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IN-SILICO DOCKING ANALYSIS OF BETA LACTAMASE FROM KLEBSIELLA PNEUMONIAE WITH VARIOUS INHIBITORS.

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ABSTRACT

The Urinary Tract Infection (UTI) is a prevalent disease whose misdiagnosis may lead to kidney disease and even pyelonephritis. *Klebsiella pneumoniae* is one of the most important causative pathogens of UTIs. Penicillin derivatives and Cephalosporins are the commonly prescribed antibiotics for UTI's. Beta-lactamases are a large family of hydrolases, which catalyse the hydrolysis of the amide bond in the β-lactam ring of Penicillin and Cephalosporin. The hydrolysis product, Penicilloic Acid or Cephalosporoic Acid, is biologically inactive. Drug resistance to therapeutic antibiotics pose a challenge for the treatment of infectious diseases. In this study, *in silico* docking of various ligands/inhibitors to the Beta-lactamases has been performed by using Autodock Suite.

KEYWORDS: Urinary Tract infections (UTI), *AmpC* & *Oxa* Beta-lactamase, Multi Drug Resistant (MDR), Docking.

INTRODUCTION

Bacterial infections have been the major cause of diseases throughout the history of human population. One of the common bacterial infection is Urinary tract infection (UTI), also called as bladder infection or acute cystitis. The causal agents include *Escherichia coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Saprophyticus* and *Enterobacter*. The drug resistant bacterial strains are currently a major health concern in treating bacterial infections.[1] The β- lactam antibiotics are generally prescribed to treat UTI's, but consequent and extensive use of antibiotics has evolved multi drug resistant (MDR) bacteria. Various

mechanisms have been developed by bacteria to resist the action of β-lactam antibiotics, among them the most common is the production of β-lactamases (E.C.3.5.2.6), which destroy the antibiotics before they reach the bacterial target [2,3,4]

Beta-lactamases are a large family of hydrolases that catalyse the hydrolysis of the amide bond in the β-lactam ring of penicillin and cephalosporin. The hydrolysis product, Penicilloic Acid or Cephalosporoic Acid, is biologically inactive. Primarily due to their clinical importance, β-lactamases have been extensively studied with respect to enzyme structure, function, induction, secretion, and transfer of genetic elements.[5-6]

Use of molecular docking is a cost effective strategy for speeding up the process of drug discovery and development process. Hence, understanding binding interactions between receptor and ligand is very essential for drug discovery.[7] Auto Dock abbreviated as AD, is an automated suite of protein-ligand docking tool. It is designed to predict the protein interactions with small molecules. AutoDock4 has been used in this study. It analyses the interactions of ligand molecules at the specified target sites of the proteins.

MATERIALS AND METHODS

In silico docking involves the use of sampling algorithm and a scoring function to evaluate the proper orientation and pose of ligand molecule in relation to the binding energy. The correct identification of this binding pose of one or more related ligands is important in establishing a structure-activity relationship in lead optimization. The second use of scoring functions is to rank different ligands to predict their relative experimental activity.[8-10]

Ligand selection

In silico studies were performed using Autodock4. The ligands viz., Chlorogenic Acid, Ellagic Acid, Gallic Acid, Nalidixic Acid, Quercetin and Standard Antibiotics viz. Ampicillin, Cefazoline, Ciprofloxacin, Levofloxacin, Nitrofurantoin, Norfloxacin, Sulfamethoxazole and Trimethoprim, were docked with *AmpC* & *Oxa* beta- lactamase enzymes of *Klebsiella pneumoniae.* The ligands and the standard antibiotics were selected on the basis of reported antibacterial activity and prescribed drugs respectively.

Protein/Target Selection

The *AmpC* (PDB Id 4H0D) & *Oxa* (PDB Id 3HBR) protein structure of *Klebsiella pneumoniae* beta-lactamase was downloaded from PDB (X ray diffraction of 1.498 & 1.9

respectively) fig (1). These models were further used to analyze and compare the effect of binding efficiency of Beta-lactamase towards commonly prescribed antibiotics as well as various inhibitors.[11] Next, the PubSum database yielded the ligands with their Ligplots. Ligplots give interacting sites of the *AmpC* and *Oxa* of *Klebsiella pneumoniae* beta-lactamase fig (4, 5). The structure of ligands were downloaded from Pubchem (chemical structure data base) online portal and drawn in Marvin Sketch version 5.8.1.Fig (2, 3).

After docking, the results were analyzed on the basis of their binding energy and their interactions.[11]

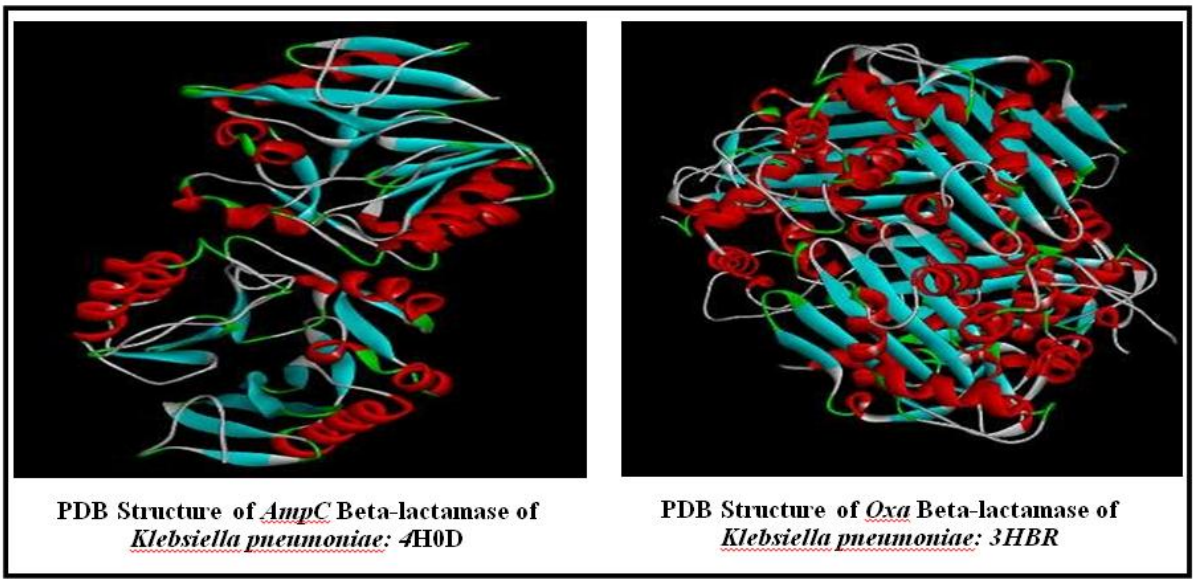

Fig. (1): PDB structure of *ampc* and *oxa* protein of *klebsiella pneumonia.*

Ligand preparation

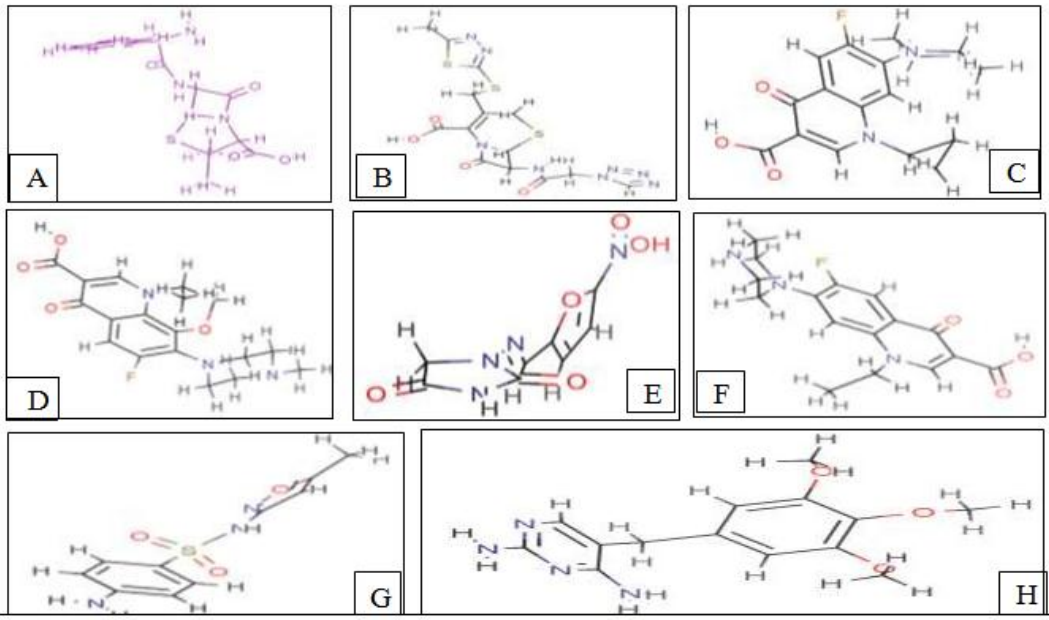

Fig (2): STRUCTURES OF ANTIBIOTICS: Ampicillin (A), Cefazoline (B), Ciprofloxacin (C), Levofloxacin (D), Nitrofurantoin (E), Norfloxacin (F), Sulfamethoxazole (G), Trimethoprim (H).

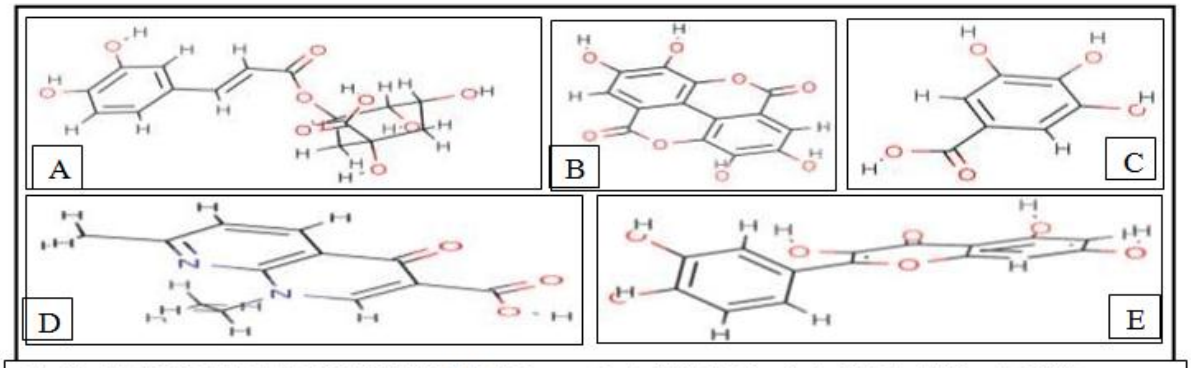

Fig (3): STRUCTURES OF INHIBITORS: Chlorogenic Acid (A), Ellagic Acid (B), Gallic Acid (C), Nalidixic Acid (D), Quercetin (E).

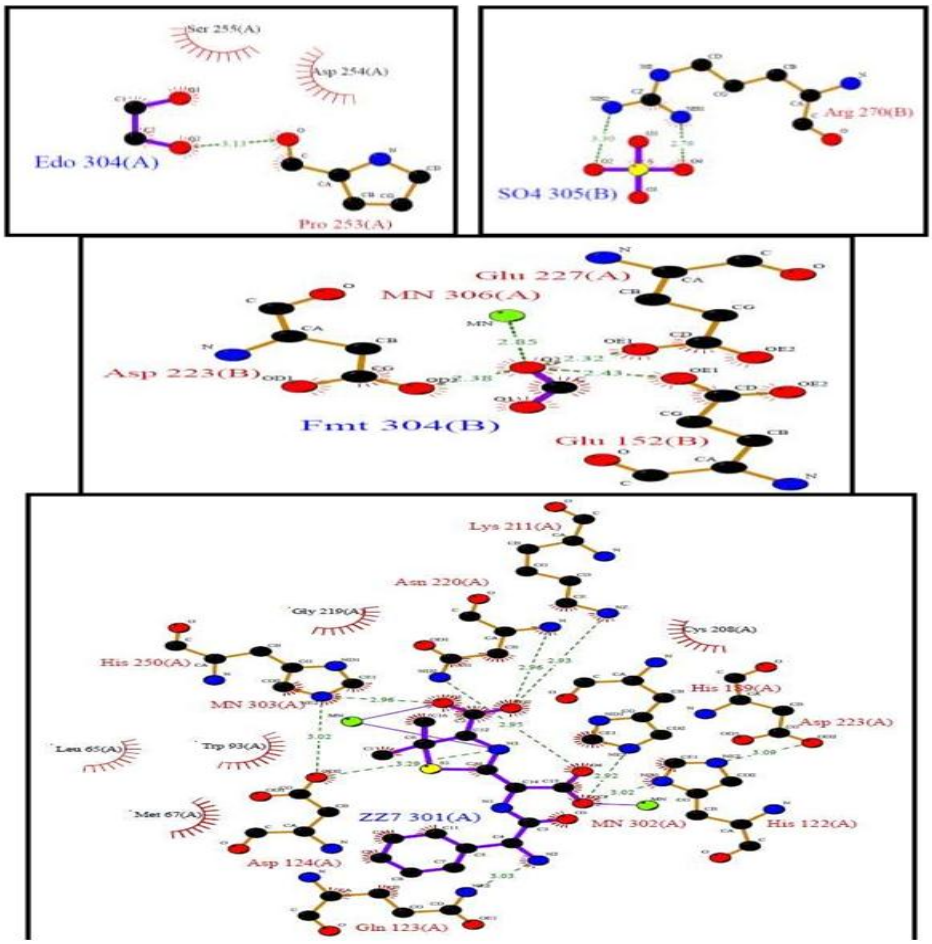

Fig (4): Ligplots of *AmpC* Beta-lactamase of *Klebsiella pneumoniae*. Ligand Edo 304(A) - (Pro253). SO4 305(B) - (Arg270). Fmt 304(B) - (Glu152, Asp223, Glu227). ZZ7 301(A) – (His122, Gln123, Asp124, His189, Lys211, Asn220, His250). Interactions are shown by green dotted lines.

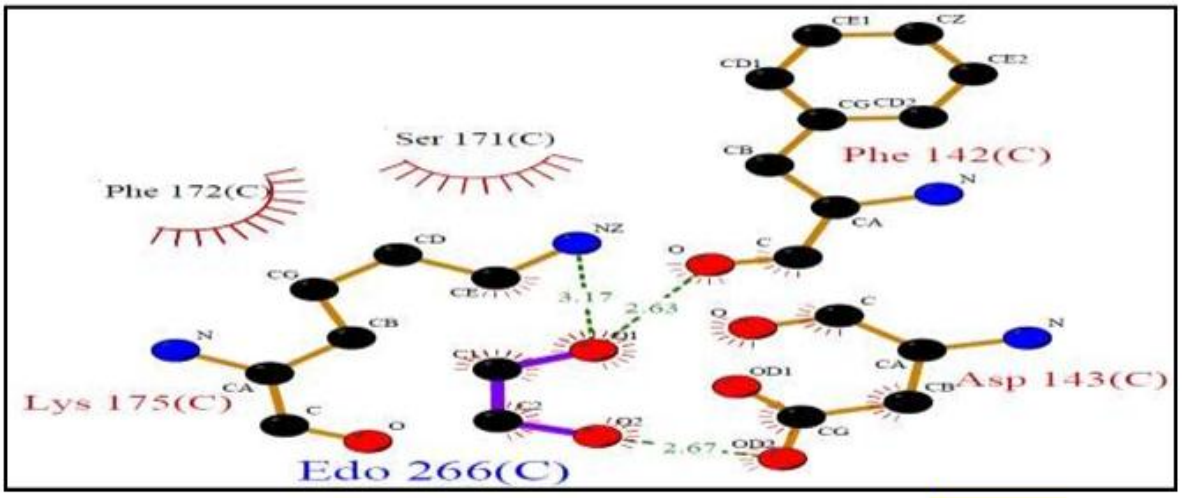

Fig (5): Ligplot of *Oxa* Beta-lactamase of *Klebsiella pneumoniae*. Ligand Edo 266(C) – (Lys175, Asp143, Phe142). Interactions are shown by green dotted lines.

Preparation protein/target

The Ligands viz., Chlorogenic Acid, Ellagic Acid, Gallic Acid, Nalidixic Acid and Quercetin and Standard Antibiotics viz., Ampicillin, Cefazoline, Ciprofloxacin, Levofloxacin, Nitrofurantoin, Norfloxacin, Sulfamethoxazole and Trimethoprim were prepared for docking. The ligands have exhibited prominent antibacterial activity towards isolated multidrug-resistant bacteria and hence were selected for molecular docking analysis.[11] The structures of *AmpC* & *Oxa* were opened in Biovia Discovery Studio 2016 version 16.1.0.15350. The structure of protein was cleared (i.e. the extra groups which includes water molecules, ligand groups were removed) by deleting the heteroatoms present in the protein.[12] Only the protein and active site for docking is required, hence was saved in the PDB format. The structure of ligands were downloaded from Pubchem and drawn in Marvin Sketch View version 5.8.1 and cleaned in 2D and 3D. This cleared the 2 dimensional and 3 dimensional structure of the Ligands. For docking, the protein structure was obtained in PDB format and Ligands in tripos-Mol format or PDB format.[12]

Grid Formation by autodock

Grid points generate the coordinates or interaction points where the ligand is docked. The grid box was generated at 60x60x60 A^0 to cover all the active site residues, and allowed the flexible rotation of ligands. The GA (genetic algorithm) and number of generation were set to 10 and 27000 for *AmpC* & *Oxa* respectively. The Lamarckian genetic algorithm was followed for ligand confirmation. All the above parameters decide the different confirmation of ligand in which the ligand will be docked. Other parameters for example, free energy, rotatable bonds (number of rotatable bonds varies depending on the ligand structure), number of torsions (12) etc. were used as default.[12]

RESULT AND DISCUSSION

Energy minimization

Docking studies revealed the interaction of the two selected proteins with the selected ligands, with respect to binding energy, type of interaction and amino acids involved in interactions. Binding energy should be ideally negative. More negative the binding energy, better the binding affinity of the ligand and the protein.[12] Table (1) represents the binding energy of various selected ligands with *AmpC* & *Oxa* beta-lactamase.

Table 1: Binding energy of ligands on docking with *ampc* & *oxa* beta- lactamase of *klebsiella pneumoniae*.

	***AmpC* Beta-lactamase**			***Oxa* Beta-Lactamase**		
Ligands	**Binding Energy**	**Interacting Sites**	**Run**	**Binding Energy**	**Interacting Sites**	**Run**
INHIBITORS						
Chlorogenic Acid	-7.81	Asp223	4	-4.16	Asp143	7
Ellagic Acid	-9.12	---	4	-5.60	Asp143	6
Gallic Acid	-6.72	Asp223, Glu227	9	-5.80	---	7
Nalidixic Acid	-8.56	Asp223, Glu227	8	-5.20	Lys175	9
Quercetin	-9.70	Asp223	7	-5.02	---	8
ANTIBIOTICS						
Ampicillin	-8.39	Asp223, Glu227	9	-5.34	Asp143	3
Cefazoline	-8.42	Asp223, Glu227	1	-6.83	---	8
Ciprofloxacin	-9.65	---	3	-5.46	---	5
Levofloxacin	-10.16	---	1	-5.81	---	4
Nitrofurantoin	-8.16	---	8	-5.91	Phe142, Lys175	1
Norfloxacin	-9.44	---	5	-5.82	---	8
Sulfamethoxazole	-8.33	Asp223, Glu227	9	-5.38	---	10
Trimethoprim	-8.27	Asp223, Glu227	5	-5.40	Phe142, Asp143	2

Inhibitors (Chlorogenic Acid, Ellagic Acid, Gallic Acid, Nalidixic Acid and Quercetin) and Standard antibiotics (Ampicillin, Cefazoline, Ciprofloxacin, Levofloxacin, Nitrofurantoin, Norfloxacin, Sulfamethoxazole, and Trimethoprim) were docked and the results obtained provide a comparative insight into the potency of inhibitors and standard antibiotics through analysis of their binding capacities.

The binding energies of *AmpC* beta-lactamase of *Klebsiella pneumoniae* with Ellagic Acid, Quercetin, Ciprofloxacin, Levofloxacin, & Norfloxacin shows lowest binding energy than other Ligands. The docking of *AmpC* beta lactamase of *Klebsiella pneumoniae* with standard Antibiotics when compared with Inhibitors shows lower binding energy especially with Quercetin and Ellagic Acid which show higher affinity. From Table (1), it is clear that standard antibiotic viz., Levofloxacin is more effective than the inhibitors docked.

The binding energies of *Oxa* Beta-lactamase of *Klebsiella pneumoniae* with Ellagic Acid, Gallic Acid, Cefazoline, Levofloxacin, Nitrofurantoin & Norfloxacin shows lowest binding energy than other ligands. The docking of *Oxa* Beta lactamase of *Klebsiella pneumoniae* with standard antibiotics when compared with inhibitors shows lower binding energy especially with Gallic acid and Ellagic Acid which show higher affinity**.** From Table (1), it is clear that standard antibiotic viz., Cefazoline is more effective than the inhibitors docked. But

Klebsiella pneumoniae has emerged resistant to these antibiotics.[11]

As these ligands have proven antibacterial (Sulfamethoxazole and Trimethoprim,[13] Penicillin[14] Cephalosporin,[15]) antimicrobial (Gallic Acid[16]) and anticancer activities (Chlorogenic acid,[17] Quercetin,[17] Ellagic acid,[18] and Gallic acid[16]), these Ligands can be further used as lead compounds in treatment of multidrug resistant UTI's caused by *Klebsiella pneumoniae.*

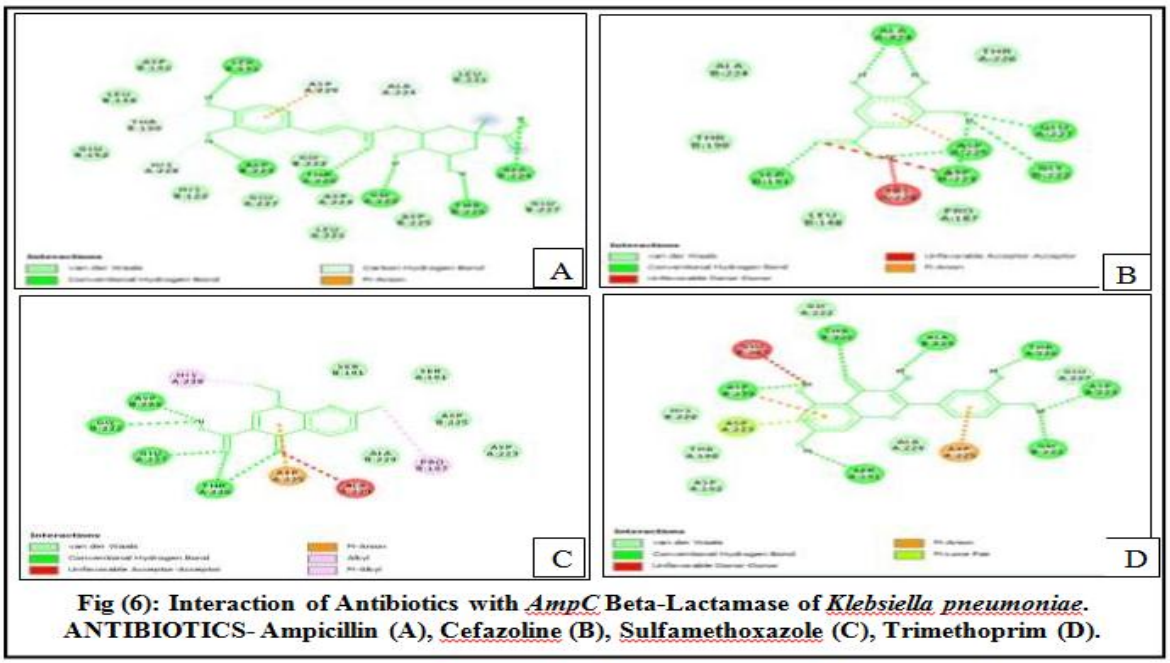

Fig (6): Interaction of Antibiotics with *AmpC* Beta-Lactamase of *Klebsiella pneumoniae*. ANTIBIOTICS- Ampicillin (A), Cefazoline (B), Sulfamethoxazole (C), Trimethoprim (D).

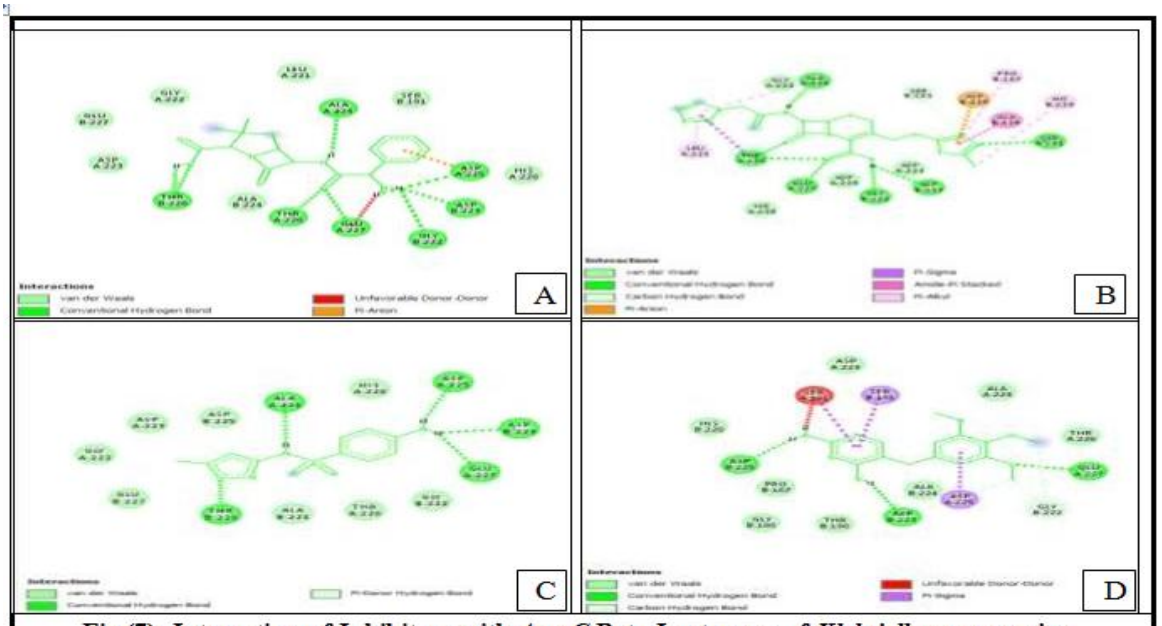

Fig (7): Interaction of Inhibitors with *AmpC* Beta-Lactamase of *Klebsiella pneumoniae*. INHIBITORS- Chlorogenic Acid (A), Gallic Acid (B), Nalidixic Acid (C), Quercetin (D).

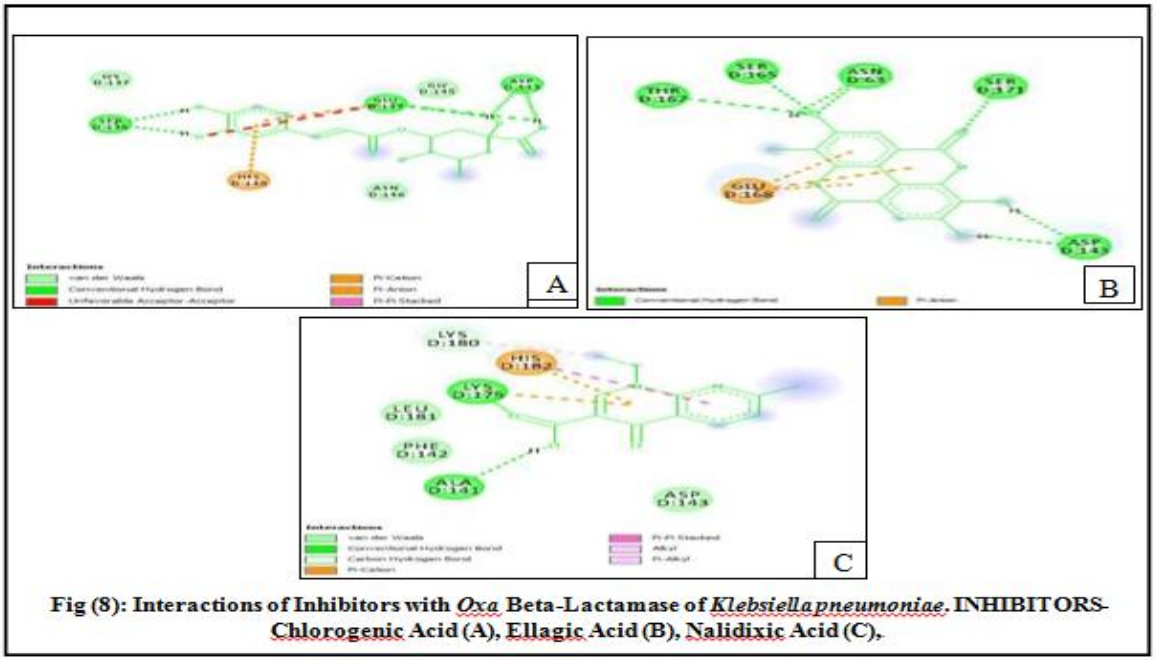

Fig (8): Interactions of Inhibitors with *Oxa* Beta-Lactamase of *Klebsiella pneumoniae*. INHIBITORS- Chlorogenic Acid (A), Ellagic Acid (B), Nalidixic Acid (C),

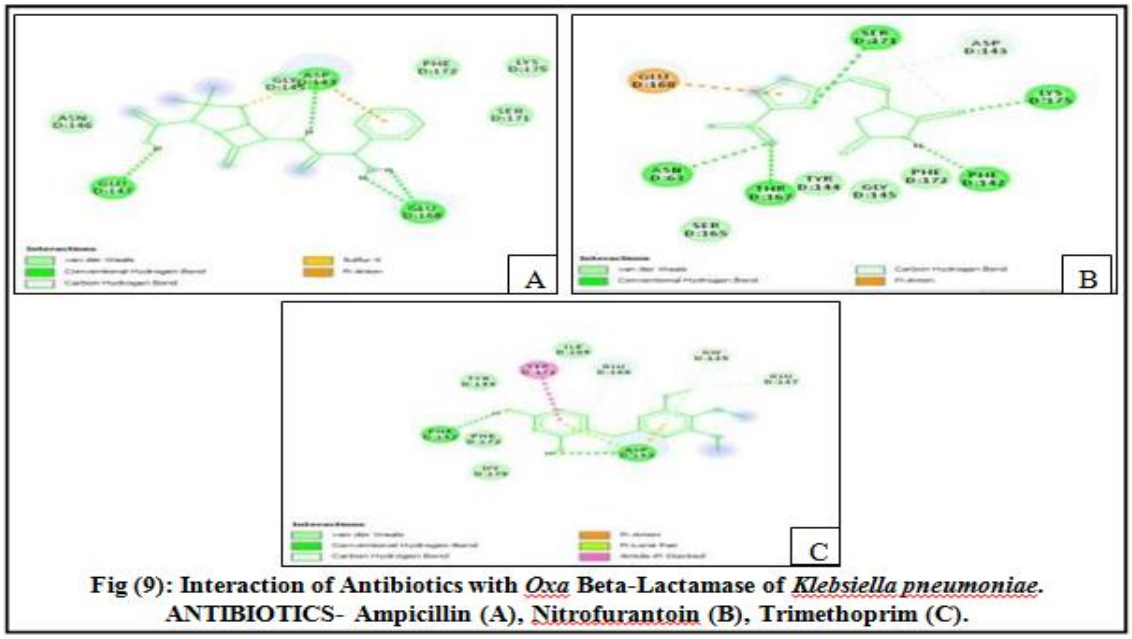

Fig (9): Interaction of Antibiotics with *Oxa* Beta-Lactamase of *Klebsiella pneumoniae*. ANTIBIOTICS- Ampicillin (A), Nitrofurantoin (B), Trimethoprim (C).

CONCLUSION

The main causal agent of UTI is *Escherichia coli* (80-85%), but other predominant infectious bacteria *Klebsiella, Proteus, Pseudomonas, Staphylococcus* also cause UTI infection. Most predominant organisms are *S. aureus* and *Klebsiella pneumoniae* generate high level of antibiotic resistance after *E. coli*. Antibiotics have been a high success till date for curbing bacterial infections. But, the widespread and uncontrolled use of antibiotics has led to the emergence of multidrug-resistant (MDR) bacteria.[11] Along with limited treatment options and increased mortality the MDR seems grave. Hence, there is an urgent need to search for a

new antibacterial agent. The molecular docking programs aid to establish new ligands/inhibitors for the selected target receptor proteins from the different available databases, based on their efficiency to bind the active sites on the receptor.[11] Molecular docking has been carried out to check the efficiency of these Ligands and Standard antibiotics to bind to the active site of the *AmpC & Oxa* Beta-lactamase respectively. Our study shows that inhibitors viz., Quercetin and Ellagic acid show best interactions and binding energy with *AmpC* Beta- Lactamase, while Gallic Acid and Ellagic Acid show best interactions and binding energy with *Oxa* Beta-Lactamase. These *in silico* studies will certainly help towards developing candidates for treatment of MDR-UTI in the future. More studies on mutations are needed for corroborating the role of Quercetin, Gallic Acid and Ellagic Acid as antibacterial agents to treat MDR *Klebsiella pneumoniae* mediated uropathological infections.

Antibiotic resistance is becoming a big chronic problem for the individuals admitted to health care centres. Out of eight antibiotics, Cefazolin and Levofloxacin in which maximum resistance was found. This suggests a need for continuous monitoring to organisms causing UTI's in all age group and testing its resistance to the different antimicrobial agents before antibiotics prescription in order to ensure adequate treatment of UTI's. *In silico* studies point out that, the docking of *AmpC* Beta lactamase of *Klebsiella pneumoniae* with standard antibiotics when compared with selected inhibitors show lower binding energy especially with Quercetin and Ellagic Acid which show higher affinity. The docking of *Oxa* Beta lactamase of *Klebsiella pneumoniae* with standard antibiotics when compared with selected inhibitors shows lower binding energy especially with Gallic acid and Ellagic Acid.

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