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Molecular docking analysis of new generation cephalosporins interactions with recently known SHV-variants

Asad Ullah Khan[1]*, Mohd Hassan Baig[1], Gulshan Wadhwa[2]

[1]Interdisciplinary Biotechnology Unit, Aligarh Muslim University Aligarh- 202002, India; [2]Department of Biotechnology,Ministry of Science &Technology, New Delhi – 110003, India; Asad Ullah Khan- Email: asad.k@rediffmail.com, asadukhan72@gmail.com; Phone: 00919837021912; Fax: 0091-571-2721776; *Corresponding author

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Abstract:
Extended-spectrum-β-lactamases (ESBLs), constitutes the growing class of betalactamses, these are enzymes produced by bacteria which impart resistance against advanced-generation-cephalosporins. SHV enzymes are among the most prevalent ESBLs. The mode of molecular interactions of recent SHV-variants to advanced generation cephalosporins has not been reported yet. This is the first time we are reporting the insilico study of these recent variants with new generation cephaosporins. Homology models for SHV-105, SHV-95, SHV-89, SHV-61 and SHV-48 were generated using MODELLER9v3. New generation Cephalosporins were selected to target the active site amino acid residues of these modeled SHV enzymes for predicting comparative efficacies of these inhibitors against the said enzymes on the basis of interaction energies of docking. The docked complexes were analyzed by using DISCOVERY STUDIO 2.5. In this study A237, S70, K234, R275, N132, R244 and S130 were found crucial to the correct positioning of drugs within the binding site of SHV enzymes in 11, 6, 6, 6, 5, 5 and 5 instances, respectively. On the basis of interaction energy and Ki calculations cefatoxime emerged as the most efficient among the other advanced cephalosporins against all the studied SHV variants, excluding SHV-48 where ceftazidime was found to be most effective drug. Furthermore, this study identified amino acid residues crucial to 'SHV-Cephalosporins' interactions and this information will be useful in designing effective and versatile drug candidates.

Keywords: antibiotic resistance, SHV, docking, extended-spectrum β-lactamases, modeling

Background:
Multidrug resistance in bacteria is becoming common, both in the community and nosocomial settings **[1].** Extended spectrum β-lactamases (ESBLs) are the enzymes produced by resistant bacteria which hydrolyze advanced-generation cephalosporin antibiotics (such as cefotaxime and ceftazidime) and cause resistance against these drugs, SHV enzymes are among the most prevalent ESBLs **[2].** ESBLs coding genes are transferred through horizontal gene transfer as they are mostly present on plasmids **[3, 4].** The frequent emergence of SHVs variants may lead us to understand the structure of newly identified enzymes so that potential and versatile drug candidates can be designed to cope up with the problem of resistance. SHV-variants up to SHV-131 have already been reported **[5].** Identification of the amino acid residues crucial to the interaction between SHV-variants (the bacterial enzymes) and β-drug molecules is a topic of priority research. This information might be useful for the scientists involved in designing SHV-resistant drugs. Effective formulations consisting of β-lactam antibiotic and SHV-inhibitor might be designed to be given as a single drug to patients infected with SHV-producing bacteria.

It has been observed earlier that hydrogen bonds play a crucial role in the binding of ceftazidime to SHV-57 **[6].** They concluded that the substitution of arginine for leucine-169 in the Ω-loop is important for substrate specificity and causes ceftazidime resistance in SHV-57 producing bacteria **[6].** This is first study reporting modeling of SHV-105/95/89/61 or 48 and their docking with advanced generation cephalosporins . Also, there was no X-ray crystallographic structure available with the Protein Data Bank for these variants of SHV-family at the time of communicating this paper.

In view of the present background, we have initiated our study to identify the mode of interaction of recent SHV-variants with advanced generation cephalosporins. Aims of the study were: (i) Modeling of recent SHV variants, (ii) Docking of advance generation cephalosporins with modeled SHV enzymes to identify amino acid residues crucial to their interaction, and (iii) predicting comparative efficacies of these drugs against the said enzymes on the basis of interaction energies of docking.

Methodology:
Homology Modeling:
The recent variants of SHV (SHV-105, SHV-95, SHV-89, SHV-61 and SHV-48) selected for the study whose structures are not available were searched from the database maintained exclusively for β-lactamase enzymes **[5]**. The sequences used in the present study appear in Swissprot **[7]** with Primary (citable) accession number:B6E133 (SHV-105), A3FFR5 (SHV-95), Q3HUP1 (SHV-89), Q2WEB8 (SHV-61) and Q83YP9 (SHV-48). The crystal structure of SHV-1 β-lactamase (Pdb : 3D4F) available at RCSB Protein Data Bank **[8]** was used as a template for constructing the 3-D models of our selected recent SHV variants. Homology modeling was done for generating structures of these recent SHV variants through Modeller9v3. The swissprot sequence with Primary (citable) accession **D2KB79** was used as a reference sequence for detecting mutations.

Energy Minimization and Model Validation:
Models generated were further subjected to energy minimization using Steepest descent algorithm with 200 steps and at RMS gradient 0.1 .The

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stereochemistry quality of the structures where validated with PROCHECK, WHATIF and Verify 3D. Quality factors for the enzyme models were calculated using ERRAT2 **[9].**

Molecular Docking study:

PDB structures of advanced generation cephalosporins were retrieved from Drug bank **[10].** The drugs were docked into each of the energy minimized modeled enzyme-structures using AUTODOCK4.0 **[11].** For the inhibitors, charges of the Gasteiger type were assigned. 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 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). The docking parameters set to perform each docking experiment was derived from 15 different runs that were set to terminate after a maximum of 2500000 energy evaluations, elitism of 1, mutation rate 0.02, cross-over rate of 0.8, local search rate of 0.06. The population size was set to 150. The best run coordinates of the drugs with enzyme were visualized and analyzed through Discovery Studio 2.5 for analysis of their mode of interaction with binding site residues.

Results and Discussion:

The genes for SHV-48 and SHV-95 were originally reported from *Acinetobacter baumannii* and *Citrobacter freundii*, respectively while those for SHV-48, SHV-89 and SHV-105 were reported from *K. pneumoniae* strains. **Figure 1** shows multiple sequence alignment of these enzymes with a reference sequence [SHV-1, Primary (citable) accession D2KB79]**.** MULTALIN alignments revealed that the SDN loop (positions 130-132) and KTG motif (positions 234-236) were conserved in all the study SHV sequences. These are typical structures of class A enzymes **[12].**

The aminoacid residues in most favoured region as revealed by Ramachandran plot were found to be close to 90% in all the generated protein structures modeled from *bla*$_{SHV}$. For instance, percent amino acid residues in disallowed regions of the Ramachandran plot for the modeled SHV enzyme were zero (data not shown). All the enzyme structures were modeled using 3D4F.pdb as template. The target sequences possessed more than 80% sequence-identity with the said template. The Errat2 expresses the overall quality of all the modeled structures was found to be above 93 in each case. 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 SHV-48, SHV-61, SHV-89, SHV-95 and SHV-105 enzymes were found to be -2.560, -2.577, -3.013, -2.752 and -2.872, respectively. More than 90% of the residues in each modeled enzymes had an averaged 3D-1D score > 0.2 (data not shown)

This is the first time our data showed the efficacies of advanced generation cephalosporin with recent SHV variants. bla$_{SHV}$ is among the most prevalent ESBLs. The drug that was showing least binding energy with the enzyme was found to have higher minimum inhibitory concentration (MIC) i.e that drug was not showing better efficacy while the drug complexed with enzyme with higher binding energy was showing lower MIC and was considered to be a better drug this has also been shown earlier **[13]. Figure 2** shows binding pocket residues and the interaction of each of the modeled enzyme structures with cefepime, ceftazidime and cefatoxime separately. Most SHV type ESBLs have the G238S substitution alone or combined with alterations at position 240. Accordingly, G238S substitution was observed in SHV-48, SHV-95 and SHV-105 while G240 was conserved in all the studied variants. It is the premier substitution that preserves penicillin and cephalosporin resistance in general and is found on the β3 strand **[14].** It was analyzed that out of the 15 docking interactions in this study, residues A237, R275, S70, K234, R244, N132 and S130 were found crucial. Of 15 docks performed, cephalosporine showed interaction with these important residues viz A237 (11 instances), R275 (6 instances), S70 (6 instances) K234 (6 instances), R234 (6 instances), R244 (5 instances), N132 (5 instances) and S130 (5 instances). Amino acid residues involved in H-bond formation with reference to each of the docked complexes studied are listed in **Table 1 (see Supplementary material).** This information might be useful for designing potential and versatile drug candidates.

Our data revealed that the cefatoxime was found to be the best antibiotic against all the variants used in this study except SHV-48 where ceftazidime was more effective. Moreover, cefapime was observed as least effective antibiotic against these variants. The interaction energies are given in **Table 1 (see Supplementary material)**. It was also found in the study that the amino acid residues at position A237, R275, S70, K234, R244, N132 and S130 were playing crucial role in the interaction of SHV variants with these antibiotics. Furthermore, our study also revealed that R275 was reported first time in such interactions. Minimum free energy of interaction or tight binding for an enzyme-drug -complex is regarded as an indicator of resistance (High MIC) against drug. Interaction energy and Ki value calculated for each of the docked complexes are shown in table 1. In earlier studies it has also been observed that the calculated binding free energy values are with inverse proportional to the corresponding MIC values for enzyme-drug complexes **[15].** Moreover, previously, authors have used interaction energies of docking to compare the efficacy of different neuraminidase inhibitors against newly evolved strains of H1N1 viruses **[16].** Our data is consistent with the earlier studies **[17, 18].** Accordingly, interaction energy and Ki calculations confirmed cefatoxime to be the most efficient antibiotic against all the studied bacterial enzymes. It was found that Cefatoxime is the drug that showed resistance against the action of SHVs and hence may be the drug of choice.

The homology models of the 3-D structures of SHV-48, SHV-61, SHV-89, SHV-95 and SHV-105 enzymes were submitted to PMDB **[19]** and were assigned the identifiers PM0076258, PM0076262, PM0076259, PM0076260, and PM0076261, respectively.

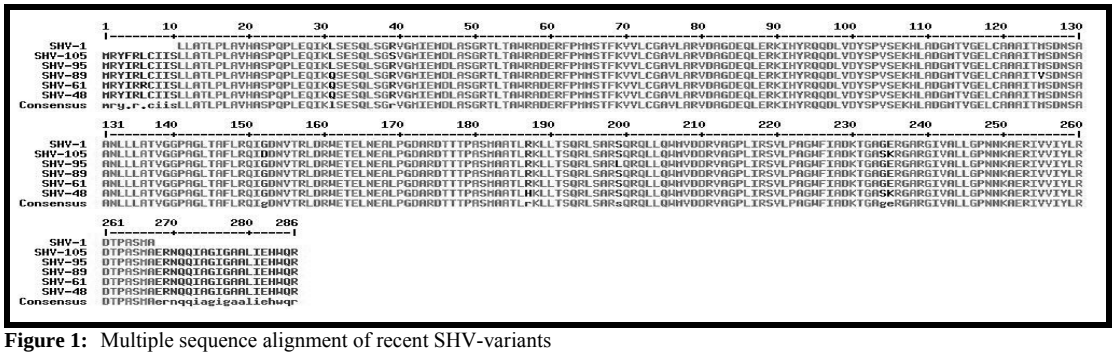

Figure 1: Multiple sequence alignment of recent SHV-variants

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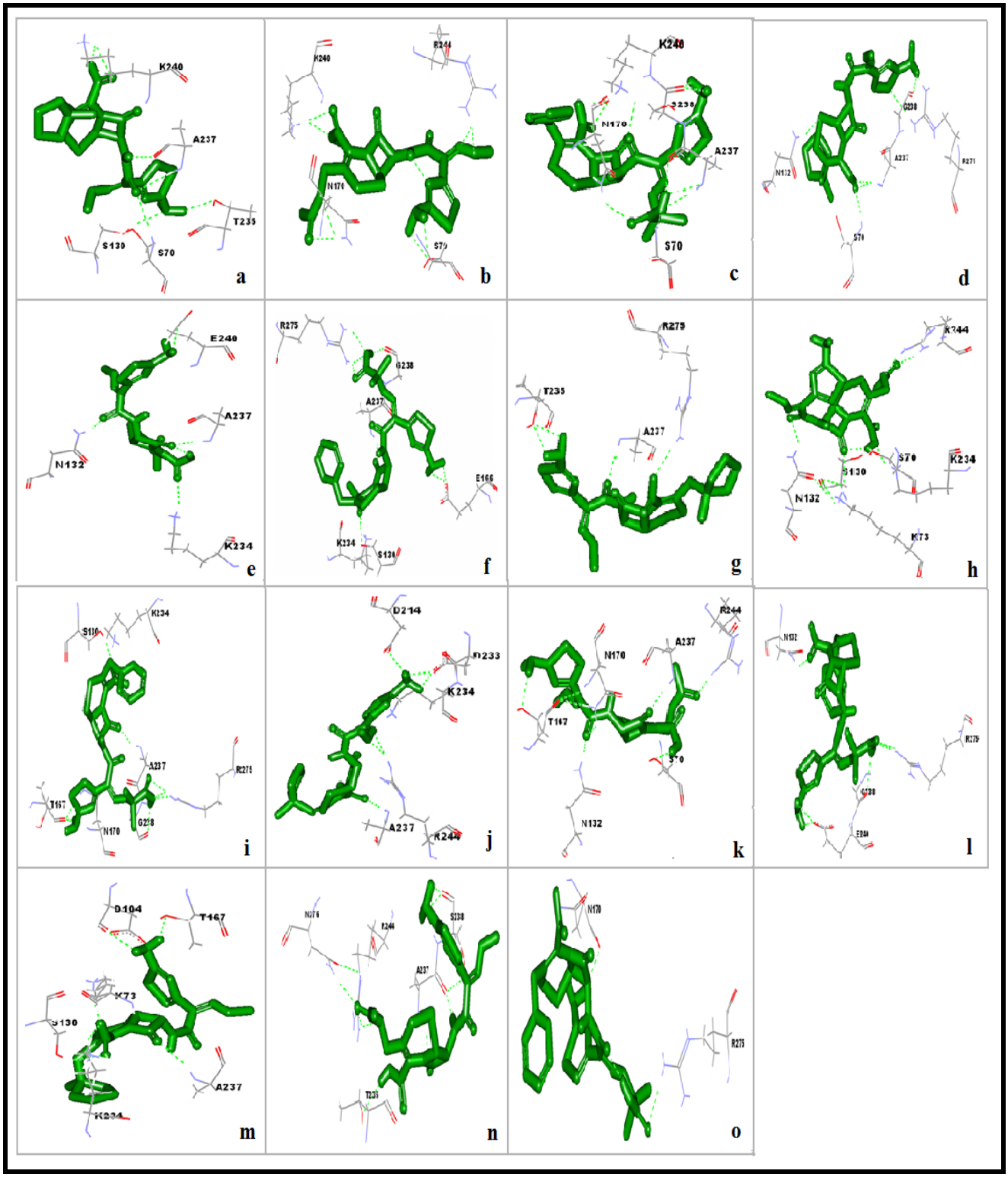

Figure 2 (a) Interaction of modeled SHV-105 with Cefepime; (b) Interaction of modeled SHV-105 with Cefatoxime; (c) Interaction of modeled SHV-105 Ceftazidime; d) Interaction of modeled SHV-95 with Cefepime; (e) Interaction of modeled SHV-95 Cefatoxime: (f) Interaction of modeled SHV-95 Ceftazidime; (g) Interaction of modeled SHV-89 with Cefepime; (h) Interaction of modeled SHV-89Cefatoxime; (i) Interaction of modeled SHV-89 Ceftazidime; (j) Interaction of modeled SHV-61 with Cefepime; (k) Interaction of modeled SHV-61 Cefatoxime; (l) Interaction of modeled SHV-61Ceftazidime; (m) Interaction of modeled SHV-48 with Cefepime; (n) Interaction of modeled SHV-48Cefatoxime; (o) Interaction of modeled SHV-48Ceftazidime

Conclusions:
This study concludes the role of crucial amino acid residues involve in 'SHV-cephalosporins' interactions. Moreover, we have first time identified a significant role of arginine at position 275 in binding site of SHV variants. This information would be useful in designing new drugs against recent SHV variants. Furthermore, on the basis of interaction energies, cefatoxime was found to be the best and most effective drug against the studied SHV enzymes.

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Authors' contributions:
MHB performed the in silico studies and write first draft of manuscript. AUK designed the problem, Rewrite the manuscript and was a guide throughout the study. GW helped in discussion.

Competing interests:
The authors declare that they have no competing interests.

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Supplementary material:

Table1: Docking details of advance generation cephalosporins with recent SHV Variants

Strains	Drugs	Binding Energy (kcal/mol)	Intermolecular Energy (kcal/mol)	Torsional Energy (kcal/mol)	Internal Energy (kcal/mol)	RMSD (Å)	Histo grams	Inhibition constant, Ki (µm)	Binding site residues	Binding Energy (kcal/mol)
SHV-105	Cefepime	-8.26	-9.85	2.39	-1.65	67.623	5	0.88541	S-70, S-130, T-235, A-237, K-240	-8.26
	Cefatoxime	-6.67	-9.64	2.98	-1.25	66.394	4	12.87	S-70, N-170, K-240, R-244	-6.67
	Ceftazidime	-8.17	-11.15	3.28	-1.43	68.616	4	1.02	S-70, N-170, A-237, S-238, K-240	-8.17
SHV-95	Cefepime	-7.56	-8.48	2.39	-2.22	73.734	7	2.86	S-70 ,N-132, A-237, G-238, R-275	-7.56
	Cefatoxime	-5.68	-8.55	2.98	-1.34	69.337	4	68.69	N-132, K-234, A-237, E-240	-5.68
	Ceftazidime	-6.45	-10.10	3.28	-0.78	72.014	3	18.84	S-130, E-166, K-234, A-237, G-238, R-275,	-6.45
SHV-89	Cefepime	-7.51	-8.98	2.39	-1.73	73.637	1	3.11	T-235, A-237,R-275	-7.51
	Cefatoxime	-6.14	-8.89	2.98	-1.46	68.666	2	31.39	S-70, K-73, S-130, N-132, K-234, R-244	-6.14
	Ceftazidime	-7.51	-10.28	3.28	-0.96	72.124	5	3.12	S-130, T-167, N-170, K-234, A-237, G-238, R-275	-7.51
SHV-61	Cefepime	-7.66	-8.77	2.39	-2.06	66.407	4	2.41	D-214, D-233, K-234, A-237, R-244	-7.66
	Cefatoxime	-5.25	-8.11	2.98	-1.36	63.997	6	141.11	S-70, N-132, T-167, N-170, A-237, R-244	-5.25
	Ceftazidime	-5.99	-8.08	3.28	-2.32	68.727	3	40.45	N-132, G-238, E-240, R-275	-5.99
SHV-48	Cefepime	-7.20	-8.25	2.39	-2.08	71.521	2	5.29	K-73, D-104, S-130, T-167, K-234, A-237	-7.20
	Cefatoxime	-6.40	-9.52	2.98	-1.09	71.259	1	20.49	T-235, A-237, S-238, R-244, N-276	-6.40
	Ceftazidime	-6.09	-8.08	3.28	-2.45	74.614	2	34.23	N-170, R-275	-6.09