



***IN SILICO* ANALYSIS OF *STREPTOMYCES* SP SECONDARY METABOLITE  
1, 2-BENZENEDICARBOXYLIC ACID, MONO (2-ETHYLHEXYL)  
ESTER WITH ESBL PROTEINS**

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**ABSTRACT**

The anti-ESBL activity of 1, 2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester (DMEHE) extracted from marine *Streptomyces* sp strain VITSJK8 was further confirmed by *in silico* analysis. A total of 10 ESBL proteins was chosen for this study from the protein data bank (PDB) and were docked with the DMEHE. The resultant atomic contact energy (ACE) was compared with dockings of known antibiotic ertapenem, penicillin G, amoxicillin, rocephin and ceftazidime. PatchDock online docking server was utilized for docking studies. LigPlot+ and PyMOL were used to evaluate the docking results obtained from PatchDock. The docking of DMEHE with ESBL proteins showed the ACE value range from -198.42 to 22.62, while docking of ertapenem showed the ACE value range from -217.67 to -71.8. DMEHE formed hydrogen bonds with AMPC, CTXM-9, TEM-1 and TEM-52 ESBL proteins. The interaction between DMEHE and ESBL protein complex was unstable and no covalent bond formation was observed, whereas ertapenem and ESBL complex was highly stable because of the covalent bond and hydrogen bond formation in the ligand-protein interaction. Similarly penicillin G, amoxicillin, rocephin and ceftazidime also demonstrated higher stability with ESBL proteins compared to DMEHE. Based on this study, it could be concluded that bacterial proteins will not involve in lysis of DMEHE and therefore DMEHE would be an effective drug for treating the drug resistant ESBL pathogens.

**KEYWORDS:** *In silico* analysis, anti-ESBL activity, 1, 2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester, PatchDock.



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## INTRODUCTION

Extended-spectrum beta-lactamases (ESBL) enzymes are mostly produced by drug resistant pathogen which causes severe infection in humans and existing threats, challenging the medical profession across the world<sup>1, 2</sup>. It usually affects the urinary tract and intestine and the treatments require different class of antibiotics. Mostly gram negative pathogens like *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella* species and other members of *Enterobacteriaceae* produces enzymes through mutated gene of plasmid and chromosome. These mutant enzymes have shown different levels of resistance to many antibiotics such as cefotaxime, ceftazidime, cephalosporins and aztreonam<sup>2, 3</sup>. The mutated enzymes are the reason for the outbreak of specific infections. Several nosocomial transmissions and outbreaks because of improper procedure have been followed for cleaning the equipments used. A remarkable resistance to antibiotics has been noted in *Escherichia coli* and *Klebsiella* species specific infection (ESBL-EK)<sup>2, 4, 5</sup>. Mutation in ESBL genes has been evolving over a period of time and antibiotic resistance increased which demands the need for new and effective drug against ESBL pathogens. Selvameenal et al., 2009 reported that *Streptomyces hygroscopicus* isolated from desert soil, demonstrated promising antibacterial activity against ESBL pathogens. Similar anti-ESBL activity was reported by Aruna et al., 2013 using secondary metabolites of actinomycetes. There are reports of antibacterial activity of squid ink on ESBL pathogens such as *Escherichia coli* and *Klebsiella pneumoniae*<sup>6, 7, 8, 9, 10</sup>. Although the hunt for novel anti-ESBL compounds are still in progress, the studies on mechanism of inhibition of ESBL proteins by the bioactive metabolites was not being reported till date. *In vitro*, *in vivo* and *in silico* procedures, combination of these techniques might possibly assist in knowing the mechanism of action of the drug. Docking is a computational simulation process used to analyse the interactions of proteins or between a protein and a compound. This *in silico* analysis has the additional advantage than *in vitro* and *in*

*vivo* procedures for the assessment of mechanism of action. *In silico* molecular docking via Autodock 4 and PatchDock has been increasing steadily in the recent past. *In silico* analysis provides a lot of hypothesis and suggestions which eventually result in several advancements in the field of drug discovery. In the present study an attempt was made to study the interaction between DMEHE to different ESBL proteins and compared with the interaction of anti-ESBL antibiotics.

## MATERIALS AND METHODS

### (i) Compounds from *Streptomyces sp. VITSJK8*

The anti-ESBL activity of ethyl acetate extract of the isolate *Streptomyces sp. VITSJK8* against ESBL producing pathogens was reported previously<sup>5</sup>. The interaction of DMEHE with ESBL drug target protein was studied with *in silico* molecular docking approach.

### (ii) Ligands and Target Proteins

The 3D structure of the ligand DMEHE was drawn using the MarvinSketch tool through Ligand-Expo. Ertapenem (03918453), penicillin-G (03871702), amoxicillin (19850111), rocephin (28467879), cefozopran (03871946) were obtained from ZINC database as MOL2 files and were converted to pdb file format using PyMOL. ZINC is a permitted database of commercially available compounds for virtual screening provided by the Shoichet Laboratory in the Department of Pharmaceutical Chemistry at the University of California, San Francisco (UCSF)<sup>11</sup>. The target proteins PER-2, SHV-1, CTX-M-9, CTX-M-14, OXA-10, TEM-52, AmpC, TEM-1, CTX-M-16 and CTX-M-27 pdb files were obtained from PDB data bank.

### (iii) Molecular Docking Studies

The ligand-protein interactions were performed using an online docking tool called PatchDock. Both the ligand and protein pdb files were uploaded in the PatchDock webserver and the docking results were obtained through the e-mail which has the details of docking score and ACE value.

Results of the highest docking score were chosen for this study. PyMOL tool was used to view the protein molecules, to remove water molecules and to change the format of ligand structures. The obtained docking solutions (PDB format) were viewed using PyMOL tool. LigPlot+ tool was used to compare the docking solutions obtained from PatchDock. Number of hydrogen and covalent bonds between docking solutions were counted using LigPlot+.<sup>12, 13, 14, 15, 16</sup>

## RESULTS AND DISCUSSION

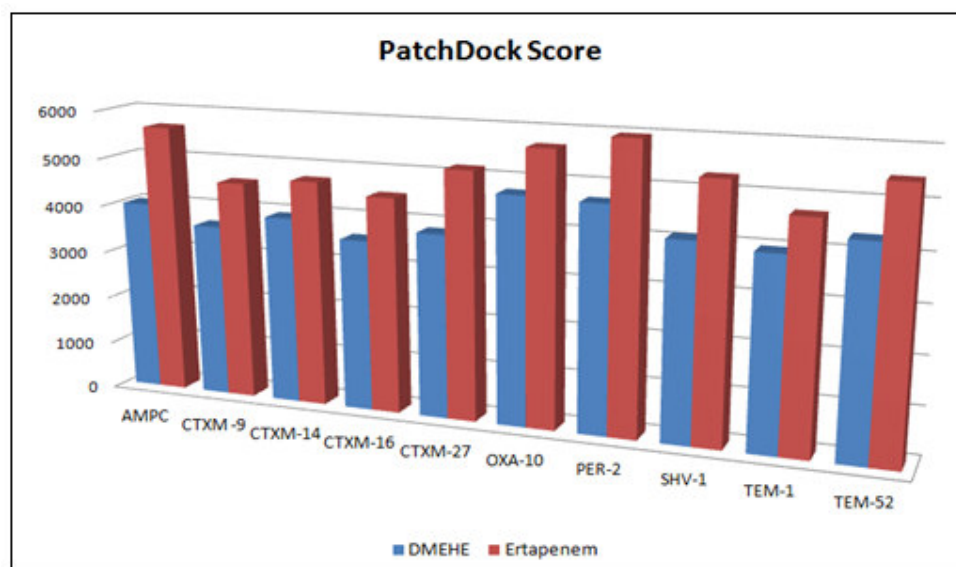
### (i) Structure of DMEHE

The structure of the compound was identified based on 90% similarity between the MS spectra of the unknown compound and reference compounds available in the MS spectra library of NIST (National Institute for Standards and Technology). The compound was identified as 1, 2-Benzenedicarboxylic Acid, Mono (2-Ethylhexyl) Ester (DMEHE, C<sub>16</sub>H<sub>22</sub>O<sub>4</sub>) and 3D structure was obtained from MarvinSketch tool through Ligand-Expo. 2D and 3D orientations were adjusted using the clean 2D and clean 3D function in MarvinSketch and saved in pdb file format.

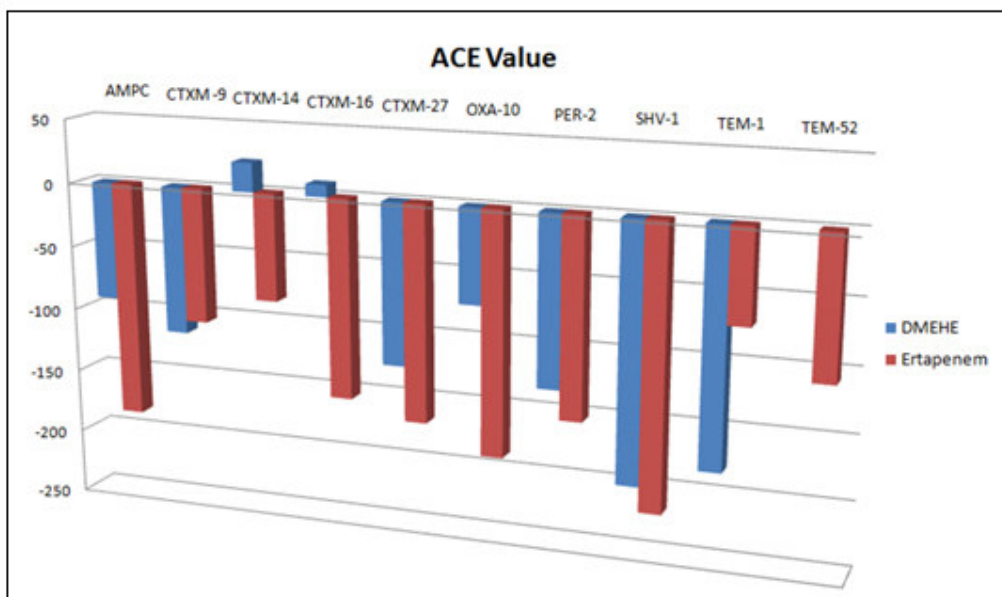
### (ii) Molecular docking studies

PatchDock scores showed the interaction of DMEHE with ESBL proteins were in the range

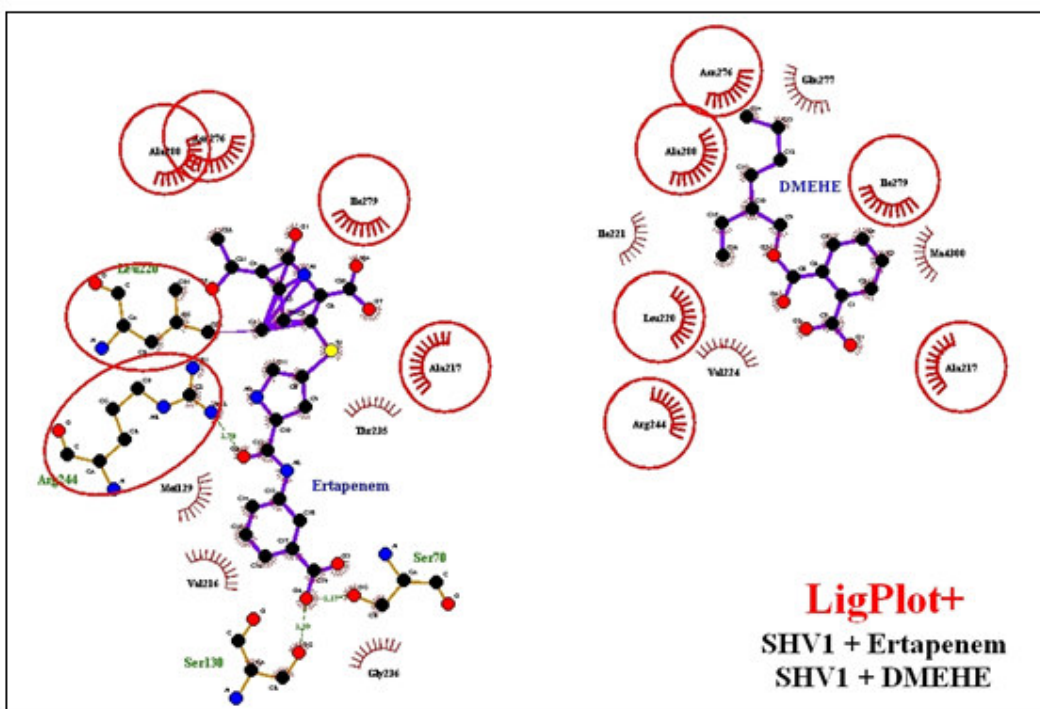
of 3582 to 4332. Interactions were considered as weaker when compared to dock score between ESBL proteins and ertapenem which were in the range of 4504 to 5964 (Fig.1). The scores were based on a rigid and/or real life docking for protein-to-protein or protein-to-drug algorithm as developed by Duhovny et al., 2002. The algorithm permits surface flexibility and also interpret intermolecular penetrations. ACE value of a docking result reveals the energy required for the interaction. Lower the ACE value, the higher rapidness for the interaction. Ertapenem showed ACE values between -217.67 to -71.8 Kcal/mol, which has higher interactions towards the ESBL proteins and DMEHE showed ACE values between -198.42 to 22.62 Kcal/mol with ESBL proteins (Fig.2). ESBL protein SHV1 showed least binding energy with DMEHE and Ertapenem. The number of hydrogen bonds formed between the ligand and protein molecule is also considered as one of the factors used for measuring the complex. The LigPlot+ showed the DMEHE and ertapenem interaction with SHV1 (Fig.3). DMEHE formed hydrogen bonds with AmpC, CTX-M-9, TEM-1 and TEM-52. Ertapenem formed hydrogen bonds with all the ESBL proteins except TEM-1 and TEM-52 and formed covalent bond with all the 10 ESBL proteins which demonstrates its stability and strength of the interaction.



**Figure 1**  
**Comparison of PatchDock score of DMEHE and ertapenem with ESBL proteins**



**Figure 2**  
*Comparison of ACE value of DMEHE and ertapenem with ESBL proteins*



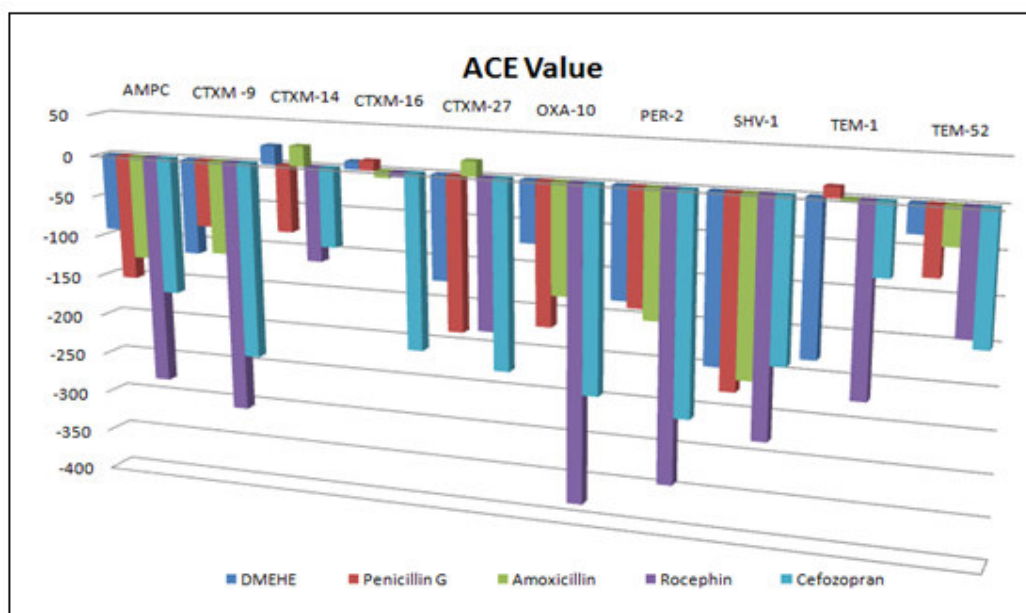
**Figure 3**  
*LigPlot+ analysis of DMEHE and ertapenem with SHV1*

DMEHE and ertapenem the respective site of interaction were observed between the AmpC, CTX-M-9, TEM-1 and TEM-52 proteins. The length of their interaction between the ligand and the amino acid of ESBL proteins was also observed respectively. DMEHE formed hydrogen bond with Val192 (2.76Å) of AmpC

protein, whereas ertapenem formed hydrogen bond with Gln120 (2.44Å) and covalent bond with Asn347 of AmpC protein. Similarly with the protein CTX-M-9, DMEHE formed hydrogen bond with Gln30 (2.70Å) and Leu59 (2.53Å), whereas ertapenem formed hydrogen bonds with Arg276 (2.89Å), Ser237 (2.78Å),

Pro167 (3.22Å) and covalent bond with Ser237. DMEHE formed hydrogen bond with Arg43 (2.67Å) of TEM-1, while ertapenem formed covalent bonds with Glu104 and Val216. DMEHE formed hydrogen bond with Ser70 (2.95Å) and Ala237 (2.48Å) of TEM-52 and ertapenem formed covalent bonds with Gln39, His26 of TEM-52. Among these interactions ertapenem showed the strongest binding energy -183.56 Kcal/mol, with AmpC. The other antibiotics penicillin G, amoxicillin, rocephin and ceftazidime too had a strong interaction with ESBL proteins when compared to DMEHE. Penicillin G had a PatchDock score of 3774 to 4892 followed by amoxicillin (3674 to 4994), rocephin (4860 to 6096) and ceftazidime (4130 to 6054), which are considerably higher than the DMEHE

score. As shown in Fig.4, penicillin G had an ACE value from 11.46 to -226.02 followed by amoxicillin (24.01 to -211.01 Kcal/mol), rocephin (-5.67 to -380.05 Kcal/mol) and ceftazidime (-83.21 to -263.19 Kcal/mol). These are highly significant compared to DMEHE, ACE value of 22.62 to -198.42 Kcal/mol. Considering the PatchDock score, ACE value, hydrogen and covalent bond formation, DMEHE had very poor interaction and stability with ESBL proteins when compared to penicillin G, amoxicillin, rocephin and ceftazidime. These four antibiotics also had shown several hydrogen and covalent bond formation with ESBL proteins similar to ertapenem signifying that these antibiotics can be hydrolysed by ESBL proteins easily.



**Figure 4**  
*Comparison of ACE value of DMEHE and antibiotics with ESBL proteins*

## CONCLUSION

Based on the results, it can be concluded that the interactions between DMEHE and ESBL target proteins suggested its mechanism of action.

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