**In vitro and in silico screening of Klebsiella pneumoniae** new Delhi metallo-β-lactamase-1 inhibitors from endophytic Streptomyces spp.

Lalitha Cheepurupalli, Aathithya Díaz, Adithya Conjeevaram Gopal, Sudarshan Singh Rathore, Vigneshwar Ramakrishnan and Jayaprada Ramakrishnan

Introduction

The discovery of β-lactam antibiotics is a boon to treat diverse bacterial infections. Unfortunately, in recent decades bacterial pathogens have evolved resistance towards antibiotics because of the wide utility owing to their clinical efficacy. The emergence of new resistant phenotypes and worsened treatment outcomes are serious clinical problems being faced in all parts of the world. The Centre for Disease Control, USA recorded approximately 600 death per year due to carbapenem-resistant Escherichia coli and Klebsiella pneumoniae (CDC, 2013). K. pneumoniae is a major nosocomial pathogen second to E. coli (Tsay et al., 2002), causing hospital-care-associated infections including bloodstream infections, wound infections, meningitis, liver abscess, bacteremia, septicemia, biofilm-related bloodstream, and urinary tract infections. Many strains of K. pneumoniae are resistant to carbapenems, the last resort of antibiotics (Gwynn et al., 2010). The major mechanism by which these strains acquire resistance is by the production of β-lactamase enzyme that hydrolyses the β-lactam ring and inactivates the drug. Klebsiella producing carbapenemase (KPC) and New Delhi metallo-β-lactamases (NDM) are the major enzymes that hydrolyse carbapenems, the last resort of multi-drug resistant bacterial infections. Of the various carbapenemases, Class B (metallo-β-lactamase) (MBL) is more challenging to tackle than the other groups – Class A (serine penicillinas), Class C (cephalosporinas), and Class D (oxacillinases) (Drawz et al., 2014). The commercially available serine β-lactamase (SBL) (Class A carbapenemase) inhibitors, such as clavulanic acid, sulbactam and tazobactam are ineffective against MBL-producing pathogens, such as K. pneumoniae, E. coli, P. aeruginosa and A. baumannii. New Delhi metallo-β-lactamase-1, which belongs to the subclass B1 of MBLs, is present widely within Enterobacteriaceae and the gene is encoded on a readily transferable plasmid which facilitates its transmission. This has, hence, become a cause for concern as carbapenems have the widest spectrum of activity among all β-lactam antibiotics and is used clinically as the last line of defense against multi-drug resistance (MDR) bacteria. Various in vitro and in silico studies had been proposed to understand the mechanism of such resistance of New Delhi metallo-β-lactamase-1...
and its variants against carbapenems. Ali et al., studied the biochemical properties of five NDM variants (NDM-1, NDM-4, NDM-5, NDM-6, and NDM-7) and found that NDM-5 has the maximum hydrolytic activity against carbapenems (Ali et al., 2019). They also observed that NDM-5 had significant variation in the secondary structure as revealed by CD spectroscopy (Ali et al., 2019). Inhibition of MBL is one potential way to combat MDR bacteria. Hence, several studies have been carried out to identify noncovalent inhibitors of β-lactamase to maintain the effectiveness of β-lactams. For instance, strategies have been developed for the discovery of non-β-lactamase inhibitors against NDM-1 type metallo-β-lactamase through multi-step virtual screening and in vitro cell assays. Efficacy of the identified inhibitors were confirmed with microbiological and kinetic studies confirming to use as the potential drug targets (Khan et al., 2017). Also, identified five potential drug candidates that can bind to New Delhi Metallo-β-lactamase-1 using a combination of in silico and in vitro methods (Rahman & Khan, 2020). Rahman and Khan adopted a virtual screening approach and identified natural compounds such as Withaferin A, beta-sitosterol, etc., which can potentially bind to and inhibit New Delhi metallo-β-lactamase-1 (Rahman & Khan, 2020). Other potential inhibitors, such as derivatives of dipicolinic acidcyclic boronic acid have also been reported (Chen et al., 2017; Hecker et al., 2015). However, presently, there are no drugs or inhibitors in the market that can effectively tackle clinically important MBLs of subgroup B1. The active site of B1 subgroup of MBLs consists of two zinc ions stabilized by histidine, cysteine and aspartate residue (Zhang & Hao, 2011). The challenge behind the discovery of new broad-spectrum MBL inhibitors is the high heterogeneity of the active site of different enzymes belonging to the same family (Drauz & Bonomo, 2010; Fast & Sutton, 2013). Hence, the reported MBL inhibitors are selective for very few MBLs and that hinders its clinical utility. Presently, clinicians prescribe the second choice of drugs; namely, colistin, tigecycline, fosfomycin to treat infections by drug-resistant strains (Poirel et al., 2017). Unfortunately, these drugs are more toxic than the first-line drugs. Discovering and launching of new antibiotics in the market is challenging as the resistant phenotypes develops more rapidly. Hence, the development of BLIs is of prime importance. Considering the limited treatment options for infectious diseases especially with the emergence of pan-drug resistant superbugs, computational and experimental approaches are being used for the identification of potential inhibitors for different classes of β-lactamases. In response to the MDR threat, the present study focused to identify MBL inhibitors from endophytic Streptomyces spp. as the bioactive compounds from this organism have greater effectiveness and versatility in nature (Strobel, 2003). The strains were isolated from Solanum trilobatum, a medicinal plant mainly used for lung disorders and respiratory diseases (Govindan et al., 1999). The isolates that exhibited β-lactamase inhibitory activity were further investigated for metabolite identification using GC–MS. This was followed by in silico investigation of the binding and the molecular interactions of the metabolites with New Delhi metallo-β-lactamase-1. These investigations identified three potential inhibitors (dodecanoic acid, DL-alanyl L-leucine and phenyl propanedioic acid). To the best of our knowledge, this is the first kind of study reporting molecular interactions of dodecanoic acid, DL-alanyl L-Leucine and phenyl propanedioic acid with New Delhi metallo-β-lactamase-1. These molecules were also found to bind with two other B1 β-lactamases, viz., IMP-1 and VIM-2.

Materials and methods

Plant collection

Healthy Solanum trilobatum plants were collected from the herbal garden at Thanjavur, Tamil Nadu, India, and transported to the laboratory in a sterile bag. Samples were processed within 4 h after collection.

Strain and storage

NDM-K. pneumoniae (MCC 2570) was procured from the microbial culture collection centre, India. The strain was maintained in chromogenic UTI agar slants at 4°C and 15% glycerol stocks at −80°C.

Isolation of endophytic actinomycetes

Roots, branches, and leaves were separated from the plant, each plant part was cut into small pieces and subjected to washing in running tap water to remove the soil particle followed by surface sterilization by 70% ethanol and then treated with sodium hypochlorite solution (0.9% chlorine) for 6 min to eliminate epiphytes and soil debris. Finally, the plant parts were soaked in 10% sodium bicarbonate for 10 min to disrupt plant tissues. Each plant part such as leaves, stem, and root was placed into starch casein agar, glycerol asparagine agar, actinomycetes isolation agar (Nanjwade et al., 2013) and root was placed into starch casein agar, glycerol asparagine agar, actinomycetes isolation agar (Nanjwade et al., 2010), tryptone–yeast extract agar and incubated at 37°C for 3 weeks (Busarakam et al., 2014). Each medium was supplemented with gentamycin (1 μg/ml) and fluconazole (50 μg/ml) to avoid bacterial and fungal growth respectively (Rathore et al., 2016). Independent actinomycetes colonies were subcultured and incubated for 7 days, further the spores were preserved in 15% glycerol at −80°C.

Identification of potential actinomycetes by iodometric assay

Each actinomycete has grown in their respective isolation media for 10 days in a shake flask at 30°C. The cell filtrate was separated by centrifuging at 4300×g for 10 min. 100 μL of each culture supernatant was incubated with 100 μL of K. pneumoniae (0.06 OD) in the presence of penicillin sodium salt (5000 U/ml) in 96 well plate at 37°C for 1 h. 50 μL of 1% of starch solution and 50 μL of iodine solution was added after the incubation period. The plates were observed for the rate of iodine consumption (Shrestha & Shamser, 2014).
Synergy test against MDR K. pneumoniae

Further, we proceeded to check the $\beta$-lactamase inhibitor activity for the crude extracts of the isolates (ABST06 and AB-2) by a modified disc diffusion method. The antibiotic discs, cefotaxime (180 $\mu$g/ml) were impregnated with and without10 $\mu$l of crude extract. The dried discs were then placed in the K. pneumoniae pre-inoculated Muller–Hinton medium. Zone of inhibition was measured after 24h incubation. The increase in zone of inhibition in the extracts was considered as positive for $\beta$-lactamase inhibitory activity (Cheepurupalli et al., 2017).

Polyphasic characterization of selected actinomycetes

The strains, ABST06 and AB-2 that showed positive results for iodometric assay and synergy test were subjected to taxonomical investigation and further studies. The cultural characteristics like aerial mycelium, melanoid, and soluble pigment formation were observed on different cultural media suggested by Shirling and Gottlieb (Shirling & Gottlieb, 1966). Few of the biochemical tests such as cellulolytic activity, H$_2$S production, starch hydrolysis, nitrate reduction, gelatin liquefaction were analyzed using different media (Gwynn et al., 2010). Spore morphology of ABST06 and AB-2 were observed under the light microscope (Nikon Eclipse Ci, Japan) and scanning electron microscope (JSM-6701F, JEOL, Japan).

16s rRNA analysis

Genomic DNA was isolated from ABST06 and AB-2 using actinomycetes genomic DNA isolation kit (Kimura, 1980). The gene fragments were amplified by using a PCR kit (GENEI Pvt. Ltd, India) and the 16S rRNA gene was amplified using an Eppendorf pro thermal cycler. Experimental conditions used for PCR amplifications include-denaturation at 95°C for 5 min, followed by annealing at 94°C for 30 s and 50°C for 30 s. And the final extension step is for 72°C for 1 min 30 s. The product obtained was electrophoresed and purified from 1.5% agarose gel using a QiAquick PCR purification kit (QIAGEN). Sequencing was performed with 8F and U1492R primers, using ABI 3100 sequencer (Applied Biosystems). The sequence was edited using Finch TV (Geospiza, Inc.) and BioEdit (Ibis Biosciences, Abbott Labs).

Phylogenetic analysis

Related Streptomyces sequences were retrieved from Genbank and aligned with ABST06 and AB-2 sequence using the BLAST program. The phylogenetic tree was constructed by using the Mega 5.2 version software program. Kimura’s two-parameter model was used for the neighbor-joining method (Cheepurupalli et al., 2017).

Production and extraction of metabolites

ABST06 and AB-2 spores were inoculated into 25 ml of tryptone–yeast extract broth and incubated at 30°C for 3 days. The seed medium was inoculated into production media composed of starch 1% (w/v), glucose 1% (w/v), yeast extract 1% (w/v), peptone 0.5% (w/v), K$_2$HPO$_4$·2H$_2$O 0.5% (w/v), MgSO$_4$·7H$_2$O (0.05%) and incubated at 30°C in continuous shaking condition for 12 days. The metabolites were then extracted from the fermented broth of ABST06 and AB-2 by solvent extraction method. Cell mycelia were separated by centrifugation at 4300×g for 10 min and the cell-free extract was mixed with an equal amount of ethyl acetate overnight, subsequently, the separated organic phase was subjected to evaporation in a rotary vacuum operator. The crude extract was subjected to GC–MS for the identification of potential compounds.

Gas chromatography–mass spectroscopy analysis (GC–MS analysis)

Ethyl acetate extract of ABST06 and AB-2 were subjected to the GC–MS analysis according to (Rathore et al., 2016). PerkinElmer Clarus 500 equipped with Elite-5 (5% Phenyl 95% dimethyl polysiloxane) capillary column of dimensions 30 m x 250 $\mu$m was used for analysis. Helium is used as a carrier gas at 1 ml/min. The mass range was 40–450 amu, operating at 70 eV. The column temperature was programmed initially at 60°C, then raised to 150°C for 2 min, and finally raised to 280°C for 5 min. The identified compounds were subjected to molecular docking studies.

In silico studies

Protein preparation

The input structure for New Delhi metallo-$\beta$-lactamase-1 of K. pneumoniae was obtained from the Protein Data Bank (PDB id: 4EXS). The protein preparation wizard in Maestro of the Schrödinger suite was used to prepare the input protein structure (Sastry et al., 2013). This procedure includes fixing the missing hydrogen atoms, ensuring proper charge/ionization state, protonation state for histidine residues, capping the termini and loop refinement through Prime, optimizing the hydrogen bonds, and energy minimization using the OPLSe force field. Water molecules beyond 5 Å of the protein surface were removed.

Ligand preparation

The chemical structure of the various identified compounds was retrieved from PubChem database or Yeast Metabolome Database (YMDB) (Jewison et al., 2012; Kim et al., 2019; Ramirez-Gaona et al., 2017). Table S1 gives the list of the various compounds along with their identifiers. LigPrep module of Schrödinger suite was used to generate the three-dimensional coordinates and conformers for all the compounds. Tautomeric states were generated with Epik which is based on the more accurate Hammet and Taft methodology (Greenwood et al., 2010; Shelley et al., 2007).
### Table 1. Cultural and biochemical characteristics.

| Name of the media                                | Streptomyces sp. ABST06                        | Streptomyces sp. AB-2   |
|-------------------------------------------------|------------------------------------------------|-------------------------|
| Inorganic salt starch agar (ISP-4)              | White colored spores                            | Grey colored mycelia    |
| Bennet’s agar                                   | No growth                                       | No growth               |
| Nutrient agar                                   | Ash colored mycelia                              | No growth               |
| Glycerol asparagine agar (ISP-5)                | Ash colored aerial mycelia                       | Ash colored aerial mycelia|
| Peptone yeast extract agar (ISP-6)              | Ash colored aerial mycelia, brown color of substrate mycelia | No growth               |
| Tyrosine agar (ISP-7)                           | Ash colored aerial mycelia, distinct brown pigment. | Ash colored mycelia, black pigment |
| Yeast malt extract agar (ISP-2)                 | Ash colored mycelia and black colored pigmentation | Ash colored mycelia    |
| Actinomycetes isolation agar                    | Ash colored, black pigment, luxuriant            | Negligible growth, white colored mycelia |
| Starch casein agar                              |                                                |                         |

**Morphological and biochemical characteristics**

|                               | Smooth surface | Spiny surface |
|-------------------------------|----------------|--------------|
| Scanning electron microscopy  |                |              |
| Starch hydrolysis             | Positive       | Negative     |
| Hydrogen sulphide (H₂S) production | Positive     | Negative     |
| Cellulase production          | Negative       | Negative     |
| Melanoid pigmentation         | Positive       | Negative     |

### Induced fit docking and ADME prediction

Induced fit docking protocol was used to dock the ligand molecules on to the protein. Grid generation was done using the Receptor Grid Generation module of the Schrödinger suite. Grid centre was determined based on the ligand in the X-ray crystal structure of the protein. The box size was set to 10 Å. The following residues in the binding pocket were constrained: His120, His122, Asp124, His189, and Cys208. The docking precision was set to XP (extra precision) and OPLSe force field was used. QikProp module in the Schrödinger suite was used to predict absorption, distribution, metabolism, and excretion (ADME) properties.

### Molecular dynamics simulations

Molecular dynamics simulations of the protein–ligand complexes were performed using Desmond (Schrödinger Release 2020-4). Each protein–ligand complex was placed in a cubic box. The size of the cubic box was set such that the distance between the outermost atom of the protein and the box was 10 Å. TIP3P water model was used for solvation. The system was neutralized by adding appropriate number of counter ions (Na⁺/Cl⁻) positioned randomly in the box. Minimization of the protein–ligand complexes were performed using minimization of the system using a hybrid method of the steepest decent and the limited-memory Brodyen–Fletcher–Goldfarb–Shanno (LBFGS) algorithms. After minimization, NPT simulations for 10 ns and final production run for 100 ns were performed using OPLSe force field parameter. The temperature of the system was maintained at 300 K and pressure of 1.01325 bar was maintained using Nose–Hoover coupling and isotropic scaling, respectively. Trajectories were written every 10 ps.

### Binding free energy

The binding free energy (MM-GBSA) was calculated using Prime-MMGBSA module of Schrödinger suite. Five snapshots from the MD trajectories (80th, 85th, 90th, 95th and 100th ns) were used as the input for performing MMGBSA analysis. The binding free energy of the ligands was given by the difference between the free energies of the protein–ligand complex (E\(_{\text{complex}}\)), apoprotein (E\(_{\text{receptor}}\)), and ligand (E\(_{\text{ligand}}\)).

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\Delta G_{\text{bind}} = E_{\text{complex}} - (E_{\text{receptor}} + E_{\text{ligand}})
\]

### Results and discussion

**Isolation and identification of potential endophytic actinomycetes**

During this study, 15 actinomycetes sp. were isolated from different parts of *S. trilobatum* using different media after three weeks of incubation. Among the 15, two strains (ABST06 and AB-2) isolated from the branches of *S. trilobatum* were shown to have *in vitro* \(\beta\)-lactamase inhibitor activity using iodometric test. The persistent blue-black colour in the wells indicates the inhibition of \(\beta\)-lactamase by the extracts ABST06 and AB-2. In addition, the synergy test indicates the positive for \(\beta\)-lactamase inhibitory activity exhibiting 17 mm and 15 mm ZOI for ABST06 and AB-2 extracts respectively, while the antibiotic control has 11 mm ZOI. The increment in the activity indicates the positivity for \(\beta\)-lactamase inhibitor activity (Figure S1). Both the strains were then selected for taxonomical investigation.

**Taxonomy of the active isolates**

**Cultural and biochemical characteristics.** Table 1 displays the taxonomy summary of the active isolates obtained. The cultural characteristics of the test strains on various media after incubation at 30 °C for 14 days were observed. From Table 1, it was clearly understood that both the isolates were different from their morphological and chemotaxonomic characteristics.

**Scanning electron microscopy**

Scanning electron micrograph of ABST06 was found to have a smooth surface and chain-like formation, while Streptomyces sp. AB-2 spore was displayed with a warty surface.
16s rRNA analysis

The results from the comparison of 16S rRNA nucleotide gene sequence of strains (1490 bp) with corresponding Streptomyces sequences showed a distinct phyletic line among the organisms in Streptomyces spp. Also, the isolate ABST06 (GenBank Accession Number MF188866) was closely related to the type strain of Streptomyces cheonanensis sharing a 16S rRNA gene sequence with 99% similarity. In addition, AB-2 (GenBank accession number: MF188865), shared 99% similarity with Actinomycetales bacterium AM030. This result from the 16S rRNA sequencing confirms that the identified ABST06 and AB-2 as a new strain belonging to Streptomyces spp.
Construction of phylogenetic tree

Evolutionary analyses and construction of a phylogenetic tree by using the MEGA5 software. The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The optimal tree with the sum of branch length = 0.02649447 was shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Zhang et al., 2015). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter model, and the tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances. The analysis involved 21 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1475 positions in the final dataset (Figure 1A and 1B).

Secondary metabolites profiles by GC–MS analysis

Ethyl acetate extract of ABST06 and AB-2 were analyzed by GC–MS. The metabolomes were compared with the National Institute of Standards and Technology spectral library. Lists of compounds that are present in potential strains are given in Table S1.

Molecular docking of ligands to NDM-1

Molecular docking of the identified ligands to New Delhi metallo-β-lactamase-1 was performed using induced-fit docking procedure. Glide score was used to rank the ligands in terms of their binding affinity. Table 2 lists the top 10 ligands arranged in decreasing order of their affinity to New Delhi metallo-β-lactamase-1. Table S2 gives Glide score obtained for all ligands investigated. Table 3 shows the Glide score for the known inhibitors and drugs against New Delhi metallo-β-lactamase-1. The maximum Glide score for known inhibitors is –8.996 kcal/mol for clavulanic acid. This is lower than the top four metabolites in Table 2 indicating that the metabolites may bind competitively and preferentially to New Delhi metallo-β-lactamase-1. DL-Alanyl L-leucine has the highest Glide score (~12.1 kcal/mol) of all the ligands in our investigation. Figure 2 shows the interaction diagram of the top 3 ligands in the binding pocket of the protein. We observe that these ligands interact with the zinc ions and water molecules present in the binding pocket. Further, the interactions of these ligands with the protein were analysed. Figures 6 and 7 show the protein–ligand interactions and the percentage of occurrence of each of the interactions in the simulations. We observe that all of the three ligands interact with the zinc ions in New Delhi metallo-β-lactamase-1.
Metallo-β-lactamase-1 throughout the simulation. While dodecanoic acid and DL-alanyl L-leucine interact with New Delhi metallo-β-lactamase-1 only via zinc, phenyl propanedioic acid makes strong water-mediated interactions with the residues Cys208 and Asn220. Also, the interaction with the standard drugs, such as cefotaroline, meropenem and l-captopril exhibits zinc mediated interactions similar to the identified ligands. Residues Cys208 and Asn220 are known to be key
residues in the functioning of New Delhi metallo-\(\beta\)-lactamase-1 (Zhang & Hao, 2011). Further, Kim et al., suggest that New Delhi metallo-\(\beta\)-lactamase-1 interacts with its substrates only via the zinc in its active site (Kim et al., 2013). These studies lend support to our finding that the ligands might bind stably via zinc or water-mediated interactions to NDM-1.

Table 4. Glide score for higher generations of known drugs.

| S. no. | PubChem ID | Compound name | Glide \(G_{	ext{score}}\) (kcal/mol) |
|--------|------------|---------------|-------------------------------------|
| 1      | 44093      | l-Captopril   | -7.286                              |
| 2      | 9852981    | Ceftaroline   | -9.529                              |
| 3      | 441130     | Meropenem     | -12.561                             |

Table 5. Per residue energy contribution identified for the top 3 ligand.

| Residues interacting with ligand | dl-Alanyl l-leucine | Phenyl propanedioic acid | Dodecanoic acid |
|----------------------------------|---------------------|--------------------------|----------------|
| Val73                            | -1.1                | -1.6                     | -1.22          |
| Trp93                            | -1.34               | -1.22                    | -1.97          |
| His122                           | 5.44                | 2.67                     | 1.91           |
| Asp124                           | 15.24               | 67.54                    | 48.36          |
| His189                           | 1.91                | 5.634                    | 3.87           |
| Cys208                           | 22.25               | 67.67                    | 42.18          |
| Lys211                           | -14.23              | -52.08                   | -26.9          |
| Gly219                           | -0.7                | -3.33                    | -1.18          |
| Asn220                           | -3.02               | -15.59                   | -2.99          |
| His250                           | 5.39                | 7.01                     | 8.28           |
| Zn302                            | -79.62              | -206.57                  | -127.86        |
| Zn303                            | -83.15              | -201.29                  | -158.46        |

Figure 4. RMSD of c-alpha atoms of the protein complexed with (A) dl-alanyl l-leucine, (B) phenyl propanedioic acid and (C) dodecanoic acid as a function of simulation time.
Figure 5. RMSD of c-alpha atoms of the protein complexed with (A) ceftaroline, (B) meropenem and (C) l-captopril as a function of simulation time.

Figure 6. Protein–ligand contact diagram for the identified ligands showing the percentage occurrence of the interactions in the simulations.
Binding free energy calculations

In addition to the interaction profile of the known drugs and the identified ligands with New Delhi metallo-β-lactamase-1 receptor, the binding free energy of the drugs and ligands was also calculated (Table 6). The order of binding free energy for the identified ligands is given by DL-alanyl-L-leucine < dodecanoic acid < phenyl propanedioic acid. Among the three identified ligands, DL-alanyl-L-leucine shows lower binding energy than any other known inhibitors (clavulanic acid, sulbactam and tazobactam). Dodecanoic acid exhibits lower binding energy than sulbactam and tazobactam whereas phenyl propanedioic acid shows lower binding energy than tazobactam. This shows DL-alanyl-L-leucine as the potent inhibitor on comparison with known inhibitors. However, the binding free energy of the identified ligands are higher than that of the higher generation drugs ceftaroline and meropenem.

The most common drug-resistant mechanism that has evolved among all the Gram-negative pathogens is the secretion of hydrolyzing enzymes such as β-lactamases that inactivate the antibiotics. Of these, NDM, produced by carbapenem-resistant bacteria is a serious threat (Gupte et al., 2014). Worldwide, researchers are bridging efforts to combat MDR by discovering new antibiotics and new enzyme inhibitors. To tackle this threat, the newer generation inhibitors avibactam and vaborbactam were introduced (van Duin & Doi, 2017). Vaborbactam in combination with imipenem having cyclic boronate pharmacophore was approved by FDA in 2017 for the treatment of complicated UTI (Lee & Baker, 2018). However, no activity was found against MBL-producing pathogens. Despite the great progress in developing inhibitors against different subfamily members of MBLs, there are no inhibitors in clinical studies now. Also, the New Delhi metallo-β-lactamase-1 gene is encoded on a readily transferable plasmid which facilitates its quick spread. This has, hence, become a cause for concern as carbapenems have the widest spectrum of activity among all β-lactam antibiotics and are used clinically as the last line of defense against MDR bacteria (King et al., 2012). The active site feature of MBLs features the presence of one or two zinc atoms. Hence the inhibitors are largely focused on the binding or chelation of the zinc ions. One such inhibitor, aspergillomarasmine A, a natural product was tested in a mice model. Andrew M. King demonstrated the activity of esters of 3-mercapto propionic acid as a covalent inhibitor that attaches to the active site of the Lys224 of B1 MBLs (King et al., 2014). Similarly, the other agent such as ebselen, a selenium compound, and bismuth sulfate is in trials. Also, VNRX-5133, a bicyclic boronate in combination with cefepime is now in phase 1 clinical trials that mimic the tetrahedral oxyanion formed during hydrolysis (Tooke et al., 2020). Despite these advancements in the development of MBL inhibitors, the specificity of the mechanism of action remains challenging. The biggest challenge in MBL inhibition is the large structural variation exhibited within and between the subclasses.
of MBL. The inhibitors of MBL were found to interact differently with different variants and thus efficient broad-spectrum MBL inhibitors even within subclass are not available so far. Therefore, to test if the ligands identified in our study can potentially bind to other MBLs in the same subclass, we docked them to VIM-2 (PDB id: 507N) and IMP-1 (PDB id: 6C6I), two other enzymes in subclass B1. Table 7 shows the docking summary for these enzymes. Both, DL-alanyl L-leucine and dodecanoic acid show lower binding energy compared to known inhibitors and drugs for both of the enzymes.

**Table 7. Summary of docking score and binding free energy for other classes of MBLs.**

| Compound name | PubChem CID | Glide Gscore (kcal/mol) | ΔG (bind) (kcal/mol) | Receptor for VIM-2 (507N) | Receptor for IMP-1 (3WXC) |
|---------------|-------------|-------------------------|----------------------|--------------------------|---------------------------|
| Clavulanic acid | 5280980     | −7.395                  | −27.19               | −8.123                   | −45.33                    |
| Sulbactam     | 130313      | −6.499                  | −25.26               | −8.116                   | −35.00                    |
| Tazobactam    | 123630      | −6.059                  | −41.50               | −8.316                   | −42.75                    |
| α-Alanyl l-leucine | 23615548    | −9.954                  | −35.69               | −9.16                    | −53.23                    |
| Phenyl propanedioic acid | 75791    | −7.615                  | −20.42               | −8.804                   | −52.06                    |
| Dodecanoic acid | 3893       | −6.889                  | −55.74               | −8.75                    | −33.25                    |
| I-Captopril   | 44093       | −6.394                  | −40.64               | −9.905                   | −59.25                    |
| Ceftaroline   | 9852981     | −12.464                 | −35.37               | −12.464                  | −124.85                   |
| Meropenem     | 441130      | −11.061                 | −48.4                | −10.964                  | −86.16                    |

**Table 8. Summary of QikProp descriptors identified for the top three ligands.**

| PubChem ID | #stars | Mol. wt | QPlogPo/w | QPlogS | QPPCaco | QPlogBB | PHOA |
|------------|--------|---------|-----------|--------|---------|---------|------|
| 23615548   | 0      | 202.253 | −1.941    | −0.66  | 9.578   | −0.898  | 33.14|
| 75791      | 1      | 180.16  | 2.07      | −1.488 | 12.494  | −0.98   | 58.695|
| 3893       | 3      | 200.32  | 3.748     | −3.695 | 284.175 | −1.014  | 92.808|

Drug-like properties of the identified ligands

The ADMET properties of the top three ligands were predicted using QikProp (Table 8). QikProp prediction for all the ligands are summarised in Table S4. Among the seven descriptors from QikProp, all the three ligands show at least four acceptable drug-like properties.

This includes the most important properties such as molecular weight, partition coefficient, water solubility and human oral absorption rate. Brain/blood barrier partition coefficient values (~−1) are very close to the higher marginal range (~−1.2). Gut blood barrier values are also better for dodecanoic acid compared to other two identified ligands. Overall, dodecanoic acid shows more acceptable drug like property than the other two ligands.

Concluding remarks

The secondary metabolites produced by the actinomycetes group are widely used to treat infections. Especially, *Streptomyces* spp. produces novel metabolites with diverse chemical structure and biological activity, widely isolated from different sources like terrestrial soil, rhizosphere, marine sediments, and endophytes (Solecka et al., 2012). We have isolated the *Streptomyces* sp from the stem portion of the medicinal plant, *Solanum trilobatum*. The isolates were initially screened for anti-Klebsiella activity and β-lactamase inhibitory activity by iodometric assay. Followed by this, the isolates ABST06 and AB-2 were identified as a new strain that belonged to *Streptomyces* spp. The metabolomes of the strains were identified by GC–MS. A total of 46 compounds were identified in the metabolome. Molecular docking and molecular dynamics simulations were done to identify which of these compounds can bind stably to New Delhi metallo-β-lactamase-1. Molecular dynamics simulations reveal that DL-alanyl l-leucine, phenyl propanedioic acid and dodecanoic acid stably bind to New Delhi metallo-β-lactamase-1 and make zinc-mediated and water-mediated interactions in the binding site of the enzyme. These top 3 compounds were shown to have greater affinity than that of the known inhibitors (sulbactam, tazobactam, clavulanic acid and I-captopril) for New Delhi metallo-β-lactamase-1. The metabolic data showed that the top 3 compounds are present in *Streptomyces* sp. AB-2, whereas ABST06 has shown to have DL-alanyl l-leucine alone. Also, when compared with ABST06, the strain AB-2 was shown to have a minimal antibacterial activity (ZOI = 10 mm) against *K. pneumoniae*. *Streptomyces* sp. with β-lactamase activity was reported to have weak antibacterial activity.

The ADMET properties of the identified ligands were also analysed. Of these three ligands, dodecanoic acid was found to have better drug-like properties as inferred from the ADMET predictions. Dodecanoic acid, more commonly known as lauric acid, is also a well-known antimicrobial agent. It has been shown to have antimicrobial properties against...
Gram-positive bacteria and Gram-negative bacteria (Matsue et al., 2019; Nakatsuji et al., 2009). Nevertheless, DL-alanyl l-leucine, phenyl propanedioic acid and dodecanoic acid were not reported to have New Delhi metallo-β-lactamase-1 inhibitory activity. Here, we propose that these compounds may be taken as lead compounds for further testing.

Acknowledgements
The authors acknowledge infrastructural support through the SERB, DST for financial support for funding this research and the DBT grant (BT/PR23795/BID/7/797/2017) to carry out computational studies and SASTRA Deemed to be University for Schrodinger software (https://www.schrodinger.com/) support.

Funding
Science and Engineering Research Board (SERB), Department of Science and Technology, New Delhi (EMR/2016/007613) to JP. DBT grant (BT/PR23795/BID/7/797/2017) Junior Research Fellowship for AD.

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