

Research Article



Drug Reprofiting: In Silico Modelling of *Haemophilus influenzae* Disease, *Staphylococcus aureus* Related Pneumonia and Urinary Tract Infections

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ABSTRACT

According to reports of ReAct forum, every year more than 750,000 patients die due to bacterial infections, making bacterial infection one of the leading causes of disease infestation in an individual. *Haemophilus influenzae* disease, pneumonia and urinary tract infections make the top of this list. *Haemophilus influenzae* is a gram-negative, commensal, facultative bacterium, which can mostly cause pneumonia along with other diseases including bloodstream infections and arthritis. Though vaccine exists for Hib infections, during infections of Hib and NTHi the patients are administered antibiotics to treat the disease. *Staphylococcus aureus* is a gram-positive versatile bacterium of the micrococcaceae family and one of the most dangerous pathogen-affecting humans as it can cause pneumonia. Pneumonia is an acute pulmonary infection, which is associated with high mortality having a more pronounced effect on young children. Urinary Tract Infection is a prevalent public health problem that is more common in women than in men. Though it can be treated by appropriate antibiotics, due to recurrent infections, they lead to a gain of resistance. Thousands of research are going on around the world to come up with a potential drug that can cure the Hi disease, *Staphylococcus aureus* related pneumonia and UTIs entirely with the least drawbacks. In this study, we present a docking-based screening using a quantum mechanical scoring of a library built from approved drugs and compounds, Amoxicillin and Cefuroxime with proteins with PDB ids 3CKM, 3EMF, 3ZU0, 4MV9, 5KCN, 5VVG, and 6XXY for Hi disease, 4RBR, 4MVN, 3KOR and 2RKZ for *Staphylococcus aureus* related pneumonia and 6QVX, 2F00, 6AYI and 2ID0 for UTIs could display antibacterial activity against the respective bacterial infections. This study needs to be evaluated and further confirmed through experimental assays and clinical trials to validate the predicted results. This finding aims to provide a better understanding and contribute to the development of a potent drug against Hi disease, *Staphylococcus aureus* related pneumonia and UTIs.

Keywords: *Haemophilus influenzae* disease, Pneumonia, Urinary Tract Infection.

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INTRODUCTION

Haemophilus influenzae is one of the leading causes of invasive bacterial infections across the world, particularly in developing countries. It is responsible for causing many diseases including sepsis, epiglottitis, cellulitis, arthritis and many respiratory disorders such as acute lower respiratory infections (ALRIs) and pneumonia¹. The pathogenesis and manifestation of the disease are high in children below 5 years and adults above 65 years old.

Haemophilus influenzae is a commensal component in the microflora of the upper respiratory tract in healthy adults. When an individual's immunity is compromised, and the pathogenicity of the bacterium increases, it can spread from the upper nasal passage to the bronchi of the lungs and cause acute and chronic respiratory infections along with other casualties. The bacterium can be grouped into

typeable and non-typeable forms². Several potential vaccines against Hib i.e. type b infections exist but unfortunately, the spectrum of all *Haemophilus influenzae* is still unknown and thus the basis of treatment for *Haemophilus influenzae* still depends on antibiotics³.

Staphylococcus aureus is a gram-positive bacteria belonging to the micrococcaceae family, it is distinguished from other staphylococcal species by the gold pigmentation observed in the colonies and positive results obtained in coagulase, mannitol fermentation, and deoxyribonuclease assays. The genome is characterized by a circular chromosome ~2800 bp in length. The pathogenicity and resistance genes are located on both chromosome and extrachromosomal elements. The cell wall is made of 50% peptidoglycan by weight which consists of alternating N-acetyl glucosamine and N-acetylmuramic acid connected by β -1,4 linkage⁴.

Pneumonia is a respiratory disease that is one of the leading causes of death in many developed countries. It can be defined as an acute infection affecting the lung parenchyma as a result of the attack by one or several pathogens. Based on this wide range of pathogens capable of causing the infection, episodes of pneumonia can be classified into four types; Hospital associated pneumonia (HAP), Ventilator-associated pneumonia (VAP), Community-associated pneumonia (CAP) and healthcare-



associated pneumonia⁵. *Staphylococcus aureus* pneumonia infection takes up about 5% of community-acquired pneumonia (CAP). It is more common in children with cystic fibrosis, after an episode of influenza infection and among intravenous drug users⁶.

Urinary tract infections (UTIs) are the most common bacterial infections affecting about 150 million people each year globally. Recent studies show that in the US alone, around 7 million hospital visits are reported due to UTIs yearly⁷. It is significantly seen in infants, older men, and females of all ages. It was estimated that about 40–50% of women experience at least one episode of UTI in their lives⁸.

Clinically, it has been classified into complicated and uncomplicated UTIs. Uncomplicated UTI is the most common type of infection affecting individuals that have no structural or neurological urinary tract abnormalities and are further differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis). On the other end, complicated ones are due to presence of an abnormal urinary tract (urinary obstruction and retention), immunosuppression, renal failure, pregnancy, and the presence of foreign bodies such as permanent catheters⁹. UTIs are caused by both Gram-negative and Gram-positive bacteria, and some fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC) seen in both community and hospital infections^{8,10}. The mechanism of action of UPEC involves the formation of fimbrial adhesions that attach to the glycolipids and glycoproteins on the epithelial surface which gives resistance for bacteria to withstand the flow of urine and reside in the urinary tract. These bacteria also produce certain toxins, hemolysin, and colony-necrotizing factors that will disrupt the epithelial integrity and allow bacterial invasion, and hence increase the risk of infection. Uropathogens also can colonize in the host epithelial cells which provide a reservoir for recurrent infection¹¹.

Repurposing or reprofiling of existing drugs can be remarkably productive as with it, high-quality medicines can be designed to battle these disease in a short period. This paper has focused on repurposing the antibiotics amoxicillin and cefuroxime against *Haemophilus influenzae* disease, *Staphylococcus aureus* related pneumonia and UTIs by targeting essential proteins that are required for the cellular survival of the bacterium.

Procedure

1. Ligand Screening

For the initial Ligand screening purposes, a web-based tool named Swiss ADME (<https://www.swissadme.ch/>) was used to eliminate a few compounds according to Lipinski's rule of five parameters. For a compound to qualify as ligand it should Have < 500 Da molecular weight, high lipophilicity i.e. value of Log P being less than 5, hydrogen bond acceptors being less than 10 and H-bond donors less

than 5. Any compound with more than 2 violations was ruled out for further study.¹²

1. 2. Protein Preparation and Active site Determination.

Required protein in PDB format was downloaded from the website rcsb.org, commonly known as the Protein Data Bank. 3D conformers of the ligand were downloaded from PubChem.

Using PyMOL (Version 2.4.1) software water molecules, as well as native ligands from the protein, were removed, defined as cleaning/purification of the protein for further application. Using a web server called Deep Site Active Pockets of the proteins were calculated. The results calculated by the webserver were in the form of different ids, centres and scores.

Scoring In deep site was using neural networking based on following instructions using DCNN architecture. <https://academic.oup.com/bioinformatics/article/33/19/3036/3859178> Center values for the grid were selected keeping a score greater than 0.98.

UCSF Chimera (Version 1.14) was used to prepare the receptor using the DockPrep function. Dock Prep prepared structures for Docking using these functions:

- deleting water molecules
- repairing truncated sidechains
- adding hydrogens
- assigning partial charges
- writing files in Mol2 format

3. In silico Docking Using Auto dock Vina

Auto dock Vina (Version 1.1.2) along with UCSF Chimera (Version 1.14) was used for molecular Docking Studies. Centre values and size of the grid of different scores were used from DEEPSITE calculations done above.

Following Parameters were set in auto dock vina:

Receptor options

- **Add hydrogens in Chimera (true/false)** – whether to add hydrogens in Chimera before calling the script. The receptor prep script will check for hydrogens and add them if they are missing. AutoDock Vina needs the polar (potentially H-bonding) hydrogens to identify atom types for scoring purposes.
- **Merge charges and remove non-polar hydrogens (true/false)** – note AutoDock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect results except for the presence or absence of nonpolar hydrogens in the processed receptor
- **Merge charges and remove lone pairs (true/false)** – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the



processed receptor (and there may not have been any lone pairs to start with)

- **Ignore waters (true/false)**
- **Ignore chains of non-standard residues (true/false)** – ignore chains composed entirely of residues other than the 20 standard amino acids.
- **Ignore all non-standard residues (true/false)** – ignore all residues other than the 20 standard amino acids.

For Ligands

- **Merge charges and remove non-polar hydrogens (true/false)** – note Auto Dock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect results except for the presence or absence of nonpolar hydrogens in the ligand output files
- **Merge charges and remove lone pairs (true/false)** – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the ligand output files (and there may not have been any lone pairs to start with)

Docking parameters

- **Number of binding modes (1-10, 10)** – maximum number of binding modes to generate
- **The exhaustiveness of search (1-8, 8)** – thoroughness of search, roughly proportional to the time
- **Maximum energy difference (kcal/mol) (1-3,3)** – maximum score range; binding modes with scores not within this range of the best score will be discarded.

The docking results were calculated by Auto dock vina using its Scoring function and results were displayed in the form of Scores and RMSD values. Docking results with the highest value score accompanied by negative signs and least RMSD values were chosen for further studies.

4. Residue Analysis

PyMOL was used for the visualization of interactions of the docked structure at the ligand sites. Discovery Studio 2020 was used to study the ligand interactions and the total number of residues. It was also used to plot the 2D structure of the interactions and residues.

5. Statistical Analysis

Descriptive, estimation and Hypothesis testing with a confidence interval of 95% was applied to data using formula 1 given below.

$$CI = \bar{x} \pm z \frac{s}{\sqrt{n}}$$

CI = confidence interval

\bar{x} = sample mean

z = confidence level value

s = sample standard deviation

n = sample size

Formula 1 used for calculation of confidence interval

RESULTS AND DISCUSSION

Molecular Docking:

The docking result was obtained from Auto dock vina in the form of a Dock score for all the fifteen proteins docked with the above-mentioned ligands

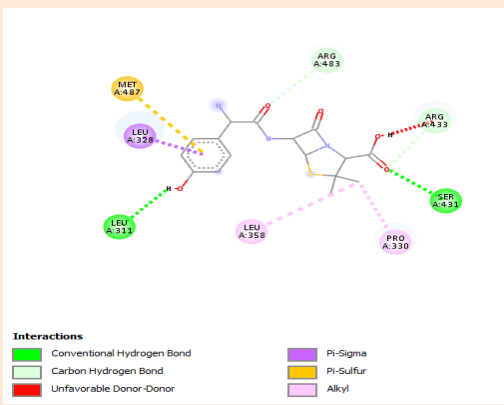
Haemophilus influenzae protein docking

PDB ID: 5VBG

For 5VBG, two active sites were selected out of which the 1st active site was selected with a Deep site score of 0.992. The selection was made based on the highest binding energy of the ligand-receptor. The docking results before statistics are shown in Table 1 and Table 2 shows the post statistical docking scores with Ligand Protein Interactions.

Ligand	Dock Score
Amoxicillin	-7.1
Cefuroxime	-7.3

Table 1:

Ligand	Dock Score	Discovery Studio Image
Amoxicillin	-7.1	

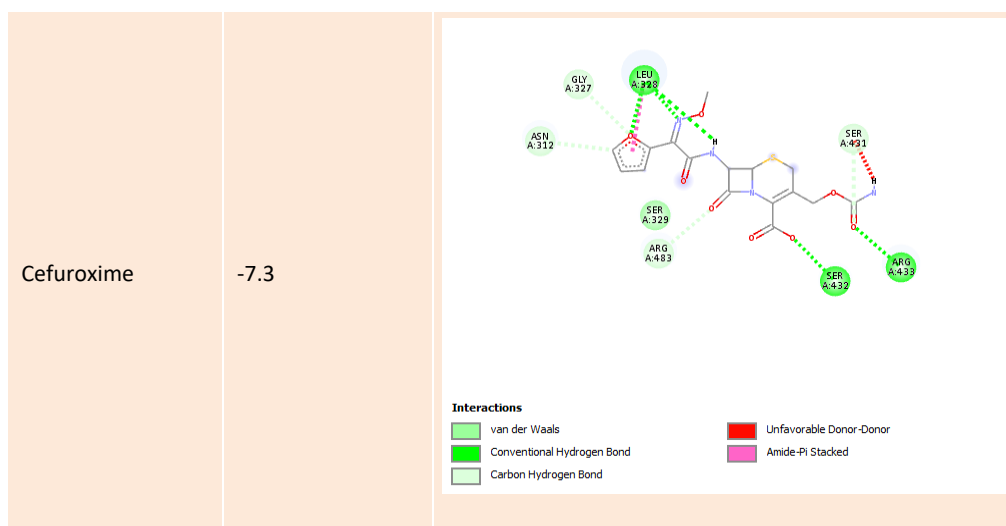


Table 2:
PDB ID: 3CKM

For 3CKM, five active sites were selected out of which the 1st active site was selected with a Deep site score of 0.991. The selection was made based on the highest binding energy of the ligand-receptor. The docking results before

statistics are shown in Table 3 and Table 4 shows the post statistical docking scores with Ligand Protein Interactions.

Ligand	Dock Score
Amoxicillin	-7.5
Cefuroxime	-7.2

Table 3:

Ligand	Dock Score	Discovery Studio Image
Amoxicillin	-7.5	
Cefuroxime	-7.2	

Table 4:

PDB ID: 3EMF

For 3EMF, five active sites were selected out of which the 1st active site was selected with a Deep site score of 0.998. The selection was made based on the highest binding energy of the ligand-receptor. The docking results before

statistics are shown in Table 5 and Table 6 shows the post statistical docking scores with Ligand Protein Interactions.

Ligand	Dock Score
Amoxicillin	2.6
Cefuroxime	7.6

Table 5:

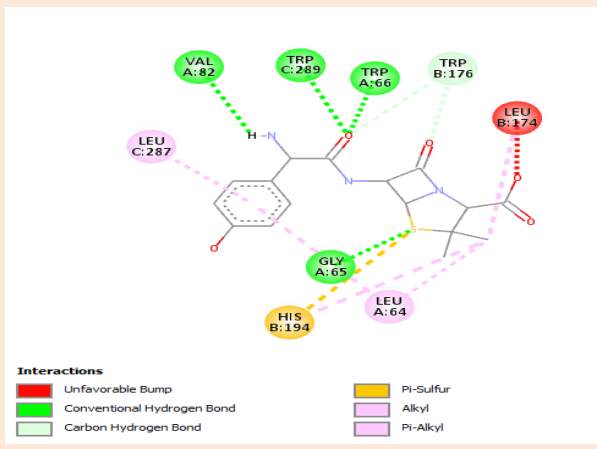
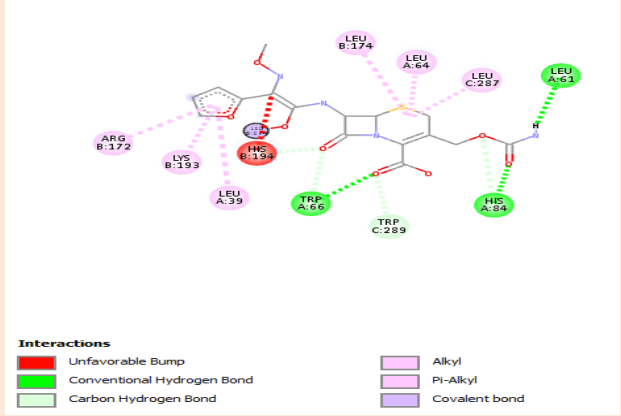
Ligand	Dock Score	Discovery Studio Image
Amoxicillin	2.6	
Cefuroxime	7.6	

Table 6:

PDB ID: 3ZU0

For 3ZU0, four active sites were selected out of which the 1st active site was selected with a Deep site score of 0.996. The selection was made based on the highest binding energy of the ligand-receptor. The docking results before

statistics are shown in Table 7 and Table 8 shows the post statistical docking scores with Ligand Protein Interactions.

Ligand	Dock Score
Amoxicillin	-9
Cefuroxime	-8.1

Table 7:

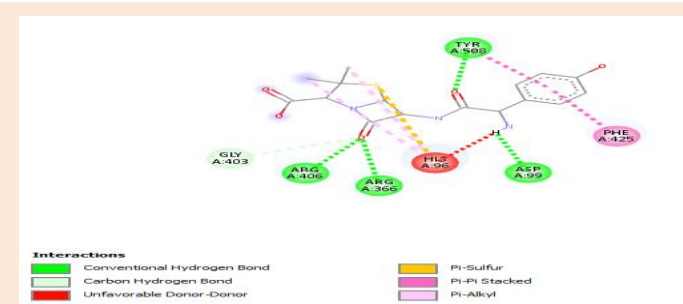
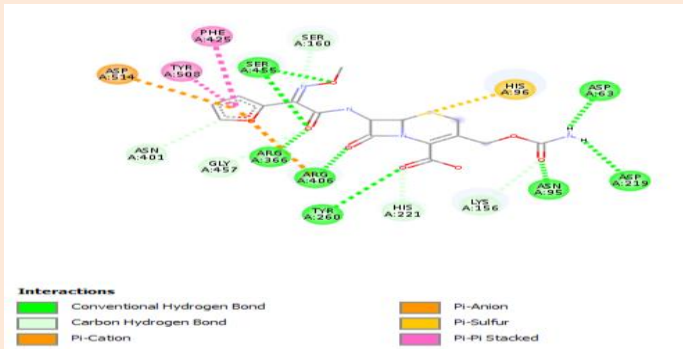
Ligand	Dock Score	Discovery Studio Image
Amoxicillin	-9	
Cefuroxime	-8.1	

Table 8:

PDB ID: 4MV9

For 4MV9, six active sites were selected out of which the 2nd active site was selected with a Deep site score of 0.993. The selection was made based on the highest binding energy of the ligand-receptor. The docking results before

statistics are shown in Table 9 and Table 10 shows the post statistical docking scores with Ligand Protein Interactions.

Ligand	Dock Score
Amoxicillin	-6.6
Cefuroxime	-6.7

Table 9:

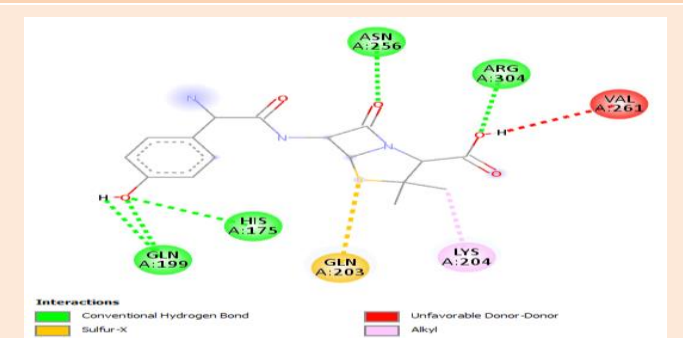
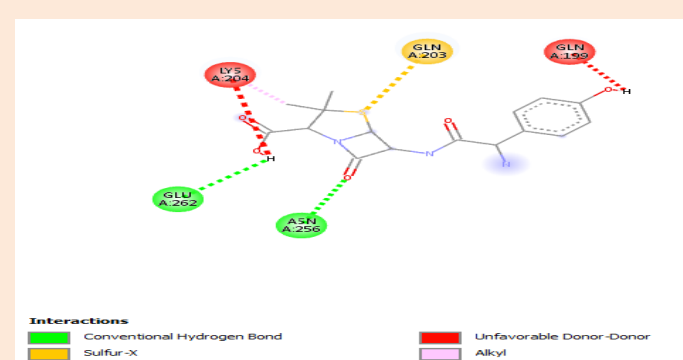
Ligand	Dock Score	Discovery Studio Image
Amoxicillin	-6.6	
Cefuroxime	-6.7	

Table 10:

PDB ID: 5KCN

For 5KCN, two active sites were selected out of which the 1st active site was selected with a Deep site score of 0.994. The selection was made based on the highest binding energy of the ligand-receptor. The docking results before statistics are shown in Table 11 and Table 12 shows the

post statistical docking scores with Ligand Protein Interactions.

Ligand	Dock Score
Amoxicillin	-7.7
Cefuroxime	-6.9

Table 11:

Ligand	Dock Score	Discovery Studio Image
Amoxicillin	-7.7	
Cefuroxime	-6.9	

Table 12:

PBD ID: 6XXY

For 6XXY, three active sites were selected out of which the 1st active site was selected with a Deep site score of 0.985. The selection was made based on the highest binding energy of the ligand-receptor. The docking results before statistics are shown in Table 13 and Table 14 shows the

post statistical docking scores with Ligand Protein Interactions.

Ligand	Dock Score
Amoxicillin	-7.9
Cefuroxime	-7.3

Table 13:

Ligand	Dock Score	Discovery Studio Image
Amoxicillin	-7.9	

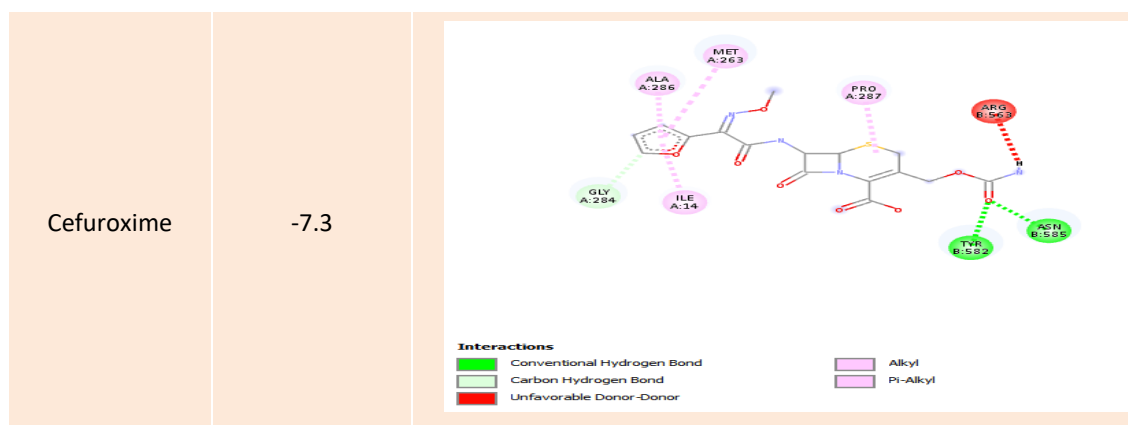


Table 14:

Staphylococcus aureus related pneumonia

PDB ID: 4RBR

For 4RBR, blind docking was done since the Deep site scores were not promising. The docking results before statistics are shown in Table 15 and Table 16 shows the

post statistical docking scores with Ligand Protein Interactions.

Ligand	Dock Score
Amoxicillin	62.7
Cefuroxime	59.8

Table 15:

Ligand	Dock Score	Discovery Studio Image
Amoxicillin	62.7	
Cefuroxime	59.8	

Table 16:

PDB ID: 4MVN

For 4MVN blind docking was done as none of the active sites had promising Deep site scores. The docking results before statistics are shown in Table 17 and Table 18 shows

the post statistical docking scores with Ligand Protein Interactions.

Ligand	Dock Score
Amoxicillin	-2.1
Cefuroxime	-1.9

Table 17:

Ligand	Dock Score	Discovery Studio Image
Amoxicillin	-2.1	
Cefuroxime	-1.9	

Table 18:

PDB ID: 3KOR

For 3KOR, 1 active site was considered with a Deep Site score of 0.994. The selection was made based on the highest binding energy of the ligand-receptor. The docking results before statistics are shown in Table 19 and Table 20

shows the post statistical docking scores with Ligand Protein Interactions.

Ligand	Dock Score
Amoxicillin	-5.6
Cefuroxime	-5.55

Table 19:

Ligand	Dock Score	Discovery Studio Image
Amoxicillin	-5.6	
Cefuroxime	-5.55	

Table 20:

PDB ID: 2RKZ

For 2RKZ, three active sites were selected out of which 1st active site was selected with a Deep site score of 0.994. The selection was made based on the highest binding energy of the ligand-receptor. The docking results before statistics are shown in Table 21 and Table 22 shows the

post statistical docking scores with Ligand Protein Interactions.

Ligand	Dock Score
Amoxicillin	-5.7
Cefuroxime	-5.9

Table 21:

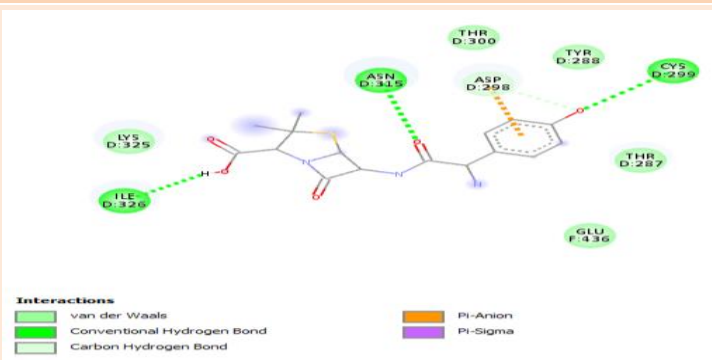
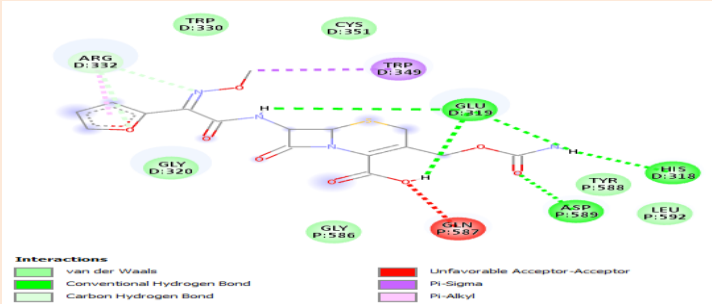
Ligand	Dock Score	Discovery Studio Image
Amoxicillin	-5.7	
Cefuroxime	-5.9	

Table 22:

Urinary Tract Infections

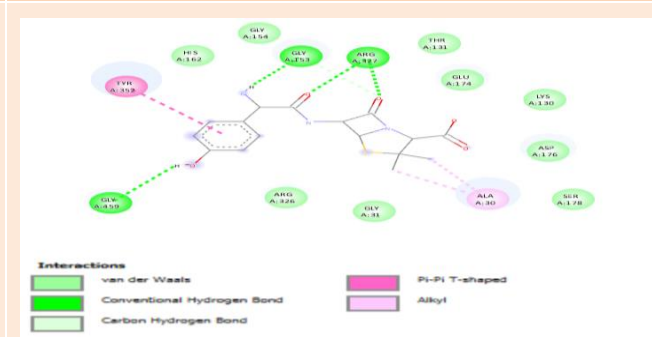
PDB ID: 2F00

For 2F00, three active sites were selected out of which the 1st active site was selected with a Deep site score of 0.997. The selection was made based on the highest binding energy of the ligand-receptor. The docking results before

statistics are shown in Table 23 and Table 24 shows the post statistical docking scores with Ligand Protein Interactions.

Ligands	Dock Score
Amoxicillin	-6.3
Cefuroxime	-6.8

Table 23:

Ligands	Dock score	Interactions
Amoxicillin	-6.3	

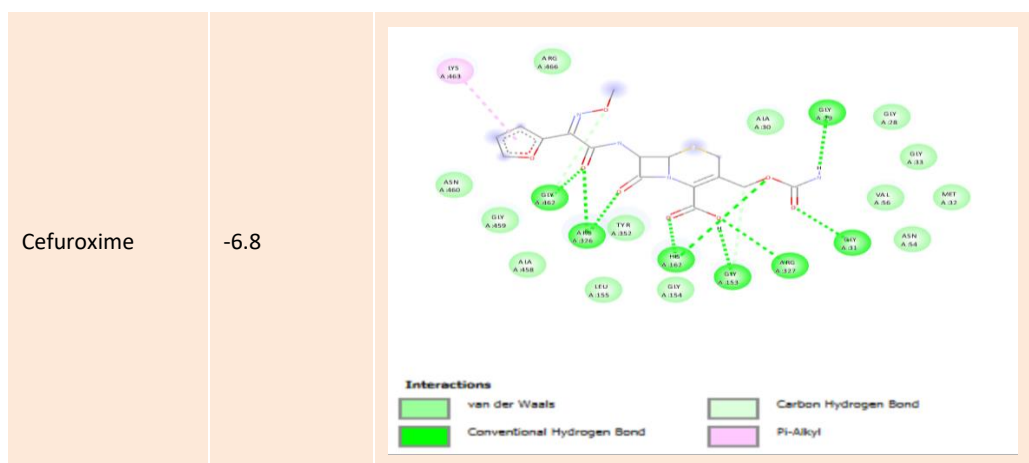


Table 24:

PDB-ID 6AYI

For 6AYI Chain A, out of the three active sites the 1st active site was selected with a Deep site score of 0.996. The selection was made on the basis of the highest binding energy of the ligand-receptor. The docking results before statistics are shown in Table 25 and Table 26 shows the

post statistical docking scores with Ligand Protein Interactions.

Ligands	Dock Score
Amoxicillin	-6.5
Cefuroxime	-5.4

Table 25:

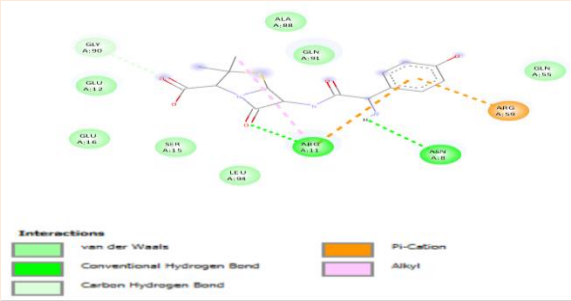
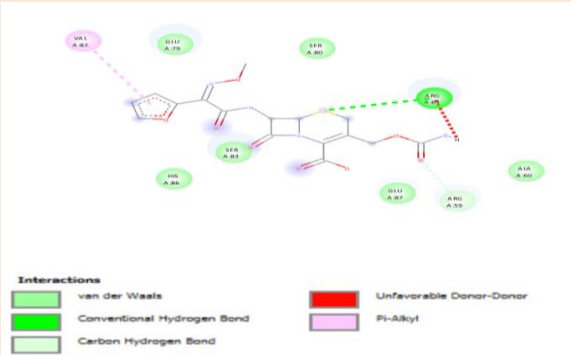
Ligands	Dock Score	Interactions
Amoxicillin	-6.5	
Cefuroxime	-5.4	

Table 26:

PDB-ID 6QVX

For 6QVX, two active sites were selected out of which 1st active site was selected with Deep site score of 0.966, the selection was made on the basis of highest binding energy of ligand-receptor. The docking results before statistics are

shown in Table 27 and Table 28 shows the post statistical docking scores with Ligand Protein Interactions.

Ligands	Dock Score
Amoxicillin	-5
Cefuroxime	-5.4

Table 27:

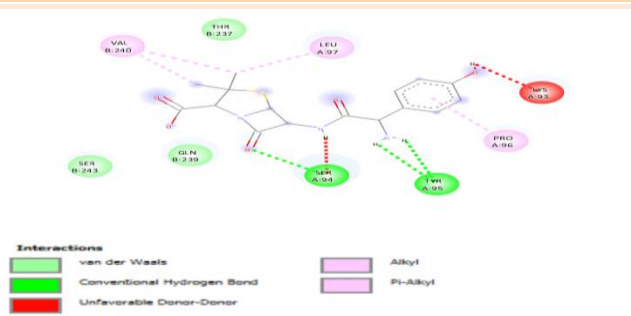
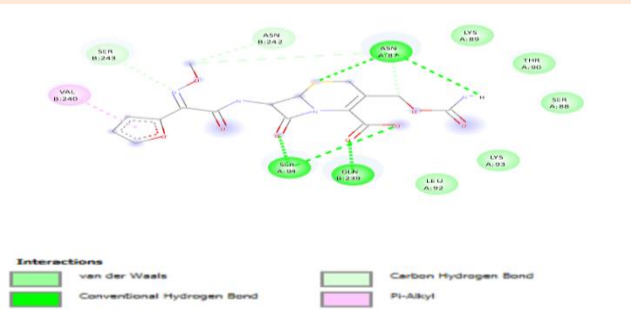
Ligands	Dock Score	Interactions
Amoxicillin	-5	
Cefuroxime	-5.4	

Table 28:
PDB-ID 2ID0

For 2ID0, two active sites were selected out of which 1st active site was selected with Deep site score of 0.993, the selection was made on the basis of highest binding energy of ligand-receptor, docking results before statistics are

shown in Table 29 and Table 30 shows the post statistical docking scores with Ligand Protein Interactions.

Ligands	Dock Score
Amoxicillin	-7
Cefuroxime	-7

Table 29:

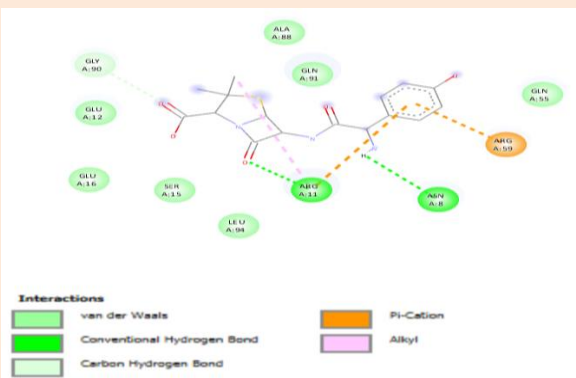
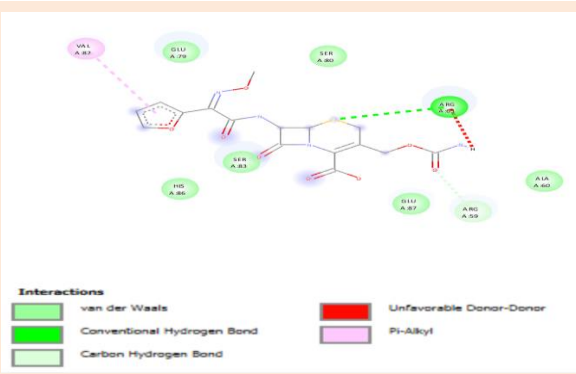
Ligands	Dock Score	Interactions
Amoxicillin	-7	
Cefuroxime	-7	

Table 30: Summary of the results showing ligands and their interacted proteins that were considered in the study for the targeted disease.

Ligand	Proteins Interacted	Targeted Disease
Amoxicillin	5VBG, 3CKM, 3EMF, 3ZU0, 4MV9, 5KCN, 6XXY	<i>Haemophilus Influenzae</i> Disease
	4RBR, 4MVN, 3KOR, 2RKZ	<i>Staphylococcus aureus</i> Pneumonia
	6QVX, 2F00, 6AYI, 2ID0	Urinary Tract Infection
Cefuroxime	5VBG, 3CKM, 3ZU0, 3EMF, 4MV9, 5KCN, 6XXY	<i>Haemophilus Influenzae</i> Disease
	4RBR, 4MVN, 3KOR, 2RKZ	<i>Staphylococcus aureus</i> Pneumonia
	6QVX, 2F00, 6AYI, 2ID0	Urinary Tract Infection

CONCLUSION

Both the ligands were studied using bioavailability radar. Our results proposed that Amoxicillin and Cefuroxime showed the best docking result for *Haemophilus influenzae* proteins with PDB IDs 5VBG, 3CKM, 3EMF, 3ZU0, 4MV9, 5KCN and 6XXY, *Staphylococcus aureus* proteins with PDB IDs 4RBR, 4MVN, 3KOR and 2RKZ and UTI related bacterial proteins with PDB IDs 6QVX, 2F00, 6AYI and 2ID0. To find the effectiveness of these results and to propose the exact mechanism, in-vitro studies are to be done on Amoxicillin and Cefuroxime targeting *Haemophilus influenzae* disease, *Staphylococcus aureus* pneumonia disease and UTIs that is discussed above to understand the mechanism and identify a potential cure.

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