Chapter 1:
Introduction and review of literature

1.1 *Klebsiella pneumoniae*

In 1882, Carl Friedlander made the original discovery of *Klebsiella pneumoniae*, a bacterium which was found to be prevalent in the surrounding environment. The initial designation of Friedlander's bacillus underwent a change in nomenclature in 1886, leading to its current classification as *Klebsiella* (Ashurst & Dawson, 2023). *K. pneumoniae* is characterized by its normal appearance as Gram-negative, straight rod-shaped measuring between 0.3um to 1.8 µm in size (De Jesus et al., 2015). It is a non-motile bacterium, a facultative anaerobe, that digests lactose and proliferates at 37 °C. They create mucoid colonies on medium rich in carbohydrates, which are thought to be caused by a capsule (Drancourt et al., 2001; De Jesus et al., 2015). *Klebsiella* species can be readily cultivated on many types of media that are appropriate for *Enterobacteriaceae* bacteria, such as nutrient agar, bromocresol purple lactose agar, tryptic casein soy agar, MacConkey agar, Drigalski agar, bromothymol blue agar, and eosin-methylene blue (EMB) agar (Reyes, 2005). Since pneumoniae is able to undergo both respiratory and fermentative metabolism, it does not require any additional growth factors (Drancourt et al., 2001). The mucoid appearance of the facultative anaerobe varies corresponding on the strain and is affected by the medium's composition (Drancourt et al., 2001; Reyes, 2005).

Klebsiella species are classified as opportunistic pathogens that typically reside within the normal flora of the nasal passages, throat, gastrointestinal tract of healthy individuals, and skin. However, they have the potential to induce various infections, such as pneumonia, soft tissue, urinary tract infections, surgical wound infections, bloodstream infections, and sepsis (Holt et al., 2015; Podschun & Ullmann, 1998). The most prevalent disease is urinary tract infection (UTI), pneumonia, and wound or soft tissue infections. *K. pneumoniae* has consistently been recognized as one of the primary pathogens responsible for hospital-acquired infections across various healthcare settings (Jones, 2010). They can colonize a diverse array of animal hosts, as well as establish associations with various plant species. It may be detected in many environments such as soil, water, and drainage. Furthermore, *K. pneumoniae* has the capability to colonize several anatomical areas inside the human body, including the respiratory system, gastrointestinal tract, nasopharynx, oropharynx, and skin (Bagley, 1985; Podschun & Ullmann, 1998).

K. pneumoniae is one of the few bacterial species that has seen a notable rise in antibiotic resistance as a result of modifications to the organism's fundamental DNA. In 1929, Alexander Fleming first identified the presence of resistance to beta-lactam antibiotics in gram-negative organisms (Fleming, 1929; Abraham, 1981). The prevalence of antimicrobial resistance in *K. pneumoniae* on a worldwide scale has escalated to a staggering 70%, while the death rate linked with infections caused by this bacterium ranges from 40% to 70% (Iredell et al., 2016). The exponential growth in the quantity of genomes archived in public databases may be attributed to the significant advancements seen in next-generation sequencing technologies (Hamamoto et al., 2015). The NCBI database now contains 62,103 genomes, with 2,573 of these being the complete genomes of *K. pneumoniae*, as of February 2024. (https://www.ncbi.nlm.nih.gov/datasets/genome/). This extensive genomic dataset enables us to augment the quantity and variety of strains, consequently facilitating the worldwide phylogenetic examination of *K. pneumoniae* (Zhou et al., 2016). The typical *K. pneumoniae* genome (isolate M50 used in this research) shown in **Figure 1.1** has a size of around 5.5 megabase pairs (Mbp) and contains around 5,500 genes. The analysis of several isolates by whole genome comparisons reveals that the core genome, which encompasses the genes shared by all *K. pneumoniae* strains, consists of less than 2,000 genes (Bialek-Davenet et al., 2014; Holt et al., 2015).

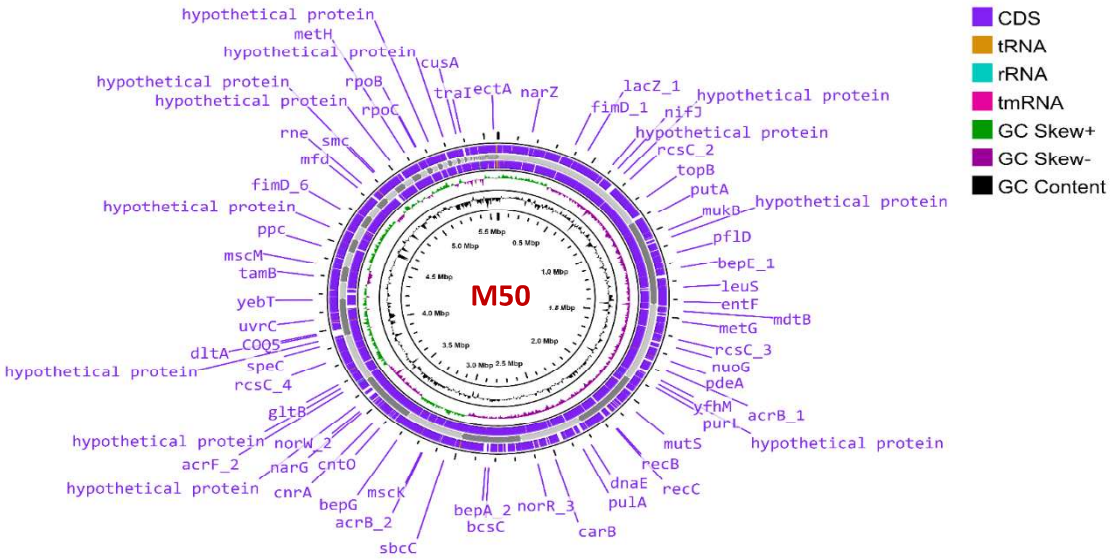

Figure 1.1 Representative image of isolate M50 shows the circular genome of *K. pneumoniae.* The genome contains coding sequences (CDSs), tRNA, rRNA, tmRNA, GC Skew $^{(+/-)}$, and GC content of the genome.

Apart from that, in *K. pneumoniae,* the accessory genome consists of acquired genes that provide resistance to antimicrobials and metals, as well as genes that contribute to virulence. It also includes plasmids and other elements that facilitate the transfer of genes between organisms. This is confirmed through functional annotation and comparative genomics and is further supported by the presence of domains with different guanine/cytosine content (Kitchel et al., 2009; Wyres et al., 2020).

1.2 Antibiotic resistance

Bacterial antimicrobial resistance has emerged as a prominent public health problem in the 21st century. It happens when bacteria undergo changes that reduce the effectiveness of medications used to treat diseases. The antimicrobial resistance review, commissioned by the UK Government, contended that by 2050, AMR had the potential to cause the deaths of 10 million people annually (J. A. Cox et al., 2017; C. J. L. Murray et al., 2022), as shown in **Figure 1.2.** This concern has also been highlighted in the reports on AMR by reputable institutions such as the US Centers for Disease Control and Prevention (CDC) and the UK Department of Health (Tang et al., 2020). As a result, the World Health Organization (WHO) designated *K. pneumoniae* as a "Priority 1: CRITICAL" pathogen in 2017 (Havenga et al., 2019). In 2021, it was also included in the Indian priority pathogen list (IPPL) (https://dbtindia.gov.in/sites/default/files/IPPL_final.pdf). This is due to the growing antibiotic resistance, which includes non-susceptibility to last-resort medications like carbapenems, colistin, and tigecycline (Osei Sekyere, 2016).

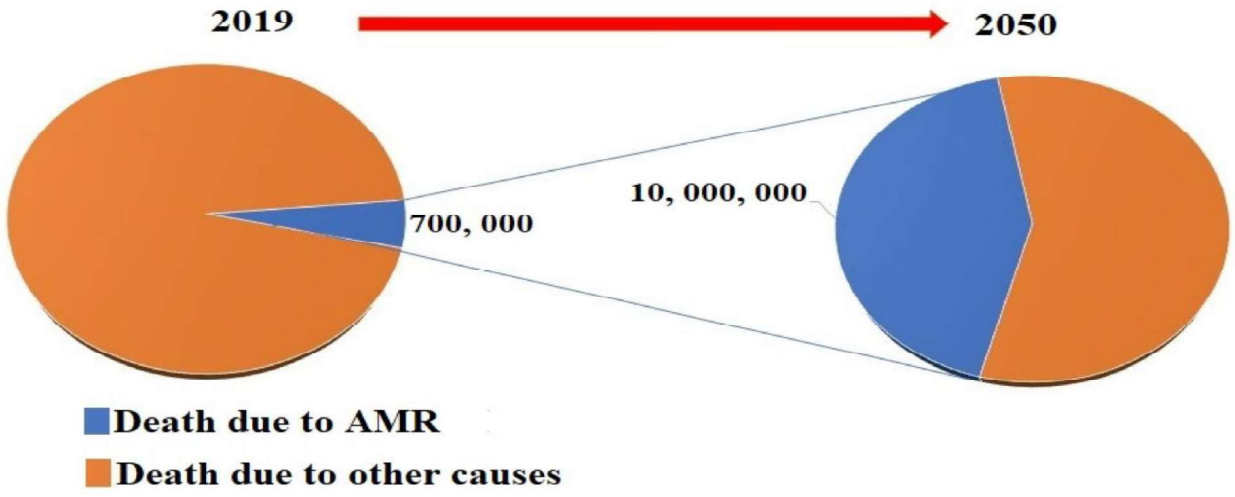

Figure 1.2 Global death prediction due to AMR. Pie chart displaying the present count of worldwide fatalities caused by antimicrobial-resistant infections, as well as the projected number of deaths due to such infections in 2050. Source: (Shinu et al., 2022).

The primary cause behind the emergence of multidrug resistance is the phenomenon of selection pressure. This phenomenon arises from the persistent and repeated exposure to a multitude of antibiotics in environments characterized by their excessive and/or inappropriate use, such as healthcare facilities and agricultural practices (Davies & Davies, 2010). In several low- and middle-income countries (LMICs), there is a growing prevalence of public exposure to multidrug-resistant bacteria. This may be attributed to the unregulated use of "antibiotic growth promoters" in animals, as well as the unrestricted availability of antibiotics for purchase without a prescription (J. A. Cox et al., 2017). Furthermore, there is a scarcity of clinical recommendations pertaining to the use of antibiotics for therapeutic purposes in animals intended for human consumption. The presence of significant budgetary limitations, limited access to diagnostic resources, and insufficient pathogen monitoring systems provide considerable obstacles to the effective implementation of infection prevention and control (IPC) measures. These challenges contribute to the emergence and spread of multidrug-resistant strains (Okeke et al., 2005).

The worldwide dissemination of multidrug-resistant *Klebsiella pneumoniae* (MDR*Kp*) is a significant concern within the realm of public health. The comprehension of the genetic composition of recently developing lineages of these pathogens would facilitate the optimization of antibiotic use for patient therapy and contribute to global endeavors in combating antibiotic resistance, as well as devising suitable infection prevention and control methods (L. Wang et al., 2017; Lepuschitz et al., 2019). The use of whole-genome sequencing (WGS) has been extensively employed in the examination of the worldwide transmission and molecular processes of *K. pneumoniae* (J. Sheng et al., 2023). The discovery of penicillin in 1929 as shown in **Figure 1.3** is appropriately acknowledged as a significant event in the medical field, and its use in clinical practice throughout the 1940s led to a profound transformation in our capacity to combat bacterial illnesses. Although there have been significant advancements in antimicrobial chemotherapy over the last 70 years since the first use of penicillin, the β-lactams continue to be the fundamental components of the antibacterial arsenal. The enduring prominence of this antibiotic class, as seen by its status as the most often prescribed and commercially significant, underscores its ongoing pivotal position in the management of bacterial infections (Fleming, 1929.; Klein et al., 2018). Interestingly, the first evidence of antibiotic resistance became evident shortly after the discovery of penicillin. In 1940s, Abraham and Chain documented that a strain of *E. coli* has the capability

to render penicillin ineffective via the production of penicillinase (Abraham, 1981) shown in **Figure 1.3.**

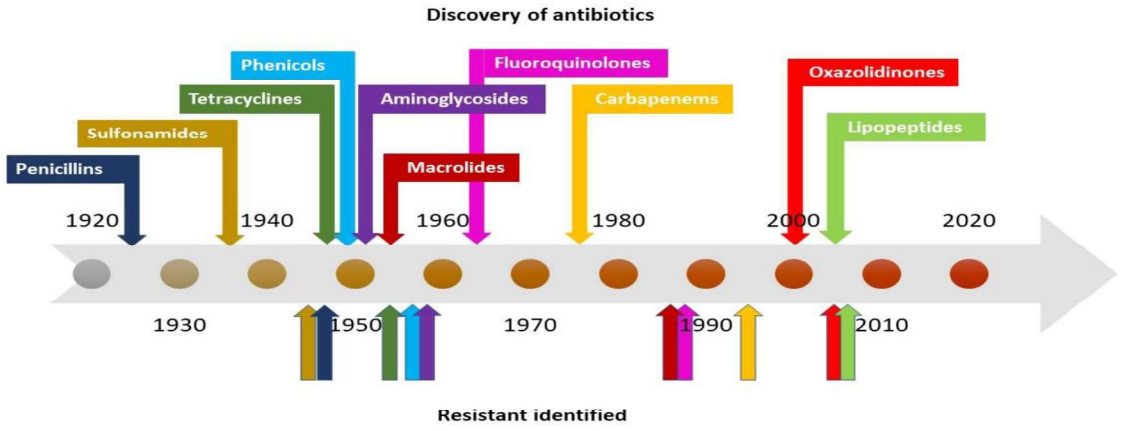

Figure 1.3 Timeline of the discovery of antibiotics and the subsequent identification of antibiotic resistance. Each arrow pointed upside indicates the drug resistant identified, and each color is filled in accordance with the respective antibiotics mentioned above.

1.2.1 Mechanism of antibiotic resistance

There are two ways that contribute to antibiotic resistance: (i) Intrinsic antibiotic mechanisms are encoded in the chromosome and consist of non-specific efflux pumps (which likely developed as a broad defense against environmental toxins), antibiotic inactivating enzymes, or mechanisms that operate as barriers to prevent antibiotic entry (G. Cox & Wright, 2013; Fajardo et al., 2008) as shown in **Figure 1.4**. An extensively researched instance of an inherent resistance mechanism is the AcrAB/TolC efflux pump in *E. coli* and *K. pneumoniae*. This pump has a wide-ranging substrate specificity and can expel many categories of antibiotics, dyes, detergents, and disinfectants (Nikaido & Takatsuka, 2009). Gram-negative bacteria exemplify inherent resistance due to the impermeable outer membrane that acts as a barrier (Arthur & Courvalin, 1993). While environmental bacteria with intrinsic mechanisms or typical commensal flora might transmit low-

level antibiotic resistance to their original host, these pathogens can become opportunistic in individuals with impaired immune systems (Wright, 2007).

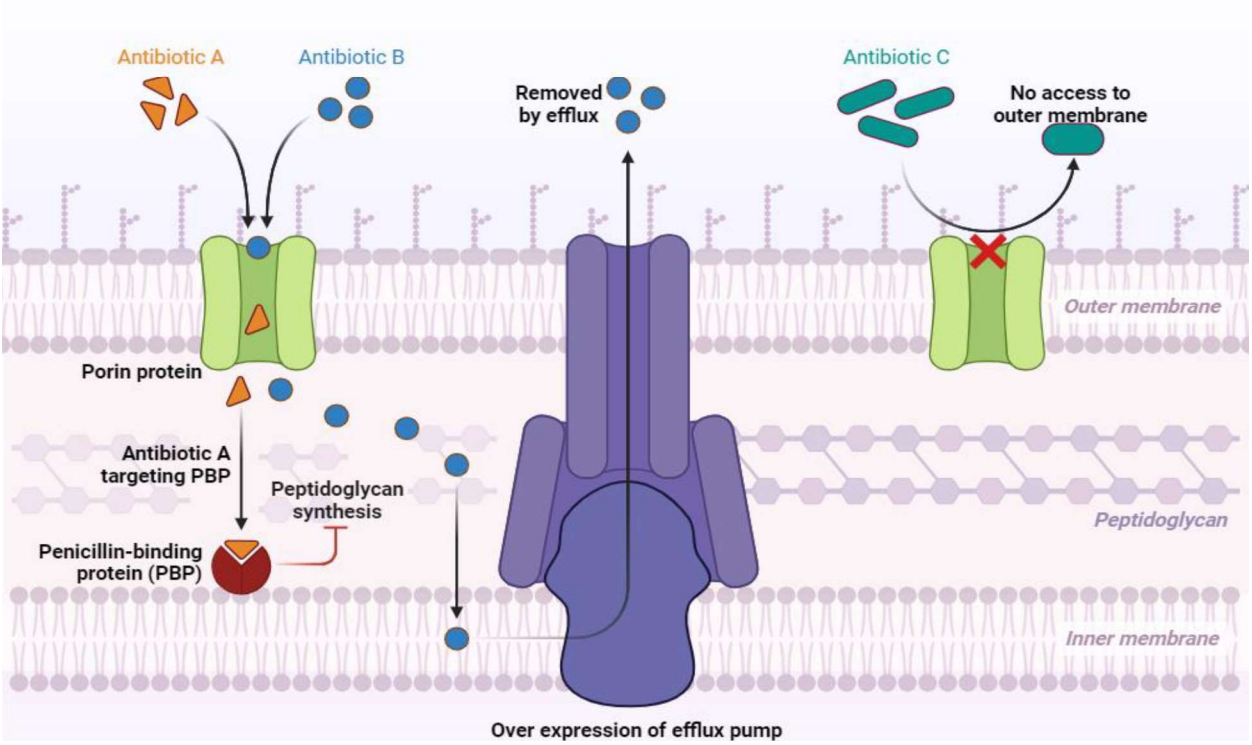

Figure 1.4 Methods of intrinsic resistance seen in Gram-negative bacteria. The image shows antibiotics' intrinsic resistance via overexpression of efflux pumps (Purple colour) and reduced porin (light green colour) permeability. (The image was created using BioRender tool).

(ii) The acquired resistance is often acquired by horizontal gene transfer (HGT), which involves the transfer of genes across organisms. These mechanisms include efflux pumps and enzymes encoded by plasmids, which may change either the antibiotic itself or its target (Bismuth et al., 1990; Van Hoek et al., 2011). The emergence of antibiotic resistance in *K. pneumoniae* has resulted in a decrease in the efficacy of conventional therapies against the bacterium. Resistance might arise because of enhanced efflux, drug inactivation, or modified binding to the target site. A multitude of *K. pneumoniae* strains exhibit Extended-Spectrum β-Lactamase (ESBL) production or engage in biofilm formation, hence increasing their resistance.

K. pneumoniae exhibits antibiotic resistance primarily via the following five mechanisms: The mechanisms of antibiotic resistance include enzymatic antibiotic inactivation and modification, changing of antibiotic targets, loss and/or mutation of porins, increased expression of efflux pumps for the antibiotic, and biofilm development (Sikarwar & Batra, 2011; Mulani et al., 2019) shown in **Figure 1.5.**

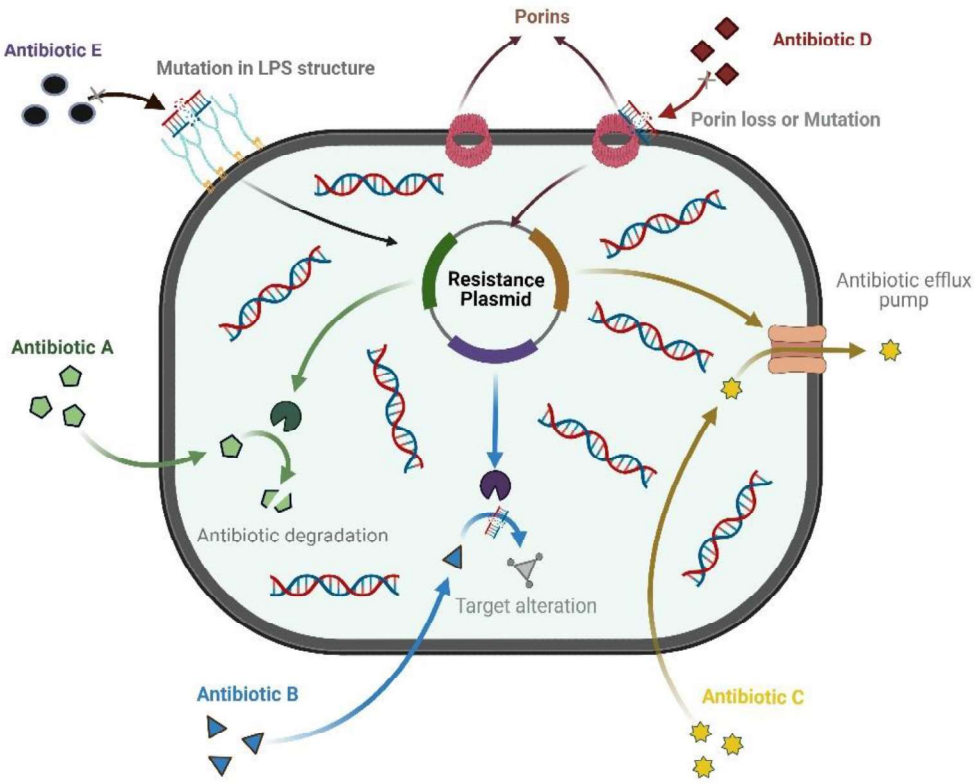

Figure 1.5 Illustration of the five primary mechanisms of antibiotic resistance in Gram-negative bacteria. The image shows key features that contribute to antibiotic resistance, including target degradation, target alteration, antibiotic efflux pumps, porin loss or mutation, and mutation in LPS structure. (The image was created using BioRender tool)

1.3 Genomics of antibiotics resistance in *Klebsiella pneumoniae*

In accordance with standard outbreak management procedures, several phenotypic and molecular techniques are often used to analyze and control the dissemination of antibiotic-resistant bacterial infections in hospitals around the globe (Van Belkum et al., 2007). Nevertheless, traditional

methods for controlling outbreaks sometimes struggle to differentiate between closely related strains causing the epidemic or identify specific traits associated to virulence or resistance (Beceiro et al., 2013; Quainoo et al., 2017). The primary reason for this is the restricted ability of traditional molecular techniques to accurately analyze the genome, as well as the specific focus of outbreak analysis methods. For instance, when dealing with infections caused by antimicrobial-resistant organisms, genotypic tests are used. However, these tests only identify antimicrobial resistance genes and not virulence genes. The detection of both types of genes simultaneously can offer supplementary phylogenetic information and enhance outbreak analysis (Leopold et al., 2014). To address the limitations of traditional outbreak management, it is necessary to use innovative technologies that provide greater genomic resolution and comprehensive genetic data for the whole bacterial genome. *K. pneumoniae* is recognized as both an origin and a storage place for antimicrobial resistance genes (Chaves et al., 2001; Nordmann et al., 2009, 2011). Many of the main groups of these genes (For example, the genes *KPC, OXA-48,* and *NDM-1* are associated with resistance to carbapenem antibiotics) were initially discovered in *K. pneumoniae* before being found in various other types of Gram-negative bacteria (Holt et al., 2015; Wyres et al., 2020). Therefore, it is essential to enhance our knowledge of the wider population of *K. pneumoniae* beyond just a few well-known strains. Whole genome sequencing has the potential to provide a much higher level of detail and comprehensive genetic data for the whole bacterial genome, including all pertinent genomic features. In addition to identifying bacteria and characterizing them at the molecular level, WGS has the potential to provide valuable information for predicting the phenotypic of the microorganism (Balloux et al., 2018). Whole-genome sequencing may include all pertinent genomic attributes, although medical professionals have historically been reluctant to incorporate WGS into conventional epidemic analysis techniques owing to exorbitant costs and the unwieldy nature of early next-generation sequencing (NGS) technology (Margulies et al., 2005; Valouev et al., 2008).

1.3.1 Multi-Locus Sequence Typing (MLST)

Genotyping plays a crucial role in identifying cases or outbreaks caused by *K. pneumoniae* and in subsequently tracing the origin and transmission of infections. The primary genotyping techniques used for *K. pneumoniae* are pulsed field gel electrophoresis (PFGE), multiple-locus variable number tandem repeat analysis (MLVA), and multi-locus sequence typing (MLST) (Diancourt et

al., 2005; J. F. Turton et al., 2010), of which MLST stands out as the most often favored option among them. The *K. pneumoniae* MLST technique was established in 2005 (Diancourt et al., 2005) and subsequently used worldwide to assess the diversity and epidemiology of clinical *K. pneumoniae* isolates. This facilitated the discovery of distinct clones that exhibit significant variations in terms of virulence and drug resistance characteristics (Brisse et al., 2009; L. Chen, Mathema, Pitout, et al., 2014; Harada et al., 2018; Y.-T. Lin et al., 2020; Y. Luo et al., 2014; Siu et al., 2011; F. Wang et al., 2019). MLST is a molecular technique used to determine the genetic link between bacterial isolates. It is primarily used for investigating the molecular epidemiology of microorganisms that are of public health concern (Enright & Spratt, 1999; Feil et al., 2004). MLST is a definitive method for classifying bacterial isolates of a species by analyzing the sequences of internal regions of typically seven (*infB, gapA, mdh, phoE, pgi, rpoB,* and *tonB*) house-keeping genes (Diancourt et al., 2005; Maiden et al., 1998). Since they can be precisely sequenced on both strands using an advanced and automated DNA sequencer, internal segments of each gene ranging from 450 to 500 bp are employed. Each bacterial species assigns diverse alleles to the various sequences of house-keeping genes. The sequence type (ST) or allelic profile of each isolate is determined by the alleles at each of the seven loci (Jolley et al., 2018; Urwin & Maiden, 2003). As of February 12, 2024, there were 6869 distinct STs of *K. pneumoniae* recorded in the PasteurMLST database (https://bigsdb.pasteur.fr/klebsiella/). MLST analysis has shown that *K. pneumoniae* has a mostly oligoclonal nature, with a wide range of sequence types identified, including ST 11, ST 14, ST 15, ST 26, ST 101, ST 147, ST 149, ST 231, ST 258, ST 627, and ST 977 (Munoz-Price et al., 2013). **Figure 1.6** displays an abundance of multidrug-resistant and hypervirulent clones of *K. pneumoniae* (Kocsis, 2023). Several STs have shown geographic specificity, with some being epidemic and/or endemic (Zhou et al., 2016). The identification and fast worldwide dissemination of high-risk lineages like as ST607, ST307, ST147, ST15, and ST14 which are associated with MDR strains, have heightened the need for global monitoring of antimicrobial resistance.

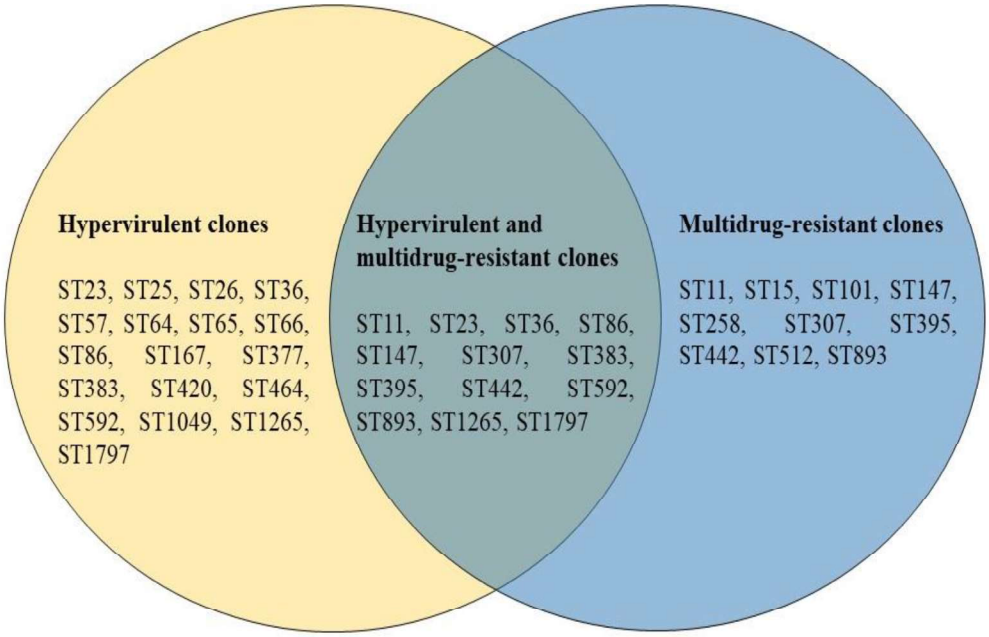

Figure 1.6 Hypervirulent and multidrug-resistant *K. pneumoniae* clones. The pale-yellow color indicates the sequence types that are associated with hypervirulent clones; the light blue color indicates the STs associated with multidrug-resistant clones; and the grey color indicates the STs associated with both hypervirulent and multidrug-resistant clones.

1.3.2 Genes involved in antibiotic resistance in *K. pneumoniae*

β-Lactam antibiotics are often used for the cure of *K. pneumoniae* infections. Once patients develop multidrug-resistant or prolonged drug-resistant *K. pneumoniae*, they must turn to alternative antibiotics such aminoglycosides, quinolones, polymyxins, tigecycline, and other similar drugs. Nevertheless, the use of these antibiotics in clinical settings might result in the emergence of drug resistance. The genes associated with antibiotic resistance were meticulously summarized, and their roles in *K. pneumoniae* are thoroughly described in **Table 1.1**.

Table 1.1 Genes associated with antibiotic resistance in *Klebsiella pneumoniae.*

Characteristic	Gene name	Gene function	References
β-Lactam	*bla*CTX-M *bla*SHV *bla*TEM	ESBLs	(Kliebe et al., 1985; Sirot et al., 1987; Li et al., 2018)
	*bla*GES *bla*KLUC-5 *bla*PER *bla*SFC *bla*TLA *bla*VEB	ESBLs and Carbapenemase (Plasmid mediated)	(Bradford, 2001; Philippon et al., 2016; P. Li et al., 2018)
	*bla*IMP *bla*KPC *bla*NDM *bla*OXA-48-like *bla*VIM	Carbapenemase	(Papp-Wallace et al., 2010; Lee et al., 2016)
	*bla*CMY *bla*DHA *bla*FOX *bla*MOX	AmpC type (Plasmid mediated)	(Jacoby, 2009; Bush, 2010)
Aminoglycoside	*aph* *ant* *aac* *16S rRNA methylase*	Plasmid mediated	(Galimand et al., 2003; Poulikakos & Falagas, 2013; Doi et al., 2016)
	AcrAB-TolC *kpnO* *kpnEF*	Efflux pump systems	(Srinivasan & Rajamohan, 2020)

Quinolone	*Topoisomerase IV* *DNA gyrase*	Quinolone-binding targets	(Nam et al., 2013)
	AcrAB *OqxAB* *kdeA* *aa(6')-lb-cr* *OmpK36*	Plasmid-mediated quinolone resistance	(Martínez-Martínez et al., 1996; Mazzariol et al., 2002; Ping et al., 2007; Ruiz et al., 2012)
Polymyxin	*phoPQ* *pmrA* *pmrD* *mgrB*	Regulatory gene	(Cannatelli et al., 2015; Poirel et al., 2015; Silva et al., 2021)
	mcr-1	Plasmid mediated	(R. Li et al., 2016; Lai et al., 2017)
Tigecycline	*AcrAB-TolC* *OqxAB* *tetA*	Efflux pump systems	(Osei Sekyere, 2016)
	RarA *RamA* *RamR* *acrR*	Regulators of efflux pumps	(Osei Sekyere, 2016)
	rpsJ	Encoding ribosome	(Villa, Carattoli, et al., 2013; Villa et al., 2014)
Fosfomycin	*fos*	Plasmid mediated	(P. Liu et al., 2020)

The presence of SHV beta-lactamase in the core genome of *K. pneumoniae* confers intrinsic resistance to ampicillin. It is worth noting that *K. quasipneumoniae* and *K. variicola* possess distinct variants of this beta-lactamase, referred to as OKP and LEN, respectively, which show

significant divergence (Holt et al., 2015). Presence of β-lactamase enzyme activity was first detected in 1940, predating the use of penicillin for therapeutic purposes (Bush, 2010). The presence of β-lactamases was spontaneously found in environmental isolates (Bush & Jacoby, 2010; Derbyshire et al., 2009). The first β-lactamase enzyme, known as the Temoneria (TEM-1) enzyme, was initially identified in 1965. Shortly after, the sulphydryl variable (SHV-1) β-lactamase was also discovered. These enzymes can provide Penicillin resistance, lacking resistance to cephalosporins (BMJ Group, 2008). In the past, the most prevalent types of β-lactamases found were TEM- or SHV-type, particularly in the United States. However, there has been a change, and now the most often identified extended-spectrum β-lactamase is of the Cefotaximase-Munich (CTX-M) type (Lewis et al., 2007; Van Der Bij & Pitout, 2012). The global spread of *Enterobacteriaceae* that produce ESBL, particularly *Klebsiella pneumoniae* and *E.scherichia coli* carrying the CTX-M gene, has been documented, showing a rising frequency over time (Hennequin et al., 2012). In order with AMR genes, a complete set of all resistance genes in a representative image from isolate M50 has been shown in **Figure 1.7.**

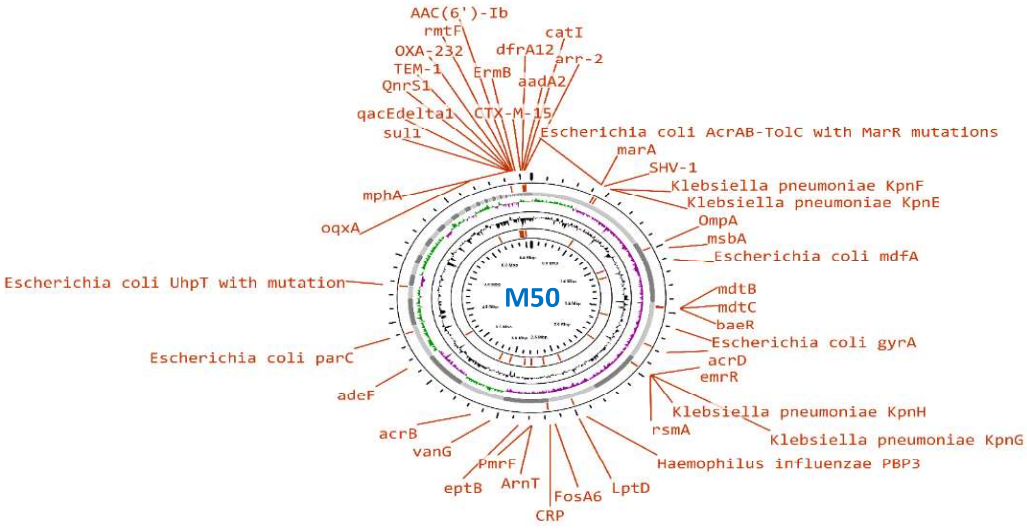

Figure 1.7 Complete set of drug resistance genes detected in genome of representative isolate M50. It shows the multiple types of AMR genes of different classes, including beta-lactamase (*OXA-232, CTX-M-15, SHV-1*, and *TEM-1*), and multiple other genes.

1.3.3 Plasmid diversity and mediated resistant in *Klebsiella pneumoniae*

The worldwide spread of drug-resistance genes is often connected with horizontal gene transfer facilitated by plasmids. Plasmid conjugation is possible in several species. Conjugated plasmids play a crucial role in transmitting clusters of antibiotic resistance genes (ARGs) in Gram-negative bacteria (Moran et al., 2019). Plasmids can be categorized into many groups based on the variations in common gene components that regulate replication or division (Novick, 1987). Additionally, each variety comprises numerous incompatible subgroups (Villa, Carattoli, et al., 2013).

Plasmid biology research has significantly advanced molecular genetics and yielded several important findings outside of the plasmid biology field (Cohen, 1993). Remarkably, the investigation of plasmids had already started prior to the elucidation of DNA's structure. The tests conducted to unveil bacterial conjugation and recombination used the plasmid F, which was then referred to as the "F factor" (Lederberg & Tatum, 1946). Subsequent investigations have shown that bacterial plasmids function vitally in determining the specific characteristics of bacteria that are significant in the fields of healthcare, manufacturing, and agriculture (Ramirez et al., 2014). The significance of plasmids in antibiotic resistance was first acknowledged in Japan when strains that were either sensitive or resistant to several antibiotics were recovered from the same patient during a single outbreak of dysentery (Watanabe, 1963)2. This observation indicated that sensitive strains were developing resistance to several drugs, not via a series of gradual genetic changes, but rather by acquiring the required genetic traits all at once. According to Watanabe and Fukasawa, this phenomenon was caused by the transfer of a plasmid (referred to as the resistance transfer factor, RTF, or R-factor) that contained the resistance genes (Watanabe & Fukasawa, 1961; Watanabe, 1963). *Klebsiella pneumoniae* is a well acknowledged source of antimicrobial resistance plasmids, renowned for its capacity to obtain and disseminate these plasmids within its own population and to other species (Wyres et al., 2020). In *K. pneumoniae* also, it was reported in many literatures that plasmids served as vehicles for both antibiotic resistance genes and genes or clusters of genes that determine features crucial or contributing to the virulence (Ramirez et al., 2014; Zhang et al., 2016; J. Turton et al., 2019; Yang et al., 2019 Aghamohammad et al., 2020; Hendrickx et al., 2020; Kopotsa et al., 2020; Booq et al., 2022; Hawkey et al., 2022; Shankar et al., 2022).

In the literature, many plasmid replicons have been reported to carry carbapenemases and ESBLs genes, listed in **Table 1.2.** Although *bla*NDM–1 has been detected on the chromosome of bacteria,

a large proportion of it is present on plasmids (W. Wu et al., 2019a). Currently 20 different incompatibility groups (Inc) of plasmids carrying *bla*NDM-1 in *Enterobacteriaceae*, including IncA/C, IncFII, IncFIA, IncFIB, and IncX3. These Inc groups represent the various ways that bacteria from common or different species can acquire *bla*NDM-1 and spread horizontally (W. Wu et al., 2019b). It is often discovered that the gene *bla*OXA-181 is situated on plasmids belonging to the incompatibility group X, which are classified as the X3 type (IncX3) (Pulss et al., 2017). The plasmids can spread several carbapenemase genes, such as *bla*NDM and *bla*KPC (Fortini et al., 2016; Ho et al., 2018).

Table 1.2 List of important carbapenemase and ESBLs genes and associated plasmid replicons

Beta-lactamase genes	Associated plasmid replicons	References
Carbapenemases		
*bla*NDM-1	IncF, IncFIIA, IncFIIk, IncFIIy, InX3, IncFIB-KQ	(Potron et al., 2011; Y. Jin et al., 2015; W. Wu et al., 2019a; Bocanegra-Ibarias et al., 2019; Toledano-Tableros et al., 2021; Hammad et al., 2023)
*bla*NDM-5	InX3, IncFII	(J. Shin et al., 2017; X. Yang et al., 2019; Kyung et al., 2022; L. Wei et al., 2022)
*bla*OXA-181	InX3, IncA/C, IncN, IncT, , IncFIIK, ColKP3, ColE,	(Villa, Carattoli, et al., 2013; X. Yu et al., 2019; C. Liu, Fang, et al., 2020; Naha et al., 2021)
*bla*OXA-232	ColKP3, ColE-type, IncF, InX3,	(Shankar et al., 2019; Nagaraj et al., 2021; M. Wang et al., 2022)

blaKPC-2	ColRNAI, IncFII, IncFIB, IncR, IncI2, IncX, ColE1, IncFIB(pQIL), IncFII(K)	(Fu et al., 2019; Stoesser et al., 2020; Sugita et al., 2021; Eda et al., 2021)
ESBLs and Others		
*bla*CTX-M-15	IncFIB(K), ColRNAI, IncFII, IncF, IncFIA, IncFIIk	(Carattoli, 2009; Zhuo et al., 2013; Agyepong et al., 2019; Kakuta et al., 2020; Y.-T. Lin et al., 2020)
*bla*OXA-1	IncFIB(K), ColRNAI, IncHI1B/FIB, IncFIB/FI, IncFII	(Agyepong et al., 2019; M. M. C. Lam et al., 2019; R. Li et al., 2016)
*bla*TEM-1	IncFIB(K), ColRNAI, IncX5, IncX6, IncFI, IncFII	(C. Liu & Guo, 2018; M. M. C. Lam et al., 2019; Agyepong et al., 2019; Y. Yang et al., 2023)

1.3.4 Prophages and their influence on AMR and pathogenicity

Prophages, which are DNA fragments derived from temperate phages, have been found in the genome of about 40-50% of microorganisms (Howard-Varona et al., 2017). The prophages replicate in coordination with the host genome, using a vertical transmission pattern. *Klebsiella pneumoniae* is positioned in close proximity to the lower 5th percentile among the species exhibiting the highest prophage abundance. This study implies that prophages are an important component of *Klebsiella* genomes and may have a significant influence on their biological activities (De Sousa et al., 2020). *Klebsiella* genomes often include a large number of prophages. *K. pneumoniae*'s genome comprises three categories of prophages: intact, questionable, and incomplete. These categories are established by their completeness ratings, which are assessed using PHASTER bioinformatics software. Regions having a total score less than 70 are considered as incomplete. A score between 70 and 90 is considered questionable. On the other side, if the score exceeds 90, it is classified as intact (Arndt et al., 2016). The use of lytic phages in phage therapy to specifically eliminate target bacteria has faced regulatory challenges, hindering the

progress of this therapeutic method. The possible use of prophage induction therapy in clinical settings entails specifically targeting antibiotic-resistant strains of bacteria and using it as a substitute for existing antibiotic-based therapies (Lakshminarasimhan, 2022a). When prophages become part of the bacterial genome, they may cause the phage genome to deteriorate or transfer genes to the host. This process can lead to the production of toxins and the emergence of antibiotic resistance. As a result, this increases the harmfulness and ability to withstand treatment of the bacteria. Prophage integration into bacterial communities is also crucial in bolstering fitness and propelling evolutionary mechanisms (Shukla, Joshi, et al., 2023).

Although prophages are generally quite stable, they may be selectively activated, resulting in the removal of DNA and the release of active phages (Oh et al., 2019; Hu et al., 2021). The activation of the lytic cycle in prophages leads to the destruction of lysogenic bacteria by lysis. An exceptional, diminutive chemical inducer specifically focuses on the lysogenic-lytic switch in prophages and has the capability to activate the lytic cycle, akin to the impacts of mitomycin C (MMC), diclofenac, ciprofloxacin, and digoxin (Sutcliffe et al., 2022). Gram-positive and gram-negative bacteria have been shown to induce the activation of bacteriophages in a rec A-dependent way when exposed to the DNA-damaging drug mitomycin C. There is a hypothesis suggesting that methylation DNA modification enzymes, also known as MMCs, might impede the capacity of a repressor protein to bind to an operator region, hence facilitating the commencement of the lytic cycle. Observation reveals that the biofilm saw a significant increase in the number of phages, leading to their aggressive reproduction. Consequently, the dispersion and diminution of the biofilm were a direct outcome of the introduction and infection of these phages (Dakheel et al., 2019). Knowledge of prophages is essential for studying the wide range of genetic variations in *K. pneumoniae* and developing a full knowledge of bacterial strains virulence and drug resistance.

1.4 Virulence factors and associated genes in *K. pneumoniae*

Klebsiella pneumoniae's ability to cause disease is linked to many virulence characteristics that enable it to resist the host's natural immune defenses. *K. pneumoniae* has several major virulence factors, such as capsules, exopolysaccharides that contribute to mucoviscosity, lipopolysaccharides (LPSs), adhesins, and iron absorption mechanisms (Riwu et al., 2022) as shown in **Figure 1.8**. These elements are essential in the adhesion, colonization, invasion, and advancement of infection. Additional virulence factors that have been later discovered include

outer membrane proteins, porins, iron transport systems, efflux pumps, and genes associated with allantoin metabolism (Paczosa & Mecsas, 2016). There are two categories of *K. pneumoniae* strains: "classic" and “hypervirulent” (Shon et al., 2013). Immunocompromised persons often have pneumonia, nosocomial infections, urinary tract infections, and newborn sepsis that are commonly associated with "classic," non-virulent *K. pneumoniae* (c-Kp) strains (Podschun & Ullmann, 1998). The emergence of hypervirulent strains of *K. pneumoniae* (hv-Kp) was first seen in Taiwan during the late 20th century. These strains were shown to be responsible for meningitis, liver abscesses, and endophthalmitis in previously healthy adult patients (Cheng, 1991; C. Liu & Guo, 2018). Most of the evidence for the high virulence of hv-Kp points to enhanced capsule formation, which may be stimulated by mucoviscosity-associated gene A (*magA*) and the regulator of the mucoid phenotype (*rmpA*) gene (V. L. Yu et al., 2007; Shon et al., 2013). The hypermucoviscous phenotype-based string test, with a string length of at least 5 mm, was often used as an indicator for hvKp. It was estimated to have an approximate accuracy of 90% for clinical strains of the bacteria (Russo & Marr, 2019a). One alternative technique to assess mucoviscosity was to measure the absorbance of the supernatants that were collected using low-speed centrifugation. This was carried out since strains of hypermucoviscous (hmv) material settle poorly, producing turbid supernatants (Walker & Miller, 2020).

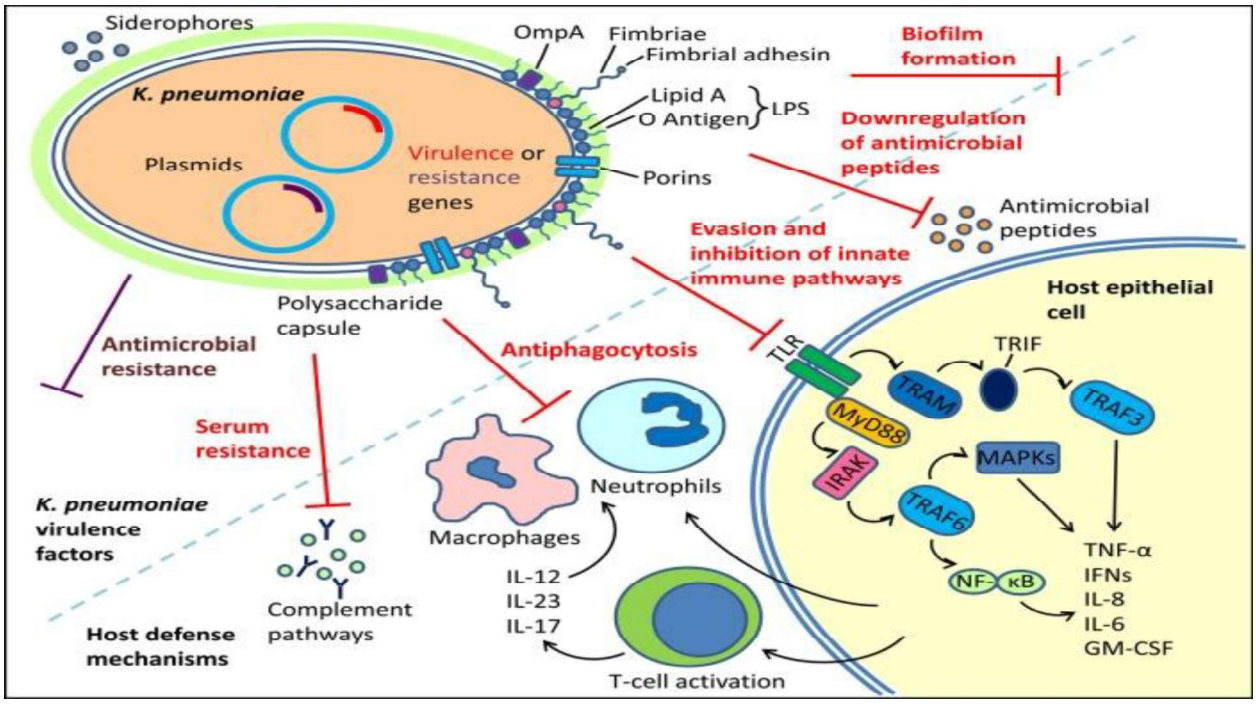

Figure 1.8 Overview of *Klebsiella pneumoniae* pathogenicity and host cell reaction. The capsular polysaccharide is essential for infection, preventing phagocytosis, and avoiding the host's innate immunological responses. Other outer membrane proteins and siderophores are also considered as additional virulence factors. Antimicrobial resistance exacerbates negative consequences, although it has little correlation with indicators of virulence. The presence of antimicrobial resistance or virulence genes expressed on plasmids has the potential to give rise to new and highly aggressive strains of carbapenem-resistant *K. pneumoniae* (Gomez-Simmonds & Uhlemann, 2017).

Evidently, a broad range of *K. pneumoniae* strains may lead to infections in people who are hospitalized (Holt et al., 2015; Runcharoen et al., 2017). Furthermore, it is worth noting that basic components contributing to the development of disease, such as type 3 fimbriae, capsular polysaccharide, lipopolysaccharide, and the siderophore enterobactin (*Ent*), are universally present in all strains of *K. pneumoniae* and remain unchanged in the chromosome as essential genes (Podschun & Ullmann, 1998; Holt et al., 2015). However, Siderophores, such as Salmochelin (*Iro*) (Müller et al., 2009), aerobactin (*Iuc*) (Nassif & Sansonetti, 1986), and yersiniabactin (*Ybt*) (Holt et al., 2015; M. M. C. Lam et al., 2018; Z. Lin et al., 2020) are three more siderophore systems that are particularly significant, that facilitate the absorption of iron by bacteria, resulting

in improved growth. In addition, the presence of *rmpA/rmpA2* enhances the functional benefit of serum resistance that is associated with hypermucoviscosity. While regulator of mucoid phenotype A (*rmpA/rmpA2*) were recently found as major virulence factors, capsular serotypes (K1/K2) and hypermucoviscosity (positive string test) have historically been viewed as virulence factors indicative of hvKp (Holt et al., 2015; Russo & Marr, 2019a).

It may be difficult to distinguish hvKp from cKp strains accurately, and there is ongoing debate about what constitutes each of these categories (Harada et al., 2018). Even though hvKp strains are hmv, mutations within the capsule locus may also cause cKp strains to possess this characteristic (wzc) (Ernst et al., 2020). HvKp is often characterized in some research as the mere existence of *iuc* genes (R. Zhang et al., 2016; C. Liu, Du, et al., 2020). Nonetheless, Russo and his colleagues conducted a comprehensive assessment of five specific genes (*rmpA, rmpA2, peg-344, iucA*, and *iroB*) as well as one phenotypic (hmv) to establish a clear definition of hvKP. This evaluation was performed on a sample of 175 isolates, consisting of both hvKP and cKP (Russo & Marr, 2019a).

There are some capsular serotypes associated with a higher degree of pathogenicity, known as "hypervirulence." (K1, K2, and K5) and accessory genes expressed by mobile genetic elements (MGEs) that are less common in the overall population of *K. pneumoniae* (Holt et al., 2015). Hypervirulent *K. pneumoniae* strains often show susceptibility to a broad spectrum of antibiotics, including cephalosporins and carbapenems, except for their inherent resistance to ampicillin. Nevertheless, there has been a recent rise in multidrug-resistant and hypervirulent (MDR-hv) strains, primarily caused by the horizontal transfer of resistance or virulence genes mediated by plasmids (C. Liu, Du, et al., 2020; Tang et al., 2020). Certain *K. pneumoniae* sequence types classified as "high risk," including the ST11, ST15, and ST383 clones, exhibited both drug resistance and virulence factor. Many components of these strains are carried on a large virulent plasmid (e.g., ST147 with *bla*NDM-1), which typically encodes heavy metal resistance genes (e.g., encoding resistance for tellurite, silver, copper, and lead), iron vector genes (e.g., aerobactin, salmochelin, and enterochelin), and capsule up-regulation genes (*rmpA* and *rmpA2*). Apart from c-Kp and hv-Kp, a third strain of *K. pneumoniae* has been identified recently, which is distinguished by a blend of antibiotic resistance and hypervirulence (Bialek-Davenet et al., 2014; Surgers et al., 2016; J. Zhang et al., 2023). The distinctive features of classical *K. pneumoniae*, hypervirulent *K. pneumoniae*, and Hypervirulent carbapenem-resistant *K. pneumoniae* (Hv-

CRKp) or carbapenem-resistant hypervirulent *K. pneumoniae* (CR-HvKp) have mentioned in **Table 1.3**. It is concerning that multiple isolates emerge with both resistance and virulence characteristics as convergent strains. With the greatest incidence in the Asian Pacific Rim, hvKP infections have expanded across Asia (Chung et al., 2007; Shankar et al., 2022b), Europe (Cubero et al., 2016; Rossi et al., 2018), Oceania (Foo et al., 2018; Sturm et al., 2018), North America (Fazili et al., 2016), South America (Coutinho et al., 2014), and Africa because of increased population migration. MDR-hvKP similarly followed a similar trend in terms of regional distribution, with most cases being reported in Asian nations despite a confirmed worldwide prevalence (W. Li et al., 2014; Tang et al., 2020). In addition to these key virulence factors, recent studies have demonstrated that clinical isolates associated with pyogenic liver abscess (PLA), such as NTUH-K2044, possess a unique chromosomal region containing genes associated with allantoin metabolism. This region allows K. pneumoniae to fix nitrogen. Furthermore, these isolates significantly expressed the allS gene, that is essential for growth in allantoin minimum media (Chou et al., 2004).

Table 1.3 Distinctive features of classical *K. pneumoniae* (cKP), hypervirulent *K. pneumoniae* (HvKP), and Hypervirulent carbapenem-resistant *K. pneumoniae* (Hv-CRKp) or carbapenem-resistant hypervirulent *K. pneumoniae* (CR-HvKp)

Parameters	**Classical *K. pneumoniae***	**Hypervirulent *K. pneumoniae***	**Hypervirulent carbapenem-resistant *K. pneumoniae* or carbapenem-resistant hypervirulent *K. pneumoniae***	**References**
Typical infections	Pneumonia, bacteremia, UTIs	Pneumonia; myositis; cellulitis; osteomyelitis;	Joint infections caused by HvKP and cKP	(Korvick et al., 1991; Meatherall et al., 2009;

		septic arthritis; kidney, lung, and neck, abscesses; meningitis; endophthalmitis; necrotizing fasciitis		McLaughlin et al., 2014; Patel et al., 2014; Fodah et al., 2014; Paczosa & Mecsas, 2016; L. Wang et al., 2017)
Susceptible populations	Immunocompromised people (diabetics, cancer patients, transplant recipients, bedridden people)	Diabetics, otherwise healthy individuals	Combined populations	(Fodah et al., 2014; Ku et al., 2017; McLaughlin et al., 2014a; Lan et al., 2019; M. Xu et al., 2019)
Serotypes	K1-K79	Primarily K1 and K2, occasionally K5 and K57	K2, K20, K47, and K64	(Korvick et al., 1991; Podschun & Ullmann, 1998; Fodah et al., 2014;

				Prokesch et al., 2016)
Siderophores (% prevalence)	enterobactin (100%), yersiniabactin (17-46%), aerobactin (6%), salmochelin (2-4%)	enterobactin (100%), yersiniabactin (90%), aerobactin (>93%), salmochelin (>90 %)	enterobactin (100%), yersiniabactin (90%), aerobactin (>93%), salmochelin (40%)	(Yeh et al., 2007; Holt et al., 2015; Pan et al., 2015; Follador et al., 2016; I. R. Lee et al., 2016)
Distribution	Global	Primarily the Asia-Pacific Rim, the global trend	Asia in particular, with a global trend	(McCabe et al., 2010; Bachman et al., 2011; El Fertas-Aissani et al., 2013; Moore et al., 2013; Fodah et al., 2014; Patel et al., 2014; Prokesch et al., 2016; Mgbemena et al., 2017; Odouard et

				al., 2017; Sturm et al., 2018; Paterson et al., 2022)
Prevalent form of infection	Generally nosocomial	Acquired by the community	Often nosocomial and seldom community-acquired	(Fodah et al., 2014; McLaughlin et al., 2014; Rossi et al., 2018; J. Turton et al., 2019; Effah et al., 2020)
Drug-resistance	Often (carbapenemase and ESBLs producing)	Rare apart from penicillin resistance.	Bears carbapenemase	(Fodah et al., 2014; McLaughlin et al., 2014; Rossi et al., 2018; J. Turton et al., 2019; Effah et al., 2020)

1.4.1 Capsule

It is often known that hypermucoviscosity and hypervirulence are related, although there are several situations in which this is not the case. The outer covering of *K. pneumoniae's* surface, known as the capsule, is the main factor responsible for its pathogenicity, which is composed of a complex layer of polysaccharides that are closely connected with the surface. The specific composition of the capsule varies significantly depending on the strain and the development of its viscous phenotype (Schembri et al., 2005). Capsule polysaccharides have a role in causing disease by helping the pathogen evade phagocytosis and survive in the presence of serum (Williams et al., 1983). Other tasks include safeguarding against dehydration and assault by phages. The majority of *Enterobacteriaceae* members possess the ability to generate a capsule, which has been closely linked to extraintestinal diseases, including septicemia, meningitis, and urinary tract infections (UTIs) (Tarkkanen et al., 1992; Podschun & Ullmann, 1998). In hvKp strains, the capsule size is greater than the normal range of cKp. In addition, it is very common to observe that hvKp bacteria obtained from the cerebral fluid exhibit a mean capsule size exceeding 2 mm (Ku et al., 2017). According to an investigation of an intranasal infection model, mice infected with acapsular strains of *K. pneumoniae* exhibited significantly greater survival rates and decreased bacterial density in the lung and blood compared to mice infected with capsular strains. This implies that a capsule's existence might provide defense against immune reactions (Lawlor et al., 2005; Yamaguchi et al., 2000). The resistance of capsules to bactericidal actions is mostly protective in nature, as opposed to aggressive. By preventing bacterial adherence, *K. pneumoniae* employ capsules as a defensive mechanism to avoid complement, antimicrobial peptides, phagocytosis, and certain antibodies. However, few reports exist on the rare instances of active suppression and immune cell assault caused by capsule use (Domenico et al., 1994; Llobet et al., 2011; Paczosa & Mecsas, 2016). Historically, the process of identifying and categorizing *K. pneumoniae* relied on capsular serotyping, often known as K typing. Nevertheless, this method has other drawbacks, such as significant labor requirements, frequent occurrence of cross-reactivity across different capsular groups, and the inability to classify strains that lack a capsule (Cryz et al., 1986). However, recent advancements in molecular type techniques have made it possible to classify isolates more effectively. These methods depend on analyzing the cluster of genes carrying the capsular antigen, namely by sequencing the wzc or wzi genes and typing the cps locus amplified by polymerase chain reaction (PCR) using phage-depolymerase mapping (Brisse et al., 2009, 2013; Pan et al.,

2015). All known *K. pneumoniae* capsule variants are synthesized by the Wzx/Wzy polysaccharide polymerization machinery, which is encoded by a single capsule polysaccharide (cps) biosynthetic locus (Whitfield, 1995; Pan et al., 2015). The variability of virulence characteristics among different capsule types of *K. pneumoniae* has been shown to be noteworthy. *K. pneumoniae* has a minimum of 79 distinct capsule serotypes that have been determined by serological methods (Mori et al., 1989; Pan et al., 2015), and over 140 serotypes have been found via the process of comparative genomics (Pan et al., 2015; Wyres et al., 2020). The variety of serotypes is a consequence of the rapid evolution of capsular loci, which occurs at a quicker pace relative to the rest of the genome. This is due to the high frequency of homologous recombination and horizontal gene transfer (Croucher et al., 2015; Holt et al., 2015; Wyres et al., 2020). There are primarily eight distinct capsular types of hvKp, namely K1, K2, K5, K16, K20, K54, K57, and KN1, with K1 and K2 being the most often seen (Pan et al., 2015; Wen & Zhang, 2015; I. R. Lee et al., 2016). Moreover, some analyses have demonstrated that K1 and K2 strains display higher levels of pathogenicity in comparison to populations associated with other serotypes. hence, serotype is considered a potential virulence determinant, perhaps because of distinct compositions that confer advantages in survival (Yeh et al., 2007; G. Wang et al., 2020). The application of multilocus sequence typing demonstrated that the variation in sequence types (ST) was more prominent in the capsule type of K2 serotype as compared to the K1 serotype. More precisely, it was demonstrated that K1 had a strong association with ST23, in contrast to K2 which was associated with ST25, ST86, ST375, and ST380, in that order (Struve et al., 2008; I. R. Lee et al., 2016).

Other than capsular polysaccharide, exopolysaccharides (EPS) are significant substances released by *Klebsiella* that may be seen outside the cell as a loosely linked slime layer or as a tightly bonded capsule. They serve a crucial function in combating desiccation, cell recognition, phagocytosis, antibiotics or toxic chemicals, phage assault, and osmotic stress (Angelin & Kavitha, 2020). EPS, or extracellular polymeric substances, have multiple functions. The consequences incorporate promoting bacterial interactions with their environment, protecting against harmful conditions and toxic agents (such as bile salts, hydrolyzing enzymes, lysozyme, digestive and pancreatic enzymes, ions of metal, and antimicrobial agents), coping with environmental pressures (such as fluctuations in the pH level, heat, or the salinity), evading immunity in animals, and defending against phage attacks (Castro-Bravo et al., 2017; Lynch et al., 2018).

1.4.2 Lipopolysaccharides (LPS)

The bacterial membrane of *K. pneumoniae* is anchored by lipopolysaccharides (LPS). The structure of LPS consists of three components - Lipid A, the bacterial membrane is anchored by a component consisting of an oligosaccharide core and a terminal side chain known as the O antigen. The O antigen, located at the outermost region of LPS, consists of recurring units of an oligosaccharide biopolymer. The O1 antigen is the predominant O antigen reported in clinical isolates of *K. pneumoniae* (Hansen et al., 1999). The *wb* (previously *rfb*) gene cluster governs the production, organization, and transmission of many structurally distinct O antigens. The structural differences of LPS are attributed to the varied repetitions of O antigens (Schnaitman & Klena, 1993; Whitfield, 1995). LPS O-antigens may bind to complement component C3b during *K. pneumoniae* infections, which hinders complement-mediate killing and increases bacterial survivability (Paczosa & Mecsas, 2016). Nevertheless, lipopolysaccharide may trigger inflammation by binding lipid A to TLR4, so initiating an inflammatory cascade that leads to the production of chemokines and cytokines to combat bacterial infection (Bengoechea & Sa Pessoa, 2019; Patro & Rathinavelan, 2019). In O-typing, which involves the *wzm* or *wzt* genes responsible for O-antigen transport, is also often used for O-locus typing process (Fang et al., 2016a; Follador et al., 2016). At present, 11 serotypes of O-antigen exist, each with a unique molecular structure: O1, O2a, O2c, O2aeh, O2afg, O3, O4, O5, O7, O8, and O12 (Patro & Rathinavelan, 2019), the variety is largely determined by variations in the content and sequencing of sugar monomers. While the O1-antigen is more common in clinical isolates of *K. pneumoniae* (Trautmann et al., 1997; Pennini et al., 2017), it has been shown that the O2-antigen is more abundant in strains that are resistant to several drugs. Specifically, the subtype O2afg of the O2-antigen provides enhanced survival in human blood serum (Szijártó et al., 2016; Pennini et al., 2017).

1.4.3 Siderophores

Siderophores are small molecules that have a strong attraction to iron and are used by bacteria to acquire iron for their growth and to enhance their ability to cause disease. The secretion of siderophore by *K. pneumoniae* during infection may affect the localization of tissues, the spread throughout the body, and the survival of the host (Holden et al., 2016). Iron is a crucial element for hosts and bacteria, necessary for important metabolic activities. However, the scarcity of iron in the extracellular fluid makes it challenging for bacteria to acquire it. This scarcity acts as a non-

specific immune defense mechanism. To ensure their survival and growth, bacteria must utilize strategies to overcome the challenge of acquiring iron. Human hosts typically need a siderophore-dependent iron acquisition mechanism due to the insolubility of free iron Fe^{3+} under normal physiological settings (J. Zhu et al., 2021). Siderophores are essential components for the development of *K. pneumoniae* infections (Paczosa & Mecsas, 2016). On the other hand, cKp and HvKp have different amounts of siderophores. Russo et al. demonstrated that hvKp produces a higher quantity of siderophores compared to cKp (Russo et al., 2014). In addition, they observed that elevated levels of siderophore production were linked to severe sickness or mortality in mouse sepsis models (Russo et al., 2021). The effectiveness of bacterial growth is enhanced by these four siderophores (Choby et al., 2020). The genes responsible for production of enterobactin (ent) are present in nearly all strains of *K. pneumoniae*. However, the biosynthesis genes for aerobactin (*iuc*) and salmochelin (*iro*) are often found in strains of hypervirulent hvKP. The yersiniabactin (*ybt*) biosynthesis genes are found in some hvKP and cKP isolates. These genes are often linked to the colibactin (*clb*) genotoxin genes, forming integrated and conjugative components like ICEKp10 (M. M. C. Lam et al., 2018). Prior studies have also shown that the hypervirulent strain has a characteristic 6- to 10-fold increase in siderophore production (Enterobacin, Salmochelin, Yersiniabactin, and Aerobactin) in comparison to cKp (Russo et al., 2021).

The production of obtained siderophores enhances the pathogenicity of *K. pneumoniae* via many methods. However, the absorption of iron through the often-conserved siderophore Ent is hindered by neutrophils and epithelial cells because of the secretion of lipocalin-2 (Lcn2). Lcn2 binds to iron-loaded enterobactin, hence hindering bacterial uptake of iron (Goetz et al., 2002). However, yersiniabactin, salmochelin, and aerobactin do not bind to Lcn2. Salmochelin is a glycosylated form of enterobactin, whereas yersiniabactin and aerobactin have a completely different structure from enterobactin. The capacity of salmochelin to counteract Lcn2 binding is crucial for proliferation of bacteria and empirically demonstrated to be linked with increased pathogenicity in a murine sepsis model (Fischbach et al., 2006). The correlation between aerobactin and virulence has been well discussed, as several studies have shown its crucial involvement in enhancing iron uptake, bacterial development, and/or pathogenicity in different murine models, blood, and human ascites fluid (Nassif & Sansonetti, 1986; Russo et al., 2021). Even in strains that include all four genetic regions responsible for producing siderophores, aerobactin seems to have the most crucial function in causing disease both in laboratory settings and in living organisms

(Russo et al., 2011) and acts as a significant indicator for detecting highly virulent isolates (Patel et al., 2014). Further genes involved in the metabolism of iron and other metabolic processes have been linked to the pathogenicity of *K. pneumoniae*. In mice, *kfu* is connected to invasive infection, enhanced virulence, and iron acquisition (Ma et al., 2005).

1.4.4 Type 3 Fimbriae

Type 1 and 3 fimbriae are integral parts of the cell membrane of *K. pneumoniae*. They have a crucial function in facilitating the attachment of bacteria to host cells, tissues, and environmental surfaces. The fimbriae facilitate this adhesion by binding to both biotic and abiotic surfaces (Sebghati et al., 1998; Langstraat et al., 2001; Struve et al., 2008). Originally identified in *Klebsiella*, type 3 fimbriae are now commonly observed in various groups within the *Enterobacteriaceae* family. There are two genes in the type 3 fimbrial genetic set: *mrkA,* responsible for encoding the main fibrial subunit, and *mrkD,* responsible for encoding an adhesin polypeptide that aids in attaching to collagen and cells of epithelial tissue in the basement membrane (Sebghati et al., 1998). The *mrkABCDF* operon encodes Type 3 fimbriae, commonly found in highly encapsulated *K. pneumoniae* isolates. These fimbriae have a significant impact on the production of biofilms (Jagnow & Clegg, 2003; C.-C. Wu et al., 2012). Analysis indicates that the majority of *K. pneumoniae* isolates obtained from hospital-acquired infections in the urinary or respiratory tract are capable of generating functional type 3 fimbriae (Ong et al., 2010). Furthermore, the ability of *K. pneumoniae* to establish and survive in mice was reduced when the presence of type 3 fimbriae was removed (Murphy et al., 2013). The secondary messenger produced by bacteria, c-di-GMP, controls the upregulation of virulence genes and the production of biofilms (Tamayo et al., 2007). Numerous investigations into *K. pneumoniae* have shown that the *mrkHIJ* gene cluster, which is located next to the type 3 fimbriae, is engaged in the regulation of type 3 fimbriae production as well as the sensing and modulation of cyclic di-GMP (c-di-GMP) (Wilksch et al., 2011; J. Yang et al., 2013; Murphy et al., 2013).