is “interaction energy,” contributing to efficiency. In our study, amongst drugs, cefepime showed higher interaction energy than cefotaxime and cefixime. On the other hand, among inhibitors, clavulanic acid with higher interaction energy proved to be an efficient inhibitor than tazobactam and sulbactam. It is noteworthy that PNK (D-benzyl penicilloic acid) molecule generated by the COACH server showed greater inhibition when compared to other molecules with higher interaction energy (Table 4). Previous studies reported that Benzylpenicillin has higher hydrolytic activity ( $k_{cat}$  230  $s^{-1}$ ) and affinity ( $K_m$  50  $\mu M$ ) to CTX-M-5 suggesting that our results are in coherence with

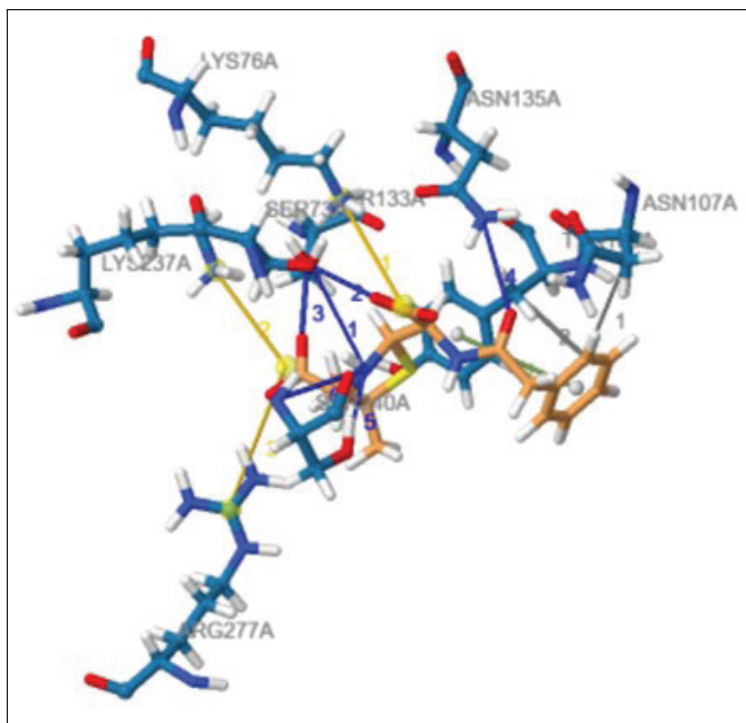
their studies. So, PNK can be considered as strong inhibitor that can efficiently inhibit CTX-M-5.

The 3D interactions of (PNK) ligand and CTX-M-5 were displayed by PoseView as shown in Figure 8. Protein-Ligand Interaction Profiler was used to obtain detailed bond interactions between the PNK and CTX-M-5 (Figure 9) and were tabulated Table 5). It was found that the amino acid residues Asn173, Ser240, Arg277, Gly239, Ser73, Thr238, Lys237, Thr219, Lys76, Ser133, Asn107, Glu169, Tyr108, and Asn135 enable correct positioning of the drugs and inhibitors within the active site of CTX-M-5.



**Figure 8.** The 2D Diagrams Show the Interactions of the Amino Acid Residues in the Active Cavities of CTX-M-5 with Ligand (PNK). Directed Bonds Between Protein and Ligand Are Drawn as Dashed Lines and the Interacting Protein Residues and the Ligand Are Visualized as Structure Diagrams. Hydrophobic Contacts Are Represented More Indirectly by Means of Spline Sections Highlighting the Hydrophobic Parts of the Ligand and the Label of the Contacting Amino Acid.

**Source:** Created by Authors using PLIP Web Server.



**Figure 9.** 3D View of Interactions Ligand and CTX-M-5 (Image Generated with [Protein-Ligand Interaction Profiler] PLIP Web Service Provided by TU Dresden/Redvia).

**Source:** Created by Authors using PLIP Web Server.

## Discussion

Fluoroquinolones are commonly used antibiotics to treat invasive infections caused because of *Salmonella*. Misuse and overuse of fluoroquinolones led to antibiotic resistance. As a result, other antibiotics such as azithromycin and third-generation cephalosporins were used as the first-line therapy for treatment.<sup>30</sup> Increased cases of quinolone and cephalosporin resistant *Salmonella* serovars especially *S. typhimurium* in hospitals and communities are of great global concern in the management of *Salmonella* infections. The commonly reported extended-spectrum  $\beta$ -lactamases in different *Salmonella* serovars were majorly derived from SHV, TEM, and CTX-M type  $\beta$ -lactamase families.<sup>15,31</sup>

CTX-M is highly prevalent among different serovars of *Salmonella*. CTX-M-5 one such plasmid-mediated ESBL belonging to CTX-M-2 group has been reported widely in *S. typhimurium*.<sup>10,11,32</sup> In addition to CTX-M-5, the other members of the CTX-M-2 group include Toho-1, CTX-M-20, CTX-M-7, CTX-M-6, CTX-M-4L, CTX-M-4, CTX-M-2, CTX-M-65, and CTX-M-55.<sup>5,33,34</sup> Intriguingly, CTX-M-5 is found only in human-associated *S. typhimurium* and confers a high level of resistance to cefotaxime. *S. typhimurium* causes gastroenteritis in humans and typhoid-like fever in a mouse model. Although the CTX-M-5, variant of CTX-M-2 was first reported in a gastroenteritis outbreak in children in Latvia in 1990, several nosocomial outbreaks involving CTX-M-5 ESBL *S. typhimurium* were reported in European countries such as Latvia (1991–1998), Russia (1996–1997), Hungary (1996–1998), Greece (1991–1998), Belarus (2007), the USA (2000, 2003, and 2007), and Russia, Belarus, and Kazakhstan (1994–2003). *S. typhimurium* is considered a zoonotic infection, i.e., it originates from animal sources, and, hence, it is assumed that transmission of CTX-M-type cephalosporinases/ESBL carrying *S. typhimurium* occurs from animals or animal products to humans via food chain. In contrast, CTX-M-5 is found only in human *S. typhimurium* isolates, and, hence, person-to-person transmission occurs.<sup>15</sup> Traditional  $\beta$ -lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam, as well as new inhibitors such as LN1-255 and NXL-104, reduce the activity of these enzymes.

Previously, various *in silico* studies on “CTX-M-drug” and “CTX-M-inhibitor” interactions were undertaken to identify the critical amino acid residues for enzyme interactions with medications and inhibitors. Shakil and Khan<sup>19</sup> used AutoDock for molecular modeling and docking of CTX-M15 with cefotaxime. They reported that the amino acids Asn 104, 132, Gly 227, Thr 235, Gly 236, and Ser237 are important for positioning cefotaxime in the active site of CTX-M-15 and Ser237 and Arg276 residues in CTX-M-14  $\beta$ -lactamase.<sup>35</sup> Mutational analysis and a high-resolution crystal structure of CTX-M-14 suggest that amino acid residues Ser237 and Arg276 play a crucial role in catalytic specificity.<sup>36</sup> Docking study of CTX-M-9, 14, and 27 was done using Schrodinger by Kumar et al.<sup>18</sup> and found that the

amino acids Asp 101, Asn 136, Lys 137, Asp 101, Glu 166, Ser 130, Asn 132, Thr 235, Ser 237, and Asp 240 are crucial for interaction with drug and inhibitor. Cefotaxime, cefixime drugs, tazobactam, and clavulanic acid inhibitors were shown to interact with the CTX-M-9 family of enzymes with higher energy.<sup>18</sup>

In a similar molecular docking study done using Discovery Studio by Shakil and Khan,<sup>35</sup> using CTX-M variants (CTX-M-15, CTX-M-53, CTX-M-71, CTX-M-82, and CTX-M-89), it was observed that ceftazidime drug interacted with the amino acid residues of L/Y291, G290, D289, T288, R285, W232, S231, A/T230, P229, L228, G227, and A226 of CTX-M-type 2009 variants. Additionally, among the different inhibitors studied, sulbactam was binding most efficiently with high interaction energy.<sup>19</sup>

Bethel et al.<sup>37</sup> studied the inhibition of CTX-M-9 by sulbactam, tazobactam, clavulanate, meropenem, doripenem, ertapenem, and 6-methylidene penem using electrospray ionization mass spectrometry. They reported that the amino acid residue Arg276 of the CTX-M-9 contributes to the region of positive charge near the catalytic site, which is very essential for CTX-M-enzyme activity. More specifically, c3 carboxylase inhibitors are recognized by the Arg276 amino acid residue of CTX-M-9.<sup>37</sup> Danishuddin and Khan<sup>38</sup> modeled and studied the docking of PCR amplified CTX-M  $\beta$ -lactamase with traditional inhibitors such as sulbactam, clavulanic acid, and tazobactam followed by novel  $\beta$ -lactamase-inhibitors such as LN1-255 and NXL-104 using the software, GOLD 5.0. Among the tested inhibitors, compound LN1-255, and tazobactam have shown to be the best inhibitor against the enzymes CTX-M-15, SME-1, and IMI-1. Amino acid residues Gly237, Thr236, Lys235, Ser130, and Ser70 were reported to be essential for the formation of enzyme-inhibitor complexes by hydrophobic interactions and hydrogen bonding.<sup>38</sup> Ali et al.<sup>39</sup> have studied the interaction of non- $\beta$ -lactam inhibitors against the CTX-M-15-type  $\beta$ -lactamases through a virtual screening protocol. Three non- $\beta$ -lactam inhibitors such as RF04517, BTB04243, and RJC01340 interacted with CTX-M-15 very tightly and interaction is stabilized through hydrogen bond and hydrophobic interaction by Asn104, Asn132, Ser237, and Asp240 amino acid residues. Ser237 creates a strong hydrogen connection with the acyl-amide cefotaxime chain. These compounds were experimentally validated against *E. coli* strain carrying CTX-M-15  $\beta$ -lactamase.<sup>39</sup> Furthermore, Maryam et al.<sup>40</sup> showed the importance of amino acid residues Asn-247 and Arg-64 located in the proximity of the catalytic site of the CTX-M-15-type  $\beta$ -lactamase by site-directed mutagenesis. *E. coli* cells expressing Asn247Val and Arg64Leu mutant CTX-M-15-type  $\beta$ -lactamases have shown reduced cefotaxime minimum inhibitory concentrations of 512- and 128-fold, respectively. Similarly, in *in vitro* kinetic studies, the catalytic efficiencies of the CTX-M-15 (N247V) and CTX-M-15 (R64L) mutant  $\beta$ -lactamases were reduced by 89.66% and 71.11%, respectively.<sup>38</sup>

## Conclusion

In the current study, CTX-M-5 of *S. typhimurium* was modeled and validated using 3D structure validation tools. Besides this, conserved sites and the ligand-binding sites were also determined to predict the active site of the protein. Of the drugs and inhibitors used, Cefipime and clavulanic acid were found with higher interaction energies and effective. Above all, the COACH server predicted that PNK (D-benzylpenicilloic acid) molecule competently inhibited CTX-M-5 with greater negative energy. Previous studies reported that the PNK molecule, which is a diastereomer of benzylpenicillin is high affinity compound. So, we infer that our docking results are compatible with the wet lab studies. We also identified the amino acid residues of the active site responsible for the positioning of drugs and inhibitors. With the information above, alterations in the amino acid residues could aid to develop more potent and adaptable inhibitors. This is the first time to report an in silico modeled 3D structure of CTX-M-5, conserved sites of CTX-M-5, and all the commonly found amino acid residues that are crucial in hydrogen bonding interactions. These conserved sites can be further used in the rational design of novel drugs and inhibitors.

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## References

1. Chaudhuri D, Roy Chowdhury A, Biswas B, et al. *Salmonella typhimurium* infection leads to colonization of the mouse brain and is not completely cured with antibiotics. *Front Microbiol* 2018; 9: 1632. doi: 10.3389/fmicb.2018.01632.
2. Majowicz SE, Musto J, Scallan E, et al. International collaboration on enteric disease 'burden of illness' studies. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin Infect Dis* 2010 Mar 15; 50(6): 882–9. doi: 10.1086/650733.
3. Umadevi S, Kandhakumari G, Joseph N, et al. Prevalence and antimicrobial susceptibility pattern of ESBL producing gram negative bacilli. *J Clin Diagn Res* 2011; 5: 236–239.
4. Tooke CL, Hinchliffe P, Bragginton EC, et al.  $\beta$ -lactamases and  $\beta$ -lactamase inhibitors in the 21<sup>st</sup> century. *J Mol Biol* 2019; 431(18): 3472–3500. doi: 10.1016/j.jmb.2019.04.002.
5. Bonnet R. Growing group of extended-spectrum beta-lactamases: The CTX-M enzymes. *Antimicrob Agents Chemother* 2004; 48(1): 1–14. doi: 10.1128/AAC.48.1.1-14.2004.
6. Santos AG, Da Rocha GO, and De Andrade JB. Occurrence of the potent mutagens 2- nitrobenzanthrone and 3-nitrobenzanthrone in fine airborne particles. *Sci Rep* 2019; 9(1): 1. doi: 10.1038/s41598-018-37186-2
7. Bush K. Past and present perspectives on  $\beta$ -lactamases. *Antimicrob Agents Chemother* 2018; 62(10):e01076-18. doi: 10.1128/AAC.01076-18
8. Cantón R, González-Alba JM, and Galán JC. CTX-M enzymes: Origin and diffusion. *Front Microbiol* 2012; 3: 110. doi: 10.3389/fmicb.2012.00110
9. Bauernfeind A, Casellas JM, Goldberg M, et al. A new plasmidic cefotaximase from patients infected with *Salmonella typhimurium*. *Infection* 1992; 20(3): 158–163. doi: 10.1007/BF01704610
10. Bradford PA, Yang Y, Sahm D, et al. CTX-M-5, a Novel cefotaxime-Hydrolyzing  $\beta$ -lactamase from an Outbreak of *Salmonella typhimurium* in Latvia. *Antimicrob Agents Chemother* 1998; 42(8): 1980–1984. doi: 10.1128/AAC.42.8.1980
11. Gazouli M, Sidorenko SV, Tzelepi E, et al. A plasmid-mediated beta-lactamase conferring resistance to cefotaxime in a *Salmonella typhimurium* clone found in St Petersburg, Russia. *J Antimicrob Chemother* 1998; 41(1): 119–121. doi: 10.1093/jac/41.1.119
12. Tzouveleakis L. Emergence of resistance to third-generation cephalosporins amongst *Salmonella typhimurium* isolates in Greece: Report of the first three cases-J. *J Antimicrob Chemother* 1998; 42(2): 273–275. doi: 10.1093/jac/42.2.273
13. Tassios PT, Gazouli M, Tzelepi E, et al. Spread of a *Salmonella typhimurium* clone resistant to expanded-spectrum cephalosporins in three European countries. *J Clin Microbiol* 1999; 37(11): 3774–3777. doi: 10.1128/JCM.37.11.3774-3777.1999
14. Tapalski D, Hendriksen RS, Hasman H, et al. Molecular characterisation of multidrug-resistant *Salmonella enterica* serovar *Typhimurium* isolates from Gomel region, Belarus. *Clin Microbiol Infect* 2007; 13(10): 1030–1033. doi: 10.1111/j.1469-0691.2007.01795.x
15. Kozyreva VK, Ilina EN, Malakhova MV, et al. Long-term dissemination of CTX-M-5-producing hypermutable *Salmonella enterica* serovar *Typhimurium* sequence type 328 strains in Russia, Belarus, and Kazakhstan. *Antimicrob Agents Chemother* 2014; 58(9): 5202–5210. doi: 10.1128/AAC.02506-14
16. Egorova S, Kaftyreva L, Grimont PA, et al. Prevalence and characterization of extended-spectrum cephalosporin-resistant nontyphoidal *Salmonella* isolates in adults in Saint Petersburg, Russia (2002–2005). *Microb Drug Resist* 2007; 13(2): 102–107. doi: 10.1089/mdr.2007.712

17. Sjölund M, Yam J, Schwenk J, et al. Human *Salmonella* infection yielding CTX-M  $\beta$ -lactamase, United States. *Emerg Infect Dis* 2008; 14(12): 1957–1959. doi: 10.3201/eid1412.080494
18. Kumar GP, Sharmila JS, and Murugan S. Docking of ctx-m-9 group of enzymes with drugs and inhibitors and their evolutionary relationship. *Asian J Pharm Clin Res* 2014; 7: 237–242.
19. Shakil S and Ullah Khan A. Interaction of 2009 CTX – M variants with drugs and inhibitors: A molecular modeling and docking study. *J Proteomics Bioinform* 2010; 03(4): 130–134. doi: 10.4172/jpb.1000131
20. Tamura K, Stecher G, Peterson D, et al. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013; 30(12): 2725–2729. doi: 10.1093/molbev/mst197
21. Lovell SC, Davis IW, Arendall WB, et al. Structure validation by Calpha geometry: Phi, psi and Cbeta deviation. *Proteins* 2003; 50(3): 437–450. doi: 10.1002/prot.10286
22. Colovos C and Yeates TO. Verification of protein structures: patterns of nonbonded atomic interactions. *Protein Sci* 1993 Sep; 2(9):1511–9. doi: 10.1002/pro.5560020916.
23. Wiederstein M and Sippl MJ. ProSA-web: Interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Res* 2007; 35(Web Server issue): W407–410. doi: 10.1093/nar/gkm290
24. Volkamer A, Kuhn D, Grombacher T, et al. Combining global and local measures for structure-based druggability predictions. *J Chem Inf Model* 2012; 52(2): 360–372. doi: 10.1021/ci200454v
25. Ashkenazy H, Erez E, Martz E, et al. ConSurf2010: Calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. *Nucleic Acids Res* 2010; 38(Web Server issue): W529–533. doi: 10.1093/nar/gkq399
26. Celniker G, Nimrod G, Ashkenazy H, et al. ConSurf: Using evolutionary data to raise testable hypotheses about protein function. *Isr J Chem* 2013; 53(3–4): 199–206. doi: 10.1002/ijch.201200096
27. Yang J, Roy A, and Zhang Y. Protein–ligand binding site recognition using complementary binding-specific substructure comparison and sequence profile alignment. *Bioinformatics* 2013; 29(20): 2588–2595. doi: 10.1093/bioinformatics/btt447
28. Bernstein HJ. Recent changes to RasMol, recombining the variants. *Trends Biochem Sci* 2000 Sep; 25(9):453–5. doi: 10.1016/s0968-0004(00)01606-6
29. Mayrose I, Graur D, Ben-Tal N, et al. Comparison of site-specific rate-inference methods for protein sequences: Empirical Bayesian methods are superior. *Mol Biol Evol* 2004; 21(9): 781–791. doi: 10.1093/molbev/msh194
30. Ahamed Riyaz AA, Perera V, Sivakumaran S, et al. Typhoid fever due to extended spectrum  $\beta$ -lactamase-producing *Salmonella* enterica serovar Typhi: A case report and literature review. *Case Rep Infect Dis* 2018; 2018: 4610246. doi: 10.1155/2018/4610246
31. Sabry MA, Abdel-Moein KA, Abdel-Kader F, et al. Extended-spectrum  $\beta$ -lactamase-producing *Salmonella* serovars among healthy and diseased chickens and their public health implication. *J Glob Antimicrob Resist* 2020; 22: 742–748. doi: 10.1016/j.jgar.2020.06.019
32. Sjölund-Karlsson M, Howie R, Krueger A, et al. CTX-M–producing non-Typhi *Salmonella* spp. isolated from humans, United States. *Emerg Infect Dis* 2011; 17(1): 97–99. doi: 10.3201/eid1701.100511
33. Nadimpalli M, Fabre L, Yith V, et al. CTX-M-55-type ESBL-producing *Salmonella* enterica are emerging among retail meats in Phnom Penh, Cambodia. *J Antimicrob Chemother* 2019; 74(2): 342–348. doi: 10.1093/jac/dky451
34. Brown AC, Chen JC, Watkins LKF, et al. CTX-M-65 extended-spectrum  $\beta$ -lactamase–producing *Salmonella* enterica serotype Infantis, United States. *Emerg Infect Dis* 2018; 24(12): 2284–2291. doi: 10.3201/eid2412.180500
35. Shakil S and Khan AU. Interaction of CTX-M-15 enzyme with cefotaxime: A molecular modelling and docking study. *Bioinformation* 2010; 4(10): 468–472. doi: 10.6026/97320630004468
36. Adamski CJ, Cardenas AM, Brown NG, et al. Molecular basis for the catalytic specificity of the CTX-M extended-spectrum  $\beta$ -lactamases. *Biochemistry* 2015; 54(2): 447–457. doi: 10.1021/bi501195g
37. Bethel CR, Taracila M, Shyr T, et al. Exploring the inhibition of CTX-M-9 by  $\beta$ -lactamase inhibitors and carbapenems. *Antimicrob Agents Chemother* 2011; 55(7): 3465–3475. doi: 10.1128/AAC.00089-11
38. Danishuddin M and Khan AU. Molecular modeling and docking analysis of beta-lactamases with inhibitors: A comparative study. *In silico Biol* 2011; 11(5–6): 273–280. doi: 10.3233/ISB-2012-0443
39. Ali A, Danishuddin Maryam L, Srivastava G, et al. Designing of inhibitors against CTX-M-15 type  $\beta$ -lactamase: Potential drug candidate against  $\beta$ -lactamases-producing multi-drug-resistant bacteria. *J Biomol Struct Dyn* 2018; 36(7): 1806–1821. doi: 10.1080/07391102.2017.1335434
40. Maryam L, Khalid S, Ali A, et al. Significant role of Asn-247 and Arg-64 residues in close proximity of the active site in maintaining the catalytic function of CTX-M-15 type  $\beta$ -lactamase. *RSC Adv* 2019; 9(10): 5325–5337. doi: 10.1039/c8ra10313e