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Pangenome Analysis of *Klebsiella pneumoniae* and Vaccine Candidates Identification

by

Asma Bibi

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I dedicate this thesis to my parents, my teachers and my husband

CERTIFICATE OF APPROVAL

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Abstract

Klebsiella pneumoniae (*k.pneumoniae*) is an opportunistic pathogen that can cause life threatening infections. The rapid increase of multidrug resistantance in *Klebsiella pneumoniae* and the fact that there is no currently licensed vaccine available against *Klebsiella pneumoniae* warrant the need for vaccine development. Reverse vaccinology is a techniques it involves whole genome sequencing of pathogen and identification of potential antigen by using different bioinformatics tools, and this is very good approaches for identifying vaccine candidates. So we performed pangenome analysis to find out the core proteins of *Klebsiella pneumoniae* then we use Reverse vaccinology approach to determine the subcellular localization, antigencity, host and gut flora ,transmembrane helices, physicochemical properties and immunogencity of core protein to find out the potential vaccine candidates.The vaccine candidates were then subjected to epitope maping to predict the exposed antigenic epitopes that have ability to connect with major histocompatibility complex I/II (MHCI/II) molecules.These vaccine candidates as well as epitopes will form a library components for the development of a univversal or polyvalent vaccine against *Klebsiella pneumoniae*. It was discovered that the 126 complete proteomes of K. pneumoniae contained 15319 protein families, with 3,864 protein families (≈63.63% of the pangenome) making up the core proteins. This indicates a low level of intraspecies genetic diversity. After screening nonhost homologous membrane and extracellular proteins from the pool of core proteins,8 vaccine candidates were identified. ultimately determined by their antigenicity, physicochemical characteristics, and additional variables. These included three proteins involved in the drug efflux and toxin export, four proteins involved in the facilitate the passive diffusion of small molecules,one outer membrane protein involved in pilus assembly and bacterial adhesion. The protein data bank (PDB) contains a 3D structure that has been experimentally verified for each of the vaccination candidates.The MHC I and MHC II epitopes with the highest immunogenicity were exposed on the protein surface, according to epitope mapping of the candidates, suggesting that they may be utilized to create a vaccine with

polypeptides. Consequently, it was employed an analytical approach that combines pan-genome analysis with Reverse vaccinology to produce a peptide antigen library that facilitates the creation of universal or multivalent vaccinations against *Klebsiella pneumoniae* and Sequencing of more Pakistani strains to understand the genomic diversity of *K. pneumoniae* in Pakistan can be used in the creation of additional vaccines.

Contents

List of Figures

List of Tables

Abbreviations

ABC	ATP-binding cassett
CPKP	Carbapenemase-producing K. pneumoniae
CPS	Capsular PolySaccharides
ESBL	Extended- Spectrum BetaLactamase
HGT	Horizontal Gene Transfer
LPS	LipopolySaccharide
MATE	Multidrug and Toxic Compound Extrusion
MDR	Multi Drug resistance
MFS	Major facilitator superfamily
NGS	Next-generation Sequencing Technologies
RND	Small Multidrug Resistance

Chapter 1

Introduction

1.1 Background

Klebsiella pneumoniae is categorized as a Gram negative, encapsulated, non-motile bacteria that have been correlated to patients with alcohol related disorders and diabetic patients with pneumonia. The bacteria dwell in the moist areas of the human body particularly the oropharynx and the mucosa of the gastrointestinal tract [1]. After isolating the bacteria from the lungs of patients that succumbed to pneumonia, Carl Friedlander initially described the bacterium as an encapsulated bacillus in 1882, naming it *Klebsiella pneumoniae*. It was formerly classified as Friedlander's bacillus and the term Klebsiella was not adopted until 1886. At the current time, it is believed that *Klebsiella pneumoniae* is the most common pathogen in the development of pneumonia that is acquired in the hospital setting in the United States, and this pathogen is accountable for 3% to 8% of all nosocomial bacterial infections [2]. Especially, one of the strains of the *K. pneumoniae* is the primary bacterium responsible for the world's increasing problem of antibiotic resistance, and at the same time, it is known to be a part of the biofilm-forming microbiota that is naturally present in human body . Originally, there were interactions with the host immunity and *K. pneumoniae* providing crucial involvement in the human metabolism. The ways that *K. pneumoniae* utilizes to

evade and subvert immune responses, these are molecular mimicry, allowing antiallergic agent and producing biofilms. That's also true for the metabolic process and glycolysis which this bacterium also affects [3].

Hospital patients, who have weakened immune systems and are more susceptible to bacterial infections, are one place we cannot coexist with them. A coterie of bacteria that are resistant to the deadly effects of antibiotics has arisen, even though the majority of bacteria are still sensitive to its effects. The superbugs known collectively as the ESKAPE bugs include the *Enterobacter faecium, Staphylococcus aureus, Klebsiella pneumoniae*, *Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species are equally prevalent in both the developed and poor countries' hospitals [4].

K. pneumoniae is a pathogen of severe danger because of the co-occurrence of hypervirulent strains in community-acquired illnesses and widespread and elevated antibiotic resistance. A recent report from the World Health Organization (WHO) suggests that antibiotic resistance in *K. pneumoniae* severe HAIs across the globe ranges to about 50%. As with other facilities, there have been cases of resistance associated with KPC-3, porin mutations or the use of multiple carbapenems even with the recent emergence of new anti-microbial agents for treating KPC producing *K. pneumoniae*. These options include ceftazidime-avibactam, being a cephalosporin antibiotic, which is approved with a fresh beta-lactamase inhibitor [4].

Under the influence of antibiotics, bacteria can quickly evolve and become resistant to them, mostly due to genetic changes in the target genes of the antibiotic that reduce or block their antimicrobial action. Other subtypes or types of bacteria can also assimilate genes that exist in genetic elements that have been mobilized through horizontal gene transfer (HGT) which enhances the attainment of resistance genes and hence the sprouting of multidrug resistant (MDR) bacteria. If a bacterial strain or a strain is resistant to at least one agent from three or more antimicrobial classes, then it is said to be multidrug resistance (MDR). Because there are currently few effective treatment options for MDR-bacterial infections,

these infections are becoming more common and a serious healthcare problem. Resistant bacteria are thought to be the cause of around 670,000 illnesses in Europe, which result in over 33,000 deaths annually [5].

1.2 Drug Resistance Mechanism in *K. pneumoniae*

It is demonstrated the clinical consequence of colistin resistance, showing that it is linked to a higher risk of in-hospital death. These results reinforce the threat to public health that is posed by colistin resistance and emphasize the significance of comprehending the variety of processes behind medication resistance. The most prevalent and efficient resistance mechanism to colistin, based on its mode of action, is lipid modification. Lipid A contains a negative charge and colistin binds to this part of the LPS layer, adding 4-amino-4-deoxy-L-arabinose (L-Ara4N) negates the charge leaving the lipid A with a neutral charge which means that colistin cannot bind to the lipid A.

Thus, by increasing the net charge of lipid A from -1. 5 to -1, addition of phosphoethanolamine (pEtN) putatively provides another layer of resistance. As mentioned earlier, the efficiency of L-Ara4N over pEtN substitution in terms of increasing the lipid A net charge is somehow higher than Focalin [5]. In addition, the new functional modifications which have been lately identified are palmitoylation and lipid A hydroxylation. Since there are little colistin resistance genes are sought after in resistant isolates and because it is not a usual procedure to confirm these observations or change in the isolates with susceptible phenotypes, some of these is likely to be phylogenetic marker not related to resistance. Therefore, there is an increasing need to understand the genetic history of genes linked to colistin resistance in isolates that are susceptible to colistin. Also, the *K. pneumoniae* increases its resistance due to the overexpression of capsule polysaccharides, efflux pumps, or the mobilization of plasmid-encoded genes [6].

1.3 The Capsule

As mentioned earlier, *K. pneumoniae* has an outer membrane which has this extra layer called lipopolysaccharide capsule. This showed that down-regulation of drug at cell surface through drugs reduction due to increase of capsular polysaccharides (CPS) provides protection against colistin. On the other hand, no difference in resistance was determined between two strains that only varied for the amount of CPS. The two tests had certain differences; the researchers pointed out that the *K. pneumoniae* strains that was utilized had amazingly low constructs of CPS. In addition, from the above-discussed points, suggestions have been made such that due to site-specific and dispersal characteristics of anionic CPS by *K. pneumoniae*. Thus, its surface can chelate with colistin (cationic molecule). These CPSs are bound to the LPSs which in return are anchored by divalent cations. These cations are displaced by the binding of colistin to lipid A which damages the LPS bridges and frees up CPS molecules and in the process reduces the interaction of colistin with lipid A and the amount of colistin that gets to the surface [7].

1.4 Pumps for Efflux

In the case of many antibiotics, the formation of efflux pumps can be counted among most common forms of the antibiotic resistance through which the antibiotic is expelled. On the other hand, efflux pumps are present in Gram +ve as well as Gram -ve bacteria and in eukaryotes also. They consist of transporter proteins with a large molecular transport activity through which concentration gradient can be established across the membrane. These transporter proteins of the five superfamilies are ATP-binding cassette (ABC), major facilitator superfamily (MFS), resistance nodulation division (RND), small multidrug resistance (SMR), and multidrug and toxic-compound extrusion (MATE). Bacteria have been able to employ these proteins in order to also decrease the efficiency of several antimicrobial agents. *K. pneumoniae* has the efflux pumps belonging to all five superfamilies [8].

For most types of antibiotics, the evolution of efflux pumps is one of the common resistance mechanisms which enable the antibiotic to be expelled. In fact, efflux pumps are found in Gram-positive and Gram-negative bacteria and other eukaryotes are also present. They are composed of transporter proteins that exhibit a large number of transport activities at the molecular level necessary to develop a concentration difference across the membrane. These transporter proteins are present in five superfamilies namely, ATP-binding cassette (ABC), major facilitator superfamily (MFS), resistance nodulation division (RND), small multidrug resistance (SMR) and multidrug and toxic-compound extrusion (MATE) and which bacteria have co-opted into modulating the activity of several antimicrobial agents. *K. pneumoniae* has efflux pumps belonging to all the five superfamilies [8].

1.5 Fimbriae Subunits

There are two primary forms of fimbriae in *K. pneumoniae*: type I and type III. Type I fimbriae are linked to adherence to mucosal membranes and the epithelium, and they are produced by the fim gene cluster . There are still unidentified fimbriae clusters some of which belong to the auxiliary genome and vary in distribution between species [9]. Mrk gene cluster derived fimbriae produce Type III fimbriae subunits that are involved in the process of adhesion and biofilm formation on biotic as well as abiotic surfaces. The principal virulence factor of the organism is its polysaccharide capsule since it shields the bacteria from being swept away by the host's scavenger cells and subsequently killed by serum. Seventy-seven different capsular serotypes have been studied so far and Klebsiella species with reduced virulence are said to be those that do not have a capsule. the second engulfment toxic factor is Lipopolysaccharides that is located at the surface of the bacteria. Sepsis and septic shock arise mainly due to the activation of an inflammatory response by lipopolysaccharides that is noticed by the host organism. Fimbriae, another factor of past in the pathogenicity of bacteria [9]. Despite being of source to animals, K. pneumonia's primary host is humans.

1.6 Biofilm Formation

K. pneumoniae forms biofilms on abiotic surfaces such as medical devices and catheters, as well as on host tissues like the respiratory, urinary, and gastrointestinal tract mucosa.Biofilm is a strong and dynamic structure that confers a broad range of advantages to its members, such as adhesion/cohesion capabilities, mechanical properties, nutritional sources, metabolite exchange platform, cellular communication, protection and resistance to drug

1.7 Strategies for *K. pneumoniae* Infection Treatment

One major obstacle to efficiently treating *K. pneumoniae* infections is the emergence of particularly virulent and multidrug-resistant forms of the illness. Since *K. pneumoniae* antibiotic resistance is rising, it's critical to understand the risk factors that go along with it, take preventative action, and investigate other treatment options to deal with these serious illnesses. This section will focus on four efficient strategies, which include the pharmacological intervention, immunological therapy, biological treatment, and vaccination for the management or prevention of the *K. pneumoniae* infections.

One of the most efficient ways to prevent infection by *K. pneumoniae* is to locate and eradicate its source. Finding and removing the virus source still presents significant obstacles, though. For the majority of hospitals, the main technique for checking for the presence of *K. pneumoniae* is still specimen culture. According to reports, multiplex polymerase chain reaction may be used to detect chromosomal genes using molecular techniques . Preventing the hvKp and CRKP sources is mandatory to discover Enterobacteriaceae generating carbohydrase, especially for CRKP in asymptomatic carriers, the Carb NP test and molecular identification have been used [10].

K. pneumoniae spread can be control by multimodal intervention, identification, education, and extensive screening. Identifying affected people as soon as possible and taking all necessary measures, such as donning masks, gowns, and gloves, are important aspects of exposure avoidance. To reduce future exposure to uninfected people, contact tracing must be utilized wherever feasible, in both hospital and community settings. Medical professionals also need to be educated about hand cleanliness. These antibodies and vaccines against the capsular polysaccharides are still being developed but with some challenges because of the fact a total of seventy-seven different capsules and nine different types of LPS serotypes are present in the *K. pneumoniae*. Some other strategies that can increase the immunity in humans include getting enough sleep, quitting smoking, avoiding too much alcohol, maintaining a healthy weight, exercise regularly means healthy lifestyle [11].

Vaccines develop against different forms and variety of bacteria such as Neisseria meningitides, Streptococcus pneumoniae, and Haemophilus influenzae and deem very useful. For the first time in 1985 the vaccine was design against *K. pneumoniae* K1capsule polysaccharide CPS and then in 1986 polyvalent *K. pneumoniae* CPS were reported. But up to date there they are no clinical *K. pneumoniae* vaccine available [11].

While much research is going into the development of efficient means of vaccinations, at this point, there are no accredited vaccines for the *K. pneumoniae* infection. For *K. pneumoniae* only two antigens are possible for the vaccine, they include the LPS (O-antigen) and capsular polysaccharides (K-antigen). The current strategies being studied entail conjugate vaccines whereby PS-protein or LPS-protein complexes, inactivated whole cell vaccines, polysaccharide/LPS vaccines, protein vaccines, and outer membrane vesicles having multiple substances that cause virulence [11].

The progress of *K. pneumoniae* K1 capsule polysaccharide (CPS) vaccines in 198,560 and polyvalent *K. pneumoniae* CPS vaccines in 1986 (six-valent) and 1988 (24-valent) were acclaimed. However, no vaccines have been approved for use at clinical level till now [12].

Another relatively new and highly promising area, although both with landmark achievements and challenges area is the interconnection between *K. pneumoniae* infection, immune system, and metabolism. Even more profound knowledge of how *K. pneumoniae* copes with the immune response mechanisms and alters the metabolic processes could be helpful in guiding the development of more effective medications. A rather engaging topic is determining how *K. pneumoniae* comes into contact with the immune system and front and center the metabolisms. Knowledge of the molecular processes that are occurring during the infection may be provided by the advanced techniques of transcriptomics, proteomics, and metabolomics. Other aspects of disease progression might also be disclosed by establishing the physiologically relevant animal models and in vitro models of human infection. A promising and one of the main focuses of modern scientific activity, therefore, is the search for novel therapeutic strategies in *K. pneumoniae* [13].

This includes investigating novel immunotherapeutic strategies, such as antibody therapy, both immunization and phage treatment. Thus, it explained that the increasing prevalence of antibiotic-resistant bacteria stressed the imperative need for the development of novel antibiotics against *K. pneumoniae*. Finding new targets for pharmaceuticals and creating inventive Drug delivery systems have a great deal of potential to improve treatment results. While advancements have been achieved in comprehending *K. pneumoniae* infections, there are still difficulties in understanding the intricate relationships during infection between the immune system, metabolism, and other physiological functions.

A multidisciplinary strategy is needed to address these issues, using knowledge from immunology, microbiology, biochemistry, and allied domains. Connecting the dots between basic science discoveries and their practical use in medicine is.drug resistance genes from the environment are known to be trafficked by *K. pneumoniae* to bacteria that are therapeutically relevant.The lack of approved vaccinations or potent medications to treat the infection which in certain cases may even be fatal complicates the issue even more.There is an increasing amount of *K. pneumoniae* genome sequences available in public biology databases. A technique for comparing multiple genomes is pangenome analysis.The global gene repertoire

of the strains of bacteria. By using the genome data that is currently accessible, it is now feasible to identify potential drugs and vaccines in silico using techniques from subtractive proteomics and reverse vaccinology, respectively. The proteins which are located on the exterior of the bacterial cell include the external and outer membrane proteins, these proteins are able to interact with the host immune system hence, vaccination is anticipated for these proteins while the proteins which are located in the bacterial cytoplasm are thought to offer the best bet for drugs.

1.8 Problem Statement

Klebsiella pneumoniae is a significant clinical because it can lead to variety of healthcare associated diseases and infection. Treatment of *Klebsiella pneumoniae* has grown more difficult in recent times due to rise of multi drug resistance strains which is serious world wide problem or illness. As *K. pneumoniae* is part of ESKAPE pathogens, therefore to design effective control strategies, it is imperative to comprehend the pangenome of multidrug resistance *K. pnemoniae* and identification of candidates vaccine targets could help in infection prevention.

1.9 Aims and Objectives

The project is design with aim to identify core proteins of *K. pneumoniae* and to evaluate the potential of these core genes as potential vaccine candidates.In this regard following objectives are design.

1. To identify core proteins in *K. pneumoniae* from different resources by Pangenome analysis.
2. To determine the physicochemical properties and immunogenicity of core proteins.
3. To determine of exposed antigenic epitopes.

Chapter 2

Literature Review

2.1 *Klebsiella pneumoniae*

Carl Friedlander initially characterized *Klebsiella pneumoniae* in 1882. After removing the bacteria from the lungs of patients who had passed away from pneumonia, he referred to it as an encapsulated bacillus. The bacteria were formerly known as Friedlander's bacillus, and Klebsiella was not officially used until 1886. An environmental gram-negative, encapsulated, non-motile bacteria called *Klebsiella pneumoniae* has been linked to pneumonia in patient groups suffering from diabetes mellitus or alcohol use disorders. The most common cause of sepsis, one of the most enigmatic and ancient illnesses and a significant medical problem is pneumonia. Over 750,000 cases of sepsis, or 10% of all ICU patients, occur in the United States alone each year due to a lack of appropriate therapeutics; depending on certain risk factors, this results in a fatality rate ranging from 20 to 50%. The opportunistic pathogen *Klebsiella pneumoniae* in particular is responsible for 5–20% of cases of Gram-negative sepsis [14].

Concerns about the rapidly diminishing supply of antibiotics have been raised by the significant rise in antibiotic-resistant *K. pneumoniae* strains in clinical settings. Therefore, knowledge of pathogen-mediated modification of host immune responses and host immune responses themselves will probably lead to new treatment targets. Neutrophils play a crucial role in the first protective response by

being the first cell type to reach the infection site. In fact, it has been demonstrated that neutrophil-mediated responses are crucial for the early management of the infection in mouse models of K Pneumoniae infection. The pneumoseptic *K. pneumoniae* infection's ongoing inflammation is caused by a persistent buildup of neutrophils and their excessive activation. It has also been observed that neutrophils serve as a reservoir for this pathogen and facilitate the infection's systemic spread as this [14].

Because of the rise of hypervirulent strains and antibiotic resistance, K Pneumoniae has recently drawn attention as a "successful" pathogen. The multitude of virulence characteristics this virus exhibits and employs to shield itself from the host immune response has contributed to the increasing difficulty in treating the wide variety of infections it causes in both immunocompromised and immunecompetent individuals. *K. pneumoniae* is classified as an "evader" pathogen instead of a "offender" pathogen based on these virulence parameters [14].

In clinical settings, an increasingly critical issue related to extended-spectrum beta lactamase (ESBL)-producing Klebsiella pneumonia (ESBL-KP) carriers with silent intestinal carriers has emerged. To ascertain clonal relatedness among carriers, several epidemiological investigations are being carried out. Using multiple locus variable number tandem repeat analysis (MLVA) and multi-locus sequence typing (MLST), clonal relatedness was examined. The major sequence types (STs) found in ESBL-KP isolates were ST147, ST15, and ST16, according to the MLST study. MLVA was used to characterize the isolates into 4 miniclusters and 11 singletons. Elevated variability among ESBL-KP isolates suggested that this bacterium might be readily transmitted and colonized in various locations. The community and hospital carrier screening program may aid in the management of infections in these settings. Human mucosal surfaces of the oropharynx and gastrointestinal (GI) tract are usually colonized by the bacteria. In the United States, hospital-acquired pneumonia is thought to be most frequently caused by *K. pneumoniae* in these days [15].

2.2 Taxonomic Hirarchy

An enclosed, nonmotile, rod-shaped class of oxidase-negative, Gram-negative bacteria is represented by the genus Klebsiella. Ten strains of this genus were initially discovered in the late 1800s, and Trevisan (1885) gave them the name Edwin Klebs (1834–1913), in honor of the German scientist. Klebsiella is a member of the Enterobacteriaceae family, which includes several different biochemically unique genera [16].

Consequently, due to their immense level of antibiotic resistance, gram-negative bacteria (GNB) are considered one of the world's most dangerous pathogens. It is therefore important in current patients since they raise the patient risk and enhance the morbidity and mortality in the intensive care unit (ICU) scope of these bacteria. Without an exterior layer, the cell wall is thin. There is a significant presence of lipids. It has every kind of amino acid, with less muramic acid, Streptomycin, and has no teichoic acid or magnesium ribonuclease [16].

TABLE 2.1: Classification of *K. pneumoniae* [16]

S.No	**Domain**	**Eukarya**
1	*Kingdom*	Bacteria
2	*Phylum*	*Protobacteria*
3	*Class*	*Gammaproteobacterial*
4	*Order*	*Enterobacteriales*
5	*Family*	*Enterobacteriaceae*
6	*Genus*	*klebsiella*
7	*Specie*	*Klebsiella pneumoniae*

2.3 Origin

K. pneumoniae is a non-motile, capsulated, gram-negative bacterium and belongs to the Enterobacteriaceae family. There are many different type of virulent factor that involve in virulency of bacterium. Current under study are 77 different capsular forms and the less pathogenic are the species of Klebsiella without a capsule [17].

One of the few bacteria with a high incidence of antibiotic resistance now is *Klebsiella pneumoniae*, which is caused by changes to the organism's basic DNA. In 1929, Alexander Fleming made the initial discovery of beta lactam antibiotic resistance in gram- negative bacteria. Since then, *K. pneumoniae* has undergone extensive research, and it has been demonstrated that the bacteria produce a beta-lactamase that hydrolyzes the beta-lactam ring in antibiotics. In 1989, *K. pneumoniae* was shown to contain extended spectrum beta-lactamase (ESBL) in the United States and in Europe in 1983 [18].

Over 80% were caused by *K. pneumoniae*. The alterations of the outer membrane, the up regulation of efflux pumps, and the increased synthesis of ESBL enzymes within the organism have all been associated with carbapenem resistance [19].

2.4 Natural Habitat

In terms of its relationships with habitats, the genus Klebsiella appears to be widespread. Klebsiella is a frequent permanent or transitory flora pathogen that preys on humans and other animals, especially in the gastrointestinal system. Sewage, soils, surface waterways, industrial effluents, drinking water, and plants are examples of additional habitats. Nearly every one of these Klebsiella species was previously thought to be *K. pneumoniae*. However, phenotypic and genotypic research has revealed that the genus "*K. pneumoniae*" is really composed of at least four species, each with unique traits and environments. Klebsiella species are generally associated with the following habitats: *K. oxytoca* frequent association with most environments; K. terrigena-unpolluted surface waters and soils, drinking water, and vegetation; K. planticola-sewage, contaminated waters and soils, humans, animals, and sewage [20].

2.5 Pathogen Virulence Potential

Immunocompromised people have been the primary target of *Klebsiella pneumoniae*-related infections. But even those who are healthy and immune-compromised are becoming affected by its appearance and spread. Treatment for *K. pneumoniae* infections is particularly challenging due to the strain's growing resistance to antibiotics. Human mucosal surfaces are easily colonized by *K. pneumoniae*, particularly those of the GI tract and oropharynx, where its colonization appears to have benign effects (3–5). These locations provide *K. pneumoniae* strains access to other tissues, where they can infect people severely. The best offence for a pathogen is not always a strong defense, as *K. pneumoniae*, an incredibly resilient bacterium, appears to operate under the theory that "the best defense for a pathogen is a good defense."

The fact that these bacteria may thrive at many locations within hosts and escape and survive several immune system components, instead of aggressively suppressing them, serves as an example of this. This review concentrates on aspects related to *K. pneumoniae* pathogenicity that have been thoroughly examined [21].

In order to avoid being detected by the host's innate immune system, the pathogenicity of *K. pneumoniae* bacteria is linked to many virulence factors. Adhesins, lipopolysaccharides (LPSs), microvasculitis-associated exopolysaccharides, iron absorption systems, and capsules are among the virulence factors of *Klebsiella pneumoniae*. Both K. pneumonia's capacity to induce nosocomial infections in people and its resistance to certain antibiotics are variables that exacerbate the illness. Additionally, the preceding instance had six Asian patients who were hospitalized in the US for a *K. pneumoniae* liver abscess; in one of the instances, the gastrointestinal system was suspected of being the entrance point [22].

Furthermore, meningitis, pneumonia, urinary tract infections, and blood infections are all brought on by *K. pneumoniae* [23]. Most doctors agree that community-acquired bacterial pneumonia is caused by *Klebsiella pneumoniae*. Hospitalization of immunocompromised people and those with serious conditions is mostly caused by opportunistic pathogens. Nosocomial *Klebsiella* infection is mostly caused by

Klebsiella pneumoniae, a necrotic process that primarily affects the frail. Moreover, *K. pneumoniae* can infect healthy people with endophthalmitis, meningitis, liver abscesses, and other localized illnesses [23].

Global public health is seriously threatened by food borne illnesses brought on by pathogenic microorganisms. Prevalent food borne pathogens like *Salmonella, Campylobacter, Escherichia coli, Shigella, Listeria monocytogenes, Staphylococcus aureus,* and *Vibrio parahaemolyticus* were the focus of the majority of food borne bacterial research conducted up to now. Conversely, as *K. pneumoniae* is not typically identified as a foodborne pathogen, not much information has been found regarding foodborne *K. pneumoniae*. On the other hand, marketed fresh vegetables prawns traded internationally and farm-raised chicken have all been linked to antimicrobial-resistant *K. pneumoniae* strains. According to a recent study, foodborne *K. pneumoniae* may be the source of a nosocomial outbreak. Additionally, a number of *K. pneumoniae* resistance genes are found in genetic components that are transferable to other bacteria. Consequently, *K. pneumonia*'s potential contribution [24].

Public health organizations, the food sector, and consumers are all still concerned about the appearance and reemergence of foodborne diseases. The appearance is caused by a number of variables, such as altered consumer and microbe behavior, altered animal husbandry and agricultural techniques, increased international travel, global food distribution, and altered climate conditions. In addition, additional infections are being identified due to developments in molecular technologies and pathogen detection techniques. New viruses that can infect food are Government organizations, the food industry, and other organizations concerned in food safety must form international alliances to reduce the incidence of foodborne illnesses and the emergence/re-emergence of pathogens. Often zoonotic in origin, they might be viruses, parasites, bacteria, or both Gram-positive and Gram-negative. Prioritized foodborne pathogens could [25].

Numerous *K. pneumoniae*-related foodborne outbreaks have been documented in a number of nations in recent years. The expression of many acridity factors,

including as capsule, endotoxin, siderophore, iron scavenging system, and adhesins, is a critical aspect of the pathophysiology of *Klebsiella pneumoniae* [26].

The protective effect of the capsule against phagocytosis and its direct suppression of the susceptible host response makes it a noteworthy acridity factor that contributes to at least two pathogenic pathways [27].

Furthermore, the reproduction of harmful bacteria depends on iron. Microorganisms, akin to bacteria and fungus, bury composites called siderophores, or iron transporters, to transfer iron within their cell membranes. They possess a more sophisticated iron magnet than the transferrin, the host transport protein [28]. The bacterium that causes *Klebsiella pneumoniae* infection produces extended-spectrum beta-lactamases (ESBLs) and is resistant to carbapenems [29, 30]. The pathogenicity of bacteria is mostly caused by lipopolysaccharides and CPS. Lipopolysaccharides comprise antigens that microbes need in order to reject complement-mediated payoff, including lipid A, core, and O-polysaccharide. By functioning as a physical fence, CPS, the pathogen's most distant subcaste, mainly contributes to resistance against phagocytosis by polymorphonuclear cells [31].

Consequently, for microbes to propagate throughout the circulation and induce sepsis, both conditions must be met. The way that these two variables interact with *K. pneumoniae* is not well understood, though. The experimental confirmation indicates that *K. pneumoniae* conformation may be influenced by CPS because of the active vaccination against the experimentally persuaded *K. pneumoniae* using pure CPS-defended mice [32].

In addition to having a high death rate, *K. pneumoniae* can cause significant lung scarring in up to 50% of survivors Furthermore, meningitis and other extrapulmonary symptoms are relatively uncommon. Given that *Klebsiella* is the most commonly isolated gram-negative bacillus and that gram-negative bacterial pneumonia accounts for more deaths than any other nosocomial infection, it is evident that immunological methods are necessary to control *Klebsiella* pulmonary infections. The immunological mechanisms that defend against *K. pneumoniae* pneumonia are comparatively little understood [32].

Similar findings were made in a recent investigation about the reduction of *K. pneumonia*'s hematogenic spread and inflexibility by monoclonal antibodies against Klebsiella CPS. Moreover, it has not been adequately studied how CPS and LPS may contribute significantly to the formation of necrotic lesions [33].

In addition to blocking complement-mediated lysis and opsonization, the polysaccharide capsule also inhibits phagocytosis. Whole LPS will provoke a potent seditious reaction, contributing to the transfer of C1q to bacteria and activating the complement system. Additionally, certain strains of Klebsiella have the ability to alter LPS such that it is not used by susceptible cells, while other strains may utilize the capsule to aid in the detection of LPS by the toll-like receptor (TLR4) [34].

The process of neutrophil reclamation is facilitated by the induction of IL-17 and IL-8 products, which is facilitated by interleukin (IL)-23. By using IFN-, IL-12 increases the expression of IL-17. The seditious pathway for nucleotide oligomerization domain (NOD)-like receptors, which resemble the pyrin receptor, is activated to produce IL-1, another type of cytokine [35].

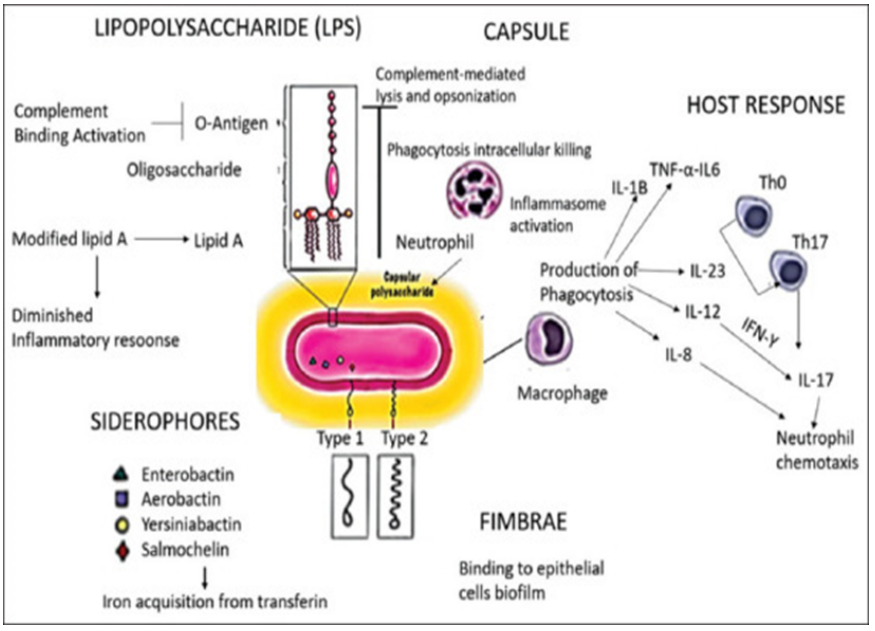

FIGURE 2.1: Pathogen virulence Potential [35]

2.6 Clinical Importance of *Klebsilla pneumoniae*

Researchers in Taiwan discovered the unusual illness of a monomicrobial *Klebsiella pneumoniae* pyogenic liver abscess in the middle of the 1980s in people who were not suffering from biliary tract diseases but were frequently diabetics. Afterward, community-acquired *K. pneumoniae* liver abscesses have been raised as a serious health concern in a number of regions in Asia and has been reported as the cause of 80% of pyogenic liver abscess in Taiwan and Korea. There has also been what appears to be sporadic cases of these abscesses in North American, Australia, Europe as well as Asia [36].

Hypermucoid strains that belong to the capsular K1 (or less frequently K2) serotype of *K. pneumoniae* dominate infections. Even though secondary metastatic infections, like endophthalmitis and meningitis, have been reported in 10-16% of patients, the mortality rate is approximately 5%, close to the one observed in patients with non-*K. pneumoniae* liver abscess [36].

Even the two case studies published in the US revealed that half of the patients were of Asian ethnicity. The majority of patients had community-acquired illnesses, which are primarily documented in Asian nations like Taiwan and Korea. No host genetic variables that could account for the increased frequency among Asians have yet to be found by studies. As per previous studies, it has been observed that *K. pneumoniae* can be recovered from healthy individuals in Asian countries in about 75% of cases and, in Taiwan, among the total typeable, isolates, 23% were belonging to K1/K2 serotype. Among 1,175 patients who underwent stool sample collection, 248 (21. 1 percent) were identified as *K. pneumoniae* isolates, and of those 57 (248) or 23 percent were K1 serotypes as reported in Korean study. One of the representatives in size said that *K. pneumoniae* was present and widespread [37].

It is thought that one of the risk factors for a liver abscess caused by *K. pneumoniae* is diabetes mellitus (DM). In the Taiwanese case series, up to 63% of patients were found to have DM, compared to 5–33% of patients with liver abscesses that were not caused by *K. pneumoniae*. One theory indicates that poor glycemic control

may impede neutrophil phagocytosis of K1 and K2 capsular serotypes despite the fact that the specific details regarding the process have not been established. There have been reports linking endophthalmitis patients to unfavorable visual outcomes. Moreover, some individuals analyze the interconnection between the cases of using antibiotics like ampicillin and amoxicillin within 30 days before developing *K. pneumoniae* liver abscess. For example, in an ancillary animal investigation of the effect of sildenafil on pulmonary vessels in rats [37].

Hospital settings, long-term care facilities like nursing homes, and, less frequently, the community can all be the source of *K. pneumoniae* infections (also known as non-nosocomial healthcare-associated infections). Apparently, 3-8% of hospital-acquired bacterial infections are attributed to Klebsiella species, of which UTIs, pneumonia, and primary bacteremia are the most common manifestations. Several medical care items are considered as the intermediate risks for these infections which include bladder catheters, endotracheal tubes and intravenous catheters as well as past use of antibiotics. The multidrug resistance that *K. pneumoniae* is exhibiting is also a result of widespread antibiotic usage [38].

2.6.1 Clinical Symptoms

The main host of *K. pneumoniae* is humans. As it is observed that Klebsiella species are rarely identified in skin of human body, therefore the carrier rate of K.pneumoniae in the population shows that the rate in stool sample is 5-38% and in nasopharynx is 1-6%. In alcoholically fermented ambulatory patients, as shown by previous reports, higher rates of nasopharyngeal carriage have been noted [39]. The ethnicity of Chinese people might be an important risk factor in the colonization of intestine; stool carrier rates of *K. pneumoniae* among the normal healthy Chinese was found to be between 19% in Malaysian and 88% in Japanese population. ICUs are the most frequent locations where outbreaks originate [possibly because patients in these areas are exposed to a multitude of feasible colonization/infecting hazards]. Patients' ability to resist exogenous bacteria may be reduced and the possibilities of cross infection may increase due to such factors as more serious

condition of the patient, his age, improper or irrational use or abuse of antibiotics, multiple manipulations, and high invasiveness of the procedure [40].

2.6.2 Hospital Acquired Pneumonia

K. pneumoniae is one of the relatively frequently occurred pathogens of hospital acquired pneumonia [41]. The fifth, seventh, and the eighth AMR organisms were *K. pneumoniae* and it accounted for 8. This is equal to 4% of cases of Ventilator associated pneumonia VAP, 7. Consequently, only 1% of non-ventilated hospital-acquired pneumonia cases and 7% of non-ventilated CAP are presumed to have been acquired within hospital. 6% of cases of healthcare-associated pneumonia [42].

The clinical signs and symptoms of hospital pneumonia caused by *K. pneumoniae* are comparable to those of ventilator-associated or nosocomial pneumonia generally; they include leukocytosis, fever, coughing up phlegm, and a new pulmonary infiltration. When patients are hospitalized, nosocomial colonization of the upper respiratory tract is prevalent, especially when those patients need to be admitted to an intensive care unit or are on a ventilator. Thus, the presence of Klebsiella in a hospitalized patient's sputum without any further pneumonia-related symptoms may not always indicate an infection [43].

2.6.3 Community Acquired Pneumonia

The community-acquired pneumonia (CAP) infection rate that was caused by *K. pneumoniae* differs depending on the geographic area (with greater rates in Asian nations). In the US and Europe, it is an uncommon cause that mostly affects people with severe chronic obstructive lung disease, diabetes, and alcoholism [44]. *K. pneumoniae* was an uncommon cause in investigations of CAP patients in countries like Iran and the Netherlands [45].

But among the top three isolates in a study of 225 persons with CAP in Shanghai, China, *K. pneumoniae* was shown to be the culprit.Clinical signs and symptoms of

K. pneumoniae resemble those of other bacterial pneumonia causes, such as fever, coughing, pleuritic chest discomfort, dyspnea, tachypnea, production of sputum, crackles on physical examination, and leukocytosis. Sputum that is thick, mucoid, and tinged with blood—a condition known as "currant jelly"—can result from severe inflammation and necrosis linked to *K. pneumoniae* [46].

Radiologically, lobar pneumonia caused by community-acquired *K. pneumoniae* generally resembles that of Streptococcus pneumoniae, in contrast to the results in nosocomial infections [46]. A tendency exists for the right upper lobe's posterior section to be involved. Excessive consolidation might cause the fissure to bow downward, resulting in the bulging fissure indication. Based only on radiographic evidence, Klebsiella cannot be excluded as the etiology of community-acquired pneumonia [46].

2.6.4 UTI

An infection may result from the entry of *Klebsiella pneumoniae* bacteria into your urinary system. If you had a catheter inserted during a hospital visit, this is more likely to happen. Life-threatening UTIs can result from *Klebsiella pneumonia*, particularly in patients with underlying medical conditions. It's critical that you notify your doctor of any UTI symptoms, particularly if you've been admitted to the hospital [47].

2.6.5 Meningitis

A frequent cause of nosocomial meningitis is *K. pneumoniae* . *K. pneumoniae*, for instance, was responsible for 13 cases (8.6%) out of 151 cases reported from the United States [47]. A brain abscess is a potentially fatal infection that originates from distant or para-meningeal sites. While *Klebsiella pneumoniae*-caused brain abscesses that spread from liver abscesses have been more common in recent years, streptococci have historically been the most often reported organisms. Young patients and those with diabetes mellitus were more likely to have brain abscesses.

The most common causes of community-acquired brain abscesses in Taiwan are *K. pneumoniae* and Streptococcus species. The most likely cause of a *K. pneumoniae* brain abscess is a liver abscess [48]. They established that the prevention of gram-positive pathogens with prophylaxis increased the rates of post-neurosurgery meningitis caused by gram-negative microbes like *K. pneumoniae*. Meningitis acquired in hospitals is mainly associated with neurosurgery [49].

However, *K. pneumoniae* can exceptionally be regarded as one of the main pathogens in community-acquired bacterial meningitis and brain abscesses in case the mentioned diseases appear as metastatic complications of primary *K. pneumoniae* liver abscesses that are prevalent predominantly among the Taiwanese population belonging to East Asia. A bacterial pathogen may be a gram-negative bacterium, like *K. pneumoniae*, in the case of meningitis. This type of infection will result in fever and symptoms of central nervous system pathology, for example altered level of consciousness, seizure activity or stiffness of the neck [50].

2.6.6 Soft Tissues and Skin Infection

K. pneumoniae can cause necrotizing fasciitis [51] or necrotizing myositis which are potentially lethal infections that proceed from severe cellulitis with or without crepitation. In western nations, *K. pneumoniae* is an uncommon source of these illnesses; nevertheless, in Taiwan, it is more prevalent [52] and was the most frequently isolated pathogen in one dataset. Initially, intense pain without any cutaneous signs may be the only symptom in certain individuals with necrotizing soft tissue infections. Other symptoms that may be present include systemic toxicity, discomfort, swelling, and erythema at the afflicted location [53].

2.6.7 Intraabdominal Infections

Liver abscess often polymicrobial and linked to underlying hepatobiliary disease or cholangitis, pyogenic liver abscesses are the source of *K. pneumoniae* isolates .

K. pneumoniae was the most frequent cause of pyogenic hepatic abscess in case studies from New York and San Diego [54].

A study of the clinical aspects of 171 instances of pyogenic liver abscesses was conducted in the New York report [55]. Fever (90%), right upper quadrant ache (72%), chills (69%) and other symptoms were the most often reported ones. Only 43% of patients exhibited the triad of increased alkaline phosphatase, right upper quadrant discomfort or soreness, and fever; however, 86% of patients had at least two of those symptoms. The clinical features of the individuals with liver abscesses of various or unknown etiologies were comparable to those of the 23 of 54 (41%) hepatic abscesses in whom *K. pneumoniae* was identified [55, 56].

An ascitic fluid infection without a visible intra-abdominal medically curable cause is known as spontaneous bacterial peritonitis (SBP). It nearly invariably happens to ascites and cirrhosis patients. The ascitic fluid usually has an increased polymorphonuclear leukocyte count, and patients frequently arrive with fever and stomach discomfort. Gram-negative intestinal flora is responsible for about 60% of cases of SBP in individuals with positive ascitic fluid cultures [57, 58]. In around 10 to 15 percent of cases, *K. pneumoniae* is found, after E. coli, which is the most prevalent. This is true for nosocomial infections as well as those acquired in the community [59].

2.7 Evolution of Resistance towards Large Spectrum Antibiotics

The use of antibiotics has transformed medical care throughout the years, paving the way for the eventual eradication of once-fatal bacterial illnesses [59]. But there have been drawbacks to this victory as well. The extensive application and mishandling of antibiotics has resulted in significant selection pressure on bacterial populations, leading to the emergence of resistance characteristics. In this arms race for resistance, *K. pneumoniae*, a member of the Enterobacteriaceae family, has been leading the charge. It has been progressively accumulating genetic

determinants that make it resistant to our strongest medicines . In *Klebsiella pneumoniae* is a potent enemy that contributes significantly to the problem of antibiotic resistance. Effective treatment of infections is hampered by this bacterium's well-known capacity for adaptation and resistance to a broad spectrum of antibiotics. Since *K. pneumoniae* continues to evade conventional therapies, it is imperative that novel approaches be taken to counteract the escalating problem of antibiotic resistance. Healthcare practitioners and the scientific community must work together on this [60].

2.7.1 Polymyxin-Resistant Genes

By attaching to the negatively charged lipopolysaccharides (LPS) and causing cell lysis, polymyxin disturbs the integrity of the membrane by dislodging the cations (Ca+2/Mg+2) in the outer membrane. Since *K. pneumoniae* was not as widely used in human treatment during the 1980s and 2000s due to its known toxicity, the pathogen's history of polymyxin resistance is shorter than that of others. Around the start of use, the first clinical isolation of *K. pneumoniae*, which is now known as pneumoniae and resistant to colistin, was discovered. Polymyxins were frequently the last choice of medication in the early 2000s due to the rising prevalence of XDR carbapenemase-producing *K. pneumoniae* (CPKP) strains [60].

The "LPS modification system" refers to the primary chromosomal mechanism that *K. pneumoniae* uses to modify its targets in order to become resistant to polymyxin. The LPS structure is changed in strains that have this complex system, which reduces the anionic charge that interferes with the binding of polymyxins. Mutations affecting multiple core genes, including lpxM and its regulator ramA, which are in charge of lipid A maturation, and pagP, pmrE, pmrC, and amino arabinose, which are responsible for neutralizing lipid A, are the sources of these alterations in LPS. More LPS-modifying gene regulators, including phoPQ, pmrA, and pmrD, are also more active during resistance. PMrB overexpression or mrgB deactivation caused by a mutation in one of the other two regulation genes is already sufficient [60].

In recent decades, there has been a significant and fast rise in the worldwide prevalence of multi-drug-resistant *Klebsiella pneumoniae* (MDRKP), which poses an immediate and critical threat to public health.

The observed increase in prevalence highlights the pressing necessity of prompt attention and intervention tactics to adequately tackle this growing issue, which presents substantial obstacles to healthcare systems around the globe [61].

The capacity of *Klebsiella pneumoniae* to withstand the effects of several antimicrobial drugs is largely attributed to its intricate and varied antibiotic resistance pathways. The acquisition of genes producing enzymes that may alter or inactivate antibiotics is the main cause of high-level antibiotic resistance in *K. pneumoniae*. Penicillin's and cephalosporins are among the broad-spectrum β-lactam antibiotics that are resistant to extended-spectrum β-lactamases (ESBLs) [62].

2.8 Antibiotic Resistance in *Klebsiella pneumoniae*

Klebsiella pneumonia's resistance to antibiotics Given the growing importance of multidrug-resistant *Klebsiella pneumoniae* (MDR *K. pneumoniae*), it is essential to understand the characteristics of its population and the relationship between these features and the genetic variability associated with antibiotic resistance. We still don't know much about the origins of MDR *K. pneumoniae* and how it travels across nations in relation to hospital infections, despite our increased understanding of the global variety of this bacterium and outbreaks within specific healthcare facilities [63].

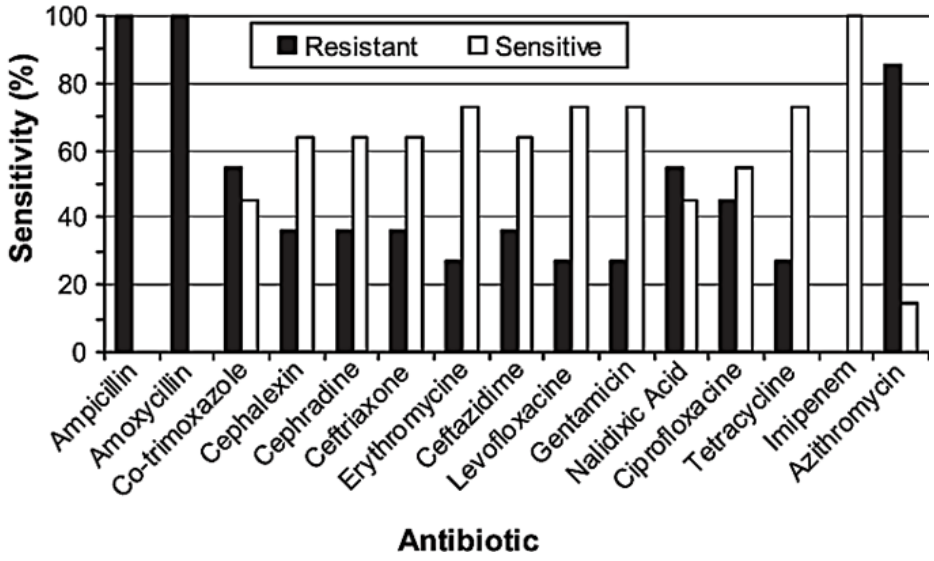

FIGURE 2.2: Antibiotic resistance/sensitivity pattern of the *Klebsiella pneumoniae* isolates (n = 22) recovered from UTI patients [63]

Currently, a particular class of bacteria called *Klebsiella pneumoniae* is facing a significant increase in antibiotic resistance, mostly due to changes in its basic genetic composition. The first evidence of beta-lactam antibiotic resistance in gram-negative organisms was discovered by Alexander Fleming in 1929, which is when this resistance initially emerged. Research on the production of beta-lactamase enzymes by *K. pneumoniae* has been ongoing for some time now. These enzymes are responsible for the breakdown of the important beta-lactam ring in antibiotics [63].

The emergence of Extended-Spectrum Beta-Lactamase (ESBL) producing *K. pneumoniae* strains was documented in Europe in 1983 and later in the United States in 1989. ESBL possess the ability to enzymatically degrade oxyimino cephalosporins, rendering third generation cephalosporin antibiotics ineffective in treating infections caused by these strains. Consequently, clinicians turned to carbapenem antibiotics as a treatment alternative for ESBL producing *K. pneumoniae*. However, a concerning trend emerged, as evidenced by data from the Centers for Disease Control and Prevention (CDC) in 2013.

Among approximately 9,000 reported infections due to carbapenem-resistant Enterobacteriaceae, roughly 80% were attributed to *K. pneumoniae*. This rise in carbapenem resistance has been linked to various factors within the bacterium,

including the upregulation of efflux pumps, alterations in the outer membrane structure, and augmented production of The human population is the primary reservoir for *Klebsiella pneumoniae*. In the community at large, a significant proportion of individuals, ranging from 5% to 38%, carried the bacteria in their stool, and 1% to 6% carried it in their nasopharynx. Stool of infected patients and hands of health care workers are considered the main sources of infection in nosocomial outbreaks. It is noteworthy that individuals with chronic alcoholism and people of Chinese ethnicity have higher rates of colonization. In hospital settings, the prevalence of *K. pneumoniae* carriage far outpaces that seen in the general community. Interestingly, in one study, carrier rates of up to 77% were found [64].

2.8.1 Mechanisms of Resistance to Antibiotics

The organisms resistant to several drugs were previously mostly found in clinical settings, but they are becoming more and more common in everyday contexts. This change suggests that there exist microorganisms with resistance to antibiotics outside the walls of medical facilities. Bacteria's capacity to respond to the antibiotic "challenge" is a prime illustration of their adaptability and highlights the highest point of their evolutionary history [65].

Bacterial pathogens possess remarkable genetic flexibility, which gives birth to the idea of "survival of the fittest". Because of its flexibility, several reactions are triggered, which can result in mutational modifications, the acquisition of new genetic components, or regulation of gene expression. Eventually, these responses lead to resistance to almost all commercially available antibiotics. Consequently, obtaining a thorough understanding of the biochemical and the relevance of the genetic foundations of antibiotic resistance acquires critical importance. A strategy to stop the creation and spread of resistance as well as the development of novel therapeutic approaches to combat multi drug-resistant organisms are based on this concept [65].

2.8.2 Resistance to Beta-Lactams UTI

Because it produces beta-lactamases, carbapenemases, and other beta-lactamase enzymes, *K. pneumoniae* is well-known for having a strong resistance to beta-lactam drugs. Before the emergence of extended-spectrum β-lactamase (ESBL) or carbapenemase-producing hvKP in China in 2014, hvKP and MDR tcp were distinct; however, many of them were found to be clonal [66]. The widespread threat posed by extended-spectrum beta-lactamase strains with various ESBL genes, including the dominant CTX-M-15 type, is worldwide. The quick horizontal transmission of ESBL genes by plasmids has accelerated their expansion. While *K. pneumoniae* strains that produce ESBLs and are hypervirulent have been investigated extensively worldwide, examining their genomes will give important insights into the processes behind drug resistance and guide focused approaches to counter this growing danger [67].

In a worrying epidemic of multi-drug resistant *Klebsiella pneumoniae* that affected five out of seven newborns in a NICU in an Indian hospital, Pathak et al.'s study was published. The isolates had carbapenems' genes such as blaNDM-1, blaNDM-5, and blaOXA-232, together with extended-spectrum antibiotic resistance [68]. *Klebsiella pneumoniae* and many strains of K. pneumonia is a serious healthcare problem that has been clarified by research done in India by Sikarwar & Batra [68]. Clinical isolates were gathered from several parts of India, mostly from diseases related to the respiratory, urinary tract, and pus systems. The fact that over half of the isolates had multidrug resistance is noteworthy and illustrates how common this problem is in Indian healthcare systems. Significant resistance to antibiotics including ampicillin, ofloxacin, piperacillin, cotrimoxazole, ampicillin, and chloramphenicol was revealed by statistical analysis using the SPSS software. Some drugs, such cefotaxime and tetracycline, showed mild resistance [68].

Their research brought attention to the widespread worry about antibiotic resistance, highlighting how it not only raises healthcare expenditures but also presents serious risks to the treatment of patients. More thorough study in this field is required, as suggested by the conclusion, which calls for concentrating on the genetic

composition of multidrug-resistant bacteria to comprehend gene alterations and their consequences on antibiotic resistance. Important first measures in resolving this issue are promoting quick detection techniques for pathogenic microorganisms and bolstering laboratory and monitoring capabilities. Other crucial tactics to lessen the escalating issue of antibiotic resistance in India and throughout the world include encouraging sensible antibiotic usage and teamwork among medical experts, pharmacists, and laboratory staff [68].

2.8.3 Aminoglycosides Resistance

Bacteria may become resistant to aminoglycosides due to the advent of aminoglycoside-modifying enzymes (AMEs). These enzymes change amino-glycoside antibiotics chemically, making them inert. When treating serious infections brought on by Gram-negative bacteria, such as *Klebsiella pneumoniae*, aminoglycosides are commonly utilized in combination therapy. There will be fewer therapeutic options available when these strains grow resistant to aminoglycosides. Since people in healthcare settings are frequently more susceptible to infections, aminoglycoside resistance is an additional worry in places where *Klebsiella pneumoniae* is a prominent source of hospital-acquired illnesses [69].

In research, 37 (72.5%) of the 51 strains of Klebsiella spp. that generated extended-spectrum beta-lactamases (ESBLs) were discovered to carry integrons. PCR was used to detect the presence of integrase genes and cassette regions. Later PCR and 51 isolates of Klebsiella spp. that generated extended-spectrum beta-lactamases (ESBLs) 37 were discovered to have integrons in 72.5% [70].

PCR was used to detect the presence of integrase genes and cassette regions. AadB and AadA2 gene cassettes, which both give resistance to different aminoglycosides, were found to be present in the cassette areas after amplicon sequencing and PCR were performed. More specifically, a class 1 integron on the 28-kb pES1 plasmid was linked to aadB. It is noteworthy that this plasmid not only carried the insertion sequence IS26 but also the blaSHV-12 gene. This discovery emphasizes the role

that integrons play in ESBL-producing Klebsiella species as carriers of antibiotic-resistance genes and the possibility that genetic factors like pES1 might spread multidrug resistance inside. A nosocomial epidemic brought on by multidrug-resistant Klebsiella pneumonia was investigated in the research [71].

In most epidemic strains, the SHV-5 ESBL gene was found and clonality was verified using genetic typing. The identification of the aminoglycoside resistance genes aadB and aadA2 inside variable integrons is noteworthy. The study emphasizes the difficulty of identifying low-level ESBL expressions and recommends customized screening for maximum sensitivity depending on ciprofloxacin MICs. It underscores the necessity of all-encompassing measures for controlling outbreaks, such as isolation protocols, enhanced hand hygiene, environmental cleanliness, and the reform of antibiotic policies [71].

2.8.4 Resistance to Fluoroquinolones

The impact of fluoroquinolone resistance in *Klebsiella pneumoniae* on treatment choices and patient outcomes is an increasing issue in the healthcare industry. Target site mutations, overexpression of the efflux pump, and plasmid-mediated resistance genes are some of the processes that might give rise to this resistance. Treatment options are restricted, particularly for severe infections, when *Klebsiella pneumoniae* develops resistance to fluoroquinolones. Overuse and abuse of fluoroquinolones, as well as the hospital setting, which might promote the propagation of resistant strains, are risk factors for resistance. Fluoroquinolone resistance can be avoided by using antibiotics sparingly, adhering to strict infection control protocols in healthcare facilities, and conducting continuous research on novel antibiotics with distinct modes of action. The synthesis of cephalosporinase and extended spectrum β-lactamase (ESBL) enzymes is well-known for *Klebsiella oxytoca* and *Klebsiella pneumoniae*. These bacteria, which have an alarmingly high frequency of ESBL positive and fluoroquinolone resistance, represent a serious hazard in both non-hygienic community settings and hospital-acquired (HA) illnesses. Patients have major worries about the wide range of antibiotic resistance, which includes

important medications like imipenem and ciprofloxacin [72]. There is an urgent need for comprehensive policies to address the worldwide problem of antimicrobial resistance. These efforts should include the wise use of antibiotics, strict infection control, and the development of other forms of care, in order to tackle this escalating risk to public health. Variou quinolone resistance determining region (QRDR) mutations were found in the genes gyrA and parC. The multidrug resistance was further complicated by the discovery of a high correlation between TMQR and β-lactamase genes such as blaCTX-M and blaTEM [72].

2.9 Pangenome Analysis

Next-generation sequencing technologies (NGS) have made genome reconstruction simpler and more accessible than it was in the past. In terms of bacterial research, it is simple to get and analyze more than 10 distinct genomes from the same species, which offers sufficient information for comparisons. These new capabilities led to studies of pangenomes, which more truly represent the concept of bacterial species. To further determine the variety and makeup of the global gene repertoire, it is highly suggested that research use several genomes. It has been characterized as the whole gene repertoire of research as the pangenome [73].

Three sections typically make up a pangenome the core genome, which contains all the genes shared by all research strains; the secondary, or accessory genome, which include genes that are shared by two and n-1 strains as well as unique genes that are found in just one strain. Numerous aspects, including the resistome, mobilome, and global metabolism, may be studied within the pangenome [73].

The pan-genome is a collection of genes that could differ in frequency within the microorganisms under study . Genes fall into one of three categories according to how frequently they occur. Core genes are those that are present in every microbe investigated and can be discovered in their genomes. Accessories are genes that are present in only a portion of the genomes under investigation. Unique genes, which are exclusively present in single genomes, comprise the third group of genes that comprise the pan-genome [74].

In a pan-genome examination of a single species, unique genes may be strain- or species-specific, depending on the scope of the investigation. The genes belonging to the subtype indicated earlier, which were discovered by pan-genome research, have distinct functions in the development of microorganisms. It is thought that the genes in the core are in charge of the housekeeping, cell division (replication), and homeostasis processes that are fundamental to bacterial cells. In contrast to core genes, accessory and unique genes function as a supporting cast. The virulence of pathogenic bacteria and the growing environment of a bacterial species are associated with these genes. The aforementioned genes are obtained via horizontal gene transfer, a phenomenon that may bestow an advantageous trait, and their existence may also play a role.

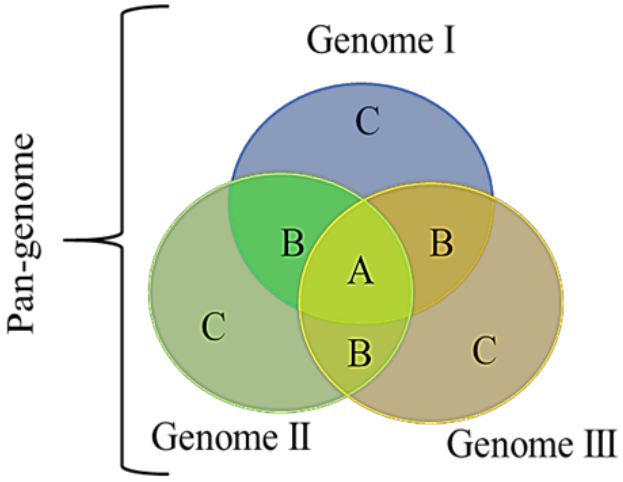

FIGURE 2.3: A Venn diagram that represents the three types of genes present in the pan-genome: A –The genes set that is incorporated in essential cellular processes of all analyzed bacteria species; B –The genes set which is concerned with specific functions but is present only in some of the analyzed species; C –The genes that can be unique for the certain species [74]

2.9.1 Pan-genome Types

The next step in characterizing the pan-genome is to ascertain the ratio of core genes to other genes, once the number of genes has been identified and assigned to distinct subtypes. Additionally, the quantity of fresh unique when more genomes are added to the pool under analysis, genes are noticed. The two types of pangenomes—open and closed—are determined by the outcomes of the second analysis. An open

pan-genome occurs when the number of unique genes in the pool under analysis is increased by adding another genome. The pan-genome, on the other hand, is referred to as closed when the number of genomes added does not enhance the pool of unique genes [74].

Rarefaction curve construction is a straightforward method for determining if the pan-genome is closed or open. When additional sampling would not result in a greater number of newly found species, ecologists typically utilize this information to graphically illustrate the situation. Gene counts are performed using comparable methods when more genomic sequences are included in the analysis. An overall pan-genome gene count versus the total number of genomes examined is used to illustrate the findings. We refer to a pan-genome as closed if the curve hits a plateau. A defining feature of open pan-genomes is the steady increase in the number of genes with each additional genome. Determining the openness of a pan-genome analysis can also be done by using Heap's law. The Heap Law quantity of unique words relative to the length of the document. The formula n = k × N–$\alpha\alpha$ is used to represent it [74].

According to a pan-genome study: For a given number of genomes, n is the predicted number of genes; k and α are free parameters that are experimentally determined; and N is the number of genomes. The pan-genome is regarded as closed when α is greater than 1, and as open when α is less than 1, according to Heap's law [74].

2.10 Emerging Therapeutic Strategies

K. pneumoniae antibiotic resistance is rising, it's critical to understand the risk factors that go along with it, take preventative action, and investigate other treatment options to deal with these serious illnesses. This section will examine four treatments that show promise in either treating or preventing *K. pneumoniae* infections: medication, immunological therapy, biological treatment, and immunization [75].

2.10.1 Bacteriophage Therapy

The scientific community is beginning to pay more attention to bacteriophage therapy and consider the best therapy for bacterial health problems that are cause by *K. pneumoniae*, as an alternative to conventional antibiotics. To tackle pathogenic *K. pneumoniae* strains, this novel strategy selectively eliminates dangerous bacterial cells using bacteriophages, viruses that particularly target bacteria [76].

2.10.2 Photodynamic Treatment

Infections brought on by *K. pneumoniae* that produce β-lactamase (ESBL) can now be treated with photodynamic treatment (PDT). In photodynamic therapy (PDT), photosensitizers (PS) are exposed to visible light at the proper wavelength. There are some reactive species like oxygen species (ROS) are produced when photosensitizer comes in active form. Then these ROS become the cause of bacterial death , hence resistance to PDT is unlikely to develop [76].

2.10.3 Phage Therapy

Because of the widespread usage of antibiotics in healthcare systems, *K. pneumoniae* has developed a strong resistance to many of them, severe pathogenicity, and the capacity to build biofilms. These strains are particularly hospital-acquired. Alternative therapies are desperately needed since this restricts the number of effective medicines that may be used for people with *K. pneumoniae* infections. Prokaryotic viruses called phages, or bacteriophages, can replicate within a bacterial host before lysing (lytic phages) the host. The treatment of multidrug-resistant bacterial infections, such as *K. pneumoniae*, has shown considerable promise with phage therapy [76].

2.11 Reverse Vaccinology

In terms of saving lives and enhancing health, vaccinations are also the most economical option. New technologies like chemical conjugation and recombinant DNA have revolutionized the vaccination sector during the last three decades. Reverse vaccination, structural biology, and systems biology are some of the more contemporary techniques used in the search for novel antigens and in the study of vaccine reactions. The functional blind selection of potential vaccines has been made possible by genome-based technologies, which have also prompted the identification of new pathogen virulence factors in addition to the discovery of novel protection antigens. Therefore, the pathogenesis-to-vaccine paradigm has been turned on its head in several instances, and the creation of vaccines frequently results in a better knowledge of pathogenesis, which has led to unique approaches in studying not only the organism itself but also the plan for the designing more successful vaccines [77].

The previously used realistic approaches of vaccination involve inactivation of the viruses, attenuation of their functions, and blocking of their functions with our help like capsular polysaccharides, toxins, and other cell surface proteins. This has been done in consultation with the knowledge on the processes that the microbial pathogens undertake to cause disease. However, with the emergence of newer technological platforms and vaccines, the emphasis is on true blind identification of the antigens via parallel functional assays of the pathogen's proteins and genes. Through the use of these techniques, several interesting vaccine candidates have been discovered to be connected to pathogenic processes such host colonization or serum resistance [77].

2.12 Way Ahead to Vaccinology

The discovery of several potential vaccine candidates has been made possible by the application of reverse vaccinology. The following step is to go through tens of thousands of new antigens that have been discovered and to select the best

candidate antigens to go to the next phase of development for clinical trials. The identification of the best candidates will be mainly based on using methods such as systems biology to improve immunogenicity pattern and correlatives of protection and structural vaccinology to design right antigens [78].

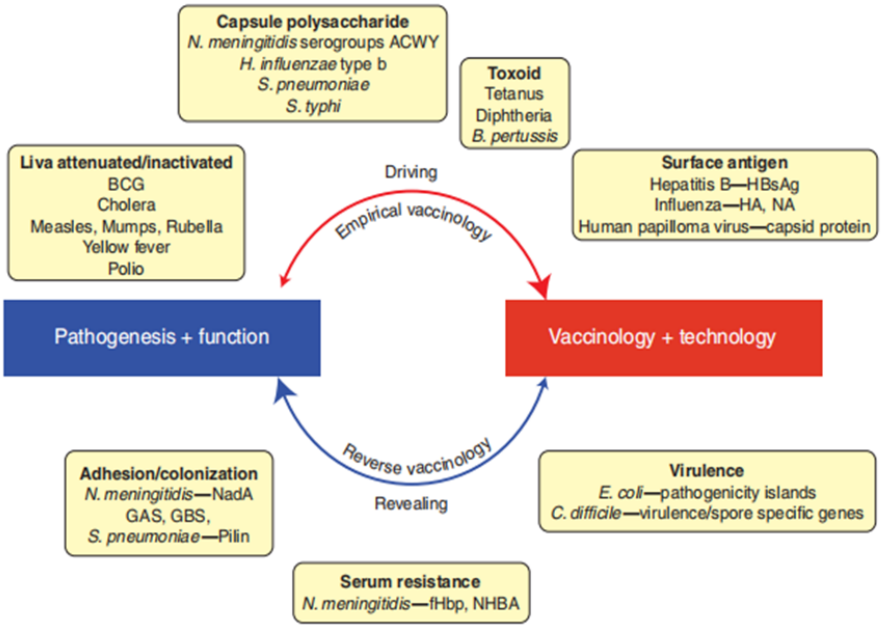

FIGURE 2.4: Core aspects of microbial pathogenesis and vaccine development along with their interaction. [77]

2.13 Structural Vaccinology

Even though bacterial genome sequencing has produced several promising targets for potential vaccinations, the sequence variability of antigens frequently poses a significant obstacle to their usage and development as widely protective vaccine component. The resolution of an antigen can be attained from structural biological studies and fixing immunodominant epitopes of a single molecule and other epitopes of others different forms of the same antigen into a logically planned single molecular champion makes the concept of structure-based design and the broad immune response against different variants of proteins. Similar to the forming of a team that is made up of three fighters; one can integrate all immunogenic [78].

2.14 System Vaccinology

An interdisciplinary field known as "systems biology" aims to anticipate a biological system's behavior by methodically examining the intricate relationships and structure among its many components. Since its inception, vaccinology is moving towards a systems-based approach with two mains.

First, it is the mechanistic aspect to understand how, in fact, vaccines elicit protective immunity; second, to identify biomarkers of immunogenicity or vaccine efficacy [79].

The areas of focus of the development of successive generation of vaccines involves gain of basic principles of the pathogen and immunology which is progressing due to the scientific progresses in high-throughput (HTP). DNA sequencing and screening technologies, which are enabling researchers to obtain more information about the genetic diversities of both the pathogen and the human host. correspondingly, to the reaction. It becomes possible to identify the pathogen's protective antigens and the host immune response signatures that confers protection with its help. These two concurrent fields of vaccination and the research along with the technological improvements in the formulation and delivery methods are essential for the development of next-generation vaccines [79].

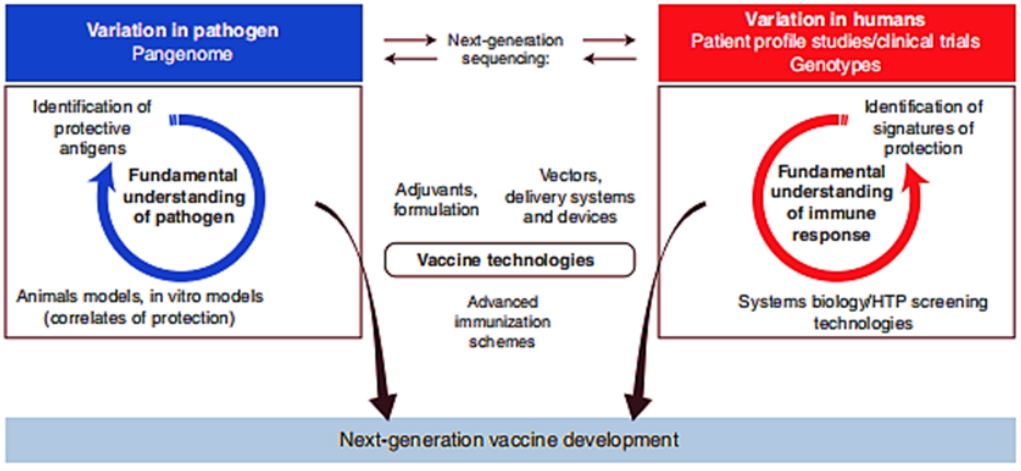

FIGURE 2.5: Key Areas of next generation vaccine development [79]

Chapter 3

Material and Method

3.1 *K.pneumoniae* Genome Retrieval

The initial stage of the process involved gathering genomic data for study. This step is divided into three parts: choosing strains based on available literature, retrieving bacterial sequences from databases, and choosing a reference genome for additional investigation .

3.1.1 Sequence Retrieval

A total of 154 complete genome including draft genome sequences of *K. pneumoniae* were retrieved from PATRIC database (www.bv-brc.org), yet the majority of them are either incomplete or only exist as scaffolds. Another characteristic that was taken into account was the ambiguity of the source of isolation; strains with a full genome but an unclear source of isolation was not taken into consideration. Total 126 *K. pneumoniae* complete global genome and 28 genome from Pakistan were taken for analysis. All the genomes were downloaded from Batch Entrz www.ncbi.nlm.nih.gov.

3.1.2 Quality Assessment

K. pneumoniae strains was then subjected for CheckM analysis for further evaluation and the genomes found low quality sequences were discarded from the dataset.

3.2 Structural Annotation of Genome

Then, the compiled genomes were subjected to structural annotation with the use of Prokka pipeline set to default parameters. Prokka is bioinformatics software used effectively for accurate and fast annotation of bacterial genomes through employing gene prediction, identification of coding regions and functionally annotating to the genomics parts. Often considered as a vital tool for the analysis of microbial genomes, the automation feature of Prokka adds to the tool's great effectiveness. This annotation process generated a Protein FASTA file, a Nucleotide FASTA file as well as an Annotation file that is in GFF3 format. Later on, through the aid of the Rapid Annotation employing Subsystem Technology (RAST) server, functional annotation was done. RAST is a bioinformatics program that gives a broad and simplistic view of the annotation and analysis of the genomes. It is also used in the process of annotating microbial genomes; thus, helping researchers to identify genes, functional parts, and pathways that are hidden in the DNA. Also, the automated and curated functions of RAST enhance the expedition of the understanding of microbial genes and biological systems. The format of the output files created during the process are the GenBank and EMBL formats.

3.3 Pangenome Analysis

The pangenome, which consists of the core and dispensable genomes together, represents the whole gene repertoire of a particular species. The common genes in the core genome of a certain species are essential for the bacterial growth. A strain's unique characteristics, like as pathogenicity, stress tolerance, and stimulus sensitivity, may be controlled by its disposable genome, which is not shared by

all genomes. The Pathosystems Resource Integration Centre (PATRIC), a comprehensive bioinformatics resource for downloading genomic data, provided 126 complete global genome sequences of *K. pneumoniae* and 28 genomes from Pakistan used for the pangenome analysis. Using the Roary version 3.13.0 used in the backend of Pangenome module (PGM) of PanRV pipeline, the pangenome analysis was performed on 126 strains with primarily global and local genomes. Pangenome analysis is done by Roary, who can read GFF3 format. Roary produces three output formats that are easily reusable and has a single graphical output for simple visualisations. Output file options and identity matrix were set to 90% for the desired outputs, but the rest of the parameters were left default.

3.4 Vaccine Candidates and Epitope Prediction

The Core proteins obtained from the PGM module of PanRV were passed through the RV module of the current version of PanRV software. Several filters were used which are listed below:

3.4.1 Subcellular Localization

Regarding membrane location of the potential vaccines, it is stated in the previous studies that the potential vaccine candidates are located in the membrane of cytoplasm, periplasm, the outer membrane and in the extracellular spaces; therefore, the proteins eluted during the first step of the method were screened using version 2. All these regions were defined with high confidence using PSORTb v 0 of PanRV. Selected proteins included proteins that are part of outer membrane and those found in the extracellular regions in addition to proteins whose location was unknown and were further filtered by version 3. of PSORTb 2.

3.4.2 Host Homology

For minimizing the risk of autoimmune diseases, three steps are taken with the core proteome: 1) human homology as well as 2) gut homology investigation. To filter out the minimum level of similarity based on Swiss-Prot BLAST and RefSeq, PanRV pipeline extends itself to find the human homologs. For the human gut flora safety, the exclusion criteria used was: % identity > (35%) Bit score > 100 and the E value <1.

3.4.3 Trans-Membrane Helices

The core proteins are further purified using the version 2. 0 of HMMTOP (Tusnády and Simon, 2001) incorporated in the PanRV. Proteins with less than 2 helices were used for prediction of the vaccine candidates.

3.4.4 Essentiality and Virulence Assessment

The important *K. pneumoniae* survival genes were identified through the BLASTp analysis of selected proteins with Database of Essential Genes (DEG) present in PanRV. This is an approach which can be used to prevent microbial functions and therefore, appropriate therapeutic intervention. It can be noted that vaccines can help target the virulence factors that are involved in the pathogenesis of the bacteria. To identify these factors, BLASTp of the selected proteins was done against the Database of Virulence Factor (VFDB) and the Database of microbial virulence factors (MvirDB).

3.4.5 Molecular Weight Determination

The putative antigens with molecular weight less than 110 kDa are chosen for vaccine construction with the aid of built-in JAVA program in PanRV, which considers molecular weight calculated based on amino acid sequences of proteins and also verified by UniProt.

3.4.6 Similarity to Gut Flora Proteins

To stop the induced immune response from harming the host, proteins that differ markedly from the gut flora of the host will be choose. SmartBLAST was utilized to conduct a homology search of the gut flora for essential proteins. Any candidate antigen that exhibited more than 70% homology to a gut flora protein will eliminate.

3.4.7 Functional Annotation

These selected proteins are then analyzed using BLASTp tool for annotation of proteins. These produced accurate results at the top with 100% similarity, 100% query coverage, and a zero or negative E-value, which was retained.

3.5 Subtractive Epitope Scrutinization

3.5.1 In Silico Prediction of B and T Cells Epitopes

The selected proteins were subjected to Immune Epitope Database (IEDB) B Cell Epitope Prediction Tools (tools.iedb.org/main/bcell) for predicting the epitopes of B cells on the basis of artificial neural networks. While IEDB T Cell Epitope Prediction Tools (tools.iedb.org/main/tcell) were used to predict the T cells of both kinds; MHC1 refer to CD8+ while MHC II for CD4+ respectively. The T cells derived from B cells were selected for further analysis.

3.5.2 Protein Antigenicity

The T cell epitopes were then subjected to AntigenPro for prediction of antigenicity based on features of amino acid sequences, and VaxiJen v.2.0 predicts the ability of an antigen to induce an immunological response when it is encountered by human body on the basis of amino acid sequences. These two tools were

used to predict antigenicity. Candidate antigens are defined as those that have a prediction score greater than 0.5 for both techniques.

3.5.3 Predicting Allergenicity of T Cell Epitopes

The T cell epitopes were used to predict allergenicity by using AllerCatPro. Its functionality is based on sequence alignment, domain mapping, and the machine learning models based on allergen and non-allergen datasets with the label 'predicted to be allergenic/not predicted to be' and respective prediction scores.

3.5.4 Prediction of Toxicity

T cell epitopes were then further analyzed for toxicity in ToxinPred. ToxinPred offers quantitative toxicity predictions together with qualitative annotations of the toxic regions.

3.6 Prediction and Visualization of Epitope Structure

PEP FOLD3 web portal was used to MODEL the 3D structure of the epitopes which models, predicts and analyzes protein structures. The selected proteins were then computationally mapped to give the required epitopes which were then visualized by UCSF Chimera 1.1. Below is the complete flow chart methodology Figure 3.1

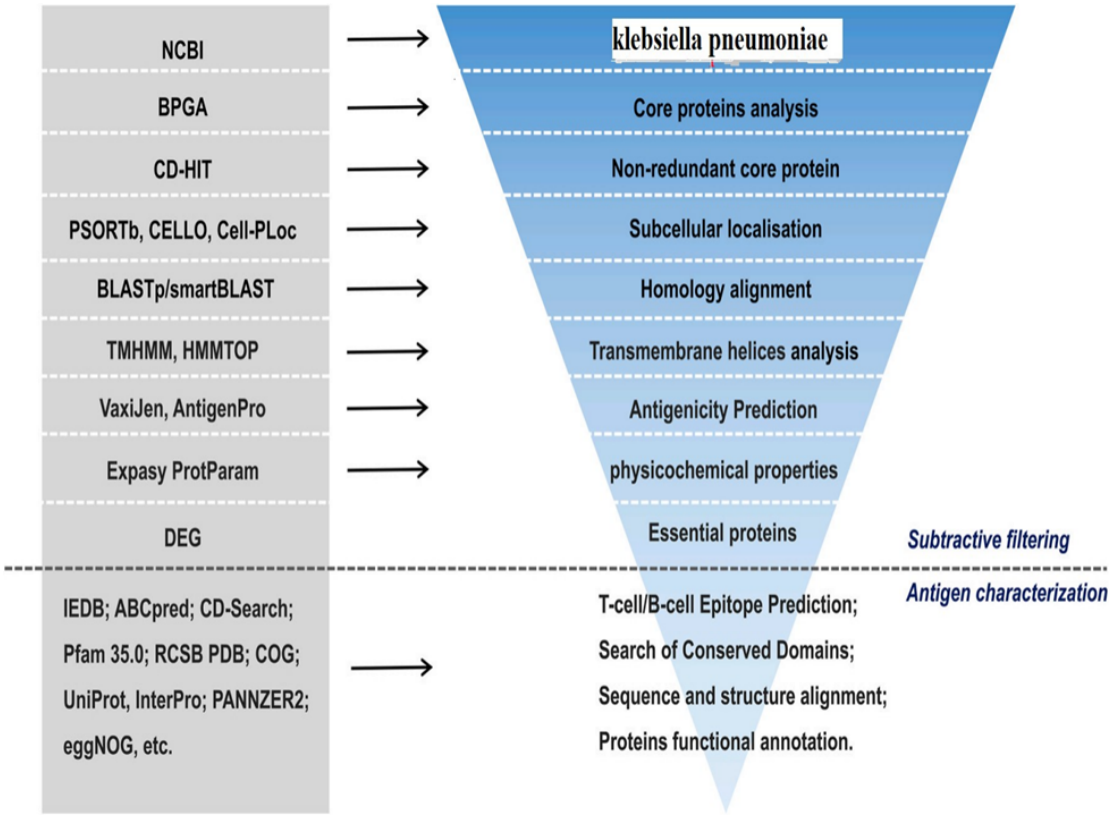

FIGURE 3.1: Overview of Methodology

Chapter 4

Results

4.1 Sequence Retrieval

From NCBI totally, 126 completely sequenced and 28 draft genomes of *K. pneumoniae* were searched on 27 MAY 2024 with an average genome size of about 5.5 Mb. Based on the obtained results, it can be estimated that the GC content in the pathogenic strains retrieved amounted to roughly 50%.

4.2 Pangenome Analysis

PanRV identified total 15,319 genes in which 3864 genes are core genes which are conserved with 99-100% strains, 310 genes are soft core genes which are conserved with 95-99% strains, 1995 genes are cloud or unique genes which are present only in single strain and 9150 are shell or accessory genes which are present in two or more strains. These statistics are depicted in below Figure 4.1, Figure 4.2.

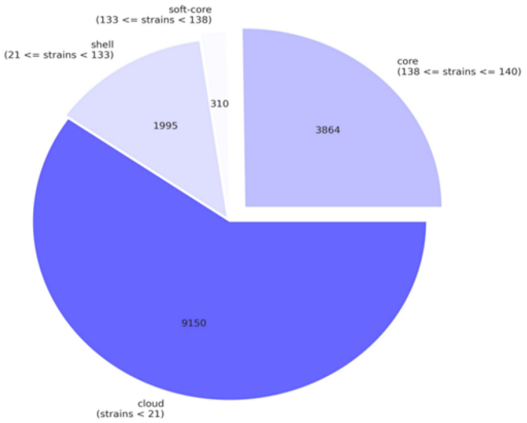

FIGURE 4.1: Global strain Pie chart of gene clusters in *K. pneumoniae* pangenome shows Core genes, soft core genes and Accessory genes

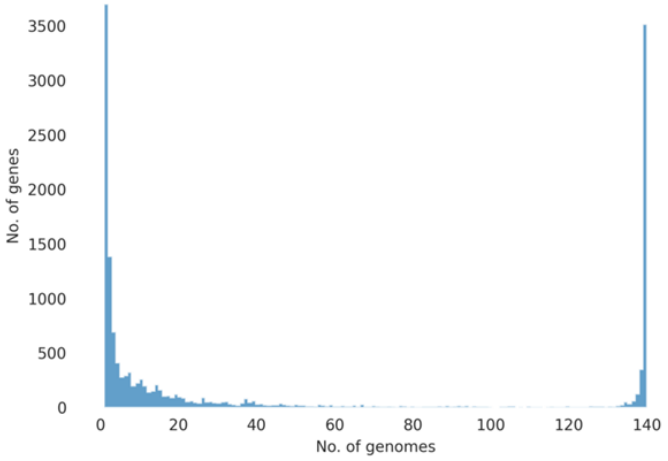

FIGURE 4.2: Globally based frequency graph of genome

The pangenome analysis of 154 Klebsiella pneumoniae strains, which revealed an open pangenome, no. of pangenome and no. of unique genes with respect to the increasing genome number shown in Figure 4.3, Figure 4.4, Figure 4.5. The analysis using Roary generated a pangenome matrix, with core proteins indicated in solid blue, and unique genes visualized along side a phylogenetic tree shown in Figure 4.6. Additionally, Roary produced a frequency graph and an open pangenome graph.

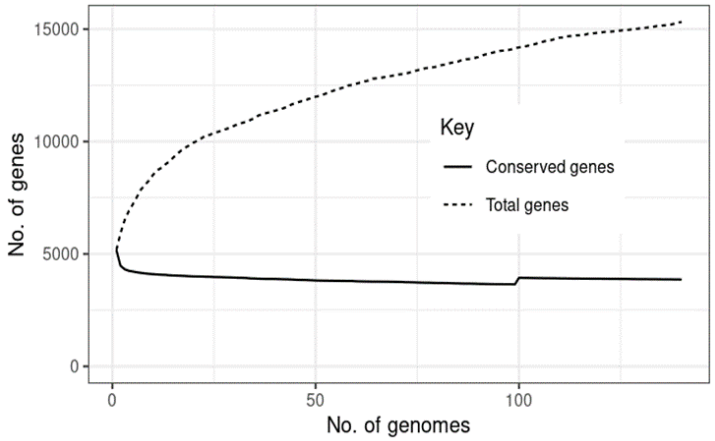

FIGURE 4.3: Graph shows increase in number of Total with increase in number of *K. pneumoniae* Genomes and conserved genes showing to be reduced which indicates open pan-genome

This analysis shows increase in number of total with increase in number of *K. pneumoniae* genomes but conserved genes shows to be reduce which indicates that this open pan-genome Which help to discover what effects genetic variants have, and to develop treatments for conditions linked to those variants shown in figure 4.3.

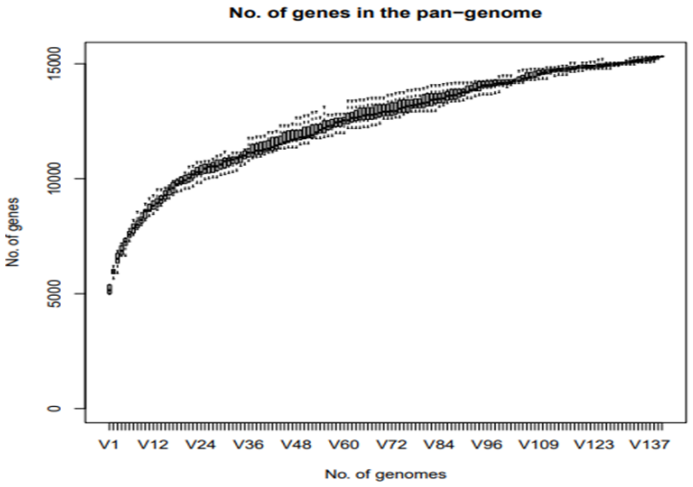

FIGURE 4.4: Graph shows increase in number of pangenes/ total genes with increase inumber of *K. pneumoniae* Genomes which indicates diversity and adoptability of isolates.

The pan genome represent the whole set of genes within a species consisting of a core genome containing sequences shared between all individual of the species and dispensable genome and increase in pangens depends on the number of genomes which shows the diversity increase shown in figure 4.4.

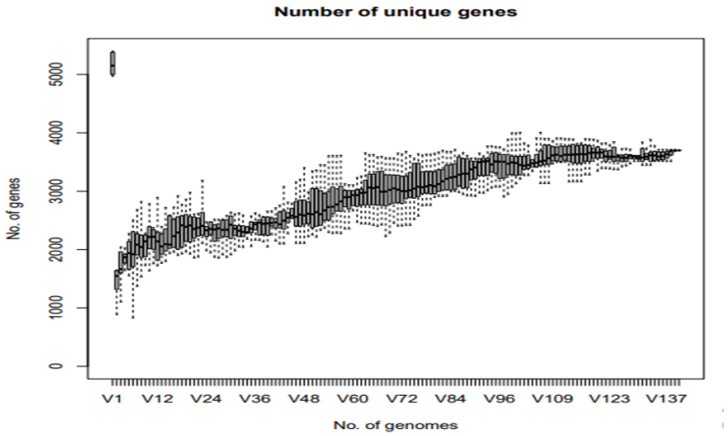

FIGURE 4.5: Graph depicting the concept of how the number of unique genes tends to rise with the rising number of genomes, whereas the number of newly identified genes shoots up at a sudden point and then remains steady as the number of genomes rises further.

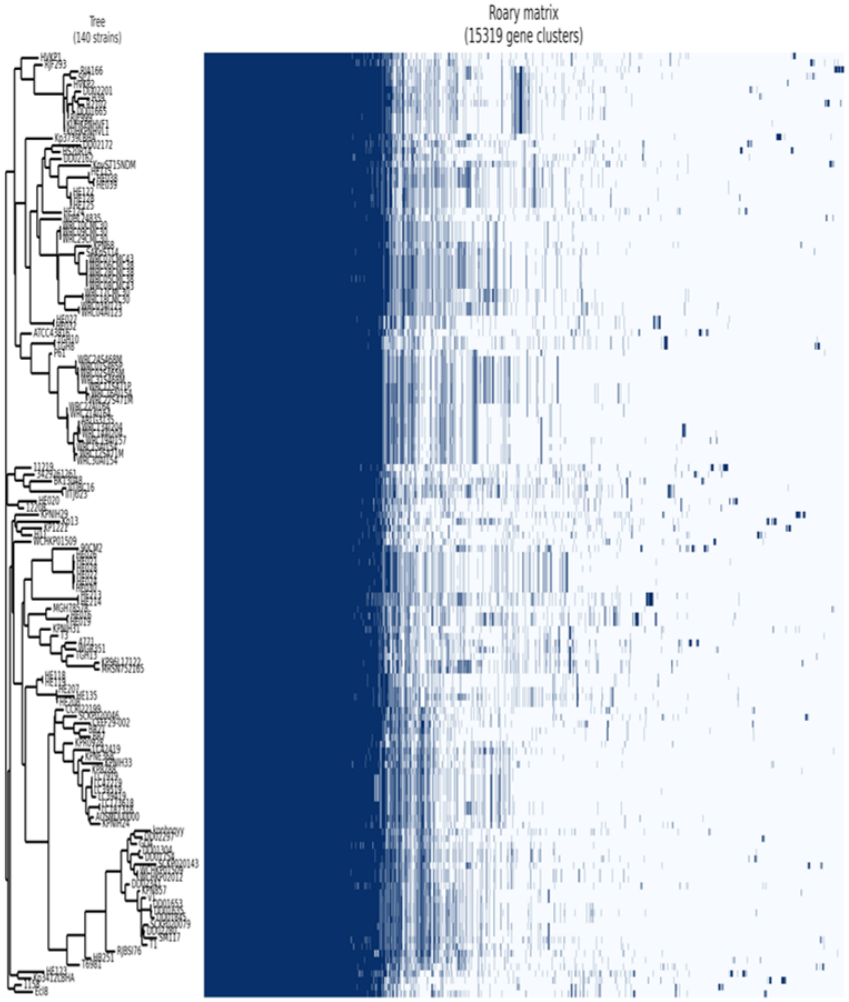

FIGURE 4.6: Pan genome tree compared to the matrix with presence/absence of core and accessory genes among *K. pneumoniae*

4.3 Prioritizing Core Vaccine Targets

Total 126 complete genomes and 28 genomes from draft genomes calculated by pipeline PanRV was used for prioritizing vaccine candidates. Of these *K. pneumoniae* strains, 3864 core proteins were obtained.

The above-listed set of protein was subjected to RV module of PanRV for prediction of the vaccine candidates. The first aspect is to list all homologous proteins that can be obtained from non-human organisms and to ensure that proteins homolog to the host organism are not included. Out of the compared proteins, 3864 were found to be non-host homologous. Then sub-cellular localization prediction is being performed and the result obtained indicate that there are 86 proteins of outer membrane and extra-cellular. Incorporation of DEG in PanRV platform predicted 1699 core proteins. The virulent proteins screening was also conducted because identifying the virulent factors is crucial in the development of vaccines and 887 virulent proteins were identified. From the proteomics data it was determined that 2994 proteins were expressing less than 2 trans membrane helices. Most vaccine candidates must have less than 2 transmembrane helices because if it's more that would complicate protein expression, colonization and purification of recombinant protein. From the above sequence of filters, the RV module jointly identified a total of 8 potential vaccine candidates. All the results are shown in Table 4.1.

4.4 Prediction of Biological Processes and Molecular Functions Associated with Vaccine Candidates

These predictions were performed by using online web-based stand-alone tool CELLO2GO. Through the COG (Clusters of Orthologous Groups) database incorporated in PanRV, seven probable vaccines candidates were selected based on their molecular functions. These include FimD/PapC or TolC and the general

TABLE 4.1: Filtered potential vaccine candidates

No.	Protein Name	Human Non Homolgy	Gut Non Homology	Localization	Essential	Virulent	Helices	Mol-Weight (Da)	Annotated
1	oprM	√	√	√	√	√	√	√	√
		0	0	Outer Membrane	1	*70*	IN:0	48216.4651	92.16%
2	ompC	√	√	√	√	√	√	√	√
		0	0	Outer Membrane	2	*40*	IN:1	40634.8868	92.16%
3	*ompN*	√	√	√	√	√	√	√	√
		0	0	Outer Membrane	2	40	IN:1	4242.16289	79.17%
4	yedS	√	√	√	√	√	√	√	√
		0	0	Outer Membrane	2	40	IN:1	39509.614	66.67%
5	oprM	√	√	√	√	√	√	√	√
		0	0	Outer Membrane	1	2	OUT:0	49964.4824	34.76%
6	phoE	√	√	√	√	√	√	√	√
		0	0	Outer Membrane	2	40	OUT:0	38723.481	98.86%
7	cusC	√	√	√	√	√	√	√	√
		0	0	Outer Membrane	1	71	IN:1	50608.416	75.98%
8	fimD	√	√	√	√	√	√	√	√
		0	0	Outer Membrane	3	193	OIT:0	43777.5436	46.00%

porin proteins. FimD/PapC is an usher protein located at the outer membrane of bacteria and participates in pilus assembly and bacterial adhesion to host tissues; it is classified under COG N, known as Cell motility and secretion. TolC is involved in drug efflux and toxin export; it falls under the COG category V which is Defense mechanisms. General porins that allow the passive diffusion of small molecules across the membrane are found to be a part of the COG category M, which deals with cell envelope biogenesis – outer membrane. The implication of these proteins in adhesion, efflux, toxin export, and molecular movement make sows them to be promising targets for vaccine. All the biological processes of protein with their molecular function listed in below Table 4.2.

TABLE 4.2: Potential vaccine candidates with their biological & molecular functions, location and COG category.

No	**COG Category**	**COG Class**	**Location**	**Protein Name**	**Molecular Function**
1	COG3188	N	Outer membrane protein	FimD/PapC	Outer membrane usher protein involved in pilus assembly and bacterial adhesion
2	COG1538	*M*	Outer membrane protein	TolC	Outer membrane protein involved in drug efflux and toxin export
3	COG3203	*M*	Outer membrane protein	(porin)	Outer membrane proteins that facilitate the passive diffusion of small molecules
4	COG3203	*M*	Outer membrane protein	(porin)	Outer membrane proteins that facilitate the passive diffusion of small molecules
5	COG1538	*M*	Outer membrane protein	TolC	Outer membrane protein involved in drug efflux and toxin export
6	COG3203	*M*	Outer membrane protein	(porin)	Outer membrane proteins that facilitate the passive diffusion of small molecules
7	COG3203	*M*	Outer membrane protein	(porin)	Outer membrane proteins that facilitate the passive diffusion of small molecules

Table 4.2 continued from previous page

No	COG Category	COG Class	Location	Protein Name	Molecular Function
8	*COG1538*	M	Outer membrane protein	TolC	Outer membrane protein involved in drug efflux and toxin export

4.5 B-Cells and Their Derived T-Cells Epitopes

An epitope also known as antigenic determinant is the part of an antigen that is identify by immune system.Hence the IEDB analysis generated total 4 epitopes of B-cell and 38 epitopes of the derived MHC I which were list down in the below table and further was prioritized for vaccine candidates.

Table 4.3: B cell epitopes with predicted MHC class I epitopes.

No.	B CELL	MHC1	Alleles	Start	End	Length
1	KEMLPDSLHGFA	EMLPDSLHG	27	2	10	9
		EMLPDSLHGF	27	*2*	11	10
		KEMLPDSLH	27	*1*	9	9
		KEMLPDSLHG	27	*1*	10	10
		LPDSLHGFA	27	*4*	12	9
		MLPDSLHGF	27	*3*	11	9
		MLPDSLHGFA	27	*3*	12	10
2	*FEINDLYATGSA*	EINDLYATG	27	2	10	9
		EINDLYATGS	27	2	11	10
		FEINDLYAT	27	1	9	9
		FEINDLYATG	27	1	10	10
		INDLYATGS	27	3	11	9
		INDLYATGSA	27	3	12	10
		NDLYATGSA	27	4	12	9
3	LAILQREGQLD	AILQREGQL	27	2	10	9
		AILQREGQLD	27	2	11	10
		ILQREGQLD	27	3	11	9
		LAILQREGQ	27	1	9	9
		LAILQREGQL	27	1	10	10

Table 4.3 continued from previous page

No.	B CELL	MHC1	Alleles	Start	End	Length
4	QDFVDNSSTQRD CCTQSGRTKGR	CCTQSGRTK	27	13	21	9
		CCTQSGRTKG	27	13	22	10
		CTQSGRTKG	27	14	22	9
		CTQSGRTKGR	27	14	23	10
		DCCTQSGRT	27	12	20	9
		DCCTQSGRTK	27	12	21	10
		DFVDNSSTQ	27	2	10	9
		DFVDNSSTQR	27	2	11	10
		DNSSTQRDC	27	5	13	9
		DNSSTQRDCC	27	5	14	10
		FVDNSSTQR	27	3	11	9
		FVDNSSTQRD	27	3	12	10
		NSSTQRDCC	27	6	14	9
		NSSTQRDCCT	27	6	15	10
		QDFVDNSST	27	1	9	9
		QDFVDNSSTQ	27	1	10	10
		QRDCCTQSG	27	10	18	9
		QRDCCTQSGR	27	10	19	10
		RDCCTQSGR	27	11	19	9
		RDCCTQSGRT	27	11	20	10
		SSTQRDCCT	27	7	15	9
		STQRDCCTQ	27	8	16	9
		STQRDCCTQS	27	8	17	10
		TQRDCCTQS	27	9	17	9
		TQRDCCTQSG	27	9	18	10
		TQSGRTKGR	27	15	23	9
		VDNSSTQRD	27	4	12	9
		VDNSSTQRDC	27	3	11	9

Thus, total 18 epitopes were predicted as MHC II were obtained from IEDB which are mentioned in the below table and they were further passed through the process of prioritization of vaccine candidates.

TABLE 4.4: B cell epitopes with predicted MHC class II epitopes

No.	**B CELL**	**MHC II**	**Alleles**	**Start**	**End**	**Length**
1	KEMLPDSLHGFA	EMLPDSLHGFA	27	2	12	11
		KEMLPDSLHGF	27	*1*	11	*11*
2	FEINDLYATGSA	*EINDLYATGSA*	27	*2*	12	11
		FEINDLYATGS	27	*1*	11	*11*
3	*LAILQREGQLD*	*LAILQREGQLD*	*27*	*1*	*11*	*11*
4	QDFVDNSSTQRDCC TQSGRTKGR	*CCTQSGRTKGR*	27	*13*	23	11
		DCCTQSGRTKG	27	*12*	22	*11*
		DFVDNSSTQRD	*27*	2	12	11
		DNSSTQRDCCT	27	5	15	11
		FVDNSSTQRDC	27	3	13	11
		NSSTQRDCCTQ	27	6	16	11
		QDFVDNSSTQR	27	1	11	11
		QRDCCTQSGRT	27	10	20	11
		RDCCTQSGRTK	27	11	21	11
		SSTQRDCCTQS	27	7	17	11
		STQRDCCTQSG	27	8	18	11
		TQRDCCTQSGR	27	9	19	11
		VDNSSTQRDCC	27	4	14	11

4.6 Epitope Prioritization

Following the prediction of allergenicity, antigenicity, and toxicity, 33 MHC class I candidates and 14 MHC class II candidates were identified as antigenic, non-allergenic, and non-toxic. The details of these candidates are provided in the table below, and their 3D structures are shown in the accompanying figure.

TABLE 4.5: MHC I and MHC II epitopes predicted for antigenicity, allergenicity and toxicity.

NO	MHC I	ANTIGENCITY	ALLEREGENCITY	TOXICITY
1	EMLPDSLHG	Probable ANTIGEN .	NON ALLERGEN	NON TOXIC
2	EINDLYATG	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
3	EINDLYATGS	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
4	FEINDLYAT	Probable ANTIGEN	NON ALLERGEN	NON TOXIC

Table 4.5 continued from previous page

NO	MHC I	ANTIGENCITY	ALLEREGENCITY	TOXICITY
1	EMLPDSLHG	Probable ANTIGEN .	NON ALLERGEN	NON TOXIC
2	EINDLYATG	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
3	EINDLYATGS	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
4	FEINDLYAT	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
5	FEINDLYATG	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
6	INDLYATGS	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
7	INDLYATGSA	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
8	CCTQSGRTK	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
9	CCTQSGRTKG	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
10	CTQSGRTKG	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
11	CTQSGRTKGR	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
12	DCCTQSGRT	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
13	DCCTQSGRTK	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
14	DFVDNSSTQ	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
15	DFVDNSSTQR	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
16	DNSSTQRDC	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
17	DNSSTQRDCC	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
18	FVDNSSTQR	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
19	FVDNSSTQRD	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
20	NSSTQRDCC	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
21	QDFVDNSSTQ	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
22	QRDCCTQSG	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
23	QRDCCTQSGR	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
24	RDCCTQSGR	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
25	RDCCTQSGRT	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
26	SSTQRDCCT	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
27	STQRDCCTQ	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
28	STQRDCCTQS	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
29	TQRDCCTQS	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
30	TQRDCCTQSG	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
31	TQSGRTKGR	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
32	VDNSSTQRD	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
33	VDNSSTQRDC	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
34	VDNSSTQRD	Probable ANTIGEN	NON ALLERGEN	NON TOXIC
35	VDNSSTQRDC	Probable ANTIGEN	NON ALLERGEN	NON TOXIC

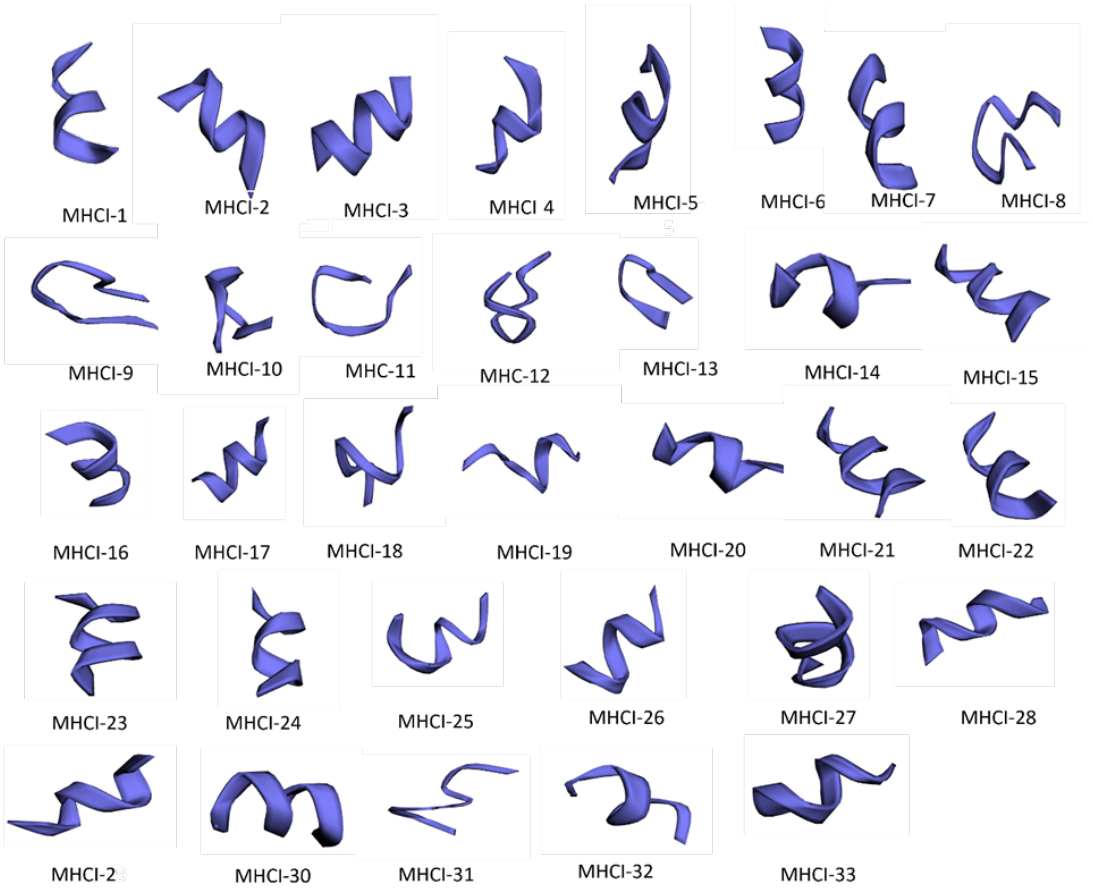

FIGURE 4.7: 3D modeled structures for MHC I

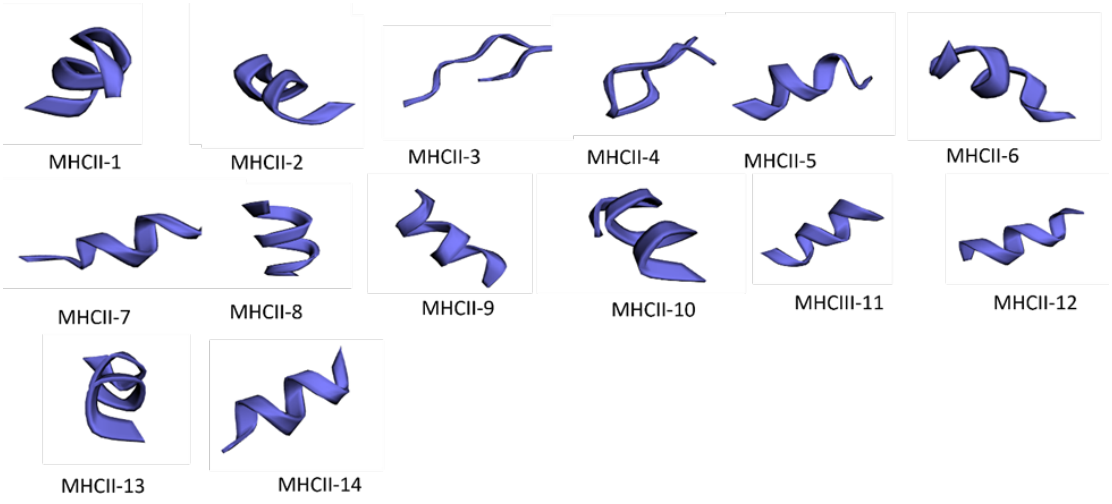

FIGURE 4.8: 3D modeled structures for MHC II

The MHC class I present antigen from intracellular proteins and expressed on surface of almost all nucleated cells.These are recognized by CD8+ T cytotoxin T cells which destroy cells presenting the antigen shown in figure 4.7.

The MHCII present antigen from Extracellular proteins and these are expressed on APCs , dendritic cells , macrophages and B cells which also have MHC II .These are recognized by CD4+ ,T Helper cells,which activate other cells to fight the infection shown in figure 4.8.

Chapter 5

Discussion

In the generation of a vaccine against *K. pneumoniae* infection, attempts have been made and are still underway. Among the different cell constituents, two surface components are mainly being discussed as candidates for an anti-*Klebsiella* vaccine: The two parties involved are the Local Public Sector (LPS) and the Central Public Sector (CPS). On the other hand, serious side effects characteristic of LPS containing vaccines remain a significant issue in developed vaccines. The CPS based vaccines which are nontoxic as well as immunogenic, however the antigenic types increase with the number of K-types (77 different antigens) prove to be rather challenging A 24 valent CPS vaccine has been started, but further studies must be conducted in human beings . By creating completely new therapeutics in *K. pneumoniae*, conserved protein vaccines can be formulated. According to persevered protein vaccines, it is possible to develop associated medicines against *K. pneumoniae* used proteome-level analysis to evaluate if *K. pneumoniae* was immunogenic. The identification and development of vaccines using the conventional or traditional method is not easy and it includes growth of bacteria, identification of pathogen specific immunogen, inactivation of the same, immunization of relevant host to prove that it induces the required immune response: all these make the whole process time consuming and very expensive and at the same has a very low success rate. Genome sciences, whole genomic sequencing, systems biology, Genomics, Proteomics has been enriched the knowledge on pathogenesis and in silico vaccine design. Lack of evidential and safe antibiotics has made vaccination

the only remaining efficient tool to subdue MDR pathogens; despite this, there is no licensed commercial vaccine available for *K. pneumoniae* to this date. Moreover, all the candidate *K. pneumoniae* vaccines indicated to have some drawbacks by being instable or having a short protective period or being reactogenic. From this evidence, it can be inferred that there is a need to look for new targets of the vaccine in *K. pneumoniae* genomes that could provide desired long-lasting immunity. For this purpose, the pangenome analysis along with the RV approach was used in the present study to recognize possible vaccines in *K. pneumonia*.

Chapter 6

Conclusion and Recommendation

Global public health is seriously threatened by *K. pneumoniae* infections. Previously, *K. pneumoniae* was mostly thought to be an opportunistic pathogen; yet, this bacteria is now ranked among the top pathogens because to the sharp rise in community-acquired Klebsiella infections, including strains that are resistant to drugs. As a result, several nations now prioritize developing vaccinations against *K. pneumoniae*. As demonstrated earlier for a number of diseases, vaccination is a successful means of preventing disease, particularly when taking into account individuals who are more vulnerable the elderly, children, and those with impaired immune systems. Numerous strategies have been investigated to stop *K. pneumoniae* infections, such as ribosome vaccinations, whole cells and cell extracts, and virulence factors such proteins, LPS, OMVs, and capsular polysaccharides all these formulations have been successful and progressed to clinical trials.

Although there are limitations to all vaccination techniques, such as the higher reactogenicity of whole cell vaccines or the poorer immunogenicity of subunit formulations, there is growing evidence that *K. pneumoniae* vaccines can, in the upcoming decades, come to pass. In particular, conjugate vaccines, which include O antigens linked to protein carriers and/or capsular polysaccharides, offer a promising substitute. Developing effective vaccines with broad coverage requires careful identification of the most clinically relevant capsular and O serotypes.

Here it was an analytical methodology that combines an RV method with pangenome analysis. By using selection criteria to identify reasonable vaccine targets, this technique drastically cuts down on the amount of time needed to produce a vaccine. This technique allowed us to identify 47 antigenic epitopes and a manageable list of 8 possible vaccine candidates. These are the proteins.significantly linked to the virulence and survival of bacteria. Testing these proteins' immunogenicity and degree of protection will require additional experimental confirmation.

Bibliography

[1] C. N. Jondle, K. Gupta, B. B. Mishra, and J. Sharma, "*Klebsiella pneumoniae* infection of murine neutrophils impairs their efferocytic clearance by modulating cell death machinery," Plos pathogens publication , vol. 14, no. 10, p. , 2018.

[2] S. Aghamohammad, F. Badmasti, H. Solgi, Z. Aminzadeh, Z. Khodabandelo, and F. Shahcheraghi, "First report of extended-spectrum betalactamase-producing *Klebsiella pneumoniae* among fecal carriage in Iran: High diversity of clonal relatedness and virulence factor profiles," Microbial drug resistance, vol. 26, no. 3, pp. 261–269, 2020.

[3] A. Y. Peleg, H. Seifert, and D. L. Paterson, "Acinetobacter baumannii: emergence of a successful pathogen," Clinical Microbiology Reviews, vol. 21, pp. 538-582, 2008.

[4] M. Bassetti, F. Ginocchio, and M. Mikulska, "New treatment options against gramnegative organisms," Critical care, vol. 15, p. 215, 2011.

[5] L. B. Rice, "Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE," Journal of Infectious Diseases, vol. 197, pp. 1079-1081, 2008.

[6] Z. Pang, R. Raudonis, B. R. Glick, T.-J. Lin, and Z. Cheng, "Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies," Biotechnology advances, vol. 37, no. 1, pp. 177–192, 2019.

[7] E. L. Fonseca, N. da V. Ramos, B. G. N. Andrade, L. L. C. S. Morais, M. F. A. Marin, and A. C. P. Vicente, "A one-step multiplex PCR to identify

Klebsiella pneumoniae, Klebsiella variicola, and Klebsiella quasipneumoniae in the clinical routine," Diagnostic microbiology and infectious disease, vol. 87, no. 4, pp. 315–317, 2017.

[8] L. J. Rojas et al., "Colistin resistance in carbapenem-resistant *Klebsiella pneumoniae*: Laboratory detection and impact on mortality," Clin. Infect. Dis., vol. 64, no. 6, pp. 711–718, 2017.

[9] J. D. Gawronski, S. M. S. Wong, G. Giannoukos, D. V. Ward, and B. J. Akerley, "Tracking insertion mutants within libraries by deep sequencing and a genome-wide screen for Haemophilus genes required in the lung," Proc. Natl. Acad. Sci. U. S. A., vol. 106, no. 38, pp. 16422–16427, 2009.

[10] C. U. Koser, M. J. Ellington, and S. J. Peacock, "Whole-genome sequencing to control antimicrobial resistance," Trends in Genetics, vol. 30, no. 9, pp. 401–407, 2014.

[11] Y. H. Grad et al., "Genomic epidemiology of Neisseria gonorrhoeae with reduced susceptibility to cefixime in the USA: a retrospective observational study," Lancet Infectious Diseases, vol. 14, no. 3, pp. 220–226, 2014.

[12] C. N. Jondle, K. Gupta, B. B. Mishra, and J. Sharma, "*Klebsiella pneumoniae* infection of murine neutrophils impairs their efferocytic clearance by modulating cell death machinery," PLoS Pathog., vol. 14, no. 10, p. e1007338, 2018.

[13] S. Aghamohammad, F. Badmasti, H. Solgi, Z. Aminzadeh, Z. Khodabandelo, and F. Shahcheraghi, "First report of extended-spectrum betalactamase-producing *Klebsiella pneumoniae* among fecal carriage in Iran: High diversity of clonal relatedness and virulence factor profiles," Microb. Drug Resist., vol. 26, no. 3, pp. 261–269, 2020.

[14] F. A. S., R. E. T. Buchanan, N. E. Gibbons, and Bergey, "Bergey's Manual of Determinative Bacteriology," Taxon, vol. 24, no. 2/3, p. 377, 1975.

[15] T. G. Rønning et al., "Investigation of an outbreak caused by antibiotic-susceptible Klebsiella oxytoca in a neonatal intensive care unit in Norway," Acta Paediatr., vol. 108, no. 1, pp. 76–82, 2019.

[16] A. Gamkrelidze et al., "Nationwide hepatitis C serosurvey and progress towards hepatitis C virus elimination in the country of Georgia, 2021," J. Infect. Dis., vol. 228, no. 6, pp. 684–693, 2023.

[17] E. P. Esposito et al., "Molecular epidemiology and virulence profiles of colistin-resistant *Klebsiella pneumoniae* blood isolates from the Hospital Agency 'Ospedale dei Colli,' Naples, Italy," Front. Microbiol., vol. 9, p. 1463, 2018.

[18] L. G. Naemura and R. J. Seidler, "Numerical taxonomy of *Klebsiella pneumoniae* strains isolated from clinical and nonclinical sources," Current Microbiology, vol. 2, pp. 175–180, 1979.

[19] M. K. Paczosa and J. Mecsas, "*Klebsiella pneumoniae*:Going on the offense with a strong defense," Microbiol. Mol. Biol. Rev, vol. 80, no. 3, pp. 629–661, 2016.

[20] K. G. Oikonomou and M. Aye, "*Klebsiella pneumoniae* liver abscess:A case series of six Asian patients," Am. J. Case Rep, vol. 18, no. 1, pp. 1028–1033, 2017.

[21] T. A. Russo et al., "Differentiation of hypervirulent and classical *Klebsiella pneumoniae* with acquired drug resistance," MBio, p. e0286723, 2024.

[22] Y. Guo et al., "Frequency, antimicrobial resistance and genetic diversity of *Klebsiella pneumoniae* in food samples," PLoS One, vol. 11, no. 4, p. e0153561, 2016.

[23] J. L. Smith and P. M. Fratamico, "Emerging and re-emerging foodborne pathogens," Foodborne Pathog. Dis., vol. 15, no. 12, pp. 737–757, 2018.

[24] G. S. Davis and L. B. Price, "Recent research examining links among *Klebsiella pneumoniae* from food, food animals, and human extraintestinal infections," Curr. Environ. Health Rep., vol. 3, no. 2, pp. 128–135, 2016.

[25] Clinical and molecular characteristics of high virulent Klebsiella pneumonia in infection in intensive care unit," Chin. J. Nosocomiol, vol. 26, no. 1, pp. 5056–5059, 2016.

[26] Y.-J. Pan et al., "Capsular polysaccharide synthesis regions in *Klebsiella pneumoniae* serotype K57 and a new capsular serotype," J. Clin. Microbiol., vol. 46, no. 7, pp. 2231–2240, 2008.

[27] A. M. Parrott, J. Shi, J. Aaron, D. A. Green, S. Whittier, and F. Wu, "Detection of multiple hypervirulent *Klebsiella pneumoniae* strains in a New York City hospital through screening of virulence genes," Clin. Microbiol. Infect., vol. 27, no. 4, pp. 583–589, 2021.

[28] M. Sugumar, K. M. Kumar, A. Manoharan, A. Anbarasu, and S. Ramaiah, "Detection of OXA-1 b-Lactamase Gene of *Klebsiella pneumoniae* from Blood Stream Infections (BSI) by Conventional PCR and In-Silico Analysis to Understand the Mechanism of OXA Mediated Resistance," PLoS One, vol. 2, no. 3, 2014.

[29] P. Williams and J. Tomás, "The pathogenicity of *Klebsiella pneumoniae*," Rev. Med. Microbiol, vol. 1, no. 1, pp. 196–204, 1990.

[30] S. J. Cryz Jr, E. Furer, and R. Germanier, "Immunization against fatal experimental *Klebsiella pneumoniae* pneumonia," Infect. Immun., vol. 54, no. 2, pp. 403–407, 1986.

[31] D. C. Straus, D. L. Atkisson, and C. W. Garner, "Importance of a lipopolysaccharide-containing extracellular toxic complex in infections produced by *Klebsiella pneumoniae*," Infect. Immun., vol. 50, no. 3, pp. 787–795, 1985.

[32] B. L. Meatherall, D. Gregson, T. Ross, J. D. D. Pitout, and K. B. Laupland, "Incidence, risk factors, and outcomes of *Klebsiella pneumoniae* bacteremia," Am. J. Med., vol. 122, no. 9, pp. 866–873, 2009.

[33] 112 T.-T. Huang, F.-Y. Tseng, T.-H. Yeh, C.-J. Hsu, and Y.-S. Chen, "Factors affecting the bacteriology of deep neck infection: a retrospective study of 128 patients," Acta Otolaryngol., vol. 126, no. 4, pp. 396–401, 2006.

[34] Y.-T. Lin, C.-J. Liu, Y.-C. Yeh, T.-J. Chen, and C.-P. Fung, "Ampicillin and amoxicillin use and the risk of *Klebsiella pneumoniae* liver abscess in Taiwan," J. Infect. Dis., vol. 208, no. 2, pp. 211–217, 2013.

[35] S. Jang et al., "Pleuritis and suppurative pneumonia associated with a hypermucoviscosity phenotype of *Klebsiella pneumoniae* in California sea lions (Zalophus californianus)," Vet. Microbiol., vol. 141, no. 1–2, pp. 174–177, 2010.

[36] Y.-T. Lin, C.-J. Liu, Y.-C. Yeh, T.-J. Chen, and C.-P. Fung, "Ampicillin and amoxicillin use and the risk of *Klebsiella pneumoniae* liver abscess in Taiwan," J. Infect. Dis., vol. 208, no. 2, pp. 211–217, 2013.

[37] Y.-T. Lin et al., "Seroepidemiology of *Klebsiella pneumoniae* colonizing the intestinal tract of healthy chinese and overseas chinese adults in Asian countries," BMC Microbiol., vol. 12, no. 1, 2012.

[38] A. Asensio et al., "Outbreak of a multiresistant *Klebsiella pneumoniae* strain in an intensive care unit: antibiotic use as risk factor for colonization and infection," Clin. Infect. Dis., vol. 30, no. 1, pp. 55–60, 2000.

[39] F. Okada et al., "Acute *Klebsiella pneumoniae* pneumonia alone and with concurrent infection: comparison of clinical and thin-section CT findings," Br. J. Radiol., vol. 83, no. 994, pp. 854–860, 2010.

[40] H. Kollef et al., "Clinical characteristics and treatment patterns among patients with ventilator-associated pneumonia," Chest, vol. 129, no. 5, pp. 1210–1218, 2006.

[41] A. Ono, Y. Ando, F. Okada, T. Nakayama, T. Maeda, and H. Mori, "Clinical and pulmonary thin-section CT findings in acute *Klebsiella pneumoniae* pneumonia," Chest, vol. 140, no. 4, p. 644A, 2011.

[42] L. A. Mandell et al., "Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults," Clin. Infect. Dis., vol. 44 Suppl 2, no. Supplement_2, pp. S27-72, 2007.

[43] H. Naderi, F. Sheybani, M. Sarvghad, Z. Meshkat, and M. Jabbari Nooghabi, "Etiological diagnosis of community-acquired pneumonia in adult patients: A prospective hospital-based study in Mashhad, Iran," Jundishapur J. Microbiol., vol. 8, no. 8, p. e22780, 2015.

[44] A. Ono, Y. Ando, F. Okada, T. Nakayama, T. Maeda, and H. Mori, "Clinical and pulmonary thin-section CT findings in acute *Klebsiella pneumoniae* pneumonia," Chest, vol. 140, no. 4, p. 644A, 2011.

[45] Y.-H. Ni, K.-M. Yeh, M.-Y. Peng, Y.-Y. Chou, and F.-Y. Chang, "Community-acquired brain abscess in Taiwan: etiology and probable source of infection," J. Microbiol. Immunol. Infect., vol. 37, no. 4, pp. 231–235, 2004.

[46] C. T. Fang, Y. C. Chen, S. C. Chang, W. Y. Sau, and K. T. Luh, "*Klebsiella pneumoniae* meningitis: timing of antimicrobial therapy and prognosis," QJM, vol. 93, no. 1, pp. 45–53, 2000.

[47] C.-T. Fang, S.-Y. Lai, W.-C. Yi, P.-R. Hsueh, K.-L. Liu, and S.-C. Chang, "*Klebsiella pneumoniae* genotype K1: an emerging pathogen that causes septic ocular or central nervous system complications from pyogenic liver abscess," Clin. Infect. Dis., vol. 45, no. 3, pp. 284–293, 2007.

[48] Y.-M. Liu et al., "Microbiology and factors affecting mortality in necrotizing fasciitis," J. Microbiol. Immunol. Infect., vol. 38, no. 6, pp. 430–435, 2005.

[49] R. A. DiGioia, J. G. Kane, and R. H. Parker, "Crepitant cellulitis and myonecrosis caused by Klebsiella," JAMA, vol. 237, no. 19, pp. 2097–2098, 1977.

[50] N.-C. Cheng et al., "Recent trend of necrotizing fasciitis in Taiwan: focus on monomicrobial *Klebsiella pneumoniae* necrotizing fasciitis," Clin. Infect. Dis., vol. 55, no. 7, pp. 930–939, 2012.

[51] J. Rahimian, T. Wilson, V. Oram, and R. S. Holzman, "Pyogenic liver abscess: recent trends in etiology and mortality," Clin. Infect. Dis., vol. 39, no. 11, pp. 1654–1659, 2004.

[52] J. Rahimian, T. Wilson, V. Oram, and R. S. Holzman, "Pyogenic liver abscess: recent trends in etiology and mortality," Clin. Infect. Dis., vol. 39, no. 11, pp. 1654–1659, 2004.

[53] C.-H. Lee, H.-S. Leu, T.-H. Hu, and J.-W. Liu, "Splenic abscess in southern Taiwan," J. Microbiol. Immunol. Infect., vol. 37, no. 1, pp. 39–44, 2004.

[54] J. H. Wang et al., "Primary liver abscess due to *Klebsiella pneumoniae* in Taiwan," Clin. Infect. Dis., vol. 26, no. 6, pp. 1434–1438, 1998.

[55] R. Francés et al., "Bacterial DNA in patients with cirrhosis and noninfected ascites mimics the soluble immune response established in patients with spontaneous bacterial peritonitis," Hepatology, vol. 47, no. 3, pp. 978–985, 2008

[56] R. Capita and C. Alonso-Calleja, "Antibioticresistant bacteria: A challenge for the food industry," Critical reviews in food science and nutrition, vol. 53, pp. 11–48, 2013.

[57] S. Navon-Venezia, K. Kondratyeva, and A. Carattoli, "*Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance," FEMS Microbiol. Rev., vol. 41, no. 3, pp. 252–275, 2017.

[58] A. Sharma, A. Thakur, N. Thakur, V. Kumar, A. Chauhan, and N. Bhardwaj, "Changing trend in the antibiotic resistance pattern of Klebsiella pneumonia isolated from endotracheal aspirate samples of ICU patients of a tertiary care hospital in North India," Cureus, vol. 15, no. 3, p. e36317, 2023.

[59] D. L. Paterson and Y. Doi, "Editorial commentary: A step closer to extreme drug resistance (XDR) in gram-negative bacilli," Clinical Infectious Diseases, pp. 1179–1181, 2007.

[60] D. Moradigaravand, V. Martin, S. J. Peacock, and J. Parkhill, "Evolution and epidemiology of multidrug-resistant *Klebsiella pneumoniae* in the United Kingdom and Ireland," MBio, vol. 8, no. 1, 2017.

[61] E. P. Esposito et al., "Molecular epidemiology and virulence profiles of colistin-resistant *Klebsiella pneumoniae* blood isolates from the Hospital Agency 'Ospedale dei Colli,' Naples, Italy," Front. Microbiol., vol. 9, p. 1463, 2018.

[62] J. M. Munita and C. A. Arias, "Mechanisms of antibiotic resistance," Microbiol. Spectr., vol. 4, no. 2, 2016.

[63] L. Surgers, A. Boyd, P.-M. Girard, G. Arlet, and D. Decré, "ESBL-Producing Strain of Hypervirulent *Klebsiella pneumoniae* K2, France," Emerg. Infect. Dis., vol. 22, no. 9, pp. 1687–1688, 2016.

[64] Y. Chong, Y. Ito, and T. Kamimura, "Genetic evolution and clinical impact in extended-spectrum β-lactamaseproducing Escherichia coli and *Klebsiella pneumoniae*. Infection," Genetics and Evolution, vol. 11, no. 7, pp. 1499–1504, 2011.

[65] A. S. Sikarwar and H. V. Batra, "Prevalence of Antimicrobial Drug Resistance of *Klebsiella pneumoniae* in India," Int. J. Biosci. Biochem. Bioinforma., pp. 211–215, 2011.

[66] T. Banerjee, J. Wangkheimayum, S. Sharma, A. Kumar, and A. Bhattacharjee, "Extensively drugresistant hypervirulent *Klebsiella pneumoniae* from a series of neonatal sepsis in a tertiary care hospital, India," India. Frontiers in Medicine, vol. 8, 2021.

[67] P. Peerbooms, N. Lemmens-Den Toom, M. Van Santen-Verheuvel, and H. Verbrugh, "Patterns of resistance associated with integrons, the extended-spectrum βlactamase SHV-5 gene, of Clinical Microbiology, vol. 41, no. 3, pp. 1161–1166, 2003.

[68] L. A. Jones, C. J. Mciver, M. J. Kim, W. D. Rawlinson, and P. A. White, "The aadB gene cassette is associated with bla SHV genes in Klebsiella species producing extendedspectrum βlactamases," Antimicrobial Agents and Chemotherapy, vol. 49, no. 2, pp. 794–797, 2005

[69] R. Shakti and N. Rabindra, "Prevalence of two multidrug-resistant Klebsiella species in an Indian teaching hospital and adjoining community," Journal of Infection and Public Health, vol. 7, no. 6, pp. 496–507, 2014.

[70] S. Pokhrel, A. Aryal, R. Adhikari, and C. M. Parry, "Characterization of Transferrable Mechanisms of Quinolone Resistance (TMQR) among Quinoloneresistant Escherichia coli and *Klebsiella pneumoniae* causing Urinary Tract Infection in Nepalese Children," BMC Pediatrics, vol. 23, no. 1, pp. 1–10, 2023.

[71] S. S. Costa, L. C. Guimarães, A. Silva, S. C. Soares, and R. A. Barauna, "First steps in the analysi

[72] GARDY, J. L., LAIRD, M. R., CHEN, F., REY, S., WALSH, C. J., ESTER, M. & BRINKMAN, F. S. L. 2004. PSORTb v.2.0: Expanded prediction of bacterial protein subcellular localization and insights gained from comparative proteome analysis. Bioinformatics, 21, 617-623

[73] S. J. Cryz Jr, E. Furer, and R. Germanier, "Safety and immunogenicity of *Klebsiella pneumoniae* K1 capsular polysaccharide vaccine in humans," Journal of Infectious Disease., vol. 151, no. 4, pp. 665–671, 1985.

[74] J. D. Ernst, "Toward the development of antibacterial vaccines: report of a symposium and workshop. Organizing Committee,"Clinical Infectious Diseases, vol. 29, no. 5, pp. 1295–1302, 1999.

[75] T. Farhadi, N. Nezafat, Y. Ghasemi, Z. Karimi, S. Hemmati, and N. Erfani, "Designing of complex multi-epitope peptide vaccine based on OMPs of *Klebsiella pneumoniae*: An in silico approach," International Journal of Peptide Research and Therapeutics, vol. 21, no. 3, pp. 325–341, 2015.

[76] I. A. Doytchinova and D. R. Flower, "VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines," BMC Bioinformatics, vol. 8, no. 1, 2007.

[77] J. D. Ernst, "Toward the development of antibacterial vaccines: report of a symposium and workshop. Organizing Committee," Clinical Infectious Diseases, vol. 29, no. 5, pp. 1295–1302, 1999.

[78] T. Farhadi et al., "Production of a novel multi-epitope vaccine based on outer membrane proteins of *Klebsiella pneumoniae*," Trends in Pharmaceutical Sciences, vol. 1, no. 3, pp. 167–172, 2015.

[79] P. Chawley, H. B. Samal, J. Prava, M. Suar, and R. K. Mahapatra, "Comparative genomics study for identification of drug and vaccine targets in Vibrio cholerae: MurA ligase as a case study," International Journal of Genomics, vol. 103, no. 1, pp. 83–93, 2014.