Pangenome Analysis of *Klebsiella pneumoniae* isolates Reveals Core-Drug Targets and Screening of Promising Lead Compounds for Drug Discovery

Submitted By

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Reg. Number: 00000318681

A thesis submitted in partial fulfillment of the requirements for the degree of

MS Industrial Biotechnology

Supervised by

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National University of Sciences and Technology (NUST),

Islamabad

May 2023

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Dedication

Dedicated to my Parents

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List of Abbreviations

(Kp)	*Klebsiella pneumonia*
KPC	*K. pneumoniae* carbapenemase
BPGA	Bacterial Pan Genome Analysis tool
CELLLO	subcellular Localization Prediction
DEG	*Database* of Essential Gene
ExPASy	Expert Protein Analysis System
FDA	Food and Drug Administration
ICUs	Intensive Care Units
KASS	*KEGG* Automatic Annotation Server
KEGG	*Kyoto Encyclopedia of Genes and Genomes*
LPS	Lipopolysaccharide
MDR	Multi-Drug Resistant
NCBI	National Center for Biotechnology Information
CPS	Capsular polysaccharide
US	United States
Spp	Species
HTS	High throughput screening
VFDS	Virulence Factors Database

Abstract

A Gram-negative, non-motile bacterium called *Klebsiella pneumoniae* poses a severe threat to the public's health and frequently causes opportunistic infections in hospital patients that are resistant to antibiotics. It is a widespread bacterium that is a major cause of multidrug-resistant health-related infections. *K. pneumoniae* is usually found in the gastrointestinal system in humans, with a few unusual cases in the nasophryrnx where it can infect other tissues and enter into bloodstream leading illness. In the pre-antibiotic period, *Klebsiella pneumoniae* was a significant pathogen of community-acquired pneumonia (CAP), particularly in diabetics and those who consume alcohol. Major community-acquired hypervirulent Kp disorder, commonly characterized as a pyogenic liver abscess with associated bacteremia, but also meningitis, brain abscess, or opthalmitis1, has emerged in the past three decades. Since then, *Carbapenem-resistant K. pneumoniae* (CRKP) has been regularly detected in various nosocomial settings around the world. A wide range of antibiotics, such as β-lactams and aminoglycosides, are involved in controlling and treating *K. pneumoniae*-related infections. The presence of a substantial number of fully sequenced genomes of *Klebsiella pneumoniae* has created a chance to investigate the specie's pan genomes as well as phylogenetic history and to establish fresh innovative drug targets contributing to drug discovery efforts. In order to identify primary drug targets that are susceptible to antibiotics, this study uses a bioinformatics framework that includes pan-genomics, subtractive proteomics, and reverse vaccination strategies. In this study, pangenome analysis and subtractive proteomics were performed on the 560 complete genomes that make up the pangenome of this concerning pathogen. Additionally, subtractive proteomics apply with subsequent filters such as (non-human homology, essentiality assessment, virulence evaluation, physiochemical checks, and pathways analysis).Furthermore, the conserved core genome will undergo several different filters, such as essentiality, non-homology, virulence, physiochemical analysis, localization, and pathway analysis are applied to the core proteome (617 proteins), resulting in the identification of five potential core drug targets with a wide therapeutic window. Additionally, the FDA's molecular docking analysis of these drugs targets five promising ligands that were found using both approved and unapproved ligands from the Drug Bank database namely

Phosphoaminophosphonic Acid-Adenylate Ester, formic acid, Vitamin E, Thymidine cyclophosphate, and Doxorubicin. Future research could experimentally validate the potential drug targets, helping with the development of new medications.

INTRODUCTION

Klebsiella pneumoniae is an increasingly difficult-to-treat bacterial pathogen in humans that causes infections in hospitals or the community and is associated with significant levels of antibiotic resistance (1). A gram-negative bacterium called *Klebsiella pneumoniae* can be encountered in the environment (such as water, dirt, etc.) or on the mucosal surface of animals. *K. pneumoniae* primarily inhabits the gastrointestinal system in humans, while there have been a few isolated reports of it in the nasopharynx, where it can infect other tissues or enter the circulation system and cause diseases. Prior to the development of antibiotics, *K. pneumoniae* was a significant cause of community-acquired pneumonia (CAP), particularly in diabetics and individuals who engage alcohols consumption. Subsequently, with the advent of antibiotic era it emerged as a major contributor to infections acquired within medical settings specifically hospitals (2). *K. pneumoniae* is a common cause of mastitis in dairy cows and has the potential to spread disease to a range of animal species, specifically when it becomes hypermucoid. Water, soil, and plant matter are only a few of the environments and plant hosts where it can persist (3). Upon entering into body bacterium, has capability to display significant levels of virulence and resistance to antibiotics. In the US, *K. pneumoniae* is the most prevalent cause of hospital-acquired pneumonia, accounting for 3 percent to 8% of all nosocomial bacterial infections (4).*Klebsiella pneumonia* has a substantially greater death rate than pneumococcal pneumonia: 21% in the general population and 64% in alcoholics (5). The WHO, the US Centers for Disease Control and Prevention, and the United Kingdom Department of Health all have been classified *Klebsiella pneumoniae* as (MDR) multidrug-resistant which becomes serious hazard to public health. Within the clinical system, *K. pneumoniae* infections are particularly troublesome in infants, the elderly and people with weakened immune systems, but this bacterium is also accountable for a sizable percentage of community-acquired diseases such as pneumonia and sepsis (6). The number of genome sequences of *K. pneumoniae* is continuously increasing in publicly available biological databases NCBI. Pangenome analysis is a method to compare more than one genome. The existence of a large number of fully sequenced *Klebsiella pneumoniae* genomes has offers unique chance to investigate the species pan genomes as well as evolutionary history (pan phylogeny) and to establish fresh, innovative drug targets contributing to drug discovery process. The identification of targets is the first step in the process of development of drugs and vaccines, and this opportunity has allowed researchers to

identify new novel drug targets. With the introduction of new sequencing technology and the resulting deluge of genetic data, scientists may now employ computational methods to quickly identify new targets that are both time and cost effective. In this process, computational tools (e.g. subtractive genomics) are widely used. Recently utilizing an in-silico technique to work with bacterial pathogens, many targets have recently been found that are either resistance to treatment or for which no suitable vaccination is known. In the post-genomic era, reverse vaccinology is a common and popular method for quickly identifying novel vaccine targets. Multiple bacteria that affect humans, such as *Burkholderia pseudomallei, Helicobacter pylori, and Mycobacterium TB,* Pseudomonas *aeruginosa, Salmonella typhi*, and *Neisseria gonorrhoeae*, are comparative and subtractive genomics, as well as differential genome analysis, is to identify targets. Proteins found in the cytoplasm are frequently considered good drug targets, whereas outer membrane, secretory, and extracellular proteins are considered likely vaccine candidates due to their accessibility to the host immune machinery(7).When using these methods, it is ensured that the prioritized proteins are required for pathogen survival but do not have human homologs (8). Exclusion of the host genome from pathogen essential genes aids in the search for non-human homologous targets, ensuring that drugs do not interact with humans as targets so the combination of these methods Using various modern bioinformatics tools may ensure finding of promising therapeutic targets for the majority of the Infectious illnesses (9).

1.1. Our Contribution:

Our study's emphasis on core druggable targets and pangenome analysis highlights its unique contribution to the field of antimicrobial research. By offering new perspectives on drug development and antibiotic resistance, our findings hold significant promise for shaping future therapeutic interventions and improving patient outcomes in the face of Klebsiella pneumoniae infections.

1.2. Research Objectives

- Conduct pangenome analysis to uncover the genetic diversity, variations, and unique elements within the *Klebsiella pneumoniae* population.
- Molecular docking and identifying potential lead compounds to develop drug targets.

LITERATURE REVIEW

Gram-negative, encapsulated, rod-shaped, and non-motile Klebsiella pneumoniae belongs to the 'ESKAPE' group, which comprises six of the most common antimicrobial-resistant bacteria causing nosocomial infections worldwide, including *Enterobacter spp, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterococcus faecium* (10).

This pathogen is responsible for nosocomial infections in immunocompromised patients, affecting their urinary tract, respiratory tract, and blood (11). *K. pneumoniae* has become an important pathogen linked to healthcare over the past 20 years, causing 14–20 percent of diseases of the respiratory tract, lower biliary duct, surgical wounds, and urinary tract (12). The susceptibility *to K. pneumoniae* infections significantly rises when medical apparatuses are present, like ventilator assistance devices, catheters employed in neonatal units, and extended-use urinary catheters in patient's requiring prolonged care (13).

2.1. Antibiotic resistance-A major challenge for *K. pneumoniae*

The effectiveness of antibiotics, a transformative factor in healthcare that has preserved countless lives, is now under threat due to the swift emergence of drug-resistant bacteria on a global scale (10). The antibiotic resistance crisis is attributed to the excessive and inappropriate use of antibiotics, along with the pharmaceutical industry's limited investment in new drug development due to economic constraints and rigorous regulatory demands. Presently, *K. pneumoniae* displays resistance to an extensive array of antibiotics, encompassing beta-lactams, fluoroquinolones, and aminoglycosides (12). The primary sign of resistance to beta-lactam antibiotics in Gram-negative bacteria is the active expulsion of beta-lactam molecules or the production of beta-lactamase enzymes by beta-lactam-insensitive cell wall transpeptidases. Similar to many other antimicrobial drugs, beta-lactams are rendered ineffective against bacteria due to three fundamental mechanisms of resistance. The predominant mechanism involves the generation of enzymes that degrade or modify the antibiotic even before it can reach its intended target site, representing a common strategy. In this context, the beta-lactamase enzyme family, responsible for the breakdown of beta-lactam antibiotics, is widely prevalent in both Gram-positive and Gram-negative bacterial populations. The second process involves the alteration of the antibiotic's intended target site. This condition is characterized by the involvement

of beta-lactam-resistant cell-wall transpeptidases, which are now recognized as a key source of resistance in a number of infections, including those caused by Gram-positive Staphylococcal and Streptococcal species that are challenging. The antibiotic is prevented from reaching the target by altered permeability or forced efflux, which is the last stage (13). Biofilm production is important in many bacterial illnesses and helps bacteria's ability to overcome the human defense system (14). *K. pneumoniae* has undergone thorough investigation, revealing its production of a beta-lactamase enzyme which catalyzes the hydrolysis of the beta-lactam ring in antibiotics. Extended-spectrum beta-lactamase (ESBL)-producing *K. pneumoniae* was identified in Europe in 1983 and later in the US in 1989. The presence of ESBLs leads to the enzymatic breakdown of oxyimino cephalosporins, rendering third-generation cephalosporins ineffective against infections caused by such strains. Consequently, carbapenems become a therapeutic choice against ESBL-induced resistance Nevertheless; *K. pneumoniae* has exhibited a significant role in nearly 80% of carbapenem-resistant Enterobacteriaceae infections reported to the Centers for Disease Control and Prevention (CDC) in 2013. The resistance to carbapenems has been linked to heightened production of ESBL enzymes, alterations in the outer membrane structure, and an increased expression of efflux pumps (15). Over the past ten years, *Kp* has transformed into a challenging-to-treat species with a wide range of acquired resistance determinant. It is now the most common carbapenem-resistant *Enterobacterales* pathogen (CRE). Carbapenem resistance (CR) is most prevalent in regions such as the Indian subcontinent, the US, Greece, Israel, Italy, Turkey, the Middle East, and North Africa. On a regional scale, the incidence of carbapenem resistance in *K. pneumoniae* strains exhibits variability. The statistics from the European Antimicrobial Resistance Surveillance Network (EARS-Net) reveal a diverse spectrum of country-specific rates of carbapenem resistance in *K. pneumoniae* isolates extracted from invasive infections across Europe, spanning from 0% to over 60%. Within the context of *K. pneumoniae*, Portugal and Malta exhibited Carbapenem resistance exceeding 10%, while Bulgaria, Cyprus, Romania, and Greece reported resistance surpassing 20%. Notably, Iceland documented an exceptionally high Carbapenem resistance rate exceeding 60% (18). *Klebsiella pneumoniae* is among the clinically significant microorganisms that have sparked considerable public apprehension.*Klebsiella pneumoniae* stands as a prominent member of the Enterobacteriaceae family, recognized as an opportunistic pathogen responsible for a

diverse spectrum of diseases and exhibiting a growing resistance to antibiotic treatment (19). The substantial increase in the prevalence of infections caused by multidrug-resistant (MDR) and extensively drug-resistant (XDR) Enterobacteriaceae, which are common natural residents of the human and animal microbiome, is a big economic challenge. Despite its considerable clinical importance, *K. pneumoniae* still lacks comprehensive information (20).

2.2. Antibiotics adverse reactions

Antibiotics are one of the most regularly prescribed medications in the United States. They are beneficial in the treatment of serious and potentially lethal illnesses. Their use, on the other hand, can increase germ resistance and have negative consequences. Antibiotic research has largely focused on their advantages, with little information on their risks. Nevertheless, when prescribing any medication, physicians must balance the potential advantages against the risk of adverse effects. A moderate level of risk is deemed acceptable when a drug holds significant potential benefits. Conversely, when the potential benefit is minimal, even a slight risk can become unmanageable. (21) In 2015, the antibiotic prescription rate per every 1000 persons in the United States was 838 prescriptions. (22) Because of the growing prevalence of antibiotic use, the occurrence of adverse drug responses or hypersensitivities becomes a hot topic among healthcare experts. Antibiotic hypersensitivity is frequently caused by the non-selective death of the bacteria being targeted. Symptoms such as diarrhea, nausea, vomiting, rashes, and gastrointestinal distress are among the most prevalent side effects.

2.3. Global infection burden

Klebsiella pneumoniae (KP) is indeed a significant bacterium that belongs to the Enterobacteriaceae family. It's known for causing a wide range of infections, including pneumonia, urinary tract infections, soft tissue infections, and septicemia (bloodstream infections). It's noteworthy that *Klebsiella pneumoniae* infections can be acquired both in community settings and healthcare facilities like hospitals.(23) Nielsen et al. report that, after Escherichia coli, *K. pneumoniae* is the second most common cause of gram-negative bloodstream infections (BSIs) in adults.(24) Patients with KP-BSI have been considered to have high death rates, ranging from 20 to 40% (25), but this rate has been observed to increase to 67.6% in ICU patients. (26) However, the great majority of *Klebsiella* infections are linked to hospitalization.

Klebsiella spp. is an opportunistic microorganism that targets individuals with compromised immune systems, often those hospitalized with severe underlying conditions such as diabetes mellitus or chronic lung obstruction. Among the species in this genus, Klebsiella pneumoniae stands out as the most medically significant, predominantly causing infections acquired within healthcare facilities. Notably, Klebsiella spp. contributes to approximately 8% of all bacterial infections acquired in hospitals across both the United States and Europe. It's worth noting that there aren't significant variations in its prevalence across different geographic regions. *Klebsiella* is listed among the eight frequently encountered infectious agents in US hospitals, responsible for approximately 3 to 7% of all cases of nosocomial bacterial infections (27). *Klebsiella* is responsible for 6 to 17% of nosocomial urinary tract infections (UTIs), displaying an increased prevalence in particular high-risk patient population, notably individuals with or diabetes mellitus or neuropathic bladders (28). Regarding hospital-acquired bacterial infections, *K. pneumoniae* can cause illness in various places of the body and various ways depending on how it is transmitted. 7-14 percent of pneumonia, 4-15 percent of septicemia, 2-4 percent of wound infections, 4-17 nosocomial infections in intensive care units, and 3-20 percent of all neonatal septicemia cases are caused by *K. pneumoniae*. In the United States, people who suffer from alcoholism make up 66% of people affected by community-acquired pneumonia. Notably, within the United States, 66% of individuals afflicted by community-acquired pneumonia are those grappling with alcoholism. Due to antibiotic resistance, *K. pneumoniae* is currently one of the top 8 hospital infections and is becoming more and more of a problem in hospitals worldwide (29). KPC-associated infections are mostly nosocomial and systemic, affecting patients with a variety of risk factors (30). There have been reports of therapeutic failures and negative effects on patient outcomes, with significant mortality rates ranging from 22% to 57% (31).

2.4. Pathogenesis of *K. pneumoniae* infections

Some authors use the phrases "pathogenicity factor" and "virulence factor" interchangeably, while others emphasize a clear differentiation between the two "pathogenicity" characterizes a bacterium's capability to induce illness, whereas "virulence" gauges the extent of pathogenicity inherent in any bacterial species.The urinary and respiratory tracts are the most typically affected by nosocomial *Klebsiella*

infections. Because these two body regions differ significantly in terms of host defensive systems, it is reasonable to predict that the virulence factor profile found in UTI-causing *Klebsiella* strains will diverge from that noticed in strains derived from respiratory reservoirs of patients with pneumonia. The exploration for *Klebsiella's* pathogenic processes has revealed several bacterial components that trigger the pathogenesis of these bacteria (32). *K. pneumoniae* pathogenicity is facilitated by multiple arrays of virulence factors that permit it to circumvent host innate immune defenses. Among these variables the capsule, lipopolysaccharide, adhesions, method for acquiring iron, resistance to serum, and the ability to create biofilm (33). Clinical specimens of *K. pneumoniae* produce Types 1 and 3 fimbriae and are organized on their surfaces.5Type 1 fimbria is the well-studied, found in several Enterobacteriaceae species, and is mostly made of numerous structural components known as FimA. At the fimbria's tip, there is present an adhesion named FimH, that aids in the identification of mannose, which is interacts with FimA (34). Type 3 fimbriae clumping erythrocytes treated with tannic acid-, facilitate attachment of bacteria to endothelial and bladder cell lines and are contributing in creation of biofilm development on non-living surfaces (35). The virulence variables of *K. pneumoniae* that are particularly important in inducing sepsis include lipopolysaccharide, or (LPS), and capsular polysaccharide also known as (CPS). LPS possesses lipid A, core, and O-polysaccharide antigens serving as a defense mechanism against complement-triggered destruction. The CPS, which consists of polymorphonuclear cells that produce phagocytosis resistance, is essentially the pathogen's outer layer. CPS reduced the amount of C3 on the bacterium and served as a barrier that restricted contact between macrophage receptors and their ligands on the bacterial surface. CPS is vital and affects how surfactant protein D (SP-D) interacts with *K. pneumoniae*.SP-D promotes clumping and phagocytic clearance by human alveolar macrophages. CPS prevents C3 and SP-D from attaching, leading to the removal of microorganisms in the lungs.*k.pneumoniae* generates complex an acidic polysaccharide capsular antigens that are important for the pathogenicity of this organism. Capsular antigens are divided into 77 serovars categories and are frequently consist of uronic acids. The microorganism eludes phagocytosis by using densely clustered bundles of fibrillous formation by polymorphonuclear granulocytes as a defense strategy. This strategy also hinders killing by bactericidal serum components and suppresses complement

constituents that opsonize the pathogen, such as C3b. These potent virulence factors are also capable of inhibiting macrophage development in vitro (36).

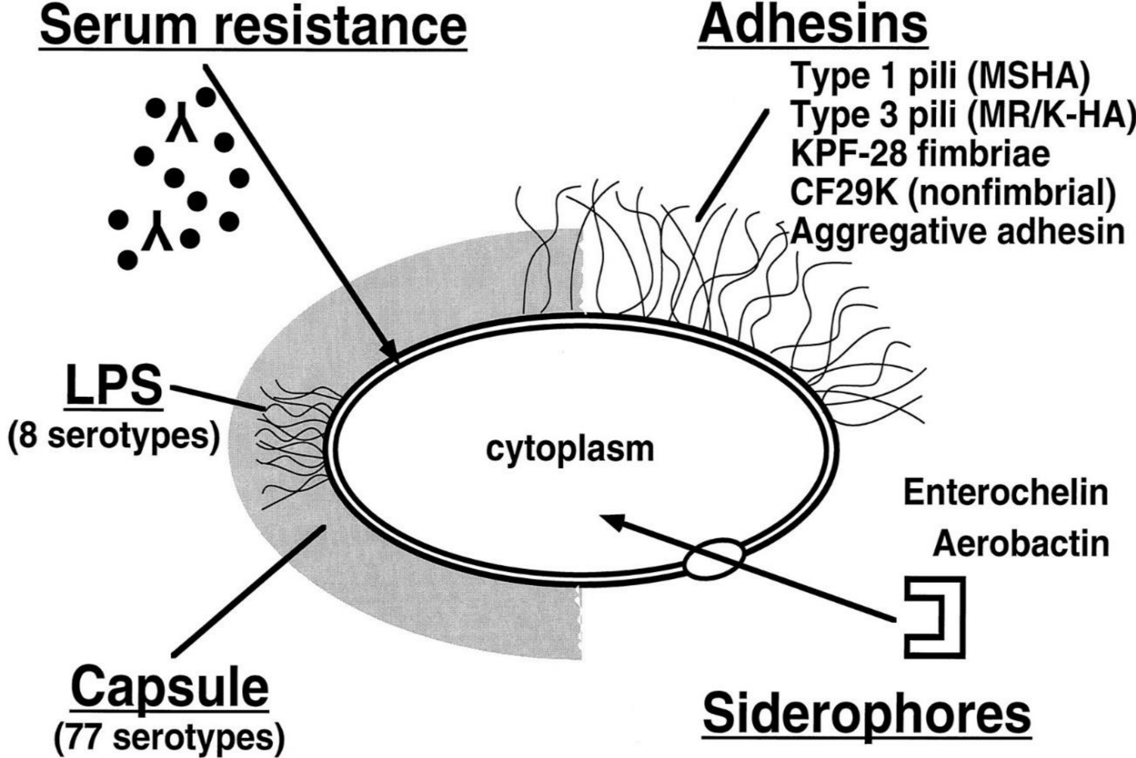


Figure.1. Schematic representation of *Klebsiella* pathogenicity factors (37).

2.5. Transmission

Klebsiella illness is spread via exposure to the bacteria through the respiratory system, leading to pneumonia, or through the blood, resulting in an infection in the bloodstream. Infections with *Klebsiella* are particularly prevalent in hospitals, where they are spread through direct contact with contaminated hands of hospital people staying there, whether they either staff members or patients. *Klebsiella spreads swiftly and easily*, though it cannot be carried by the air. Healthcare settings are more likely to contract *Klebsiella* infections due to the operations' nature, which allows microorganism's easy entry into the body and Patients who have catheters, ventilators, or surgical wounds are most susceptible to developing this severe illness (29).

2.6. Clinical impact

Klebsiella pneumoniae colonies the human gastrointestinal tract, urinary tract, skin, and upper respiratory tract. It is one of the most common organisms to cause infections that develop in hospitals and affect the bloodstream, urinary system, lower respiratory tract, surgical site, and other areas. Data from recent years have shown a rise in quantity of KP isolates that exhibit resistant to one or more major antibiotic classes. As a result, infections caused by multidrug-resistant KP (MDR-KP) are becoming increasingly linked to heightened level of illness, deaths, and healthcare costs, owing to insufficient and limited treatment options (38).According to recent research, the rate of *Klebsiella* infections varies from 5 to 35 percent in Western countries and from 18.8 to 87.7 percent in Asian region and (39). *Klebsiella* transmission rates in faecal samples can vary from 5 to 38 percent in non-hospital settings, while transmission rates in the nasopharynx vary between 1 to 6 percent (23). in ICU patients, *Klebsiella s*pecies constitute a significant source of ventilator-associated pneumonia (VAP), which accounts for 83% of hospital-acquired (HA) pneumonia (41). *K. pneumoniae* ranks are the second major source of bloodstream (BSI) infection caused by Gram-negative after only *Escherichia Coli* (42). Cancer is the most common underlying condition related to BSI acquired within hospitals, whereas liver illness and diabetes mellitus was the most common between acquired from community (CA) *K. pneumoniae* BSI (43). BSI might be a predominant infection with no known origin. On the contrary side, BSI is frequently results from recognized source spreading into the circulation and causing secondary infection.

Secondary BSI is frequently encountered through urinary system, digestive system, urinary or intravenous catheters, and pulmonary areas.The mortality rate of *K. pneumoniae*-related BSI is 20–30%, while general death rate is thought to be 1.3 per one hundred thousand individuals (44).

2.7. Risk Factors

Several elements comprising pathogenic characteristics (such as virulence attribute and antibiotic resistance), host internal factors (such as genetics, age, and immune strength), and external factors (such as antibiotic use, environmental exposure, nutrition, and alcoholism), collectively all influence susceptibility to *K. pneumoniae* infection (45).For ESBL transmission and disease risk factors includes previous antibiotic treatment, prolonged hospitalization stay, extended ICU stay, and the use of mechanical ventilation (46). Intestinal extended spectrum beta-Lactamase bacterial colonization additionally connected to ESBL infection. Particular warning variables for colonization and infection with Carbapenem resistant *K. pneumoniae* comprise antecedent antibiotic therapy, kidney failure, older age, surgical operations, and intensive care unit (ICU) hospitalization (47). Similar to native strains of *K. pneumoniae*, hospitalization appears to be an important contributor in infection. Antibiotic-resistant strains, like endemic strains, can infect a variety of body sites, but they frequently result UTIs.This could be due to *K. pneumoniae* inoculation of the urinary system from the gastrointestinal tract via the perineum (48).

2.8. Control Strategies

Detecting and removing the origin of *K. pneumoniae* infection is an efficient way to avoid infection with these bacteria. However, pinpointing and eradicating the origin of infection remains a huge difficulty. Most hospitals still use specimen culture to check for the existence of *K. pneumoniae* (49). Molecular technique like Multiplex polymerase chain reaction has been used to discover specific chromosomal genes including blaSHV, blaLEN, and blaOKP, along with their associated accessory genes such as (deoR) (50). Exposure avoidance should be carried out by identifying affected individuals as soon as possible and using basic measures like wearing gowns, protecting gloves, and face masks. Tracing contact is needed to be used wherever possible to limit additional exposure to uninfected people, both in the healthcare

settings and in the general public. Hand hygiene instructions is equally essential for healthcare workers (51)(52). Antibiotic use should be strictly governed by guidelines and principles, particularly during the first empirical treatment (53). Possible, rational, and standardized antibiotic administration, including specific indications, proper dose, enough time periods for treatment, sensitive antibiotic switching, and other interventions such as surgical drainage and implant extraction, should be followed (54).Limiting use of antibiotic in other than human situations can help to restrict CRKP's origin (55).

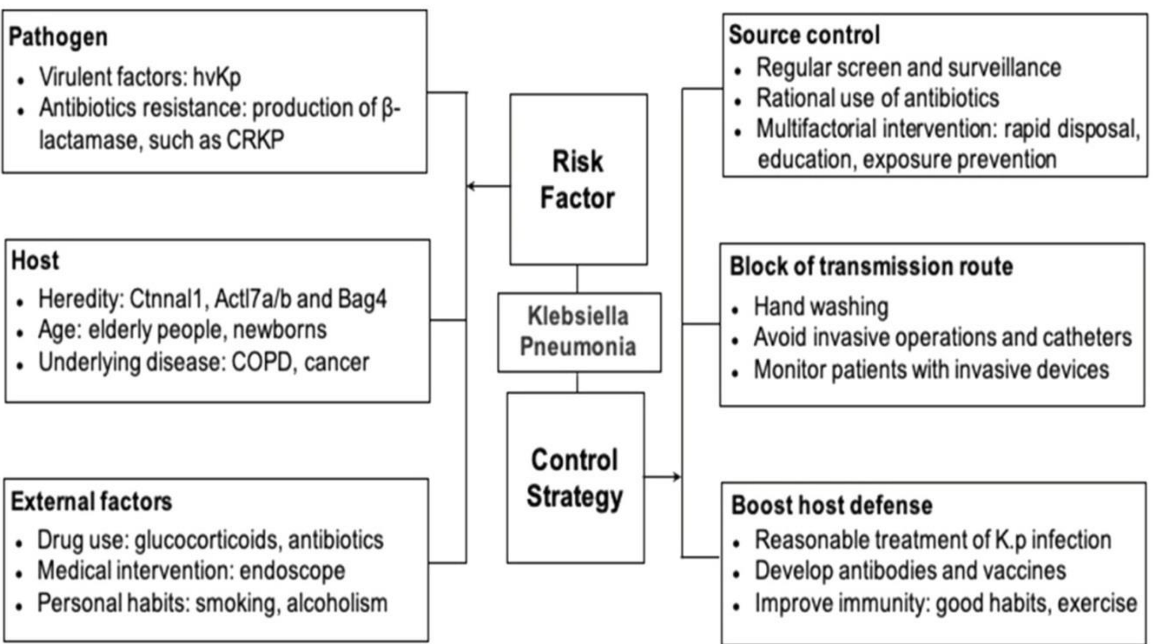


Figure 2. Risk factors and prevention methodologies (56).

2.8.1 Prevention of transmission/Block of transmission route

Among healthcare professionals, Gram negative bacteria like *Klebsiella*, a strain of Staphylococcus aureus, *C. difficile*, and other Gram-negative bacteria and other have been discovered. Close interaction with patients or exposure to contaminated surfaces with in the healthcare setting can infect healthcare workers' hands (57). Dissemination throughout patients can be prevented by checking adherence with contact restrictions and sample culture findings, which should be conveyed to healthcare provider for them to make properly and prompt selection in circumstances where unforeseen transfer is found. Touch measure for the CRKP/HvKp should be implemented, particularly in populations at elevated risk of transfer those who come into touch with the sick people (58) **.**High-risk patients

should be checked for CRKP/HvKp at admission and at regular intervals throughout their hospitalization. Regular preventive measurement should be implemented for patients with minimal risk of transmission and people who have connection with health surveillance to undiagnosed, infected, or invaded CRKP/HvKp patients. Preventive hygiene measures can be taken while the results of the entrance test is in progress, particularly for those patients brought from other institutions has been identified HvKp/CREose who come into touch with infected individuals (59).

2.8.2 Host Defense and protection of the susceptible population

K. pneumoniae is enveloped in polysaccharides, including the capsular structure and lipopolysaccharides cap; make them suitable target antigens for vaccine development or enhancing host immunity (23). Immunoglobulin and vaccines against capsular polysaccharides are being produced, although becoming problematic in *K. pneumoniae*, due to the presence of 77 unique capsules and nine diverse LPS serotypes (60). It is essential to detect extensively conserved antigens in *K. pneumoniae* strains to produce antibodies or vaccinations that provide broad coverage and protection across strains (61) **.**Other ways to boost immunity include adopting a healthy way of life, such as regular physical activity, getting adequate sleep, giving up smoking, and consuming a well balance diet abundant in fruits and vegetables. (62).

2.9. Treatment

Bacterial cultivation and subsequent drug sensitivity tests are frequently used to identify the antibiotic regimen against *K. pneumoniae* (63). Surgical procedure or interventional therapies should be undertaken on individuals with gangrene, abscess, or empyema. Empirical antibiotic therapy for community-acquired pneumonia should ensure sufficient protection against potential Gram-negative bacteria. The 3rd-generation cephalosporins or quinolones, either alone or in combination with aminoglycosides, should be administered for at least two weeks (64). Regarding individual with hospital-acquired *K. pneumoniae*, suitable antibiotic regimens, including imipenem, third-generation cephalosporin, quinolones, or aminoglycosides, should be employed for minimum of fourteen days (65).If individuals respond quickly, quinolones could be given intravenously. Carbapenem need to be considered the appropriate therapeutic option for ESBL producing stains, while intravenous

fosfomycin, colistin and tigecyclin should be used for Carbapenem-producing variant (66).

2.10. Computer-aided Therapeutic Interventions

Due to numerous earlier attempts to develop subunit or Lipopolysaccharides vaccines, there is currently no authorized FDA vaccine available for the avoidance of *K. pneumoniae*-related illnesses (lipopolysaccharides) vaccines. One such attempt at immunization was a B-cell independent vaccination Induction against *Klebsiella pneumoniae* has disadvantages because it takes time and need efficient recruitment of neutrophils, whose reduction restricts the IL-17RC signal, resulting in the abolition of vaccine-induced immunity protection (67). Reverse vaccinology is a method of identifying potential vaccine candidates (PVCs) with high accuracy and reliability by genome mining via computer-aided studies (68). This bioinformatics-based method consults a variety of tools and databases and includes a comprehensive review of the pathogen genome, virulence variables, protein relationship, and other characteristics (69)**.**The possible vaccine candidates should possess characteristics such as antigenic to the host immune system, distinct from human, gut homologous and essential for pathogen virulence (70). These innovative vaccine possibilities must additionally be tested for their subcellular placement, existence of transmembrane helices, and immunogenic epitopes.Protiens located on exterior membrane and those secreted proteins possessing elevated pathogenicity and antigenic capacity, are considered suitable vaccination targets because they can bind to host MHC (Major Histocompatibility Complexes) (71). Several researches have recently employed reverse vaccinology to identify potential vaccine candidates for multiple drug-resistant *K.pneumoniae*, such as proteomic technique to uncover several antigens that are immunogenic in *K. pneumonia* (72).In 2014 Hoppe et al, created a complementary DNA expression library and used microarrays to search for novel *Klebsiella pneumoniae* immunogenic proteins. Likewise, Dar et al, 2019 used computational vaccinology to find antigenic proteins of *Klebsiella pneumoniae* for Vaccine-development (73)**.** A separate investigation used immunoproteome-based approach to pinpoint twenty strongly immunogenic external membrane proteins against ESBL producing *K. pneumoniae* .Notably among these proteins OmpK36, OmpA FepA, OmpW. OmpK17,Colicin I receptor protein, and three newly discovered proteins were the most frequently found proteins in blood sample individual with confirm

ESBL *k pneumoniae* infection .In a murine infection model, researchers looked into two isolates of *Klebsiella pneumoniae* antigens, OmpK17 and OmpK36, along their associated fusion protein called is F36/17, as prospective vaccination contenders. Bexsero, the pioneering vaccine developed using computational vaccinology was formulated against Neisseria meningitis serogroup B (MenB) (74). Subsequently Insilco approach has been effectively employed to create vaccines against a variety of pathogenic bacteria, this include *porphyromonas gingivails,Streptococcus pneumoniae, Chlamydia pneumoniae, Bacillus* anthracis *Streptococcus agalactiae, E. coli, Neisseria meningitides serogroup B, Leishmania infantum*, and *Pseudomonas aeruginosa* (75)(76)(77)(78).

2.11. Non Traditional Approaches against MDR *K. pneumoniae* Future Potential Strategies

Innovative treatment approaches for MDR pathogens, such as C-C-RKp, will be developed in the future. The application of bacteriophages, the delivery of monoclonal antibodies targeting particular bacteria species, modification of gene, and faecal micro biome transplantation are the most promising approaches**.**

2.11.1 Bacteriophages or Phage Therapy

Bacteriophages are naturally occurring bacterial predators. Phage therapy was used in the Soviet Union since the 1920s and currently explored as potential alternative to antibiotics for future (79)(80).In animal models, studies on bacteriophages against *K. pneumoniae* demonstrated great potential. (81) However, critical issues must be addressed before implementing the bacteriophage method against bacterial disease, such as the rapid emergence of phage resistance, potential for patient to mount immune responses against the administered phages, and the the absence of specific standard regulatory protocols tailored for this method (82).

2.11.2 Monoclonal antibodies

Monoclonal antibodies offer distinct advantage due to their capacity to selectively particular specific bacterial targets, and this exceptional mode imparts attractive attributes (83) **.**Notably monoclonal antibodies do not disturb **,** thus avoiding any contribution in gut dysbiosis the gut flora, or the emergence of *C.*

difficile infection, unlike traditional broad-spectrum antimicrobials (84). Bacterial proteins such as (LPS) along with capsular polysaccharide or exopolysacchrides, proteins involved in pilus production, and components of extracellular vesicle are the most common monoclonal antibody targets. Monoclonal antibodies that target bacterial pili could be a promising future treatment for *K. pneumoniae* urinary tract infections, as they reduce bacterial attachment and biofilm formation in the urinary system (83). The absence of precise human infection models possess major issue in the current scenario for the effective utilization of monoclonal antibodies against preclinical studies bacterial infections in in preclinical studies (80)(83)(85).

2.11.3 Bacterial Gene Editing

Bacterial gene editing specifically using "clustered regularly interspaced short palindromic repeats" system (CRISPR/Cas) represent innovative approach in the fight against MDR bacteria. This approach involve to eliminate specific gene responsible for bacterial antibiotic resistance presenting novel tool for combating MDR bacteria (86).

2.11.4 Faecal microbiota transplantation (FMT)

Lastly, faecal microbiota transplantation (FMT) is a novel method for gut decolonization of MDR *Enterobacteriaceae* (87). The goal of this method is to substitute the patient's defective microbiota, which contains MDR bacteria, with a healthy donor microbiota that is free of antibiotic-resistant bacteria. FMT seems to be efficient in several situations, including colonized patients in hematologic departments, in diminishing risk of MDR pathogens causing severe challenging-to manage infections, and reducing the burden of MDR Enterobacteriaceae in these facilities (87).

2.12. Comparative phylogenetic methods to infer evolutionary relationships

A wide range of morphological and phenotypic characteristics have been used in taxonomic analyses, which are often arbitrary Because there is no clear foundation for deciding specific morphological or phenotypic attributes has led to prevailing view , that these characteristics are now widely regarded as unsuitable for generating reliable and uniform prokaryote taxonomies. Furthermore, single trait or limited number of phenotypes are unlikely to accurately and consistently represent evolutionary relationships (88). The composition of genomic G + C content, DNA-

DNA hybridization and, subsequently the 16S rRNA gene were all used as genotypic criteria for bacterial taxonomy (89).The trends has shifted toward inferring phylogenetic relationships from a larger number of genes over time, in part due to the growing accessibility and reduce cost of DNA sequencing.Addationaly, concerns have arisen about precision of evolutionary connections derived from a single gene. Multi-locus sequence analysis can be used to infer phylogeny from several universally conserved housekeeping genes (MLSA)(90) although 16S rRNA gene sequence analysis and multilocus sequence analysis (MLSA) have demonstrated to be useful phylogenetic tools, but this techniques contain notable limitations that they only rely limited dataset to characterize an entire individual. This method has acquired widespread acceptance due to the constraints posed by the time and expenses linked to genome sequencing. Recent advances in sequencing technology, on the other hand, have substantially decrease the resources necessary for genome sequencing, consequently large number of genome currently accessible in open access databases. The rapid advancement of genome sequencing has created possibilities to investigate the use of complete genomes in the study of evolutionary connections. Many methods for establishing relatedness through complete genomes have been developed (91).

2.13. Pangenome analysis and their utility in bacterial characterization

Prior to the emergence of the pan-genome concept various investigation utilizing subtractive hybridization and comparative genome hybridization (CGH) to analyze multiple strains within the same species demonstrated substantial genetic diversity. Notably, species such as *H. pylori*, *S. aureus*, and *E. coli* displayed significant genetic heterogeneity, with around 20-35 percent of gene exhibiting non conversation across all strains of the same species (92)(93). The term 'pan-genome' or ‘supragenome' refers to the collection of all genes found in the genomes of members of a species or group of organisms (94). It can be defined as the term "pan-genome" refers to a species' entire gene repertoire. It can be categories into three segments the core genome, represent set of genes that are present and conserved across isolates; the 'dispensable genome,' which includes genes that are shared by some strains but not and the strain-specific genes, which are unique in single isolate (95). The core genome frequently contains phylogenetic signals that reflect vertical mutation accumulation and can be used to assign bacterial strains to populations. (96) An open pan-genome tends to grow with each new genome added, whereas a closed pan-

genome stays at a fixed number of genes after a certain number of genomes have been added. Several queries were raised regarding origin, composition, size and finite or infinite nature of bacterial pan genome. At the same time formulating precise definition for bacterial species has proven to be persistent challenge, as the relative contribution of diverse genomics towards a potential biological characterization of bacterial species remains unclear. The issues arises from the fact that bacteria are distinct from eukaryotic genomics and species notions in that way transfer genetic materials in peculiar and uncommon way. (97)

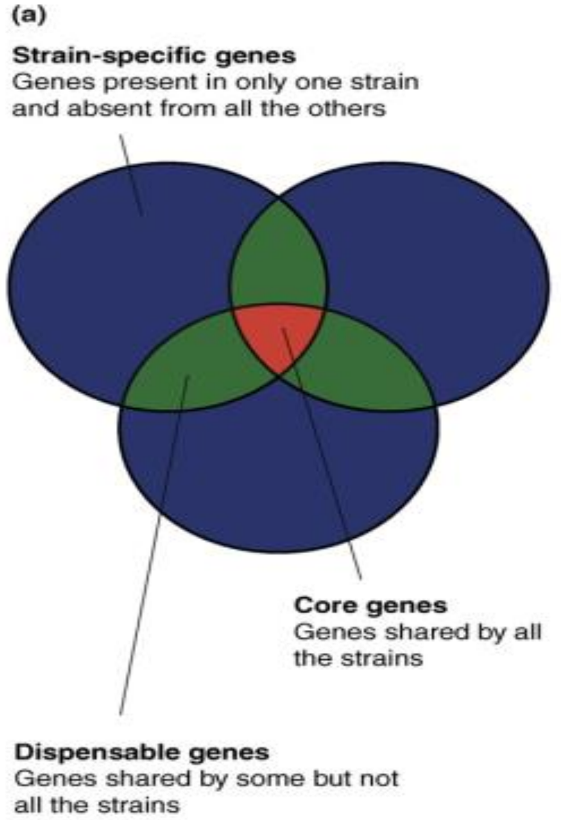


Figure:3 the species pan genome schematic image of the pan genome structure, core, dispensible,and strain specific genes are indicated in red, green and violet, respectively. Circles represent different strain. For simplicity, a comparative analysis performed on a set of three genomes is shown(98)

MATERIAL & METHODS

3.1. Genome selection

A total of 560 complete genomes of *K. pneumoniae* were retrieved from the GenBank database (NCBI) National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The National center for biotechnology information (NCBI) provide vast array of online resources for biological data and information including the GenBank nucleic acid sequence database and the PubMed database containing citations and abstracts from published life science journals. In 1988 the National center for biotechnology information (NCBI) established as a section of National Library of Medicine (NLM) at National Institute of Health (NIH) to develop system of information for molecular biology. The NCBI is responsible for variety of task including enabling the usage of databases and software and conducting research in innovative method for computer-based information processing, these involve studying the structure and function of proteins and other biologically significant molecule (99). In this present research combined the Insilco approach, a computationally based method for identifying drug targets against *Klebsiella pneumonia*, with the subtractive genomic approach to rank possible therapeutic targets (100).

3.2. Pan-genome analysis

To identify the core or conserved areas of Klebsiella pneumonia, bacterial pangenome analysis was performed using the Bacterial Pangenome Genome analysis (BPGA) tool. We kept the default sequence identity cut-off that is 50%. The clustered output has been computed to produce a tab to detect gene presence/absence binary matrix (pan-matrix), that is subsequently used to compute pan-genome profiles with default settings as well as pan-genome driven phylogeny (101) .

3.3. Subtractive Proteomics

3.3.1 Subtraction of Non-Homologous Proteins

The primary analytical step involves subjecting protein sequences to the FASTA Validator for vaccine target prediction to validate the input format. (102) to obtain the best druggable protein targets should not be identical to human proteome, as this could trigger an autoimmune response in the host.To examine whether the subtracted core proteins show similarity with human repotire the BLASTp (Basic

Local Alignment Search Tool Proteins) server was used for homology verifications. (103). the cutoff criteria used for the screening were bit Score >100, e-value <10^{-5}, and percentage identity (PI) >35%. The proteins with values higher than the specified criteria were filtered out, and those lying within the range of establish criteria were further examined (104).

3.3.2 Identification of Essential and Non-host Homologous (ENH) Proteins Targets

A subtractive genomics strategy was applied to choose conserved targets that were necessary for bacterial growth. To check the essential proteins of *Klebsiella pneumoniae*, nonhomologous proteins obtained were subsequently analyzed using the Database of Essential Genes (DEG). The VacSol pipeline's default settings were used to filter out the essential proteins for the pathogen survival The pinpointing of drug targets depends on the essentiality check because essential genes frequently help microbes to perform useful cellular processes (105) .The DEG (Database of Essential Genes) is a collection of genes that are regarded as essential for an organism's survival and, as such, as the building blocks of life. Essential genes required for the cellular survival of bacteria, archaea, and eukaryotes are included in the DEG, which is made up of experimentally verified data (106).

3.3.3 Identification of Virulence-Associated Proteins

Antibiotic resistance and the emergence of superbugs are problems that cannot be ignored. So, targeting the bacterial virulent proteome has been added as a novel parameter to our study. If antibiotic resistance develops against the predicted drug targets, this may open the door for the development of novel virulence inhibitors**.** The pathogen produces specialized chemical compounds that assist bacteria to begin infection within the host organism and these chemicals are called virulence factor for pathogens. We used two online databases in our study i.e. (VFDB: Virulence Factor Database,) also known as VFDV and microbial virulence factor database (MvirDB). The identification of virulence factor or proteins was subsequently assisted by VFDB (108). To determine the pathogenicity of the proteins for further research, the resultant proteins that had been shortlisted from the previous step were BLASTp , e-value<10^{-5} and bit score> than 100 against proteins from the "VFDB Core set A" (109).

The VFDB (virulence factor database) is comprehensive and freely accessible online databases that provide details about the virulence factors of bacterial pathogens. Microorganism virulence factors are the characteristics such as gene products that allow it to colonize or stay inside host and thereby increase its capacity to cause disease. The VFDB was established in the year 2004 and has offered latest information of virulence factors associated with wide array of clinically important bacterial pathogens. The primary goal of the virulence factor database (VFDB) (http://www.mgc.ac.cn/VFs/) is to offers researchers a convenient platform to readily access the latest information regarding virulence factors originating from diverse bacterial pathogens. VFDB is centralized gateway for storing, retrieving, searching, and updating data about virulence factors from various bacterial pathogens (110).

MvirDB (Microbial Virulence Database) is a freely accessible Online database (http://manndb.llnl.gov/) that contains information that is available to the general public, to design DNA and protein signatures, we need to quickly determine and assess specific group of genes that should be targeted, MvirDB play pivotal role in enabling this capability. We combine all publicly available ordered sequences encoding well known toxins, virulence factors, and antibiotic resistance genes into useful database to speed up the recognition and characterization of key the sequences for signature discover (111).

3.3.4 Physicochemical characterization

The selection of ideal drug targets is not solely based on criteria such as being Non-homologous to human proteins and being essential for pathogen survival several additional physiochemical properties also play an important role and can serve as valuable considerations in choosing suitable drug targets may prove useful as drug targets. Physiochemical characteristics were investigated by using the database ProtParam tool ExPasy and Protean 3D tool. The next step after the screening of essential virulent protein and core proteins was subject to Expasy and checking out physiochemical properties like the Grand Average of hydropathicity (GRAVY), aliphatic index, molecular weight, and instability index (112). The protein stability is measured by the instability index; proteins are predicted to be stable if the instability

index value is less than 40. Because they are accessible to drugs, lower molecular weight protein targets are typically regarded as ideal drug targets (113).

Expasy serves as resource portal for bioinformatics provided by the SIB Swiss institute Institute of Bioinformatics'. This extensible and integrated portal offers access to more than 160 databases software tools, created by SIB group to assist in supporting a variety of life science and clinical studies fields, including proteomics, genomics, and structural biology, along with system biology, evolution and phylogeny, , and medicinal chemistry(114).

3.3.5 Pathway Analysis

The metabolic pathway analyses of human non-homologues essential proteins of *Klebsiella pneumoniae* retrieved through Blastp was then carried out by KEGG annotation server (115). This process was used to identify the special pathways that are present in bacteria but not in humans, and they were then given the appropriate level of priority. Targeting the proteins associated with these pathways can help with the development of new therapeutics. The pathways that did not exist in humans but did in the pathogen were classified as unique to *Klebsiella pneumoniae* in the KEGG database annotations, while the remaining pathways were categorized as common pathway (116). KEGG is a database of genes and genomes that can be accessed at https://www.genome.jp/kegg/pathway.html). The primary objective of the KEGG (Kyoto Encyclopedia of Gene and Genome) database project is to give functional significance to genes and genomes at the molecular and advanced levels of biological organization. A manually curated resource, KEGG integrates 18 databases with information on systems, genomics, chemicals, and health. Additionally, it offers KEGG mapping tools, which help researchers understand how genome sequences and other molecular datasets relate to cellular and organismal functions. Based on the idea of functional orthologs, KEGG mapping is a predictive technique for creating molecular network systems from molecular building blocks (117.)

3.3.6 Sub-Cellular Localization of Drug Target Proteins

For accurate and exact drug target recognization and the development of multi-epitope vaccines, the determination of the subcellular localization of proteins is

crucial. After the KEGG analysis screened the proteins, were subsequently assessed for localization prediction, The subcellular localization of proteins was analyze in the current study using bioinformatics tools like PSORTb 3.0.3 and CELLO v2.5. The most recent version of the PSORTb programme, which is used to predict the subcellular localization of bacterial proteins, is 3.0.2.The results of subcellular localization encompass placement of protein within cytoplasm, cell wall, cytoplasmic membrane, extracellular space, and unidentified regions. Cytoplasmic proteins were thought to be potential drug targets. PSORTb (http://www.psort.org/psortb/) is popular and potent tool for determining the location of proteins produced by gram negative bacteria (118)(119).The subcellular localization of bacterial proteins is predicted by CELLO, (http://cello.life.nctu.edu.tw/). The characteristics used by CELLO include such as compositions of amino acid, the sequence composition based on physiochemical properties, partitioned amino acid composition, and di-peptide composition (120).

3.3.7 Druggability of Essential Proteins.

By analyzing the target's effectiveness in binding to potential drug candidates, the druggability of the target was evaluated. Similarly, essential nonhomologous proteins were assessed using BLASTp against the Drug Bank database (www.drugbank.ca) to ascertain their ability to finally identify novel drug targets. BLASTp was used to check for druggable compounds against the DrugBank database with the parameters of e-value $1x10^{-5}$and bit score value >100.The customized FDA (Food and Drug Administration) approved dataset of drug targets compared to the essential proteins. Proteins with a high similarity frequency (80% or more) to an FDA-approved Drug Bank database were regarded as druggable targets (121).DrugBank is a comprehensive, freely accessible online resource that provides detailed information on drugs, drugs-targets, drugs' actions, and drugs' interactions for both FDA-approved and experimental drugs undergoing FDA approval (122).

3.4. Pharmacophore Modeling

After a virtual screening of FDA-approved and experimental Drugs, the 3D structure of these ligands was searched from the PubChem database in SDF format (PubChem). (124)

3.5. Homology Modelling of Drug Targets Proteins

Protein structure complexity and function in biological systems are directly related, and visualization of protein structure facilitates understanding. Selected proteins did not yield structures for drug targets when the PDB (Protein Data Bank) was searched for the crystalline structure. Homology modelling was conducted to create a 3D structure of drug targets by employ trRosetta (transform-restrained Rosetta).TM score was used to check the quality of the modeled proteins. To calculate the TM-score (template modeling score) of the generated models was computed on the probability of the most likely predicted distance and the convergence of the best model TM-score exceeding 0.5 generally indicate a model with well predicated topology. The TM-score scale between 0 and 1where higher value signify better structure similarity between the predicted model and actual structure.

3.6. Refinement of Proteins Structure

Modeled proteins were then refined using the GlaxyRefine online webserver (http://galaxy.seoklab.org/) to further assess these proteins. Protein sequences must be supplied in FASTA format to predict their structures. For refinement purpose, user required to supply initial model structure to be refined which should be in the PDB format.addationaly the user need to define the residue number range for specific region that required refinement. A 500-residue protein's predicted structure will take 7 hours to predict, on the other hand refining 26-residue loop or terminus within protein structure is expected will take 2 hours. The five resulting models were subjected to evaluation for GDT-HA, RMSD and MolProbity score, and the best model was chosen from five models generated by the GlaxyRefine server based on the number of Ramachandran-favored residues and the absence of fewest rotamers (125).

3.7. Molecular Docking and lead identification

To analyze the interaction between small molecules and target protein within the binding site of as well as to enhance our comprehension of fundamental biochemical mechanism, the molecular docking approach was employed .In our study we used AutoDock Vina tool within PyRx was used to analyze the molecular docking of selected drug target proteins with ligands, and blind docking was accomplished by adjusting the grid box's exhaustiveness setting's. Chimera and Biovia Discovery Studio was used to choose the best pose based on factors such as root mean-square

deviation (RMSD) and their lowest binding energies. A program called UCSF Chimera was used to interactively visualize and analyze molecular structures and related data, such as density map, trajectories and sequence alignments (126).

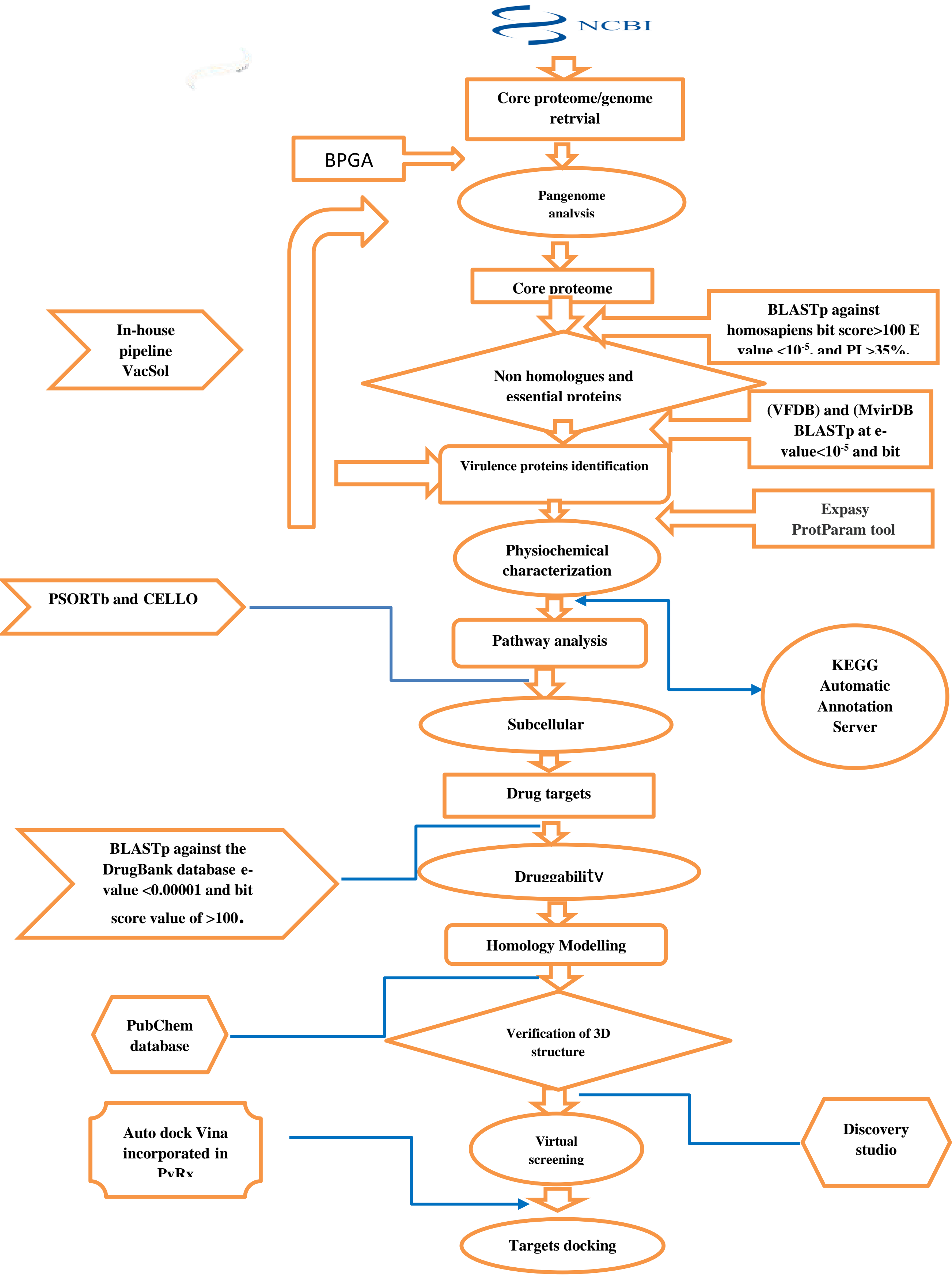


Figure 4. Workflow of the overall analysis pipeline

RESULTS

4.1. Scrutinization and Collection of Genomes

A total of 560 complete genomes and their corresponding proteomes of *K. pneumoniae* were obtained from the NCBI GeneBank Database. The source of isolation and additional metadata related with these bacterial strains were also verified, and only those pathogenic *K. pneumoniae* strains were added to the study.

Pangenome analysis of 560 *K. pneumoniae* complete genomes showed the existence of 19890 gene families within collective (pangenome), among them 617 proteins were identified as common genes present in all the genomes (core genome). The ratio between the size of the core genomes and pangenome size was determined to be 0.03, indicating that the core genome represent 3 % of the entire pangenome. This shows that the different *K. pneumoniae* strains have vast genetic diversity and little similarity. The pan-genome plot, which depict an open pan-genome, has increased as new genomes are incorporated.

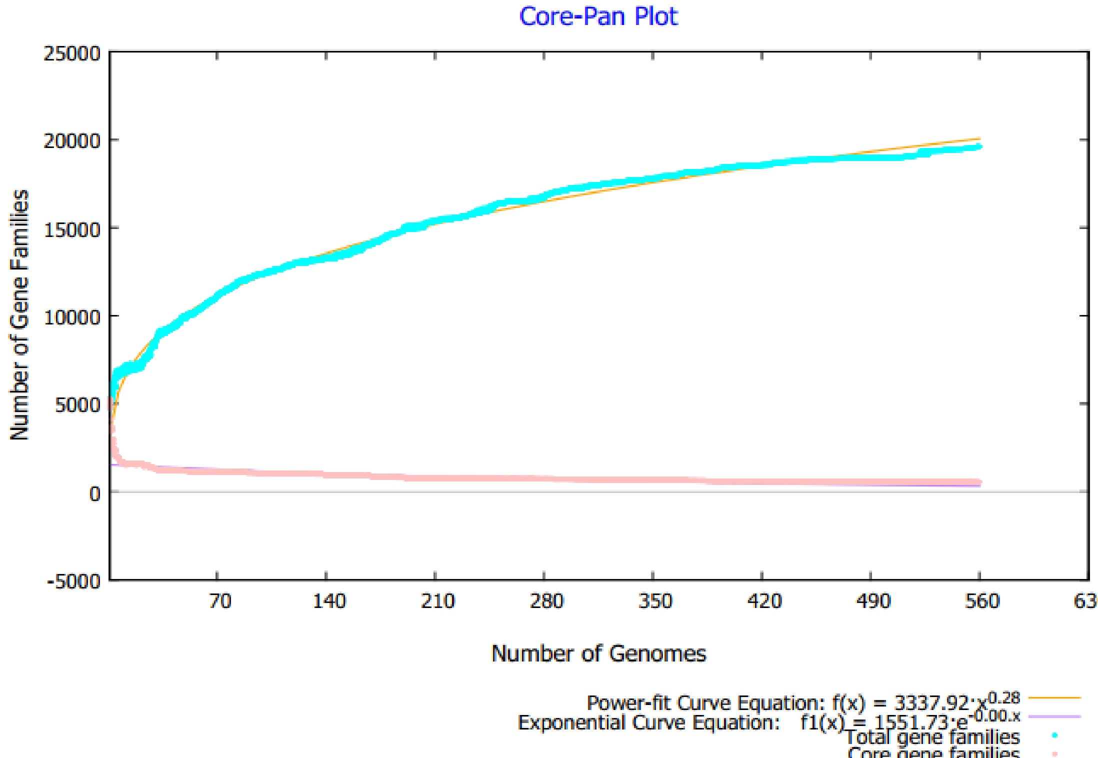


Figure 5. Analysis of the pan-core genome of *K. pneumoniae*

The pan-core plot illustrates the pan-genome characteristics of the 560 *K. pneumoniae* genomes. X-axis represents the number of genomes while the y-axis represents the number of gene families the pangenome size increased with addition of each new genome while the core genome size declined. The pan-genome curve, which is

depicted in golden brown, continues to show an upward trajectory, indicating that the pangenome is open and that the global gene pool of *K. pneumoniae* may increase in the future.

4.2. Subtractive Proteomics

4.3.1 Identification of Non-Homologous and Essential Proteins

The subtractive proteomics method was applied to the core proteome using an in-house pipeline called VacSol, Human protein database homology analysis of the core proteome revealed a total of 550 non-homologous proteins (Supplementary Table 2). The homology filter was used to reduce the autoimmune response and to get rid of any potential cross-reactivity between drug targets and the proteins in the human host (127). The essentiality of these core non-host homologs proteins was used to further filter the proteins. 280 essential proteins in total were found, and these proteins are thought to be necessary for the viability and survival of pathogenic bacteria.

4.3.2. Identification of virulent proteins

VFDB and MvirDB collectively identified total of 127 proteins associated with the pathogenicity of *K. pneumoniae* out of 280 essential proteins.

4.3.3. Pathway Analysis

KEGG predicted only 20 proteins that would participate in pathogen-specific pathways. The development of therapeutics to combat antibiotic resistance should use these proteins as ideal drug targets. Drug targets weren't chosen for those pathways that were common to both humans and bacteria.

Table. 1: Pathways of bacterial drug targets proteins

protein	KO	Pathways
ATP-binding cassette, subfamily C, bacterial CydD	K16013	ABC transporters
D-xylose transport system ATP-binding protein(xylG)	K10545	ABC transporters
general secretion pathway protein E gspE	K02454	map03070 Bacterial secretion system map0511Biofilmformation-Vibrio cholera

4-aminobutyrate aminotransferase / (S)-3-amino-2-methylpropionate transaminase / 5-aminovalerate transaminase	K07250	map00250 Alanine, aspartate and glutamate metabolism Valine, leucine and isoleucine degradation map00310 Lysine degradation map00410 beta-Alanine metabolism map00640 Propanoate metabolism map00650 Butanoate metabolism map01100 Metabolic pathways map01120 Microbial metabolism in diverse environments
MFS transporter, DHA1 family, multidrug resistance protein	K08161	NO pathway
MFS transporter, DHA1 family, multidrug resistance protein	K07552	no pathway
arginine N-succinyltransferase astA	K00673	map00330 Arginine and proline metabolism map01100 Metabolic pathways
hoxN, nixA	K07241	no pathways
oligogalacturonide transport system permease protein	K10193	abc transporters
AraC family transcriptional regulator, mar-sox-rob regulon activator	K05804	no pathways
anaerobic dimethyl sulfoxide reductase subunit C	K07308	map00920 Sulfur metabolism map01100 Metabolic pathways
polar amino acid transport system ATP-binding protein	K02028	no pathways
general secretion pathway protein O	K02464	map03070 Bacterial secretion system
thiamine transport system ATP-binding protein	K02062	map02010 ABC transporters

thiamine transport system ATP-binding protein	K03111	map02010 ABC transporters
sugar PTS system EIIA component	K02777	map00010 Glycolysis / Gluconeogenesis Starch and sucrose metabolism map00520 Amino sugar and nucleotide sugar metabolism map01100 Metabolic pathways map02026 Biofilm formation - Escherichia coli map02060 Phosphotransferase system (PTS) map05111 Biofilm formation - Vibrio cholera
diacylglycerol kinase (ATP) dgkA DGK	K00901	map00561 Glycerolipid metabolismGlycerophospholipid metabolism map01100 Metabolic pathways map01110 Biosynthesis of secondary metabolites map04070 Phosphatidylinositol signaling system map04072 Phospholipase D signaling pathway map04361 Axon regeneration map05231 Choline metabolism in cancer
urease subunit beta	K01429	map00220 Arginine biosynthesis Purine metabolism map00791 Atrazine degradation map01100 Metabolic pathways map01120 Microbial metabolism in diverse environments

4.3.4 Subcellular Localization:

In the process of finding and developing new drugs, predicting a protein's location is crucial. As a result, the subcellular locations of all detected non-homologous essential proteins were predicted using the CELLO (v2.5) and PSORTb tools separately. The subcellular localization of ten proteins (Table 2) in total was identified, and most of them were predicted to be cytoplasmic proteins. Predicting protein subcellular localization also helps in the understanding of disease mechanisms and the creation of new drugs (J. Wang et al., 2005). Table 2 displays the localization of drug targets.

Table. 2: Subcellular localization of Drug targets

Drug target protein	**Localization**
GspE	Cytoplasmic
arginine N-succinyltransferase [*Klebsiella pneumoniae*]	Cytoplasmic
Rpn family recombination-promoting nuclease/putative transposase [*Klebsiella pneumoniae]* YfaD	Cytoplasmic
MDR efflux pump AcrAB transcriptional activator RobA [*Klebsiella pnemoniae*]	Cytoplasmic
MULTISPECIES: amino acid ABC transporter ATP-binding protein [Enterobacteriaceae]	Cytoplasmic membrane
Thiamine transport ATP-binding protein thiQ	Cytoplasmic membrane
single-stranded DNA-binding protein [*Klebsiella pneumoniae*]	Cytoplasmic
MULTISPECIES: PTS glucose transporter subunit IIA [Enterobacteriaceae]	Cytoplasmic
urease subunit beta [*Klebsiella pneumoniae*]	Cytoplasmic membrane
Cysteine/glutathione ABC transporter permease/ATP-binding protein CydD [*Klebsiella pneumoniae*]	Cytoplasmic membrane

4.3.5 Physiochemical Characterization

After applying physiochemical checks like low molecular weight, a negative

GRAVY score, an aliphatic index of more than 80, and an instability index of less than 40, a total of 10 proteins were screened. Low protein molecular weight suggests that drug targets are readily accessible to drugs (128), negative GRAVY score illustrates the hydrophilic nature of the drug targets while higher aliphatic index value suggests that the drug target proteins are thermostable (129). These 10 proteins are all vital, core, and dangerous proteins. Table 3 displays the physiochemical traits of the proteins that drugs target.

Table.3 Physiochemical properties of selected essential, non-homologous, and virulent proteins

<table>
<tr><th>Protein</th><th>Grand average of hydropathicity (GRAVY)</th><th>Aliphatic index</th><th>Instability index</th><th>Mol. Weight</th></tr>
<tr><td>>CORE_REP|Org522</td><td>-0.124</td><td>106.24</td><td>38.51</td><td>55140.58</td></tr>
<tr><td>>CORE_REP|Org53</td><td>-0.167</td><td>91.57</td><td>33.2</td><td>38439.59</td></tr>
<tr><td>>CORE_REP|Org472</td><td>-0.308</td><td>100.68</td><td>24.17</td><td>37059.65</td></tr>
<tr><td>>CORE_REP|Org405</td><td>-0.380</td><td>83.39</td><td>39.78</td><td>33328.23</td></tr>
<tr><td>>CORE_REP|Org457</td><td>-0.256</td><td>88.93</td><td>34.16</td><td>30119.52</td></tr>
<tr><td>>CORE_REP|Org497</td><td>-0.080</td><td>108.93</td><td>34.1</td><td>25155.1</td></tr>
<tr><td>>CORE_REP|Org64</td><td>-0.816</td><td>65.37</td><td>37.82</td><td>20876.54</td></tr>
<tr><td>>CORE_REP|Org144</td><td>-0.175</td><td>105.44</td><td>35.21</td><td>18053.64</td></tr>
<tr><td>>CORE_REP|Org433</td><td>-0.356</td><td>76.32</td><td>21.15</td><td>11695.25</td></tr>
<tr><td>>CORE_REP|Org44</td><td>-0.215</td><td>113.88</td><td>21.52</td><td>65284.91</td></tr>
</table>

4.3.7 Druggability Assessment

The druggability test of a protein is crucial stage in the procedure of identifying potential drug targets. The druggability test was conducted under the presumption that druggable protein targets should exhibit interaction with putative drug candidates. Consequently, the DrugBank database was used to determine potential drug targets (130). During the druggability analysis, we discovered a total of 18 ligands that were FDA-approved and in the experimental stages in the DrugBank database. In total, we identified 10 potential drug targets; 5 proteins have

potential inhibitors that are readily available in drug bank databases, while 5 drug targets were novel and lack potential ligands in databases. These new drug targets include Rpn family recombination-promoting nuclease/putative transposase YfaD, MDR efflux pump AcrAB transcriptional activator RobA, (MULTISPIECS) amino acid ABC transporter ATP-binding protein from various species, MULTISPECIES: PTS glucose.

Table 4: The ligands that are highlighted in orange color are experimental drugs and the black color represents FDA-approved drugs. Similarly, the blue highlighted protein represents no ligands available in the DrugBank database.

Proteins Name	**Ligands**
[T2SS protein E] Type II secretion system protein E	Phosphoaminophosphonic Acid-Adenylate Ester
arginine N-succinyl transferase [*Klebsiella pneumoniae*] astA	Arginine N-succinyl transferase subunit alpha Formic acid
Putative transposase/Rpn family recombination-promoting nuclease [*k. pneumoniae*] YfaD	No Ligand available in the DrugBank database
MDR efflux pump AcrAB transcriptional activator RobA [*k. pneumoniae*]	No Ligand available in the DrugBank database
MULTISPECIES: amino acid ABC transporter ATP-binding protein [Enterobacteriaceae]	No Ligand available in the DrugBank database
Thiamine transporter ATP-binding protein thiQ	Vitamin E Bacitracin Roxithromycin Nelfinavir

	Terbinafine
single-stranded DNA-binding protein [*Klebsiella pneumoniae*]	TMP Cyclic 3',5'-thymidine monophosphate
MULTISPECIES: PTS glucose transporter subunit IIA [Enterobacteriaceae]	No ligand available in the DrugBank database
urease subunit beta [*Klebsiella pneumoniae*]	No Ligand available in the DrugBank database
glutathione ABC transporter permease/ATP-binding protein CydD/cysteine [*k. pneumoniae*]	Doxorubicin Roxithromycin Verapamil, Saquinavir, Nelfinavir, Indinavir, Amprenavir, Rifampicin

4.3. Homology Modelling

Selected proteins did not yield structures for drug targets when the PDB (Protein Data Bank) was searched for the crystalline structure.

4.3.1. Modeling of proteins by trRosetta

Homology modelling was conducted to create three-dimensional (3D) structures of drug targets employing trRosetta (transform-restrained Rosetta). The proteins' homology models are all depicted in Figures 5–9. Models. The selections of these models were based on the TM score and confidence score. TM score was used to

check the quality of the modeled proteins. To calculate the TM-score (Template modeling score) of the predicated models based on the possibility of the most likely predicted distance and convergence of the best models. A TM-score greater than 0.5 typically denotes a model that accurately predicts protein's topology. The TM-score varies within ranges of 0 to 1.

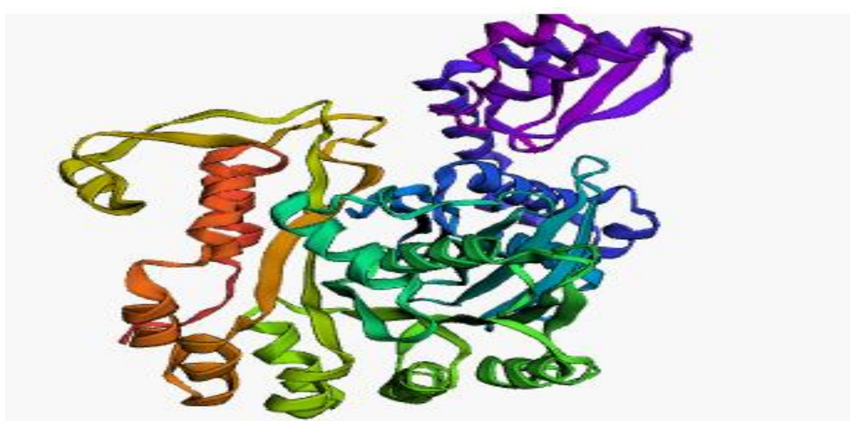


Figure 6: Structure of Type II secretion system protein E T2SS

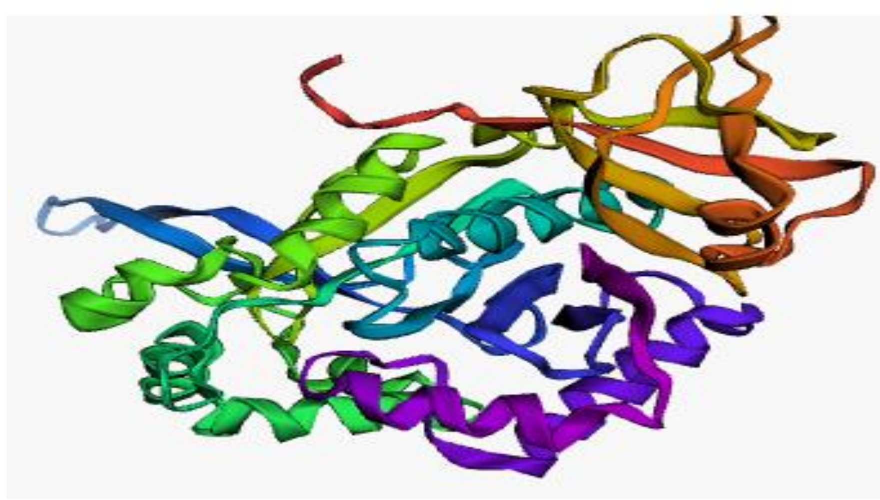


Figure: 7 Structures of protein arginine N-succinyl transferase AST

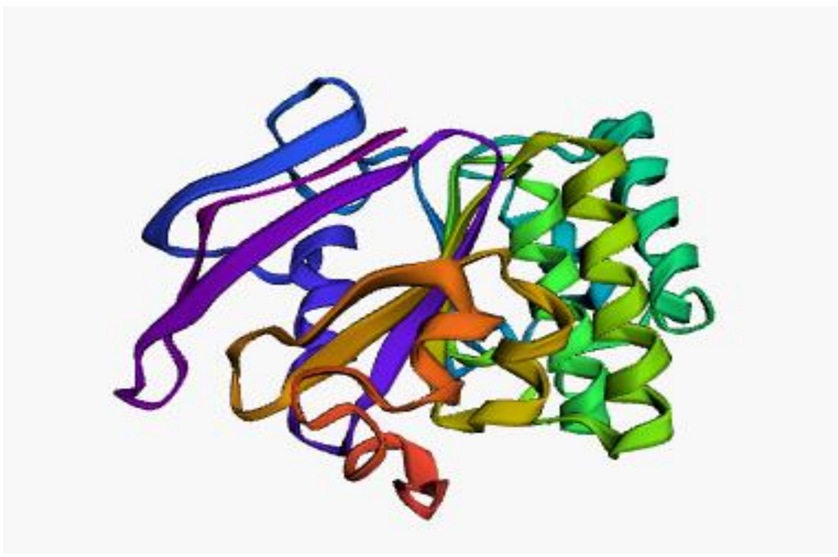


Figure: 8 Thiamine transporter ATP-binding protein thiQ

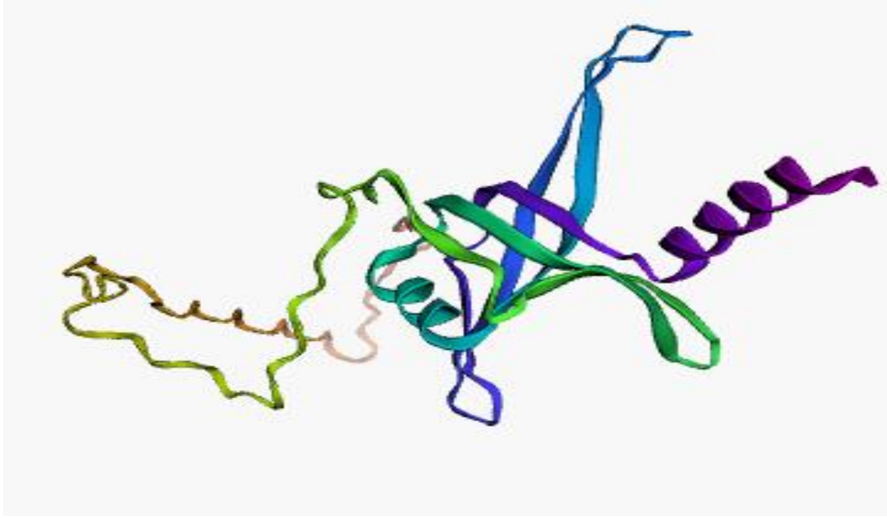


Figure: 9 Single-stranded DNA-binding protein

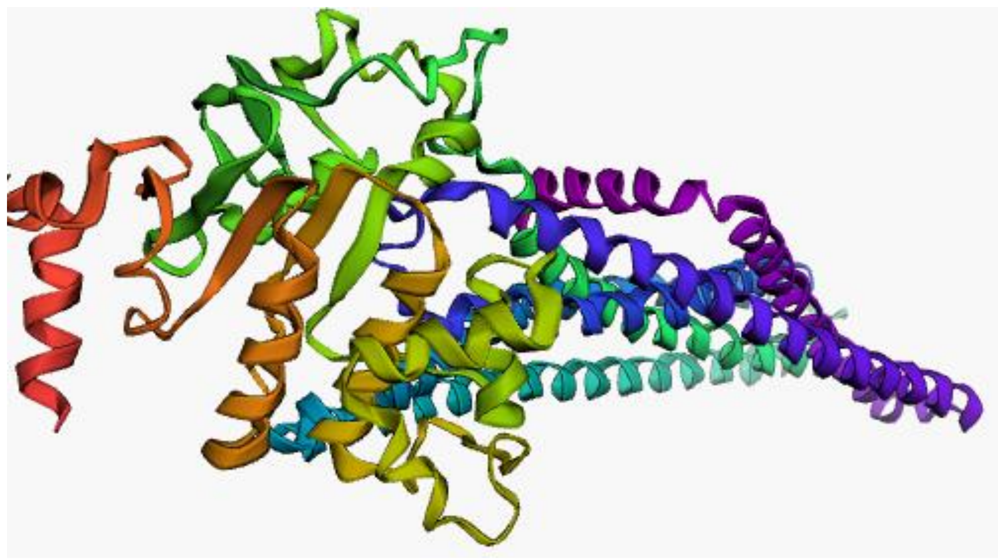


Figure: 10 glutathione ABC transporter permease/ATP-binding protein CydD/cysteine

4.4. Refinement of protein structure using GalaxyRefine

Protein	Refined Structure
[T2SS protein E]Type II secretion system protein E	Initial Rama favoured=98.6% Refined model=100%
arginine N-succinyl transferase astA	Initial Rama favoured=96.6% Refined model=98.8%
Thiamine transporter ATP-binding protein thiQ	Initial Rama favoured=99.6% Refined model=100%
single-stranded DNA-binding protein [*Klebsiella pneumoniae*]	Initial Rama favoured=99.6% Refined model=100%
glutathione ABC transporter permease/ATP-binding protein CydD/cysteine	Initial Rama favoured=98.6% Refined model=99%

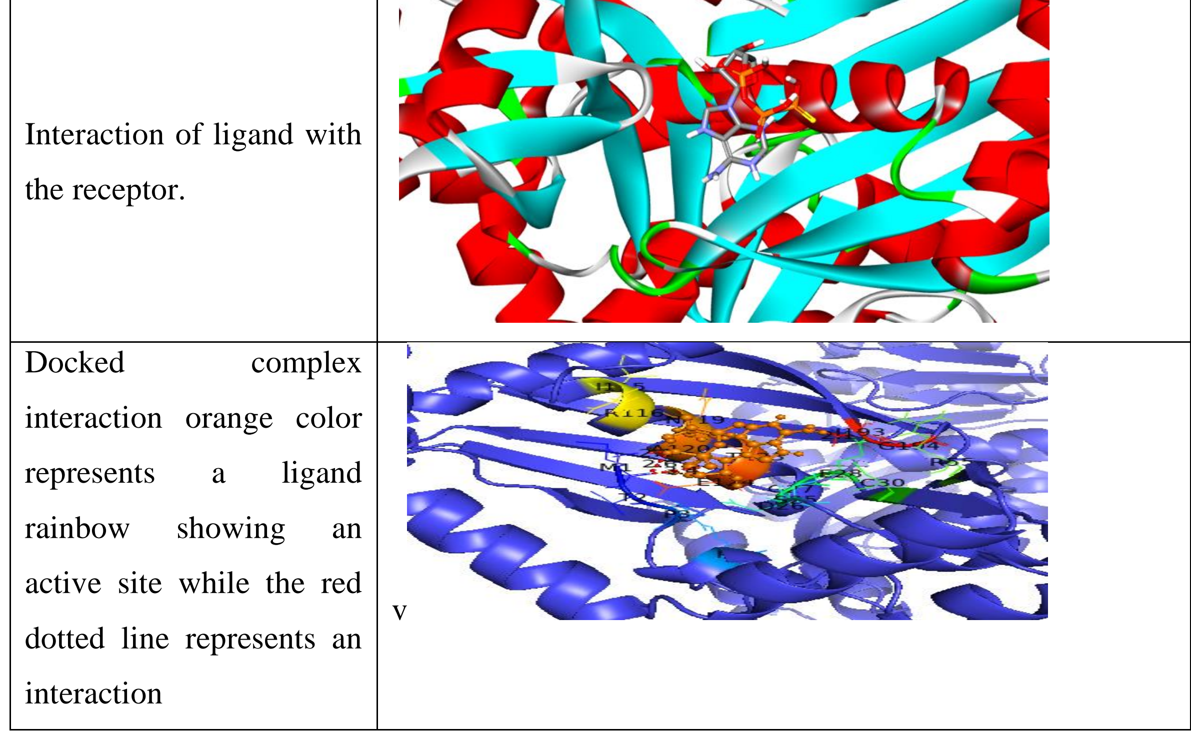
4.5. Lead Compounds

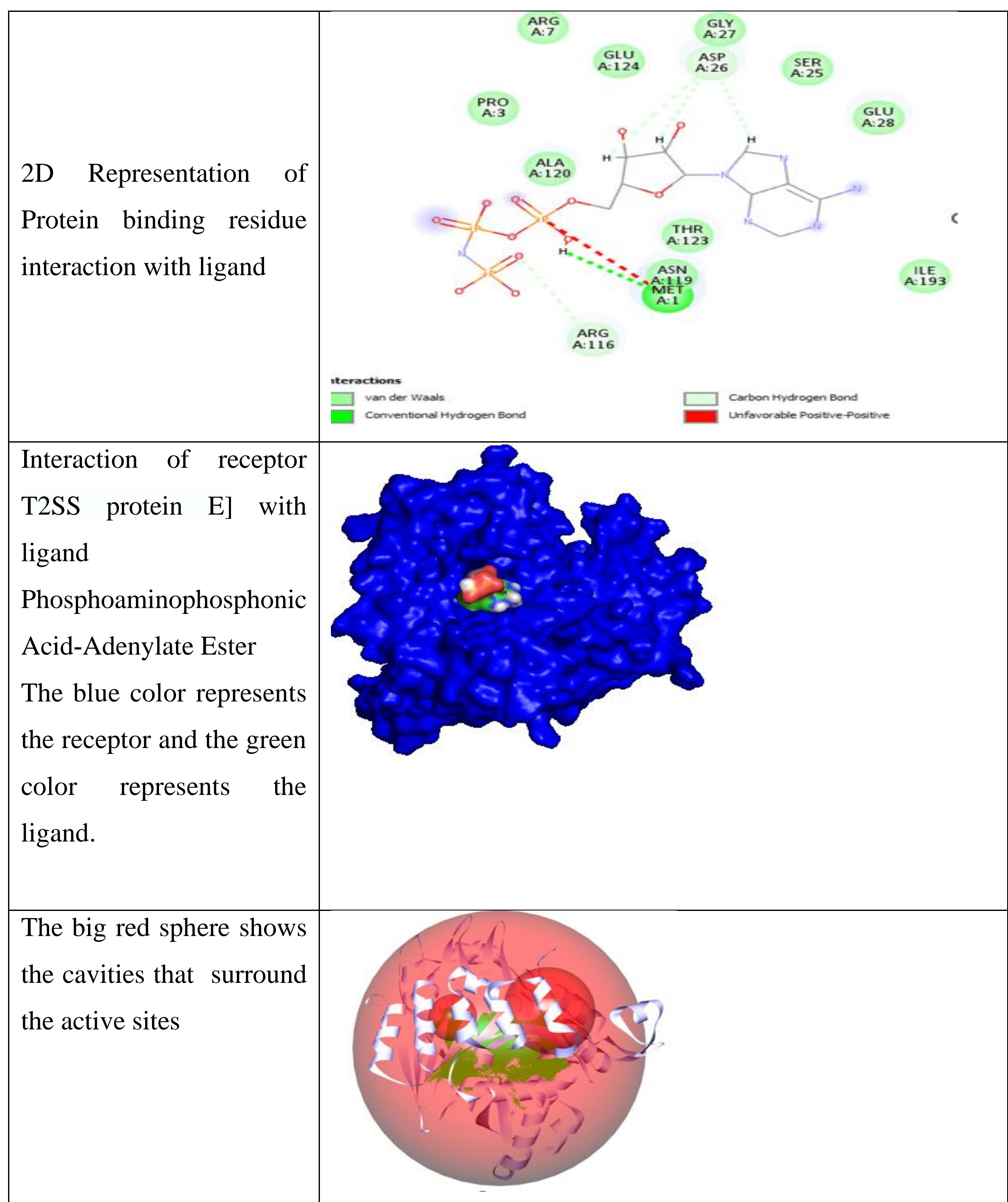
The lead compounds used in this study were those with the lowest binding energies. Consequently, promising lead compounds with potential inhibitory effects were chosen and shortlisted: namely Phosphoaminophosphonic Acid-Adenylate Ester, formic acid, Vitamin E, Thymidine cyclophosphate, **and** Doxorubicin.

4.6. Docking interaction analysis

4.7.1 Docking interaction analysis of [T2SS protein E] Type II secretion system protein E with Phosphoaminophosphonic Acid-Adenylate Ester ligand

The docking interaction of T2SSwith the ligand Phosphoaminophosphonic Acid-Adenylate Ester is depicted in Table 4.

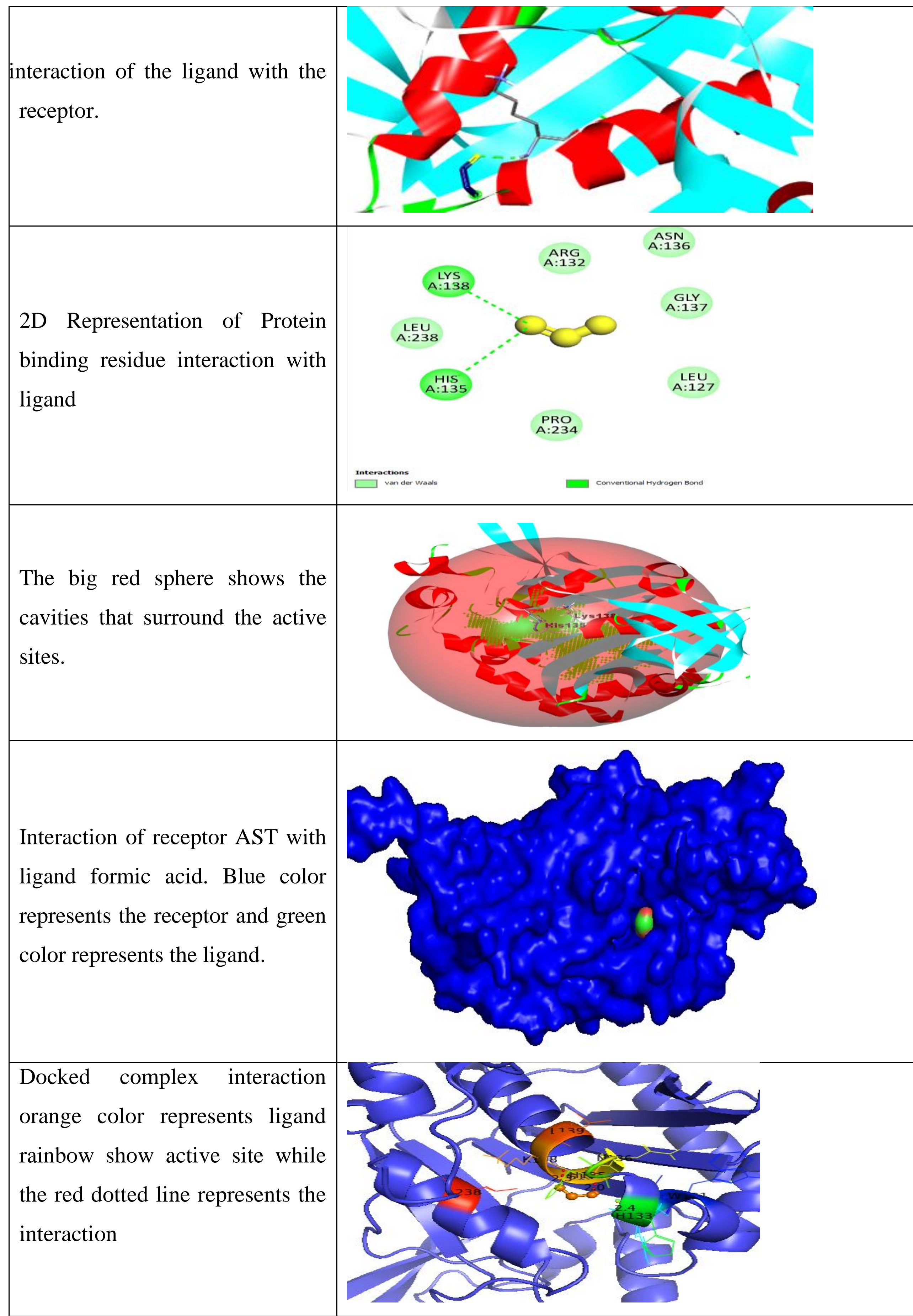
Interaction of ligand with the receptor.	
Docked complex interaction orange color represents a ligand rainbow showing an active site while the red dotted line represents an interaction	v



2D Representation of Protein binding residue interaction with ligand	
Interaction of receptor T2SS protein E] with ligand Phosphoaminophosphonic Acid-Adenylate Ester The blue color represents the receptor and the green color represents the ligand.	
The big red sphere shows the cavities that surround the active sites	

4.7.2 Docking Interaction Analysis of Arginine N-succinyl Transferase [AST] with ligand formic acid

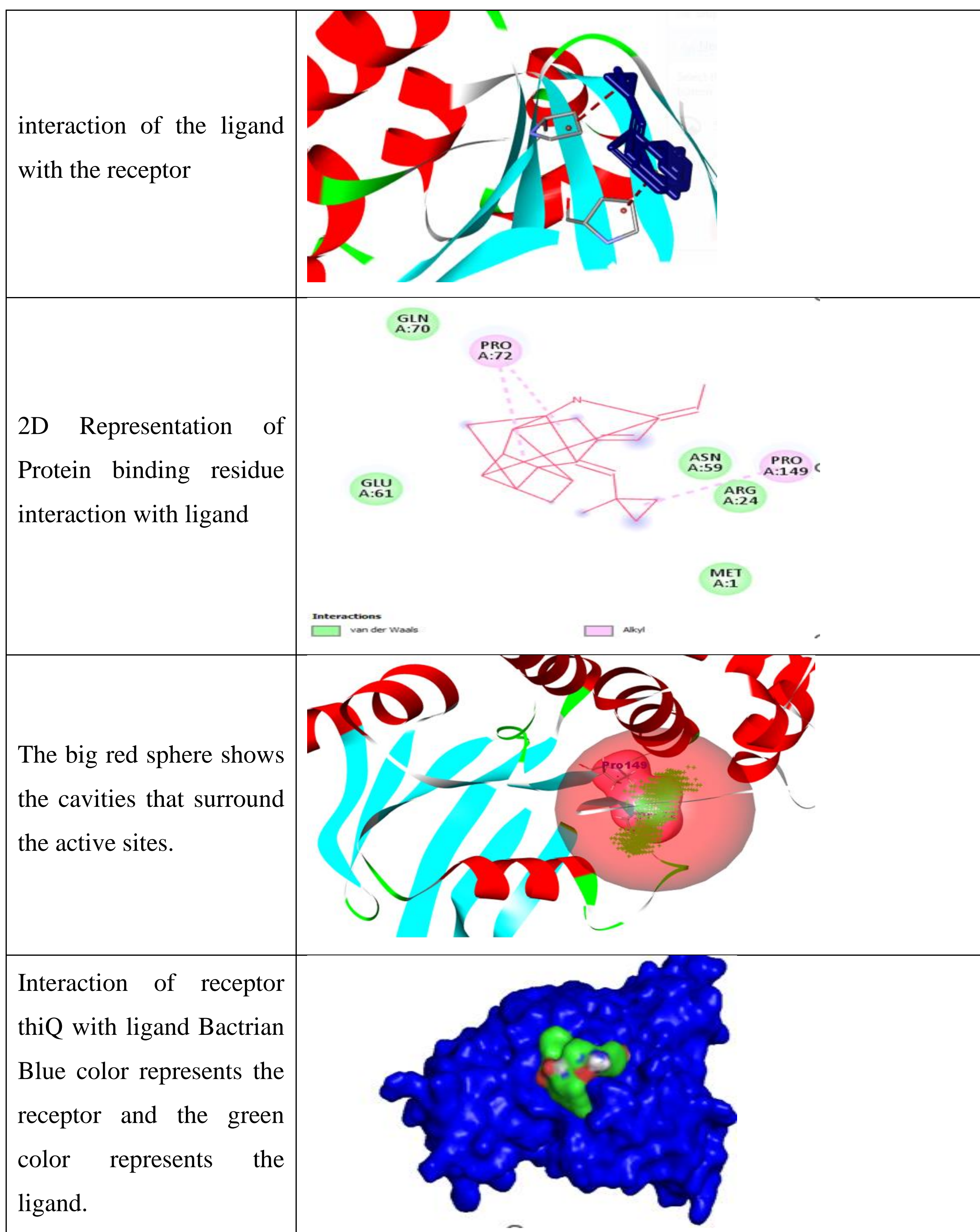
The docking Interaction analysis of astA with the lead compound formic acid is shown in the Table. 5



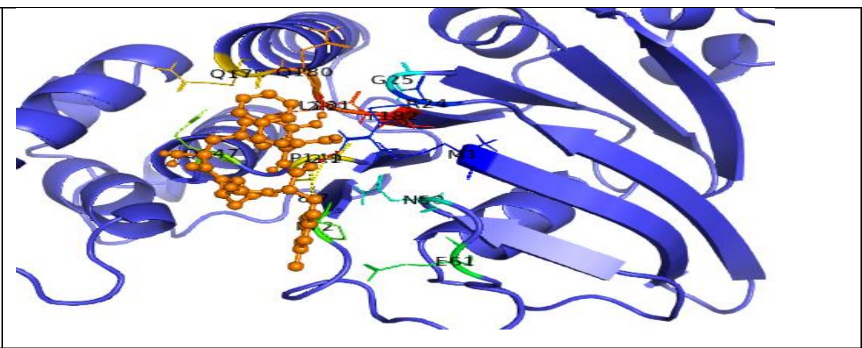
interaction of the ligand with the receptor.	
2D Representation of Protein binding residue interaction with ligand	
The big red sphere shows the cavities that surround the active sites.	
Interaction of receptor AST with ligand formic acid. Blue color represents the receptor and green color represents the ligand.	
Docked complex interaction orange color represents ligand rainbow show active site while the red dotted line represents the interaction	

4.7.3 Docking Interaction analysis of Thiamine transport ATP-binding protein thiQ with ligand Vitamin E

The docking Interaction analysis of thiQ with ligand vitamin E is shown in the Table. 6.



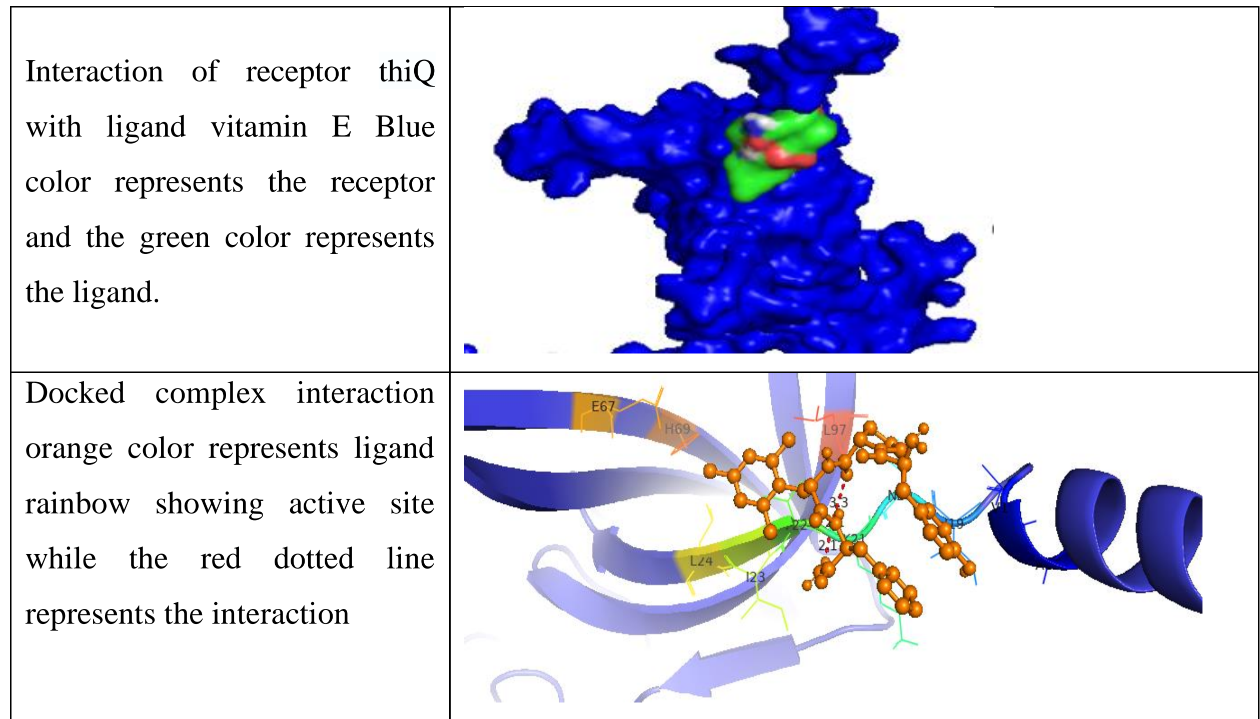
interaction of the ligand with the receptor	
2D Representation of Protein binding residue interaction with ligand	
The big red sphere shows the cavities that surround the active sites.	
Interaction of receptor thiQ with ligand Bactrian Blue color represents the receptor and the green color represents the ligand.	

Docked complex interaction orange color represents ligand rainbow show active site while red dotted line represents the interaction	

4.7.4 Docking Interaction analysis of single-stranded DNA-binding protein [SSB][*K. pneumoniae*] with ligand Thymidine cyclophosphate

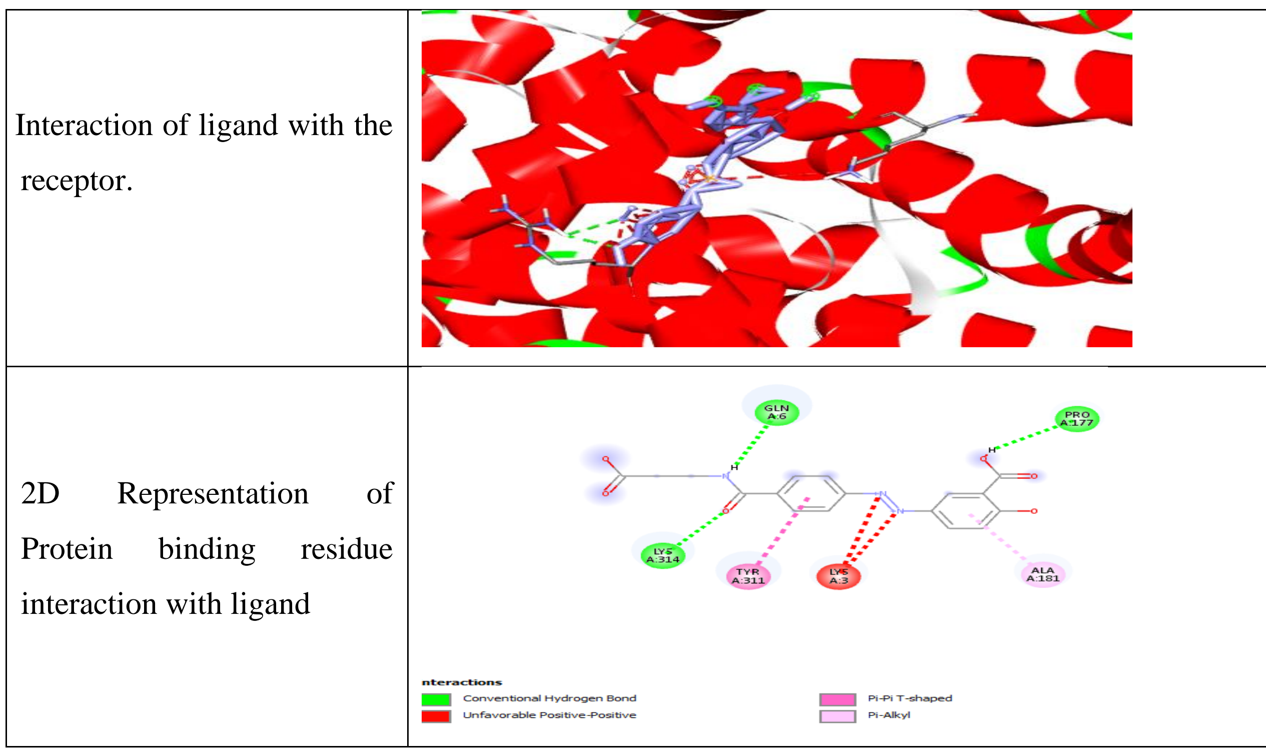
The Docking Interaction analysis of single-stranded DNA-binding protein [*Klebsiella pneumoniae*] with ligand Thymidine cyclophosphate [tmp] is shown in Table 7

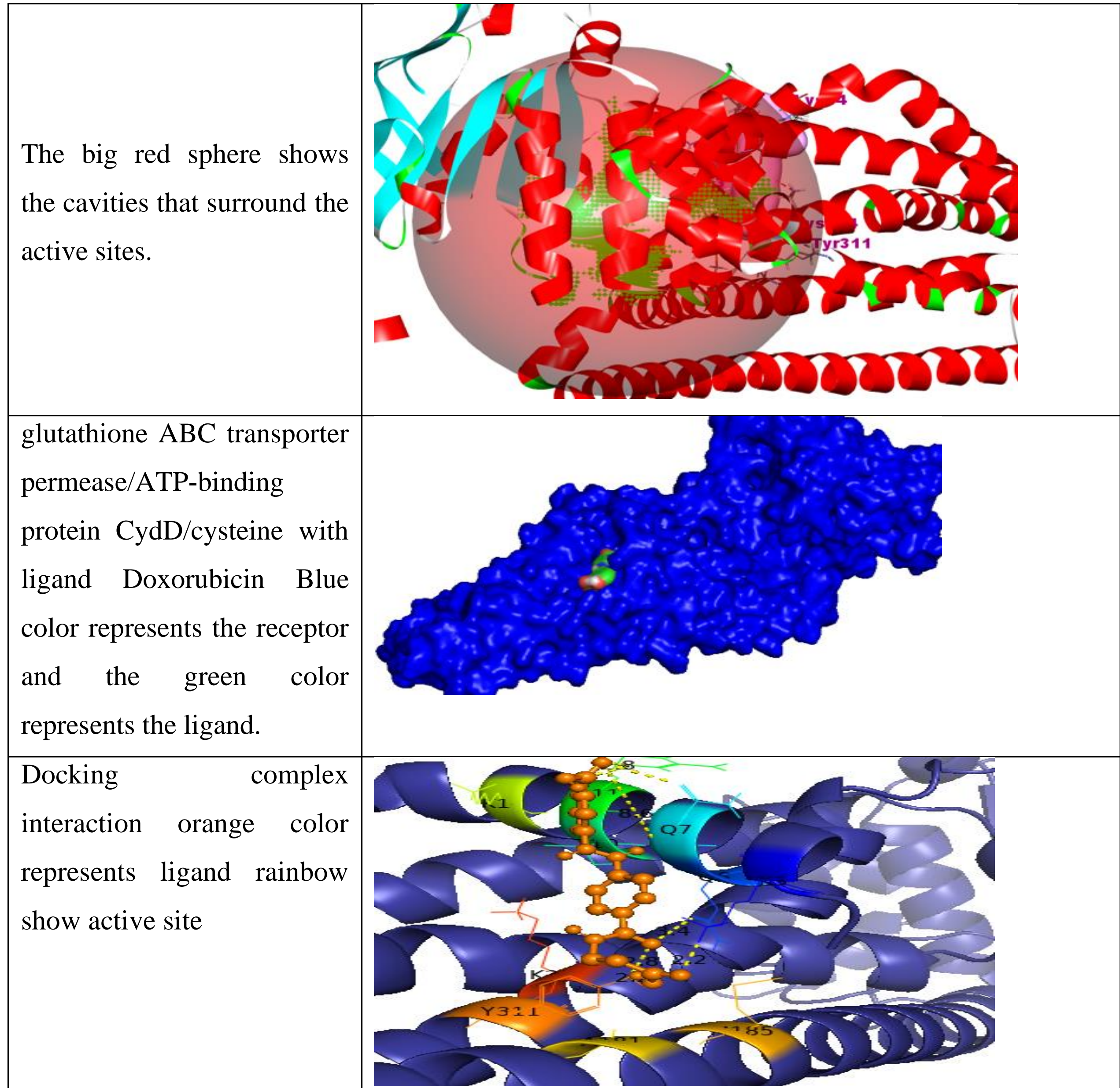
Interaction of the ligand with the receptor.	
2D Representation of Protein binding residue interaction with ligand	
The big red sphere shows the cavities that surround the active sites.	

Interaction of receptor thiQ with ligand vitamin E Blue color represents the receptor and the green color represents the ligand.	
Docked complex interaction orange color represents ligand rainbow showing active site while the red dotted line represents the interaction	

4.7.5 Docking interaction analysis glutathione ABC transporter permease/ATP-binding protein CydD/cysteine with ligand Doxorubicin.

The Docking interaction analysis glutathione ABC transporter permease/ATP-binding protein CydD/cysteine with ligand Doxorubicin is shown in Table 8.

Interaction of ligand with the receptor.	
2D Representation of Protein binding residue interaction with ligand	



The big red sphere shows the cavities that surround the active sites.	
glutathione ABC transporter permease/ATP-binding protein CydD/cysteine with ligand Doxorubicin Blue color represents the receptor and the green color represents the ligand.	
Docking complex interaction orange color represents ligand rainbow show active site	

4.7. Protein-protein interaction

Predicted drug targets were characterized by interactome analysis. These Proteins are discussed individually below:

i. Type II secretion system protein [T2SS protein E]

Type II secretion system (T2SS, previously referred to as general secretion pathway, GSP) participating for the exportation of proteins .T2SS is a mechanism employed by several Gram-negative bacteria to transport folded proteins from the periplasm across the outer membrane and into the extracellular environment. Both pathogenic and non-pathogenic species need the T2SS. STRING interactome analysis of the proteins

showed

strongest interaction with Pullulanase-specific type II secretion system factor F (0.988), *Pullulanase D protein* (0.984), Pullulanase G protein (0.979), processing enzyme; Type 4 prepilin-like proteins leader peptide cleaves type-4fimbrial leader sequence and methylates the N-terminal (usually Phe) residue (0.944), General secretion pathway protein J (0.935), Pullulanase-specific type II secretion system component C (0.927), *T2SS* protein component *L* (0.920), Type IV pilin biogenesis transmembrane protein (0.880), General secretion pathway protein M (0.875,) Pullulanase-specific T2SS component K (0.866). The interaction pattern can be visualized in Figure 10.

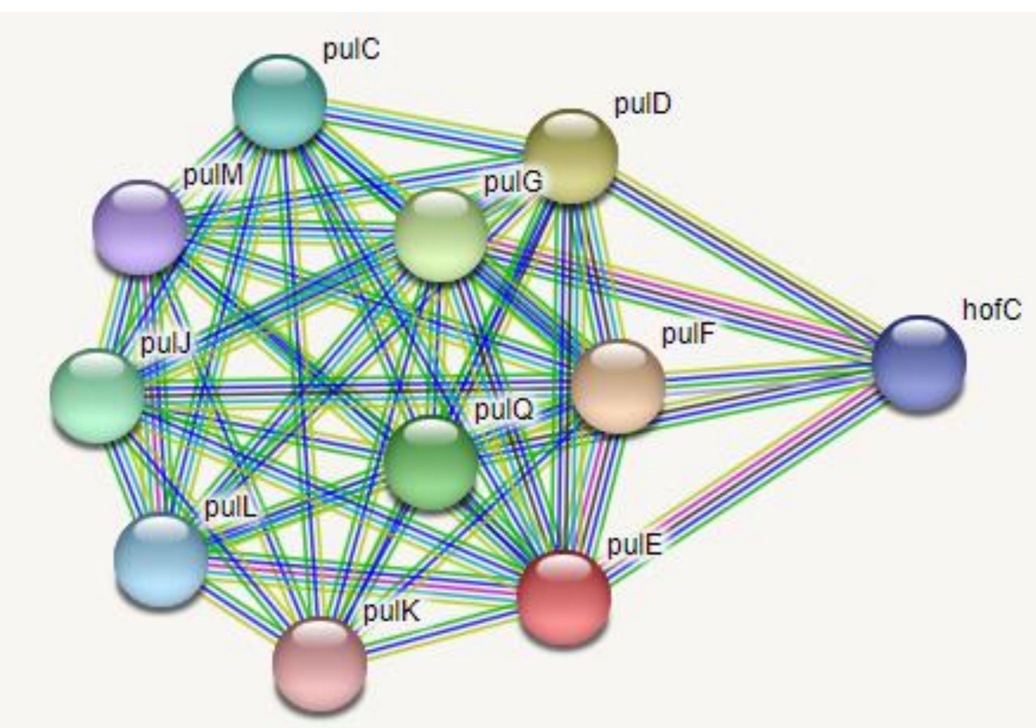


Figure 11 Protein-Protein interaction networks of T2SS.T2SS had predicted to have strongest interaction with *Pullulanase-specific T2SS component F* protein which is a secretory protein and plays an important role in the secretory pathway.

ii. astA

Arginine N-succinyl transferase is responsible for facilitating the transfer of succinyl-CoA to arginine resulting in the formation of N(2)-succinyl arginine.Arginine succinyl transferase *astA* is the first enzyme of the succinyl transferase (AST) pathway operon consisting of five genes. According to interactome analysis, there is a strong correlation of *astA* with *astB* N-succinyl arginine Di hydrolase facilitate the breakdown *of* N(2)-succinyl arginine into N(2)- succinyl ornithine,ammonia (NH3)

and carbon dioxide (CO_2) (0.998*),astD* N-succinyl glutamate 5-semialdehyde dehydrogenase; is responsible for facilitating the NAD-dependent reduction of succinyl glutamate semialdehyde into succinyl glutamate (0.982).it also interact with astE (Succinyl glutamate desuccinylase*;)* (0.970) Transforms N(2)-succinyl glutamate into succinate and glutamate. Similarly, KPN_01221 known as Acetylornithine/succinyl diaminopimelate aminotransferase participate in both the arginine and lysine biosynthetic pathways, it has interaction with *astA with a score of* (0.913) and with *KPN_01496* (N-succinyl arginine dihydrolase) facilitate the hydrolysis of N(2)-succinyl arginine into N(2)- succinyl ornithine, ammonia(NH_3) and carbon dioxide (CO_2) with a score of (0.899*) KPN_01497* (Succinyl glutamate desuccinylase) which is capable to Transforms N(2)-succinyl glutamate into succinate and glutamate with a score of (0.825). Figure 11 shows the interaction diagram.

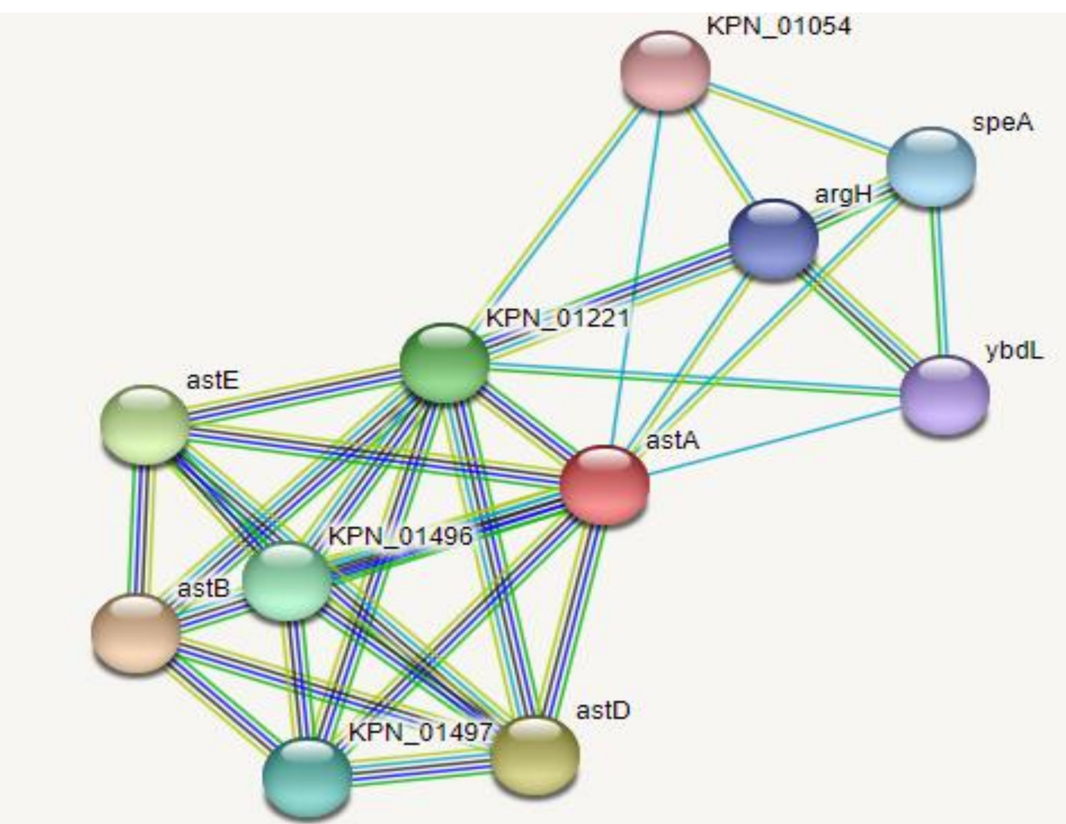


Figure 12 Protein-Protein interaction network of astA. astA was predicted to have strong interaction with astB *N-succinyl arginine dihydrolas* facilitate the hydrolysis of N(2)-succinyl arginine into N(2)- succinyl ornithine, ammonia (NH_3), and carbon dioxide (CO_2).

iii. thiQ_1

Thiamine import ATP-binding protein (thiQ) is component of the ABC transporter complex ThiBPQ ,which play a role in the import of thiamine.thiQ is primarily responsible for facilitating the energy coupling is important for the effective working of the transport system interact with *cysW* Thiamin ABC transporter transmembrane protein with a score of (0.997), *thiB* Thiamin ABC transporter(0.995), rluA_1 Ribosomal large subunit pseudouridine synthase (0.584), ffh_2 Signal recognition particle *(0.565), thiK* hiamine kinase; Catalyzes the phosphorylation of *thiamine* converting into thiamine phosphate (0.555).it also show interaction with cysS_2 Cysteine--tRNA ligase(0.551). The interaction pattern can be seen in Figure 12

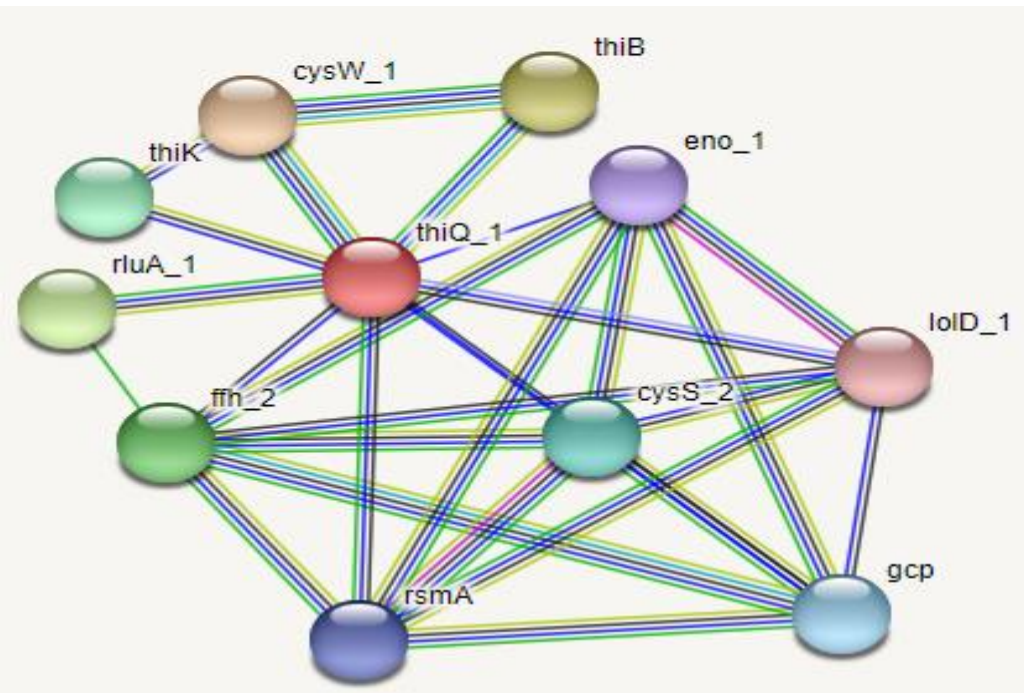


Figure 13 Interaction pattern of *thiQ*.it shows that *thiQ* is the component of the ABC transporter complex ThiBPQ which, play a role in import of thiamine. It's predicted interacting partner *cysW* Thiamin ABC transporter transmembrane protein

iv. Single Stranded DNA Binding Protein (SSB)

Single-stranded DNA-binding protein perform significant role in DNA replication, recombination, and repair. It Binds to ssDNA and interact with variety of partner protein facilitating their recruitment to respective site of action during DNA metabolism. It functions as a sliding platform that moves along DNA through

mechanism known as reptation. Interactome analysis has predicted its strong interaction of SSB with holC DNA polymerase III having (score 0.996).*holC* DNA polymerase III is a complex, multichain enzyme perform significant role in the majority of replicative synthesis within bacteria of the replicative synthesis in bacteria. The DNA polymerase also demonstrate 3' to 5' exonuclease activity, additionally it interact with *priA* Primosomal protein N that play important role in the restarting of stalled replication forks with score (0.947) as well as 30S ribosomal protein S18 also known as *rpsR* with a score of (0.937).it forms heterodimer with protein S6 binding to the central domain of the 16S rRNA, thereby aiding in the stabilization of the 30S subunit.it also interacts with 30S ribosomal protein S4 known as *rpsR*(0.871). This protein is main component in binding to rRNA and recognized as primary rRNA binding proteins, it form direct interaction to 16S rRNA where it initiating the assembly process of 30S ribosomal subunit. Similarly, *sbcB* interacts with *SSB* with a score of (0.816) and with *recA* Protein (0.816) has capability to catalyze the hydrolysis of ATP when single-stranded DNA is present. Figure 13 displays the interaction diagram.

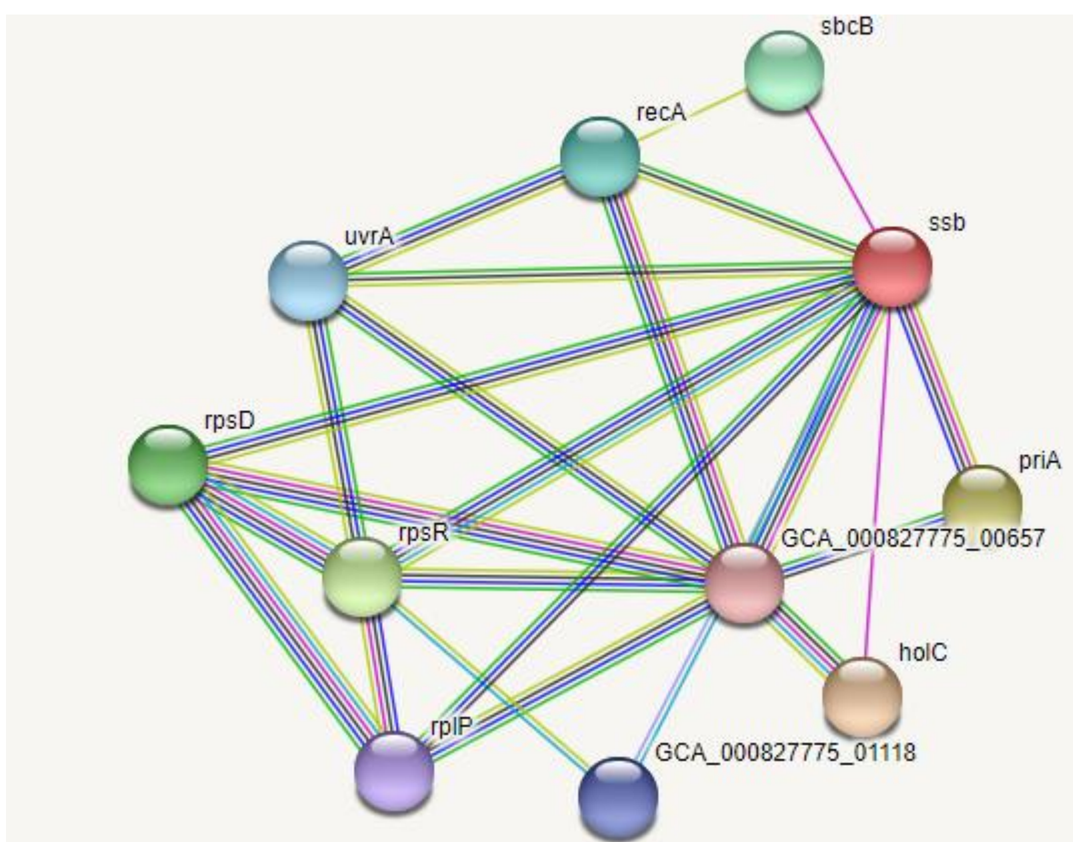


Figure 14 Protein-Protein interaction analysis of SSB.SSB indicated in the red circle has role in DNA replication, recombination, and repair having strong interaction with holC DNA polymerase III.

v. Cysteine/glutathione ABC transporter membrane/ AEW60495.1

Cysteine/glutathione ABC transporter membrane consist of both membrane component and ATP binding component. This transporter system facilitates the transport of Glutathione (GSH) which is a tripeptide composed of (γ-glutamyl-cysteinyl-glycine) and the concentration of Glutathione is maintained in millimolr within cell. It play significant role in several process encompassing post translational modification of protein, cell proliferation, apoptosis and immune responses .PPI analysis has predicated strong interaction with AEW60494.1 also known as Cytochrome-related transport system ATP-binding component with a score of (0.997) and AEW60273.1 also termed as Cytochrome d terminal oxidase polypeptide subunit II (0.888) and is important element of *E.coli* of the aerobic respiratory system.The enzyme act as a terminal oxidase, enabling the oxidation of ubiquinol-8 within the cytoplasmic membrane and changing oxygen into water. Additionally it show interaction with *aat* termed as Leucyl/phenylalanyl-tRNA-protein transferase with interaction score of *(0.757)* .This enzyme perform significant role in the N-end rule pathway of protein degradation by conjugating Leu, Phe and, less extent, Met from aminoacyl- tRNAs to the N-termini of proteins that contain an initial arginine or lysine. The interaction analysis can be visualized in Figure 13.

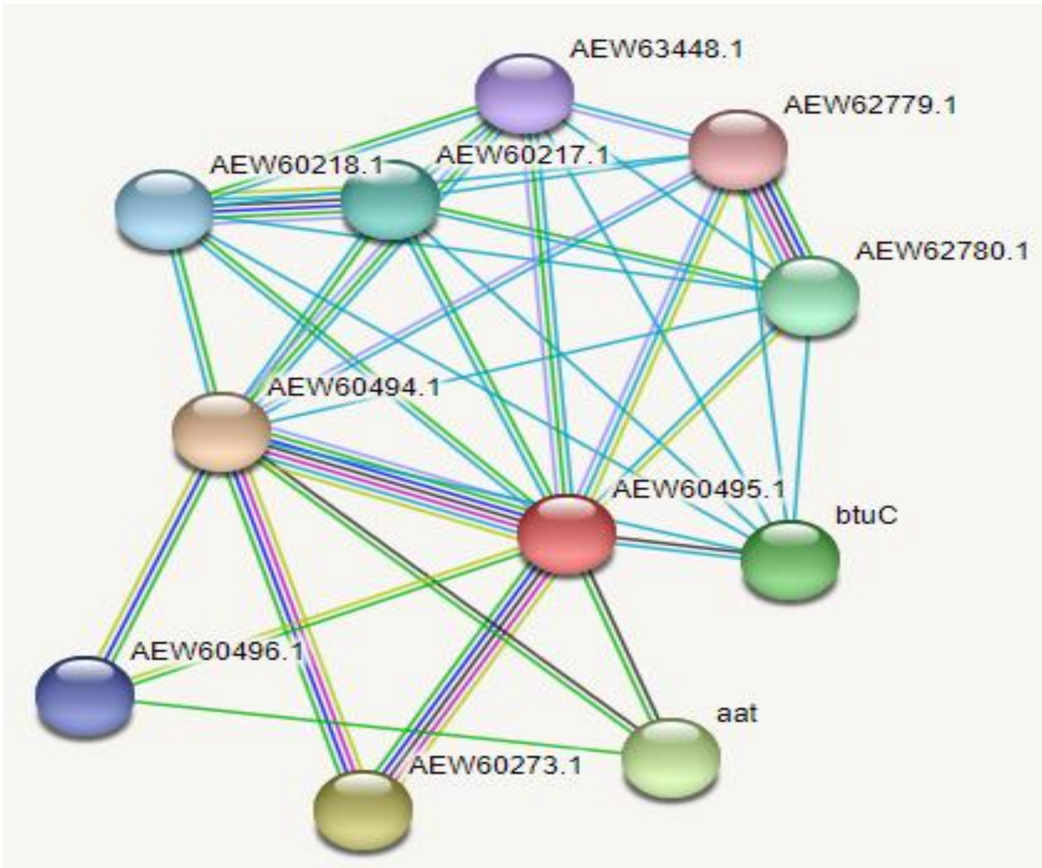


Figure 15 Interaction pattern glutathione ABC transporter membrane/ATP-binding component/cysteine It has a role in signaling processes. It's predicted interacting partner Cytochrome-related transport system ATP-binding component membrane.

DISCUSSION

The *Klebsiella* genus comprises of non-motile, aerobic, and facultative anaerobic, Gram-negative rod shape bacteria. It primarily affects people who are immunocompromised and is frequently linked to nosocomial infections. Infections of the urinary system, respiratory system, bloodstream, and surgical sites are all brought on by *Klebsiella pneumoniae* (131).A community-acquired pyogenic liver abscess is another condition that *Klebsiella pneumoniae* can cause (PLA). About 83% of hospital-acquired pneumonia can be attributed to it. Additionally, it is the main cause of pneumonia linked to ventilators. Healthy individuals are unaffected by *Klebsiella pneumoniae*, which asymptomatically lives in the typical human gut microbiota (132). The hypervirulent *Klebsiella* strain causes severe meningitis, pneumonia, endophthalmitis, cellulitis, and Plasmolytic bacterial infections [PLAs](133). Bacterial antibiotic resistance poses significant global health concern. Infections caused by such type of microorganism in hospital settings lead to more than 136,000 deaths annually in the USA and Europe(134).The limitation arising from each high-throughput (HTS) library containing a finite quantity of chemicals in a limited range of structure is significant disadvantage of conventional high-throughput screening (HTS) methods. Since this chemical space restriction is unlikely to be overcome, new strategies are required to address the growing issue of bacterial resistance to current therapies. Our research offers a design for the development and further adaptation of such novel strategies. Considering the enormous volume of high-throughput genomic, structural, and transcriptomic information from various significant bacterial pathogens freely accessible biological databases, employing a method rooted in multidimensional information integration for pinpointing of novel drug targets offers a quicker and more budget-friendly approach compared to conventional screening techniques. To support previous work has focused on in silico drug target identification within the proteomes of medically important bacteria such as Corynebacterium spp (135).Although rely solely on the use of computational techniques don't not assure precise detection of drug targets, They do facilitate the process of narrowing down potential drug targets .This effectively reduce the scope of the search of candidates that have higher likelihood of being suitable targets for either new or repurposed drug (136).The production of carbapenemase, a member of the *K. pneumoniae* antimicrobial resistance repertoire, is particularly concerning because it confers resistance to every beta-lactam. Specifically in countries like Argentina, Brazil, Greece, Colombia, Italy and Israel where KPC-2-producing *K. pneumoniae* are

widespread infections brought on by Carbapenem-resistant *K. pneumoniae* shows significant global disease burden. As an example relying on latest record from the Brazilian Health Surveillance Agency, *Klebsiella* species was considered leading microorganism accountable for resulting 3,805 cases (16.9%) catheter-related bloodstream infections among adult patients admitted to ICU in Brazil in 2015. Like such samples were represents an approximate resistant rate of 43% against Carbapenem and broad-spectrum cephalosporin's (137). So, we summarize the application of a multidimensional information integration method to identify the top drug targets within *K.pneumoniae*. We properly recognize potential proteins with essential characteristics for targets selection within *Klebsiella pnemoniae* and related pathogens by integrating diverse layers of information through multi-omics approach, which contain transcriptomic, metabolic, and protein structural data sources (135). Due to the rising drug resistance, it is now more important than ever to find new drugs and vaccines. To overcome this problem, it becomes necessary to explore the potential of finding novel drug targets within deadly *Klebsiella* strains. Nowadays, computational methods are the best option for finding new drug targets (138). Using comparative and subtractive genomic methods, researchers have identified possible therapeutics targets in a variety of disease causing bacteria (105) .To find potential drug targets in *K. pneumoniae*; we used a novel approach in this study that combined subtractive proteomics, reverse vaccinology, and pan-genomics to analyze the 560 complete genomes of the bacterium. These proteins are essential for bacteria but are not found in humans. The identification of novel therapeutic targets has been facilitated by recent developments in computational approach and omics data; including genomics, proteomics and metabolomics (139) .For finding new drug targets against different pathogenic bacteria, subtractive genomics, comparative genomic analysis, and differential genomic analysis have become popular methodologies(140).With the development of bioinformatics, it is now possible to predict new therapeutics—like vaccines—with a respectable degree of accuracy without the necessity of living cells and with a reduction in the time, expense, and labor required. As a consequent of this study, a Reverse Vaccinology method was modified to pinpoint special drug targets. Using reverse vaccinology, our strategy also focused on identifying potential immunogenic protein targets. This was accomplished by first screening the entire proteome of *K. pneumoniae* for extracellular and outer membrane proteins. Our strategy also entailing comprehensive analysis of other

analytical and fundamental features of *K. pneumoniae*. This include assessing an essentiality check, the identification of virulence factors involved in the initiation and advancement of infection, as well as exploring protein interactomes with both the host proteome and other proteins(141).The same approach was used in an earlier study to identify Pseudomonas aeruginosa vaccine candidates. Our research included a sizable number of genomes, creating a pan-genome that keeps expanding as new genomes are added. These results demonstrated that the pan-genome is still under investigation and highlighted gene gain and loss as well as persistence in human hosts throughout evolution (142).The pangenome analysis reveals that there are total gene families of 19890 (pangenome), out of which 617 proteins were common (core genome) across all examined genomes. The ratio between core and pangenome size was determined to be 0.3, signifying that the core constitutes 3% of the entire pangenome. The Pangenome has grown as new genomes have been added, while the size of the core genome has shrunk. Although pangenome curve continued to show an upward trajectory, indicating that the global gene pool of *K. pneumoniae* may increase in the future and that the pangenome is still open.In this study, only cytoplasmic proteins were taken into consideration. Essential proteins are mostly cytoplasmic, and most of them remain there to carry out their specific functions after being made there (143).

The strategy was divided into two directions after the subtractive proteomics method.Pathway analysis of these proteins, druggability testing of potential therapeutics with potential inhibitory effects, and lead compound testing. Only those proteins that play a specific and significant role in bacterial metabolism, such as those involved in ABC transporters, biofilm formation, Bacterial Secretion System Arginine, and proline metabolic pathway, were chosen for the pathways analysis. (144). In this study, a total of 10 prioritized proteins that were active in unique pathways were chosen Five of these proteins shared similarities with targets of FDA-approved drugs, suggesting that they may be druggable.The five potential drug targets were studied in the literature to understand their importance as potential drug targets.

Among the shortlisted proteins involved in unique metabolic pathways i.e. Bacteria have evolved various secretion systems to transport molecules across their cell membranes and interact with their environment. These secretion systems play critical roles in bacterial metabolism, virulence, communication, and interactions with host organisms. Specifically T2SS (Type II secretion system), also referred as the primary terminal part of the general secretory pathway (GSP), is responsible for conveying

properly folded proteins across through the outer membrane of Gram-negative bacteria (105). It facilitates the secretion of various enzymes, toxins, and virulence factors, such as proteases and lipases, involved in nutrient acquisition, pathogenesis, and biofilm formation (105). ATP-binding cassette/ (ABC) transporters are a large family of transmembrane proteins that was also one of our shortlisted pathways. ABC (ATP-binding cassette protein)/ABC transporters hold considerable significance in drug development, particularly in understanding drug Absorption, Distribution Metabolism, and Execration (ADME).These transporters are membrane proteins involved in the active transport of a wide range of molecules, including drugs, across cellular membranes.ABC transporters can be involved in drug-drug interactions by affecting the pharmacokinetics and efficacy of co-administered drugs. Some drugs act as inhibitors or substrates of ABC transporters, altering the disposition and concentration of other drugs that are substrates of the same transporter. ABC transporters expressed on the surface of certain cell types can be exploited for targeted drug delivery (145).Arginine-proline metabolism is another shortlisted biochemical pathway involved in the interconversion of arginine and proline Enzymes involved in the arginine-proline metabolism pathway can be potential targets for drug discovery. For example, enzymes such as arginase and ornithine decarboxylase, which catalyze key steps in the conversion of arginine to proline, have been targeted for the treatment of diseases such as cancer and parasitic infections. Inhibiting these enzymes can disrupt the metabolism of arginine and proline, leading to altered cell growth, proliferation, and other physiological processes. As Arginine is a precursor for nitric oxide (NO) synthesis, which performs important function in several physiological processes, encompassing neurotransmission, vasodilation, and immune system reaction. Modulating the arginine-proline metabolism pathway can impact NO production, making it a target for drug development. For example, drugs that regulate arginase activity or promote arginine availability can be used to enhance NO production for therapeutic purposes, such as managing cardiovascular diseases.

The DrugBank database's analysis of druggability also enables the identification of target proteins that correspond to the drug's active compounds. A druggability test was conducted to identify ligand binding capacity. The stability checks were yet another factor considered when ranking drug targets. Thus, it was decided that 5

proteins would make good drug targets. These proteins are all necessary for the survival of bacteria and are all involved in their metabolic and molecular pathways.

Any molecule with a potential to bind drugs is considered "druggable," according to Cheng et al. The FDA-approved and experimental drugs that have the potential to inhibit these proteins helped us identify the druggable proteins. We only chose the top 5 lead compounds with the lowest binding energies out of all the drugs that are currently being tested against the target proteins.

CONCLUSION & FUTURE PROSPECTS

In the current study, we used subtractive proteomics, and a comparative genomics strategy to identify drug targets to enhance the approach to treating bacterial diseases caused by *Klebsiella* because of the emergence of drug resistance. Pangenome analysis revealed that 560 complete genomes of *K. pneumoniae* consist of 617 core genes and the pangenome is still open. We identified 5 drug targets (puiE, ThiQ, astA, SSB, and cydD) that can be further explored and have the potential to make a significant contribution to the fight against nosocomial infections.

Future Prospects

Prioritized drug targets have distinguishing characteristics that may be used in drug development studies. The set of core proteins can be used to attack the pathogen's vital metabolic processes Additionally, virtual screening of the filtered drug targets can be done to identify alternative therapeutic antimicrobial compounds. The lead optimization and experimental validation in a suitable animal model will provide significant insights to develop new therapeutics to combat antimicrobial resistance.

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