SCREENING OF POTENTIAL INHIBITORS OF *SALMONELLA TYPHI* BETA-LACTAMASE TEM 1 USING VIRTUAL SCREENING AND DOCKING STUDIES

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Abstract- The project was undertaken to tackle one of the most alarming situations of growing antimicrobial resistance among pathogenic bacteria against various classes of antibiotics. Due to easy access of antibiotics and lack of strict drug regulations in developing countries like India, the situation is even more alarming than that of developed nations. This leads to importance and need for development of alternative and novel therapeutics. In this study we have identified four potential inhibitors (Pubchem ID's: 30331, 30340, 30514, and 25685) of S. typhi Beta-lactamase TEM 1 and compared the binding of these inhibitors with penicillin (a natural inhibitor) using in silico docking and molecular dynamics studies.

Index Terms— Antibiotic resistance, Docking, *Salmonella typhi*, Virtual screening

1. INTRODUCTION

In recent years, Multiple Drug Resistance (MDR) has emerged as a major concern in the medicine across the globe. The unsupervised and irrational use of antibiotics in poultry, medicine, and agriculture has resulted in the development of genetic mechanisms which has further enhanced the problem of resistance among gram-negative and gram-positive pathogens [1], [2]. The isolation frequency of the multiple antibiotic resistant Salmonella has been on the rise in the UK [3], and US [4]. The resistant genes are generally present on the plasmids which can then be transferred via plasmids or integrons or transposons mediated mechanisms [2]. The antibiotic resistance problem is a serious matter of concern in the case of developing countries which is due to lack of resources, unregulated use, easy access and lack of regulatory procedures have added significantly in developing resistance in developing countries which is a serious matter of concern [5]. Only a few studies have been conducted till date for the analysis of resistance levels of antimicrobials against Salmonella species in developing nations [5], [6], [7], [8], [9]. Also, the depth of resistance mechanisms in the resistant bacteria phenotypes has yet to be studied. The bacterial infection analysis from international travelers as an indirect source of information has revealed the characteristics and current scenario of the infections in the developing countries. Resistance to various classes of antibiotics such as tetracycline, ampicillin, penicillin's, streptomycin's or chloramphenicol's has been reported in concerning frequencies [10], [11]. Resistance to antimicrobial agents in Salmonella typhi [12] due to plasmid transfer mechanism [13, 14] has led the practitioners to use fluoroquinolones as an important alternative but recent reports suggest an increase in nalidixic acid (NAL) resistance strains globally which have resulted in decreased susceptibility of microbes to ciprofloxacin [15], [16], [17]. The emergence of decreased ciprofloxacin susceptibility (DCS) has lead to delayed infection clearance and treatment failures [16, 18, and 19]. It has also been reported that Salmonella strains are gaining resistance to quinolones from the samples isolated from international Indian travelers as result of using nalidixic acid for the treatment of Salmonella infections [11], [12], [13], [14], [15], [16], [17], [18], [19], [20]. The alarming situation evolved due to bacterial antibiotic resistance has resulted in urgent requirement for advancements in therapeutics.

In view of the above considerations, the current project is designed to identify novel molecules which can act as potential Beta-Lactamase inhibitors by rational drug designing using *in silico* virtual screening and docking studies.

2. MATERIALS AND METHODS

2.1 Retrieval of 3D structure of S. typhi Beta-lactamase TEM 1

The homology modeled three dimensional structure of S. typhi Beta lactamase TEM-1 was obtained from the work done by Rakesh *et. al.* [21].

2.2 Screening of the compounds based on drug-likeliness properties

All the compounds were selected for docking studies on the basis of their drug likeliness properties such as Lipinski's rule of five Ghose filters, veber filters, weighted and unweighted QED using DruLiTo tool.

2.3 Molecular docking studies of the penicillin and compounds at the active site of the receptor (Beta-lactamase TEM-1)

All the drugs were docked at the active site of the receptor i.e. Ser 68 using Autodock 4.2 [22] docking tool. Various docked poses were analyzed with DeLano Scientific PyMol 3D molecular viewer distributed by Schrodinger. Best docked poses were selected based on docking rank and various hydrogen bonded interactions were analyzed with Biovia Discovery Studio Visualizer (Dassault Systèmes, 2015).

3. RESULTS AND DISCUSSION

The docking studies of Penicillin at the active site were performed with the help of Autodock4.2 using Lamarckian Genetic Algorithm. Docking analysis (Fig. 1) revealed that the penicillin makes 7 H-bonds with the surrounding residues and the bond length of the penicillin with the active site (Ser 68) was calculated to be 2.2 Å.

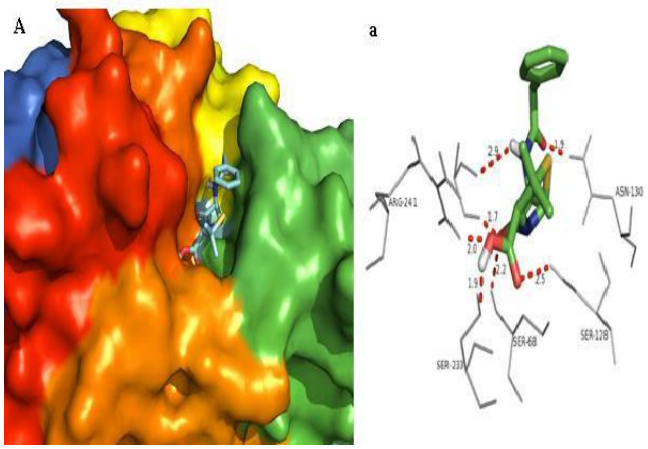

Fig. 1: Docking studies.

The docking analysis of Penicillin at the active site of *S. typhi* Beta-lactamase TEM 1 showing the best binding conformation. Surface view representing binding mode of Penicillin (A) and non-bonded interactions of Penicillin (a) with the active site residues of S. typhi Beta-lactamase TEM 1.

Total 16035 compounds were downloaded from Pubchem database for virtual screening. Out of 16035 compounds 8728 compounds were selected for docking studies on the basis of their drug likeliness properties such as Lipinski's rule of five Ghose filters, veber filters, weighted and unweighted QED.

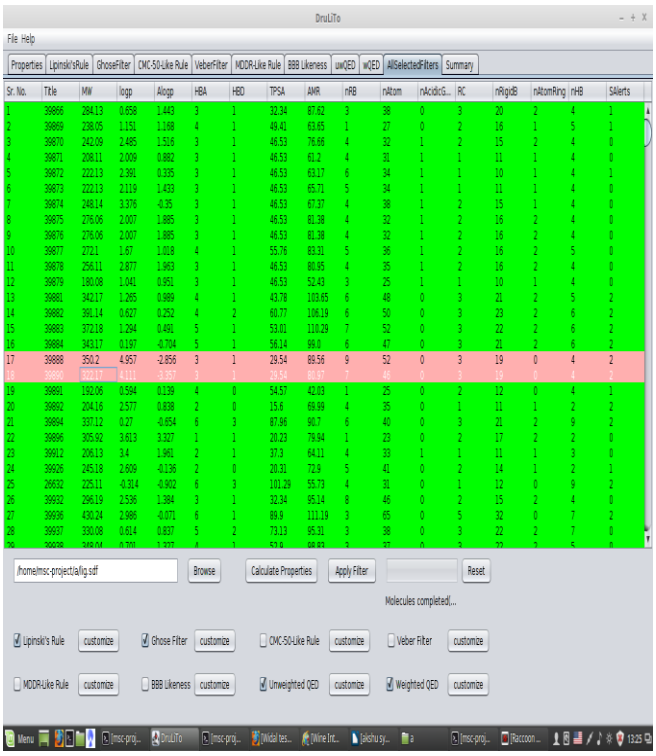

Fig. 2: DruLito screening

All the 8728 compounds were then batch docked at the active site of Beta-Lactamase TEM 1 using Autodock 4.2.

The best 50 docked compounds were selected on the basis of binding energies and H- bond distance. The docking results were then compared to penicillin–Beta-Lactamase docking parameters and 17 best docked complexes were selected on the basis of their shorter H-bond interaction with the active site and lesser ΔG values then penicillin–Beta-Lactamase complex.

Further analysis of the docking results showed that 4 compounds PMCID 30331, 30340, 30514 and 25685 had least energies ranging from -5.4 kJ/mol to -6.78kJ/mol making 5 H-bonds with the active site of the receptor. This indicate better binding of the compounds at the active site of Beta-lactamase TEM1 as compared to penicillin (ΔG= 4.7 and number of H-bonds= 5). The above results showed that all the compounds made similar number of H-bonds with the receptor but were complexes at comparably low energy than that of penicillin which indicates stability of the complex.

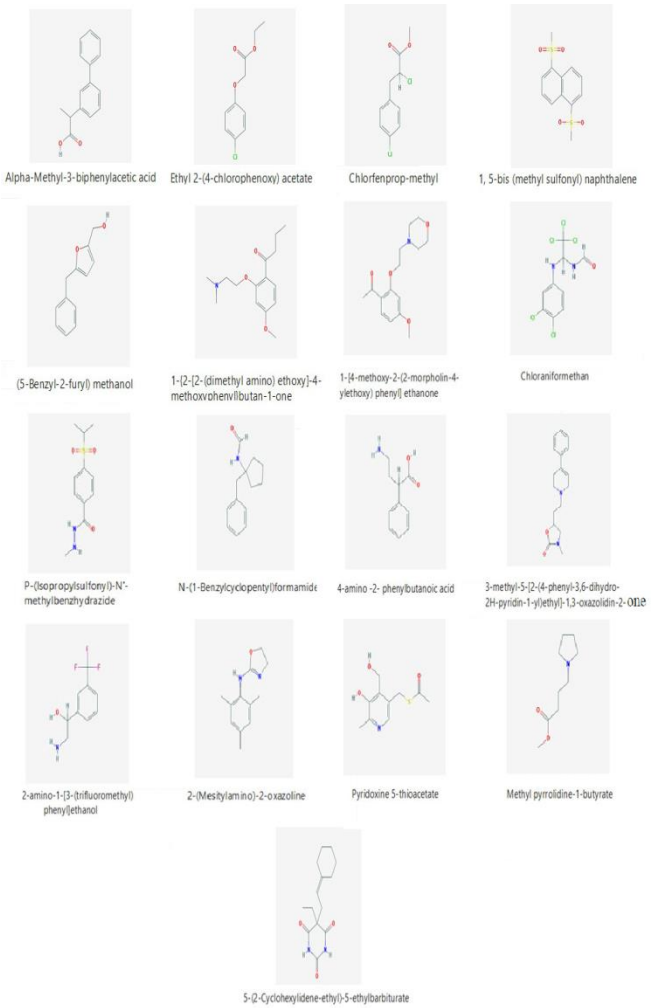

Fig. 3: 2D representation of the best docked Pubchem compounds.

This also indicates that the ligands (PMCID 30331, 30340, 30514 and 25685) can also be verified in-vitro to validate their Beta-Lactamase TEM 1 inhibitor potential and can be further developed into potential drug candidates.

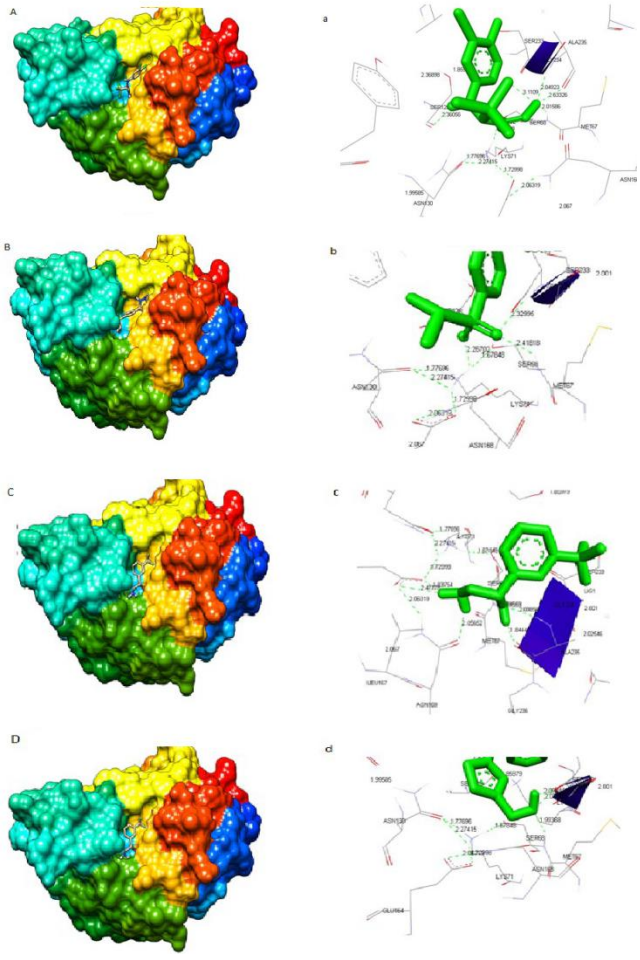

Fig. 4: In silico docking. Surface view representing binding mode of antimicrobial peptide (A) and non-bonded interactions of the antimicrobial peptide (a) with the active site residues of S. typhi Beta-lactamase TEM 1.

Table 1: List of best docked compound with number of hydrogen bonds and binding energy.

S.NO	PC ID'S	LIGAND NAME	NO. OF H-BONDS	BINDING ENERGY
1	40045	Alpha-Methyl-3-biphenylacetic acid	4	-5.2
2	26689	Ethyl 2-(4-chlorophenoxy)acetate	2	-5.29
3	26693	Chlorfenprop-methyl	2	-5.42
4	40625	1,5-bis(methyl sulfonyl)naphthalene	3	-6.4
5	30149	(5-Benzyl-2-furyl)methanol	3	-5.44
6	30275	1-{2-[2-(dimethylamino)ethoxy]-4-methoxyphenyl}butan-1-one	2	-5.53
7	30312	1-[4-methoxy-2-(2-morpholin-4-ylethoxy)phenyl]ethanone	2	-5.42
8	30331	Chloraniformethan	5	-6.3
9	30340	P-(Isopropylsulfonyl)-N'-methylbenzhydrazide	5	-6.39
10	30380	N-(1-Benzylcyclopentyl)formamide	4	-5.5
11	30514	2-amino-1-[3-(trifluoromethyl)phenyl]ethanol	5	-6.78
12	30537	2-(Mesitylamino)-2-oxazoline	1	-5.02
13	25685	4-amino -2- phenylbutanoic acid	5	-5.4
14	30822	3-methyl-5-[2-(4-phenyl-3,6-dihydro-2H-pyridin-1-yl)ethyl]-1,3-oxazolidin-2-one	2	-7.3
15	30855	Pyridoxine 5-thioacetate	4	-5.5
16	30905	Methyl pyrrolidine-1-butyrate	3	-4.9
17	30964	5-(2-Cyclohexylidene-ethyl)-5-ethylbarbiturate	4	-6.8

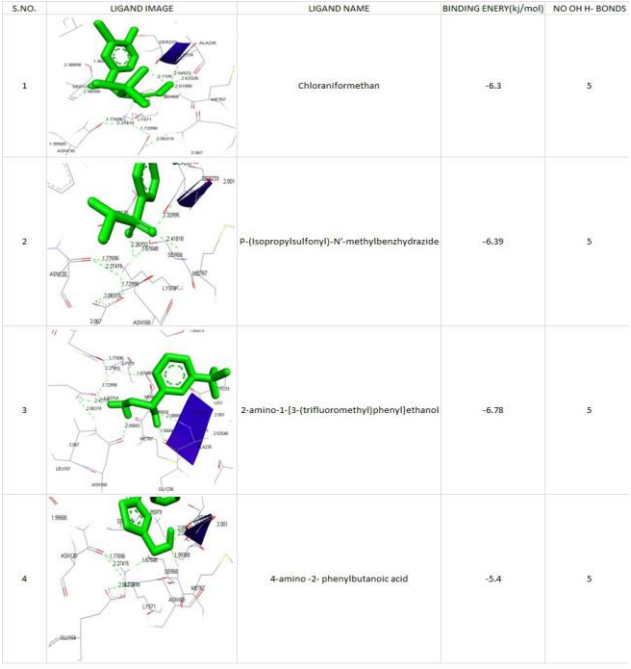

Fig. 5: Four best docked analyzed compounds representing least binding energy.

4. CONCLUSION

The project was undertaken to tackle one of the most alarming situation of growing antimicrobial resistance among pathogenic bacteria against various classes of antibiotics. Due to easy access of antibiotics and lack of strict drug regulations in developing countries like India, the situation is even more alarming than that of developed nations. This leads to importance and need for development of alternative and novel therapeutics. In this study we have identified four potential inhibitors (Pubchem ID: 30331, 30340, 30514, and 25685) of *S. typhi* Beta-Lactamase TEM 1 and compared the binding of these inhibitors with penicillin (a natural inhibitor) using *in silico* docking and molecular dynamics studies. The docking studies of penicillin and compounds were performed and the best poses were selected based on the interaction of the compounds with the active site of the receptor. The docking studies reveal that the screened compounds binds more closely to the active site cavity (fig. 5) than penicillin which indicates better efficiency of the compounds as compared to the penicillin.

It is concluded from the study that these compounds can be developed in a potential inhibitors of *S. typhi* Beta-Lactamase TEM 1. The dynamics of the complexes can be further analyzed by molecular dynamics simulation studies. It is suggested that further *in-vitro* analysis of the compounds can be performed to validate the antimicrobial activity of these compounds. It is also suggested that modifications in the above compounds can be performed to enhance their pharmacokinetic and pharmacodynamic activity.

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