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

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Abstract:
Extended-spectrum β-lactamases (ESBLs), constitutes the growing class of beta lactamases, 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 in silico study of these recent variants with new generation cephalosporins. 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 cefotaxime 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 advanced 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
respectively. More than 90% of the residues in each modeled enzymes had
Z-scores for modeled SHV-48, SHV-61, SHV-89, SHV-95 and SHV-105
allowed areas in the Ramachandran plot. Accordingly, the Ramachandran
backbone conformations of all the residues correspond to the known
modeled using 3D4F.pdb as template. The target sequences possessed
an averaged 3D-1D score > 0.2 (data not shown).

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
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.

The aminooacid 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
Ramachandran plot were found to be close to 90% in all the generated
study SHV sequences. These are typical structures of class A enzymes.

Figure 1: Multiple sequence alignment of recent SHV-variants

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, cefepime 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
found crucial. Of 15 docks performed, cephalosporine showed interaction
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.

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

### Table 1: 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) | Histograms | Inhibition constant, Ki (µm) | Binding site residues | Binding Energy (kcal/mol) |
|---------|---------|--------------------------|---------------------------------|-----------------------------|---------------------------|----------|------------|---------------------------|------------------------|-------------------------|
|         |         | Binding                   |                                 |                             |                           |          |            |                           |                        |                         |
| Cefepime| -8.26   | -9.85                    | 2.39                            | -1.65                       | 67.623                    | 5        | 0.88541    | S-70, S-130, T-235, A-237, K-240 | SHV-105                | -8.26                  |
|         |         | 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 | SHV-89                 | -7.56                  |
|         |         | SHV-61                   |                                 |                             |                           |          |            |                           |                        |                         |
| Cefepime| -7.20   | -8.25                    | 2.39                            | -2.08                       | 66.407                    | 4        | 2.41       | D-214, D-233, K-234, A-237, R-244 | SHV-48                 | -7.20                  |
|         |         | SHV-48                   |                                 |                             |                           |          |            |                           |                        |                         |
| Cefepime| -7.66   | -8.77                    | 2.39                            | -2.06                       | 66.407                    | 4        | 2.41       | K-73, D-104, T-167, K-240, A-237 | SHV-48                 | -7.20                  |

### SHV-105

| Cefepime | -6.67 | -9.64 | 2.98 | -1.25 | 66.394 | 4 | 12.87 | S-70, N-170, S-238, R-244 |
|----------|-------|-------|------|--------|--------|---|------|--------------------------|
| Ceftazidime | -8.17 | -11.15 | 3.28 | -1.43 | 68.616 | 4 | 1.02  | S-70, N-170, A-237, S-238, R-244 |

### SHV-95

| Cefepime | -5.68 | -8.55 | 2.98 | -1.34 | 69.337 | 4 | 68.69 |
|----------|-------|-------|------|--------|--------|---|------|-------------------------|
| Ceftoxime | -5.68 | -8.55 | 2.98 | -1.34 | 69.337 | 4 | 68.69 |
| Ceftazidime | -6.45 | -10.10 | 3.28 | -0.78 | 72.014 | 3 | 18.84 |

### SHV-89

| Cefepime | -7.51 | -8.98 | 2.39 | -1.73 | 73.637 | 1 | 3.11  |
|----------|-------|-------|------|--------|--------|---|------|--------------------------|
| Ceftoxime | -6.14 | -8.89 | 2.98 | -1.46 | 68.666 | 2 | 31.39 |

| Cefepime | -5.25 | -8.08 | 3.28 | -2.32 | 68.727 | 3 | 40.45 |
|----------|-------|-------|------|--------|--------|---|------|-------------------------|
| Ceftoxime | -6.40 | -9.52 | 2.98 | -1.09 | 71.259 | 1 | 20.49 |

| Cefepime | -5.99 | -8.08 | 3.28 | -2.45 | 74.614 | 2 | 34.23 |
|----------|-------|-------|------|--------|--------|---|------|-------------------------|
| Ceftoxime | -6.09 | -8.08 | 3.28 | -2.45 | 74.614 | 2 | 34.23 |

| Cefepime | -7.20 | -8.25 | 2.39 | -2.08 | 71.521 | 2 | 5.29  |
|----------|-------|-------|------|--------|--------|---|------|-------------------------|
| Ceftoxime | -6.40 | -9.52 | 2.98 | -1.09 | 71.259 | 1 | 20.49 |

| Cefepime | -6.09 | -8.08 | 3.28 | -2.45 | 74.614 | 2 | 34.23 |
|----------|-------|-------|------|--------|--------|---|------|-------------------------|
| Ceftoxime | -6.09 | -8.08 | 3.28 | -2.45 | 74.614 | 2 | 34.23 |