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Interaction of 2009 CTX - M Variants with Drugs and Inhibitors: A Molecular Modeling and Docking Study

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Abstract

Extended-spectrum-β-lactamases (ESBLs) are enzymes produced by bacteria which impart resistance against advanced-generation-cephalosporins. CTX-M enzymes have become the most prevalent ESBLs. The amino acid residues through which 2009 CTX-M-variants interact with drugs and inhibitors is not reported. Homology models for CTX-M-15 (This study), CTX-M-53, CTX-M-71, CTX-M-82 and CTX-M-89 were prepared. Ramachandran-Z-scores for models were found to be -0.449, 0.006, -0.103, -0.007 and 0.092, respectively. These models were docked with target drugs (cefotaxime, ceftazidime, cefepime) and inhibitors (clavulanate, sulbactam, tazobactam). The $bla_{CTX-M-15}$ marker was PCR-amplified from plasmid DNA of clinical *Escherichia coli* isolate. Minimum inhibitory concentrations (MICs) for drugs were tested by the microbroth-dilution-method. *E. coli* C600 cells (harboring cloned $bla_{CTX-M-15}$) were found positive for ESBL-production by the double-disk-synergy test. The $bla_{CTX-M-15}$ marker was found transmissible through conjugation. Discovery Studio analysis of the docked structures revealed that irrespective of the CTX-M-type, ceftazidime interacted with the residues A226, G227, L228, P229, A/T230, S231, W232, R285, T288, D289, G290 and L/Y291. Moreover, sulbactam was found to bind most efficiently to the studied enzymes on the basis of interaction energies. The study identifies amino acid residues crucial to 'CTX-M-drug' and 'CTX-M-inhibitor' interactions which might be useful in the ongoing search for a versatile CTX-M-inhibitor.

Keywords: Antibiotic resistance; CTX - M; Docking; Extended - spectrum β - lactamases; Modeling

Abbreviation: ESBLs: Extended spectrum β - lactamases

Introduction

Multidrug resistance in bacteria is a phenomenon of due scientific concern both in the community and nosocomial settings (Shakil et al., 2008). Extended spectrum β - lactamases (ESBLs) are the enzymes produced by resistant bacteria which impart resistance against advanced - generation cephalosporin antibiotics. ESBLs are often plasmid - associated. Hence, there can be cross - species transfer of the genes encoding ESBLs along with the plasmids through conjugation. Moreover, these plasmids often carry genes for co - resistance to other antibiotics such as aminoglycosides, fluoroquinolones, tetracyclines and chloramphenicol, to name a few. ESBLs hydrolyze the β - lactam ring present in the target antibiotics (such as cefotaxime and ceftazidime). CTX - M enzymes have become the most prevalent ESBLs (Canton and Coque, 2006). The change in activities of CTX - Ms leading to the evolution of more variants may be due to point mutations present either inside or outside of the active site omega loop (amino acid positions 161 to 179). CTX - M - variants up to CTX - M - 89 have already been reported (http://www.lahey.org/studies). Identification of the amino acid residues crucial to the interaction between CTX - M - variants (the bacterial enzymes) and target drugs (the drugs hydrolyzed by these enzymes) is a topic of priority research. This information might be useful for the scientists involved in designing CTX - M - resistant antibiotics. Variants of this bacterial enzyme are emerging at a fast pace. Hence, there is an urgent need to design a versatile CTX - M - inhibitor that might inhibit most of the emerging variants of this enzyme, if not all. Studying the mode of interaction of these recently evolved CTX - M - variants with the existing inhibitors might help in the search for a versatile CTX - M inhibitor.

In view of the present background, we found it pertinent to study the mode of interaction of 2009 CTX - M variants with target drugs and known inhibitors. This study is based on interaction energies. The objectives of the study were: (i) Homology modeling of all the sequences of CTX - M - variants submitted in 2009 (ii) Docking of drugs (cefotaxime, ceftazidime and cefepime) as well as inhibitors (clavulanate, sulbactam and tazobactam) with these modeled enzyme - structures (iii) To identify amino acid residues crucial to the enzyme - drug and enzyme - inhibitor interactions (iv) To perform an *in silico* comparison of efficacies of traditional β - lactamase inhibitors against 2009 CTX - M variants on the basis of interaction energies.

Materials and Methods

Computational methods

The database maintained exclusively for β - lactamase enzymes (http://www.lahey.org/studies) was searched for CTX - M enzyme sequences submitted in 2009 from across the globe. The five ESBL - types explored in the study included CTX - M - 15, CTX - M - 53, CTX - M - 71, CTX - M - 82 and CTX - M - 89. The sequences used in the present study appear

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in GenBank as [GenBank: FJ997864] (This study), [GenBank: DQ268764], [GenBank: FJ815436], [GenBank: DQ256091] and [GenBank: FJ971899]. GenBank sequence with accession number [GenBank: GQ339021] was used as a reference sequence for detecting mutations. Protein Data Bank (PDB) IDs of the templates retrieved for homology modeling of the study sequences are shown in Table 1. Modeling was performed using the automated mode of Swiss Model Server (Arnold et al., 2006). The models were duly verified by 'Procheck' and 'Verify3D' programmes. PDB structures of drugs (cefotaxime, ceftazidime, cefepime) as well as inhibitors (clavulanate and tazobactam) were retrieved from Drug bank (http://www.drugbank.ca/search/chemquery). In case any structure (e.g. sulbactam) was not available in pdb format with DrugBank, its 2 - D structure was retrieved from Pubchem (http://www.pubchem.ncbi.nlm.nih.gov/search). CORINA software was then employed to construct 3 - D coordinates from 2 - D structure by using 'SMILES' notation, thereby generating its 3 - D structure in pdb format. The ligand (either inhibitor or drug) was docked into each of the modeled enzyme - structures using the targeted docking mode of 'Molecular Docking Server' (http://www.dockingserver.com). The MMFF94 force field was used for energy minimization of ligand molecules. Gasteiger partial charges were added to the ligand atoms. Non - polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on the protein models. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Affinity (grid) maps of 60×60×60 Å grid points and 0.375 Å spacing were generated using the Autogrid program. AutoDock parameter set - and distance - dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the 'Lamarckian genetic algorithm (LGA)' and the 'Solis & Wets local search method'. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied. Discovery Studio 2.0 (Accelrys) was used to prepare Ramachandran plots for the modeled structures. Ramachandran Z - scores for the evaluation of these models were calculated using 'WHAT IF' package. Alignments were performed by ClustalW program using default parameters.

Microbiological methods

The gene (bla_{CTX-M}) corresponding to accession number [GenBank: FJ997864] was PCR - amplified from plasmid DNA of *Escherichia coli* strain isolated from neonatal intensive care unit of Aligarh Hospital, India. Minimum inhibitory concentrations (MICs) for piperacillin/tazobactam, ampicillin/sulbactam and amoxyclav were tested by Hicomb MIC test strips (Hi - media, India) and confirmed by microbroth dilution

GenBank Accession Number/Name of CTX-M variant/Country of isolation/Year	[GenBank: FJ997864] / CTX-M-15/India/2009 (This study)	[GenBank: DQ268764]/ CTX-M-53/France/2009	[GenBank: FJ815436]/ CTX-M-71/Bulgaria/2009	[GenBank: DQ256091]/ CTX-M-82/China/2009	[GenBank: FJ971899]/ CTX-M-89/USA/2009
PDB ID of the template retrieved for modeling	[PDB:1IYSA]	[PDB:1IYSA]	[PDB:1WE4A]	[PDB:1IYSA]	[PDB:1IYSA]
E-value	2.03e-117	3.52e-117	1.16e-118	3.73e-117	4.44e-120
Final total energy in Kj/mol (Modeling log)	-14825.173	-14555.407	-14793.956	-14708.911	-14597.197
Ramachandran Zscore	-0.449	0.006	-0.103	-0.007	0.092
Amino acid changes present in the 2009 CTX-M sequences considering the GenBank accession no. GQ339102 (Russia/2009/ CTX-M-15) as a reference	P88T, N89D	A27V, A77V, R38Q, T264I	G238C	A40P	A77V, K83Q, S86T, E87Q, P88K, N89G, N92S, K99P, V103I, S118T, L119F, A120G, V133T, V142L, A146D, S147K, Q154T, L155I, E158D, S182T, R184L, T189A, K197N, S202T, T209M, M211L, G240D, P269S, Q270E, K285R, S281A, L291Y
Docking with clavulanate (All energies are in Kj/mol)	E-total = -161.35 E-shape = -154.40 E-force = -6.95	E-total = -164.29 E-shape = -155.02 E-force = -9.26	E-total = -151.42 E-shape = -156.25 E-force = 4.83	E-total = -160.44 E-shape = -153.33 E-force = -7.11	E-total = -153.94 E-shape = -141.55 E-force = -12.39
Docking with sulbactam (All energies are in Kj/mol)	E-total = -179.59 E-shape = -144.84 E-force = -34.75	E-total = -177.07 E-shape = -114.76 E-force = -62.31	E-total = -180.62 E-shape = -135.80 E-force = -44.82	E-total = -183.43 E-shape = -146.41 E-force = -37.02	E-total = -173.10 E-shape = -137.36 E-force = -35.75
Docking with tazobactam (All energies are in Kj/mol)	E-total = -148.21 E-shape = -95.75 E-force = -52.46	E-total = -149.08 E-shape = -106.35 E-force = -42.73	E-total = -135.28 E-shape = -128.79 E-force = -6.49	E-total = -144.86 E-shape = -96.92 E-force = -47.94	E-total = -145.91 E-shape = -119.16 E-force = -26.75

Table 1: Bioinformative details of the modeled CTX-M structures and interaction energies of the docked enzyme-inhibitor-complexes.

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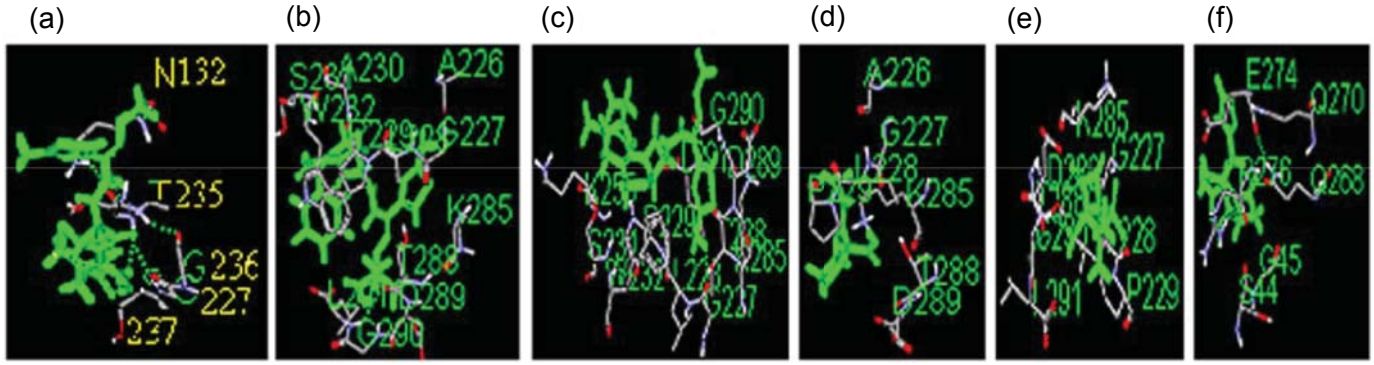

Figure 1: Interaction of modeled CTX-M-15 with (a) cefotaxime (b) ceftazidime (c) cefepime (d) clavulanic acid (e) sulbactam, and (f) tazobactam.

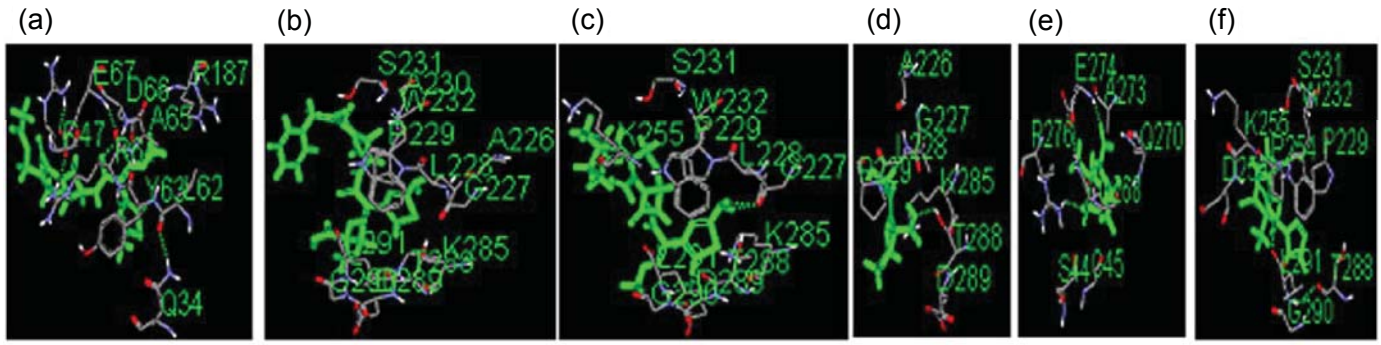

Figure 2: Interaction of modeled CTX-M-53 with (a) cefotaxime (b) ceftazidime (c) cefepime (d) clavulanic acid (e) sulbactam, and (f) tazobactam.

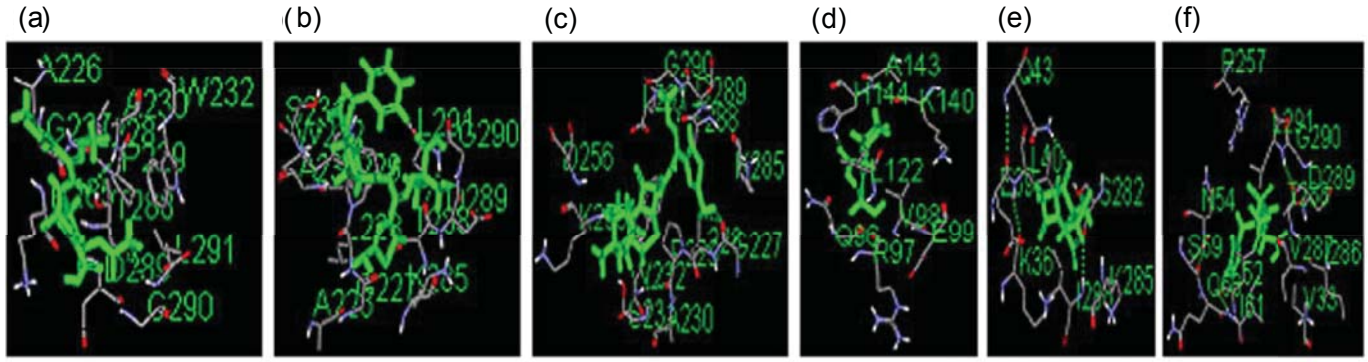

Figure 3: Interaction of modeled CTX-M-71 with (a) cefotaxime (b) ceftazidime (c) cefepime (d) clavulanic acid (e) sulbactam, and (f) tazobactam.

method as per the latest recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2009). Transmissibility of the resistance marker to *E. coli* J53 recepient cells was checked through conjugation. The gene was cloned into *E. coli* C600 cells using 'Qiagen cloningplus kit' as per manufacturer's instructions (Qiagen, USA). Sequencing was performed with an ABI 3130 Genetic Analyzer (Applied Biosystems). The donor as well as transformed C600 cells were tested for ESBL- production by the double - disk - synergy test. Briefly, the synergy test with ceftazidime, cefotaxime, ceftriaxone and cefixime was performed by disk diffusion method on Mueller - Hinton agar plates with and without 10 μg of amoxyclav. A ≥ 5 - mm increase in the zone of diameter of third generation cephalosporins, tested in combination with amoxyclav versus its zone when tested alone was considered indicative of ESBL production. *E. coli* ATCC 25922 was used as ESBL negative and *Klebsiella pneumoniae* 700603 was used as ESBL positive reference strain.

Results and Discussion

Plasmid-mediated transmission of $bla_{CTX\text{-}M\text{-}15}$

BLAST analysis of the sequence [GenBank: FJ997864] confirmed it to be blaCTX - M - 15. It possessed amino acid substitutions P88T and N89D with respect to the reference [GenBank: GQ339102] and showed 98% identity to the sequence [GenBank: FJ668785]. The donor as well as transformed C600 cells (harboring cloned $bla_{CTX\text{-}M\text{-}15}$) were found positive for ESBL - production by the double - disk - synergy test. Also, the $bla_{CTX\text{-}M\text{-}15}$ resistance marker was found transmissible through conjugation at a transfer frequency of the order 10^{-4}.

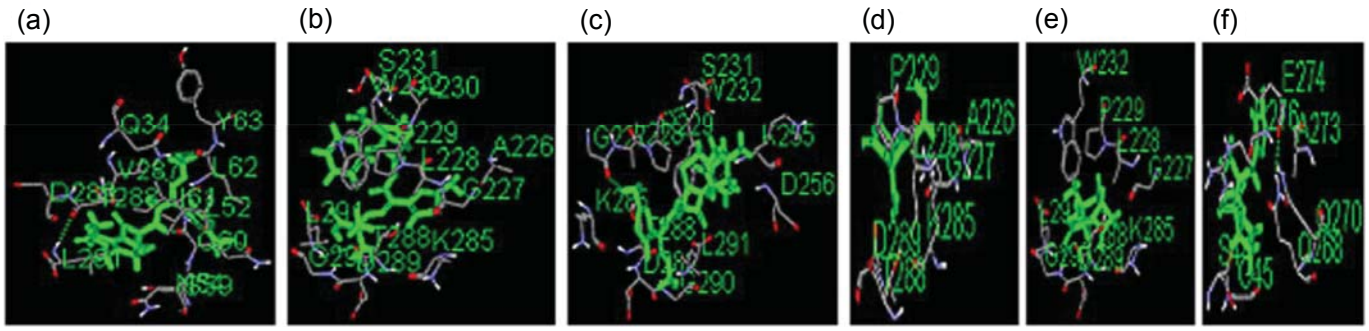

Figure 4: Interaction of modeled CTX-M-82 with (a) cefotaxime (b) ceftazidime (c) cefepime (d) clavulanic acid (e) sulbactam, and (f) tazobactam.

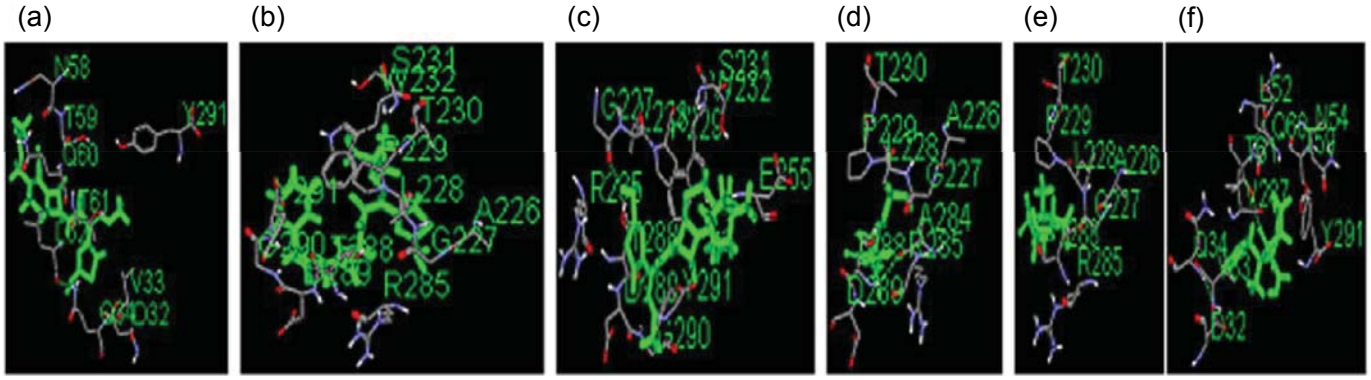

Figure 5: Interaction of modeled CTX-M-89 with (a) cefotaxime (b) ceftazidime (c) cefepime (d) clavulanic acid (e) sulbactam, and (f) tazobactam.

Evaluation of the enzyme models

ClustalW alignments revealed that the SDN loop (positions 130 - 132) and KTG motif (positions 234 - 236) were conserved in all the study CTX - M sequences. These are typical structures of class A enzymes. Over 90% of the amino acid residues in the protein structures modeled from the study *bla*$_{CTX-M}$ sequences were found to be present in the most favored regions as revealed by their respective Ramachandran plots. For example, percent amino acid residues in most favored, additional allowed, generously allowed and disallowed regions of the Ramachandran plot for the modeled CTX - M enzyme corresponding to accession number [GenBank: DQ268764] were 91.6%, 7.7%, 0.7% and 0.0%, respectively. Ramachandran Z - score expresses how well the backbone conformations of all the residues correspond to the known allowed areas in the Ramachandran plot. Accordingly, the Ramachandran Z - scores for modeled CTX - M enzymes from accession numbers [GenBank: FJ997864], [GenBank: DQ268764], [GenBank: FJ815436], [GenBank: DQ256091] and [GenBank: FJ971899] were found to be - 0.449, 0.006, - 0.103, - 0.007 and 0.092, respectively. This further confirms the accuracy of the modeled structures.

Docking results

The drugs (cefotaxime, ceftazidime and cefepime) as well as inhibitors (clavulanic acid, sulbactam and tazobactam) were docked into each of the modeled enzyme - structures. Figures (Figure 1 Figure 2 Figure 3 Figure 4 Figure5) show amino acid residues crucial to the interaction of each of the modeled enzyme structures with cefotaxime, ceftazidime, cefepime, clavulanic acid, sulbactam and tazobactam, separately. Discovery Studio analysis of the docked structures revealed that irrespective of the CTX - M - type, ceftazidime interacted invariably with the residues A226, G227, L228, P229, A/T230, S231, W232, R285, T288, D289, G290 and L/Y291. Similarly, amino acid residues found common to the interactions involving any of the CTX - M - types with cefepime included G227, L228, P229, S231, W232, K/E255, K/R285, T288, D289, G290 and L/Y 291. These informations might be useful for the future development of a CTX - M - resistant antibiotic.

In silico comparison of efficacies of traditional β-lactamase inhibitors aginst 2009 CTX-M enzymes

When a β - lactam antibiotic is given in combination with a β - lactamase - inhibitor against bacteria resistant to the antibiotic (alone), bacteria become susceptible to this combination. This is because the β - lactamase enzyme produced by bacteria is rendered ineffective with its active site occupied by the inhibitor. The drug becomes free to act on bacteria as the inhibitor forms a complex with the bacterial enzyme. Higher (negative) free energy of interaction (simply called as 'interaction energy') is regarded as an indicator of effective and tight binding for an enzyme - inhibitor - complex. Sulbactam is a suicide inhibitor, which forms a dead - end complex with β - lactamase enzymes. Among the studied enzyme inhibitor - complexes, the complexes involving sulbactam displayed the highest (negative) interaction energies (Table 1). Hence,

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sulbactam was found to be the most efficient inhibitor for all the study CTX Ms. This finding is interesting because till now it was considered a unique feature of CTX - M enzymes that they were better inhibited by tazobactam than by sulbactam or clavulanate (Ma et al., 1998). Hence, the 2009 CTX - M variants can be supposed to have broken this trend. Accordingly, for the CTX - M positive clinical *E. coli* isolate (This study), the MICs for ampicillin/sulbactam, amoxyclav and piperacillin/tazobactam were found to be 2, 4 and 8 mg/L, respectively. In a study by Banfi et al. (2006) authors observed that the calculated binding free energy values were in agreement with the corresponding MIC values for enzyme inhibitor - complexes. Moreover, in a similar 2009 study authors have used interaction energies of docking to compare the efficacy of different neuraminidase inhibitors against newly evolved strains of H1N1 viruses (Khan et al., 2009). Higher (negative) interaction energy has been associated with efficient binding in other 2009 studies as well (Danishuddin et al., 2009; Shakil et al., 2010).

Conclusions

The following are the conclusions/highlights of this study: *(i)* This is the first study to report modeling of CTX - M - 15/53/71/82/89 enzymes and their docking with target drugs and inhibitors to the best of our knowledge; *(ii)* The study identifies amino acid residues crucial to 'CTX - M - drug' and 'CTX - M - inhibitor' interactions. These informations might be useful for the future development of a versatile CTX - M inhibitor and improved drugs as well; *(iii)* On the basis of interaction energies, the study concludes that sulbactam might inhibit the 2009 CTX - M - variants more efficiently than clavulanate or tazobactam. This may be considered as a new trend for CTX - M enzymes which are generally supposed to be better inhibited by tazobactam; *(iv)* It is important to mention that the research letter being presented here is a short study which is mainly in silico. More experimental studies are needed to validate the findings presented in this letter.

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Authors' contributions

SS performed all the experiments mentioned in the manuscript. AUK designed the problem, approved the final draft of the manuscript and was a guide throughout the study.

Competing interests

The authors declare that they have no competing interests.

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