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In-silico Approach Explains Evolution of Beta-lactamases from Penicillin Binding Proteins

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Abstract

Penicillin binding proteins (PBPs) possess transpeptidase, transglycosylase as well as carboxypeptidase activities and mediates the crosslinking of N-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) of peptidoglycan, thereby promoting the cell wall synthesis. The β-lactam antibiotics bind to PBPs and inactivate them, thus killing the bacteria. During the course of evolution, bacteria have also developed differential survival strategies. Emergence of multidrug resistance resulting from β-lactamases constitutes a worldwide threat. This enzyme hydrolyzes β-lactam antibiotics and thus makes them inactive. The purpose of this study was to analyze the binding affinity of β-lactam antibiotics with PBPs and β-lactamases by computational docking studies. For this, different classes of β-lactams known till date were used as ligand for molecular interaction. Computational analysis was performed using GLIDE based on scoring functions. The binding affinity pattern was determined in terms of their binding energies. The results demonstrated that most of the β-lactam antibiotics interact with both β-lactamases and PBPs, which reveals a possible ancestry between β-lactamases and PBPs as both recognize common substrates. Therefore, present work may be helpful in the study of evolutionary relationship between β-lactamases and PBPs at catalytic level.

Keywords: β-lactamases; Penicillin Binding Proteins (PBPs); Molecular evolution; Molecular docking

Introduction

Bacteria have known to come into existence more than 3.5 billion years ago [1]. Cell wall is the primary structural feature made up of peptidoglycan and is responsible for support and maintenance of bacterial morphology. A family of enzymes collectively called PBPs i.e., Penicillin Binding Proteins (PBPs) are involved in the assembly, maintenance and regulation of peptidoglycan structure in both Gram-positive and Gram-negative bacteria [2]. These proteins are present in bacterial inner membrane with their active sites present in periplasmic space. On the basis of molecular weight, PBPs are of two types, low molecular weight and high molecular weight PBPs [3]. Based on the amino acid sequence similarities, each type is further subdivided into three classes [4]. The functions of PBPs are very diverse, that include transpeptidase, transglycosylase, and carboxypeptidase activities [5-8]. The β-lactam antibiotics bind to PBPs and inactivate them and thus kill the bacteria. PBPs undergo a chemical modification at 62nd serine residue of active site by penicillin, a commonly known β-lactam. The evolution of bacteria is very fast as they reproduce very quickly. Bacteria have developed many ways to overcome the action of a wide range of antibiotics including penicillin that render treatment of infection very difficult. The acquired resistance mechanisms comprises of alteration in the structure of PBPs, overexpression of efflux pumps etc. PBPs have high structural similarity with β-lactamases; also confer antibiotic resistance to their host organism by acquiring mutations that allow them to continue their participation in cell wall biosynthesis [9]. Main mechanism attributed to the resistance is the function of β-lactamases [10-14]. This enzyme hydrolyzes the β-lactam antibiotics and thus makes them inactive. Many β-lactamases use the same machinery as used by the penicillin-binding proteins [15]. The penicillin-binding proteins use serine residue of active site to form a covalent bond with peptidoglycan chain, and then release it as it form the crosslink with another part of the peptidoglycan network. Penicillin binds to this serine but does not release it, thus permanently blocking the active site. On the contrary, β-lactamases have acquired the ability to hydrolyse the β-lactam ring of these antibacterial agents, hence making them inactive. β-lactamases have a similar serine in their active site pocket. When penicillin interacts with this serine, it gets released but in an inactivated form. Metallo-β-lactamase act similarly but uses a zinc ion instead of serine amino acid to inactivate the penicillin. It is widely known that the genes for the ancient PBPs gave rise to those of β-lactamases [15]. However, the β-lactamases have different catalytic mechanisms and distinct PBP evolutionary progenitors [16]. Through the course of evolution, β-lactamases have gone through two major modifications so as to be specialized as antibiotic resistance enzymes. This includes the structural changes in PBPs that would impair the molecular recognition of peptidoglycan in emerged β-lactamases. Secondly, the insertion of certain amino acid residues at the binding site would abolish binding to the PBP substrate [15,16]. In the present study, the extent of interaction of β-lactams with PBPs and beta lactamases somewhere lead us to an idea of the resemblance in their active sites, implying that their evolution may be interrelated as gradual modification in the PBPs might have led to the evolution of β-lactamases without much change in the active site because both recognizes same substrate but PBP does not cleave it whereas β-lactamase does. For this study, we have selected β-lactamases from different classes such as A, B, C, and D and PBPs (namely, PBP1a and apoPBP1a). The TEM and SHV belong to the class-A β-lactamases, NDM-1 belong to class-B β-lactamases [17,18], ADC and AmpC belong to class-C β-lactamases [19,20] and OXA-58 and OXA-23 belong to Class-D β-lactamases [11,18,21,22]. The study of interaction pattern of various β-lactamases and PBPs with different

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β-lactam antibiotics may provide beneficial information regarding the evolution of beta lactamases from PBPs at catalytic level.

Methods

Selection of protein templates of β-lactamases and penicillin binding proteins

The protein structures with PDB ID-3UDI (PBP1a), 3UDF(ApoPBP1a), 1ZG4 (TEM1, Class-A β-lactamases), 3MKE (SHV, Class-A β-lactamases), 4RLO (NDM1, Class-B β-lactamases), 4NET (ADC, Class-C β-lactamases), 4LN3 (Amp C, Class-C β-lactamases), 4OH0 (OXA58, Class-D β-lactamases) and 4JF6 (OXA23, Class-D β-lactamases) were retrieved from Protein Data Bank. The crystal structures were prepared for the *in-silico* studies using Protein preparation wizard module of Schrodinger Suite v9.7 that prepares the protein by fixing errors in the protein like incomplete missing residues, missing atoms, overlapping atoms and alternate positions near the active site. The crystal structure for PBP1a comprised of overlapping atoms particularly at atom1-9860 PDB:A:137 (HZ2) and atom 2-15519 PDB:B:256 (HD2) with distance 0.79 Å. Also, alternate position error was observed in this case for glutamine at 218th position in chain A with current average occupancy as 0.5. Similarly, in case of SHV, ADC and OXA23, the alternate position error was observed. Whereas, in case of AmpC, the missing atoms from side chains were also found in glutamine at 361st position in both chain A and B. The number of heavy atoms and expected heavy atoms were found to be 6 and 9 in each case, respectively. Besides, overlapping atoms and alternate positions were also observed in AMP. In case of apoPBP1a, NDM, TEM1 and OXA51 no such errors were found. During protein preparation, all crystallographic water was first removed and appropriate bond orders and formal charges to ligands and cofactors were assigned and hydrogens were added using the Schrodinger Maestro interface. The application of protassign procedure reoriented glutamine and asparagine amide groups, where necessary in protein-ligand and also assigned the tautomeric and protonation state of histidine residues and the protonation state of Asp and Glu residues. Protassign also reoriented protein and ligand hydroxyl groups to optimize hydrogen-bonding patterns. Finally, the impref procedure was used to remove unphysical contacts and relax the complexes. This procedure comprises of short refinement stages which use increasingly smaller Cartesian restraints. Once the refined structure reaches the specified Root-Mean-Square Deviation (RMSD) to the starting coordinates of non-hydrogen atoms, here taken to be 0.30 Å, the procedure terminates and returns the structure generated in the previous refinement stage, which by definition did satisfy the RMSD limit [23].

SiteMap prediction for protein structures

Prediction of binding cavity was done for the protein structures using SiteMap version 3.0. This approach is highly efficient as it is a combination of novel and highly effective algorithms for rapid binding-site identification with easy-to-use property and visualization tools. The top-scoring site is marked by the already known ligand binding site. SiteMap also provides useful guidance as to whether a candidate site is likely to be a ligand-binding site. Moreover, SiteMap provides information about the hydrophobic, hydrogen-bond donor, hydrogen-bond acceptor, and metal-binding regions making in form of computed properties and graphical contour maps during binding site analysis. The potential ligand binding sites are identified by linking together "site points" that are close to the protein surface and protected from the solvent. The SiteScore, the scoring function, helps in accurately ranking possible binding sites and in eliminating sites that are not likely to be of pharmaceutical relevance. SiteMap predicts five potential binding cavities (5 set as default) in a protein and each cavity is assigned a score and ranked accordingly. The binding site with highest site score was taken for docking studies.

Receptor grid generation

The generation of a receptor grid for docking is performed using the GLIDE module of Schrodinger v9.7. The van der Waals radius scaling is used to soften the potential for non-polar parts of the receptor. Here only those atoms will be scaled which have the partial atomic charge less than the specified cut off i.e., 0.25. The active site of proteins was specified as the centroid of the selected residue in order to generate a grid cube that covered the entire active site.

Selection of β-lactam antibiotics from PubChem

3-D structure of penicillin, aztreonam, ceftazidime, doripenem, imipenem, oxacillin, and nitrocefin were downloaded in MOL SDF format from PubChem compound database. These antibiotics include β-lactams from all the four classes. The LigPrep module version 2.9 was used to prepare high quality, all-atom 3D structures for beta lactams. It helps in the generation of different ionization states generated at pH of 7.0 +/-2.0. The Epik state penalty is computed in units of Kcal/mol, thereby making it directly compatible with the GlideScore used for docking. It also generates tautomers and carries out the desaltation of ligands. Stereoisomers for beta lactams were generated with specified chiralities having 32 as maximum limit per ligand with atleast one low energy ring conformation per ligand. A force field of OPLS_2005 (optimized potential for ligand) was used for the minimization of β-lactams.

Docking using GLIDE (Grid based Ligand Docking using Energetics)

A computational ligand-target docking approach was used to analyze the structural complexes of PBPs and lactamases with β-lactams (ligands) in order to understand the structural basis of this protein-target specificity by using GLIDE from Schrodinger suite based on scoring functions. The minimized structures of β-lactams (SDF format) and proteins were used subsequently in docking simulation in SP mode followed by XP mode. While using XP mode, five poses (set as default) were calculated per ligand molecule and docked to the active site of proteins. The number of poses may be exceeded as per the requirement, mainly during SP docking. The resulting docked conformations were analysis using the Glide pose viewer tool. The conformation/pose that made the maximum number of interactions was considered for further analyzed [11]. The interacting residues were viewed using 2D-image viewer tool included in Schrodinger suite.

Molecular mechanics/generalized born surface area (MMGBSA) calculations

Docking energies alone are not considered the accurate computational method for determining the interaction. For this, the MMGBSA approach was used as implemented in the Prime module from the Schrodinger Suite v9.7. This calculates binding free energies for the best-docked complexes using MM force fields and implicit solvation. This method gives the relative binding energy that each docking ligand requires for the target protein. The more negative value of MMGBSA score confirms the lowest binding energy (or highest absolute value) for that ligand-receptor interaction.

Results and Discussion

PBPs are biosynthetic enzymes of bacterial cell wall assembly that

are found anchored in the cell membrane and are involved in the cross-linking of bacterial cell wall. β-lactam antibiotics target the penicillin-binding proteins (or PBPs). This group of antibiotics is characterized by four-membered, nitrogen-containing β-lactam ring at the core of their structure, which is a key to the mode of action of this group of antibiotics. β-lactams are classified into four classes i.e., penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems. Previous studies made it evident that β-lactams have evolved billions of years ago and led to the evolution of β-lactamases and PBPs. The pharmaceutical introduction of β-lactams caused the lateral transfer of resistance genes to antibiotic-treated pathogens and further modification in the β-lactamase gene [24]. In the present study, we have keen interest in determining the relatedness in molecular evolution of β-lactamase and PBPs. Hence, we performed the *in-silico* study of β-lactams with PBPs and β-lactamases and results were analyzed to determine the binding affinities keeping all the interacting residues into account. Low MMGBSA scores represent better binding (binding energy is low). It is also noted here that if a PBP has better interaction then antibiotics has more inhibitory effect on bacterium because PBP is the target of that antibiotics. In contrast, the higher binding affinity of antibiotics for β-lactamases will cause the ineffectiveness of the drug. *In-silico* interaction studies of PBPs and β-lactamases was performed using maestro v9.7. Results of the docking studies have been explained one by one. The number of hydrogen bonds formed between different selected proteins and interacting residues involved have been listed in Table 1. The binding energies of selected proteins and different β-lactams have been shown in Table 2. Visuals of docked complexes and interacting residues have been displayed in Figure 1.

Comparative interaction of Penicillin Binding protein (PBP1a) with different β-lactams

PBP1a has the transpeptidase activity required for cell wall synthesis. The calculation of binding energies using the MMGBSA approach provided the following results. Nitrocefin was observed to interact with PBP by forming four hydrogen bonds involving Ser487, Asn674, and Ser434. Its binding energy is -55.97 kcal/mol which is lowest among the group. On the other hand, penicillin showed highest binding energy of -29.80 kcal/mol among the group. Ceftazidime, doripenem, imipenem, aztreonam and oxacillin showed intermediate binding energies as -54.77 kcal/mol, -44.10 kcal/mol, -39.07 kcal/mol, -38.61 kcal/mol, and -35.90 kcal/mol respectively. Based on the binding energies, antibiotics can be arranged in the following order-

Nitrocefin>Ceftazidime>Doripenem>Imipenem>Aztreonam>Oxacillin>Penicillin

It shows that nitrocefin and ceftazidime belonging to cephalosporin class have the highest binding affinities for PBPs. Doripenem and imipenem (which belongs to carbapenem class) also shows moderately high binding with PBPs. This invokes that cephalosporins and carbapenems are effective against the PBPs. Penicillin shows least interaction with PBPs.

Comparative interaction of ApoPBP1a with different drugs

Penicillin binding ApoPBP1a found in *A. baumannii* has the transpeptidase activity required for cell wall synthesis. Ceftazidime showed the lowest binding energy of -64.24 kcal/mol. Penicillin was observed to exhibit highest binding energy of -37.29 kcal/mol. Oxacillin, nitrocefin, imipenem, doripenem and aztreonam have binding energies as -54.32 kcal/mol, -53.95 kcal/mol, -48.63 kcal/mol, -45.83 kcal/mol and -38.61 kcal/mol, respectively, ranging in between ceftazidime and penicillin. Based on the binding energies, antibiotics can be arranged in the following order:

Ceftazidime>Oxacillin>Nitrocefin>Imipenem>Aztreonam>Doripenem>Penicillin

As evident from the binding energy scores, ceftazidime, belonging to the cephalosporin class of drugs shows the highest interaction with PBPs and thus can be looked upon as better drug. The penicillin again shows the least interaction as observed in case of PBP1a.

Comparative interaction of SHV β-lactamase with different β-lactams

SHV β-lactamase belongs to class-A serine β-lactamases and is

Proteins β-lactams	PBP1a	PBP1a(Apo)	SHV	TEM1	NDM1	AmpC	ADC	OXA58	OXA23
Oxacillin	3(Ser470,Ser434, Ser487)	1(Thr670)	1(Arg671)	1(Arg178)	1(Lys216)	2(Gly320, Asn152)	3(Arg320, Asn319)	3(Ala226, Trp223)	2(Arg259, Trp113)
Penicillin	3(Ser470,Ser434, Ser487)	4(Ser470,434, Thr670, Gly709)	2(Ala146, Trp165)	2(Glu171 andArg241)	4(Lys216, Ser217, Thr34)	2(Asn289, Ala318)	3(Asn154, Asn319, Gln122)	2(Trp223, Arg263)	2(Ser126, Ser109)
Aztreonam	1(Asn489)	4(Gly709,Ser434, Ser470, Tyr707)	4(Arg161,Arg 153, Asp163)	5(Arg164,Arg178, Arg241,Glu171)	4(Lys211, Asn220,Asp212, Lys216	5(Asn152,Val121, Gly320,Ser212)	4(Asn154, Asn319, Ser66,Arg342)	4(Leu170, Trp223,Arg263	5(Arg259, Ser79, and Thr217, Ser126)
Ceftazidime	2(Arg473, Ser434)	2(Asn489, Asp675)	2(Arg178,Trp165)	2(Arg178,Trp165)	1(Gln123)	3(Val121, Asp123,Gly320)	5(Asn154, Gln122, Arg320)	6(Arg263, Ser221,Lys264)	7(Arg259, Trp217, Trp219 ,Trp113,Ser126, Asn260)
Doripenem	3(Leu486, Gly653, Ser434)	7(Leu526,Asn489, Thr672,Asn674, Tyr485,Thr670,Gly709)	3(Ala146, Asp163, Trp165)	5(Glu104, Glu240, Pro167,Glu171, Arg178)	6(Ser217, Thr34, and Lys216)	5(Ala318,Asn343, Asn289,Asn346, Thr316)	3(Gly316, Asn319, Asn289)	6(Ser221,Ala129, Ala226,Trp223, Arg263,Met215)	7(Arg259, Thr217, Leu125, Ser126, Trp219,Lys124
Imipenem	6(Ser470,Ser434, Ser487,Gly708)	5(Gly709,Ser434, Thr670,Asn489)	4(Ala177, Asp163, and Arg161)	4(Trp165, Glu168, Glu171, Arg178)	3(Glu152, Asp223, Phe70, and Asn220)	4(Ala318,Asn343, Asn289,Asn346)	5(Gln122, Asn154, Asn289, Asn319, Glu274)	5(Ser83,221, Arg263, Ala129, Trp223, Phe114)	4(Arg259,Ser126, Trp219, Met254)
Nitrocefin	4(Ser487,Ser434 ,Asn674)	3(Ser487,Asn489, \and Thr672)	2(Arg153, Asp163)	3(Arg161, Glu177, Asp176)	5(Lys216, Ser217, Lys216, and Lys211)	3(Asn289,Asn343, Gly320)	3(Asn319, Tyr224)	4(Arg263, Ser221)	8(Thr217, Arg259, Ser79,Trp219, Ser126,Met221)

Table 1: Table shows the number and interacting residues that form hydrogen bonds between proteins and different β-lactams.

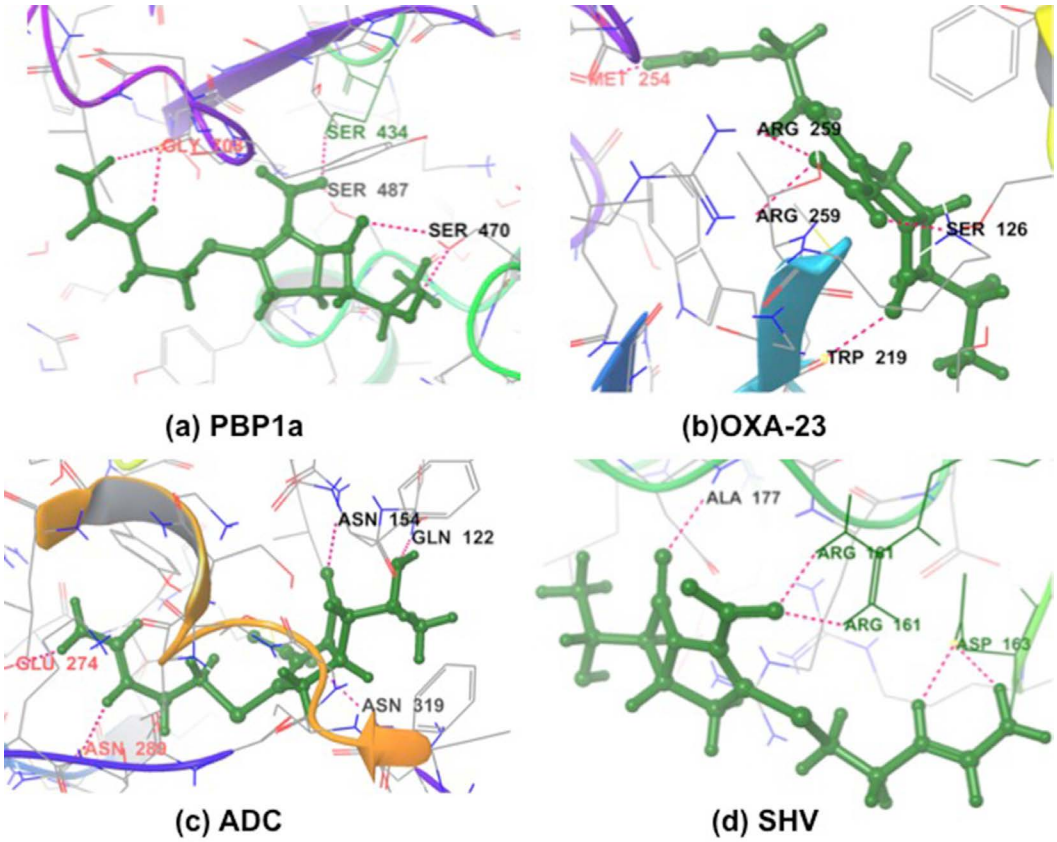

Figure 1: Representation of interacting residues of PBP's and β-lactamases with imipenem (carbapenem class of drugs).

Proteins β-lactams	PBP1a	PBP1a(Apo)	SHV	TEM1	NDM1	AmpC	ADC	OXA58	OXA23
Oxacillin	-35.90	-54.32	-35.32	-39.71	-16.54	-49.97	-37.92	-47.58	-49.97
Penicillin	-29.80	-37.29	-25.80	-9.09	-38.80	-38.80	-25.65	-45.46	-38.80
Aztreonam	-38.61	-46.93	-31.94	-19.64	-17.35	-22.17	-48.02	-58.85	-22.17
Ceftazidime	-54.77	-64.24	-36.09	-34.93	-49.57	-51.36	-26.90	-56.36	-51.36
Doripenem	-44.10	-45.83	-34.74	-30.71	-57.90	-71.58	-46.53	-65.35	-71.58
Imipenem	-39.07	-48.63	-34.80	-40.42	-41.69	-56.38	-33.92	-55.04	-56.38
Nitrocefin	-55.97	-53.95	-46.80	-45.87	-76.74	-56.37	-53.75	-59.81	-56.37

Table 2: Table shows the binding energies (kcal/mol) between selected proteins and different beta-lactams.

one of the early evolved β-lactamases from PBPs. It has been noted that β-lactamases hydrolyze the β-lactams and make them inactive therefore, a bacterium with β-lactamase exhibit resistance. Nitrocefin was observed to interact with SHV by forming two hydrogen bonds involving, Arg153 and Asp163. Its binding energy is -46.80 kcal/mol which is lowest among the group of β-lactams. This is followed by ceftazidime, oxacillin, imipenem, doripenem and aztreonam whose binding energies are -36.09 kcal/mol, -35.32 kcal/mol, -34.80 kcal/mol, -34.74 kcal/mol and -31.94 kcal/mol, respectively. Penicillin was observed to interact with SHV by forming two hydrogen bonds. It has highest binding energy of -25.80 kcal/mol among the group. Based on results, the order of binding affinity of SHV β-lactamase have been shown as follows:

Nitrocefin>Ceftazidime>Oxacillin>Imipenem>Doripenem>Aztreonam>Penicillin

Nitrocefin and ceftazidime (cephalosporins class) shows higher binding, which infer that the inactivation of these drugs by SHV β-lactamases is relatively easier. Carbapenems are showing moderate interaction which makes them comparatively better drug than cephalosporins. Almost similar binding affinity pattern was observed in the case of PBPs giving relevant information in support of their relatedness with β-lactamases.

Comparative interaction of TEM-1 β-lactamase with different β-lactam drug

TEM-1 is a class-A serine β-lactamase. Nitrocefin was observed to interact with TEM-1 by forming three hydrogen bonds involving, Arg161, Glu177, and Asp176 and has the lowest binding energy of -45.87 kcal/mol among the group. This is followed by imipenem, oxacillin, ceftazidime, doripenem and aztreonam having binding energies as -40.42 kcal/mol, -39.71 kcal/mol, -34.93 kcal/mol, -30.71 kcal/mol and -19.64 kcal/mol, respectively. Penicillin was observed to interact with TEM-1 by forming two hydrogen bonds involving Glu171 and Arg241.

It has highest binding energy among the group equal to -9.09 kcal/mol. Based on the results, the binding affinity order of TEM-1 lactamases with different ligands have been depicted as follows (higher to lower):

Nitrocefin>Imipenem>Oxacillin>Ceftazidime>Doripenem>Aztreonam>Penicillin

Here, nitrocefin and imipenem are showing higher binding affinities which mean that these drugs can be easily hydrolyzed and inactivated by TEM-1 β-lactamase. The ineffectiveness of carbapenems, a new generation β-lactams against the class-A β-lactamases may pose a serious threat. Moreover, TEM-1 β-lactamase interacts with the least affinity with penicillin group of β-lactams rendering them as a better drug.

Comparative interaction of NDM-1 β-lactamase with different β-lactams

NDM-1 (New Delhi metallo β-lactamases) belongs to class-B β-lactamases or metallo-beta lactamases. Nitrocefin was observed to interact with NDM-1 by forming five hydrogen bonds involving, Lys216, Ser217, Lys216, and Lys211 and has the lowest binding energy of -76.743 kcal/mol among all β-lactams. This was followed by doripenem, ceftazidime, imipenem, penicillin and aztreonam having binding energies of -57.90 kcal/mol, -49.57 kcal/mol, -41.69 kcal/mol, -38.80 kcal/mol and -17.35 kcal/mol, respectively. Oxacillin has the highest binding energy of -16.54 kcal/mol among the group. Based on the results, the order of affinity for β-lactams with NDM-1 β-lactamase is depicted as follows (high to low interaction).

Nitrocefin>Doripenem>Ceftazidime>Imipenem>Penicillin>Aztreonam>Oxacillin

It shows that nitrocefin and doripenem have highest binding affinity towards NDM beta lactamases, hence are likely to get easily inactivated by NDM-1. Again, the hydrolysis of most recent carbapenem class of antibiotics by the bacteria containing Class-B β-lactamases may render difficulties with the treatment in future. NDM-1 β-lactamases show least interaction with aztreonam, oxacillin and penicillin group of β-lactams making the drugs less liable to the hydrolytic cleavage by them. Therefore, monobactams and penicillin group of antibiotics may be considered more effective and useful future drugs against NDM-1 expressing bacterium.

Comparative interaction of AmpC β-lactamase with different β-lactams

AmpC belongs to class-C serine based β-lactamases. Ceftazidime was observed to interact with AmpC by forming three hydrogen bonds involving Val121, Asp123, and Gly320. Its binding energy is -63.20 kcal/mol, which is lowest among the group. This was followed by nitrocefin, doripenem, imipenem aztreonam and oxacillin having binding energies of -50.28 kcal/mol, -44.35 kcal/mol, -42.53 kcal/mol, -41.16 kcal/mol and -36.45 kcal/mol, respectively. Penicillin was observed to interact with AmpC by forming two hydrogen bonds involving Asn289, Ala318. Its binding energy is -29.22 kcal/mol which is the highest among the group. Based on the results, the order of interaction of all the β-lactams with AmpC β-lactamase is as follows.

Ceftazidime>Nitrocefin>Aztreonam>Oxacillin>Doripenem>Imipenem>Penicillin

It shows that ceftazidime and nitrocefin belonging to the same class (cephalosporins) have highest binding affinity, which means Amp-C can easily inactivate this class of drugs. Here, the carbapenems such as doripenem and imipenem are found to show relatively low interaction after penicillin (lowest interacting β-lactam) hence, these drugs cannot be easily hydrolysed by the AmpC β-lactamases harbouring bacteria. This also infers that carbapenems can be effective drugs against class C beta lactamases after penicillin.

Comparative interaction of ADC β-lactamase with different β-lactam drugs

ADC also belongs to class-C serine based β-lactamases. Nitrocefin was observed to interact with ADC by forming three hydrogen bonds involving Asn319 and Tyr224. Its binding energy is -53.753 kcal/mol being the lowest of all. Aztreonam, doripenem, oxacillin, imipenem and ceftazidime were observed to show intermediate binding energies of -48.02 kcal/mol, -46.53 kcal/mol, -37.92 kcal/mol, -33.92 kcal/mol and -26.90 kcal/mol respectively. Penicillin is observed to interact with ADC by forming three hydrogen bonds involving Asn154, Asn319 and Gln122. Its binding energy is -25.65 kcal/mol, which is highest among the group. Based on binding energies, the order of interaction between different β-lactams with ADC can be arranged in following order (higher to lower interaction).

Nitrocefin>Aztreonam>Doripenem>Oxacillin>Imipenem>Ceftazidime>Penicillin

Drugs like nitrocefin and aztreonam show highest affinity with ADC therefore, get easily hydrolyzed by ADC and becomes inactive. Older drugs like penicillin seems to be more potent in killing bacteria as it shows lowest binding with ADC. Hence, can be considered better drug for killing ADC containing bacteria.

Comparative interaction of OXA-58 β-lactamase with different drugs

OXA belongs to class-D, newly emerged β-lactamases. They are mainly expressed by carbapenem resistant strains of *A. baumannii*. Doripenem was observed to interact with OXA58 by forming six hydrogen bonds involving Arg263, Ser221, Ala129, Ala226, Trp223 and Met225. Its binding energy is -65.35 kcal/mol which is lowest among the group. Nitrocefin, aztreonam, ceftazidime, imipenem and oxacillin showed binding energies of -59.81 kcal/mol, -58.85kcal/mol, -56.36 kcal/mol, -55.04 kcal/mol and -47.577 kcal/mol, respectively. Penicillin was observed to interact with OXA58 by forming two hydrogen bonds involving Trp223 and Arg263. Its binding energy is -45.46 which is highest of all. Based on the affinity, β-lactams have been arranged in the following order (higher to lower interaction).

Doripenem>Nitrocefin>Aztreonam>Ceftazidime>Imipenem>Oxacillin>Penicillin

It shows that doripenem and nitrocefin show highest binding, which means that these drugs can be easily hydrolysed and inactivated by OXA-58. The hydrolysis of carbapenems, the recently emerged class of β-lactams, by bacteria containing Class-D β-lactamases may lead to a serious threat as the class D beta lactamases are the last generation of β-lactamases known till date and ineffectiveness of even the carbapenem is a major concern. Another carbapenem i.e., imipenem is showing comparatively less interaction. The reason for this behavior is still not understood.

Comparative interaction of OXA-23 β-lactamase with different drugs

OXA23 is a serine based class-D β-lactamase known to express more in carbapenem resistant strain of bacteria. Doripenem showed the lowest binding energy of -71.58 kcal/mol. Imipenem, oxacillin,

nitrocefin, ceftazidime and penicillin were observed to interact with OXA23 with intermediate binding energies of -56.38 kcal/mol, -51.36 kcal/mol, -56.37 kcal/mol, -49.97 kcal/mol, and -38.80 kcal/mol, respectively. Aztreonam was observed to interact with OXA23 having binding energy as -22.17 kcal/mol which is highest among the group. Based on binding affinity with OXA23, β-lactams have been arranged in following order (higher to lower).

Doripenem>Imipenem>Nitrocefin>Ceftazidime>Oxacillin>Penicillin>Aztreonam

Doripenem shows the highest binding affinity toward OXA-23 β-lactamases. This depicts that they are more prone to hydrolysis by this enzyme. The results are in resemblance with the fact that this group of β-lactamases is expressed more in carbapenem resistant strain. The highest binding affinity of carbapenems infer that these drugs can be easily inactivated by OXA23 hence making them least effective drug against bacteria containing OXA23 lactamases. Penicillin and aztreonam are showing least interaction, thus are likely to be considered as potential drugs.

Conclusion and Future Prospects

Molecular docking and binding energy calculations showed that PBPs generally use amino acids with polar uncharged R groups i.e., Ser, Thr, Asp for its catalytic activity against different β-lactam drugs. However, imipenem and doripenem also interact with Leu and Gly residues of penicillin binding proteins. Asn is involved in catalysis of ceftazidime and nitrocefin with PBP. SHV and TEM-1 β-lactamases use Arg, Asn, Glu and Trp for their interaction with β-lactam drugs. NDM-1 lactamases use mainly Ser and Thr for their catalytic activity similar to PBPs along with Lys and Phe in few cases. AmpC lactamases use amino acids with polar uncharged groups i.e., Ser, Thr, Asn and amino acids with nonpolar aliphatic groups like Gly, Ala, Val etc. ADC lactamases use Asp, Arg, Lys, Gln for their catalytic activity. OXA group of β-lactamases mostly use amino acids with aromatic and acidic side chains like Phe, Trp, Lys, and Arg for their catalytic activity with β-lactam drugs. Catalytic domains of β-lactamases are similar to PBPs and most of the beta lactamases use similar amino acid as PBPs. Moreover, from the results, the penicillin was observed to exhibit the least binding affinities for both beta lactamases and PBPs. OXA group are the latest type of β-lactamases, so they show some deviation by using aromatic and acidic amino acids in their side chain. The present study results demonstrate that most of β-lactams interact with both β-lactamases and PBPs. It was also noted that NDM-1, OXA-23 and OXA-58 have better interaction with carbapenems which shows that they may effectively hydrolyze this class of β-lactams. Therefore, bacteria with these β-lactamases may be lethal for mankind.

The result of binding energies of PBPs and β-lactamases with different β-lactams indicates almost similar pattern of interaction with PBPs and most of the β-lactamases. However, every β-lactam was found to interact with each class of β-lactamases with high or low affinity. Nitrocefin is a chromogenic yellow color substrate of β-lactamase that is converted into red color product by β-lactamases. This antibiotic is specific to the β-lactamases and is therefore considered as a control in this work. The results showed a very interesting fact that nitrocefin binds with the PBPs, hence supporting the fact that β-lactamases and PBPs might have co-evoluted. The present *in-silico* study explains the molecular evolution of β-lactamases from PBPs. This work will also be helpful in understanding the interaction of different PBPs and β-lactamases with β-lactams, which may provide evidence about evolution of beta lactamases and PBPs at catalytic level. The knowledge of binding affinities β-lactamases and PBPs would enable the identification of novel relationships, giving a pathway for the examination of other antibiotics to obtain information on their antibiotic induced evolutionary paths [24]. Further, using *in-vitro* studies on the enzyme kinetics of PBPs and β-lactamases, this process can be explained and understood with more authenticity and precision.

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Author Disclosure Statement

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