Chapter 1

Introduction and review of literature

Chapter 1

1.1 *Klebsiella* spp.

Bacteria belonging to the genus *Klebsiella* are Gram-negative, rod shape, non-motile, encapsulated, facultative anaerobes in the *Enterobacteriaceae* family. *Klebsiella* are opportunistic pathogens, mainly infect immunocompromised individuals such as patients with diabetes, alcoholism, malignancy, etc (Li et al., 2014). *Kp* colonizes human mucosal surfaces such as the gastrointestinal (GI) tract and oropharynx (Bagley, 1985). *Kp* ranks 2nd after *E. coli* in urinary tract infections (UTIs) and Bacteraemia (Song et al., 2021; Mendelson et al.,2005). *K oxytoca* is among the top 4 nosocomial pathogens found in UTI (Hoenigl et al., 2012) and commonly found in health-care associated infection (HAIs) (Martin and Bachman, 2018). *Klebsiella* spp. also cause pneumonia, bronchopneumonia, bronchitis, meningitis (Podschun and Ullmann, 1992). These bacteria reside in the environment like soil and surface waters) as well as on medical devices such as catheters and ventilators (Boucher et al., 2009). Initially, based on biochemical analysis and proteomic typing, *Klebsiella* isolates were designated three phylogenetically distinct groups or phylogroups namely, *Kp*I, *Kp*II, and *Kp*III. But now, they have been designated as distinct species: *Klebsiella* pneumoniae (*Kp*), *Klebsiella quasipneumoniae* and *Klebsiella variicola*, respectively (Holt et al., 2015; Brisse et al., 2014; Rosenblueth et al., 2004) based on Multi-locus sequence typing (MLST) and cgMLST (core genome-MLST) schemes, which can be used to differentiate among *Klebsiella* species (Brisse et al., 2009; Diancourt et al., 2005). Reports on comparative analysis of whole-genome sequences (WGS) has shown that these groups are distinguished by 3 to 4% average nucleotide divergence across the core genome. Species level identification in *Klebsiella* is difficult because of minor differences among the few species; however, recently, MALDI-TOF Mass Spectrometry is reported to be useful in identification of *Kp*, *Klebsiella* quasipneumoniae, *Klebsiella* variicola and related phylogroups (Rodrigues et al., 2018). Recent approaches use MALDI-TOF MS to identify colistin resistant *Kp* and other uro-pathogens as well (Dortet et al., 2020). *Kp* and *K. oxytoca* are the two epidemiologically and clinically important

Klebsiella species (Jonas et al., 2004). *K. planticola* and *K. terrigena*, were initially believed to be environment-restricted, but later they were also found in clinical specimens (Westbrook et al., 2000; Mori et al.,1989; Podschun and Ullmann., 1992). The average *Kp* genome is 5.5 Mbp in size and encodes 5500 genes. Core genome of *Klebsiella* includes fewer than 2000 genes while the accessory genome possess additional 3500 genes (Jolley, and Maiden, 2010).

1.2 Antibiotic resistance

Antibiotic resistance is the ability of microbes to grow in the presence of a chemical (drug) that would normally kill them or limit their growth. Antimicrobial resistance makes it harder to eliminate infections from the body as existing drugs become less effective. There are several major categories/classes of antibiotics, namely, beta-lactams, cephalosporins, aminoglycosides, fluroquinolones, quinolones, phenicols, glycycyclines, polymyxins, etc. Among these, beta-lactams are most important and broad class of antibiotics as beta-lactams are widely used to treat mild to severe infections caused by *Klebsiella.* Gram-bacteria possess various mechanisms for antibiotic resistance such as efflux activity through which antibiotics are effluxed out of bacterial cells, modification in outer layer of lipopolysaccharides (LPS), inactivation of drugs by using enzyme and by acquiring resistance genes on plasmids via horizontal gene transfer (HGT). Figure 1.1 shows different mechanism of antibiotic resistance in Gram-negative bacteria (https://www.sciencedirect.com/topics/chemistry/antimicrobial-resistance).

The increasing number of hospital and community-acquired infections by *Klebsiella pneumoniae* (*Kp*), especially by extended-spectrum beta-lactamases (ESBL) and carbapenemase-producing *Kp*, led to the declaration of *Kp* as an ‘urgent threat’ and ‘priority pathogen’ by public health agencies (CDC., 2013; WHO., 2014). Third-generation carbapenems were used to treat *Klebsiella* infection, but evolution of CRKP (carbapenem-resistant *Klebsiella pneumoniae*) was threatening. Hence, the last-resort drugs such as polymyxins (especially colistin) have been re-introduced for the treatment of carbapenem-resistant *Kp* infections (Poirel et al., 2017). However, emergence of colistin-resistant *Kp* left no therapeutic option and are associated with high mortality

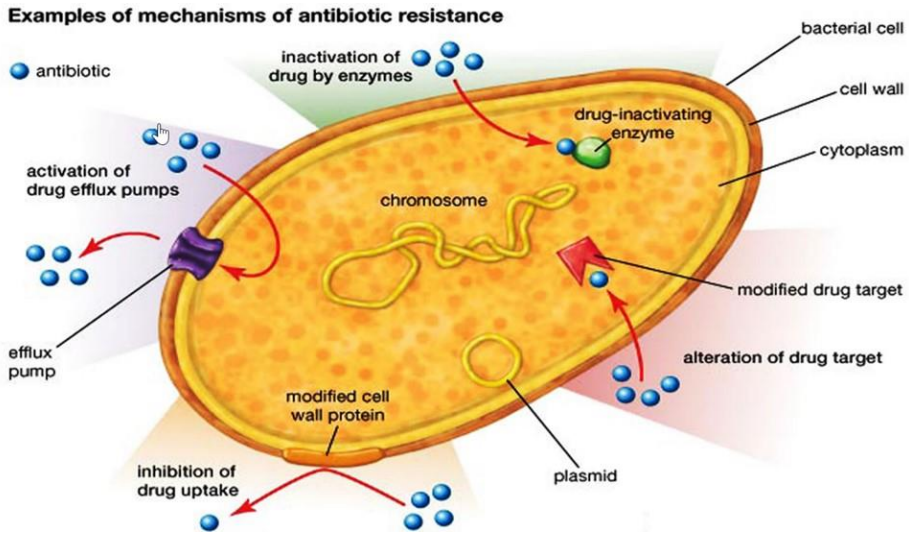

Figure 1.1 Mechanisms of antibiotic resistance in Gram-negative bacteria. Gram-negative bacteria possess various mechanisms for antibiotic resistance such as efflux activity through which antibiotics are efflux-out of bacterial cells, modification in outer layer of lipopolysaccharides (Source: https://www.sciencedirect.com/topics/chemistry/antimicrobial-resistance).

rates (Rodrigues et al., 2021; Guducuoglu et al., 2018; deMan et al., 2018; Tsala et al., 2018). In 1994, *Klebsiella* strain producing ESBL was reported, resistance to beta-lactams led to use of third generation cephalosporins against ESBL-producing *Klebsiella* strains (Paterson and Bonomo, 2005). Similarly, resistance to fluoroquinolones and other antibiotic classes was developed. Then, carbapenems are important type of beta-lactams as third generation carbapenems such as imipenem and meropenem were used as last-resort drugs (Raza et al., 2020 Jacquier et al., 2012) until the emergence of carbapenem-resistant Kp (CRKP) in 2015 (Yigit et al., 2001). Hence, to treat infections caused by CRKP, polymyxin (colistin) was reintroduced. Recently, colistin (polymyxin) and tigecycline (glycycline) are used as the last-resort antibiotics. recently, emergence of pan drug-resistant (PDR) *Kp* has developed resistance to the last-resort drugs and left no therapeutic option (Papadimitriou-Olivgeris et al., 2021; Chen et al., 2020; Bhardwaj et al., 2020; Nahid et al., 2017; Ghafur et al., 2014; Magiorakos et al., 2012). *Klebsiella* is categorized in three major categories based on the resistance pattern, namely, multidrug-resistant (MDR),

extensively/extremely drug-resistant (XDR) and pan drug-resistant (PDR) (Magiorakos et al., 2012). To categorize *Klebsiella* isolates, antimicrobial susceptibility tests such as disk diffusion methods and minimal inhibitory concentration (MIC) determination against selected antimicrobial agents belonging to various antimicrobial categories are performed.

Multiplex PCR is a rapid detection method for screening of numerous genes in one shot by using multiple sets of primers, each set corresponding to respective gene. Recently, multiplex PCR is reported to be used to identify ESKAPE pathogens (Li et al., 2020). Multiplex PCR has been used as an effective method to screen ESBL and carbapenemase producing genes in *Klebsiella* and *Pseudomonas* (Poirel et al., 2011; Ghasemi et al., 2013). Yu et al, have applied multiplex PCR for the detection of ST258 CRKP as well as ST23, ST65, ST86 hypervirulent strains (Yu et al., 2018). Other methods were also reported previously for amplification and expression of carbapenemase producing genes such as Triplex PCR (Jeong et al., 2013), qRT-PCR (Sun et al., 2010a) and PCR (Dong et al., 2015).

1.3 Genomics of antibiotic resistance in *Klebsiella* spp.

Application of genomics to study and monitor antibiotic resistance in recent years has provided very insightful information about the worldwide status of AMR (antimicrobial resistance) in *Klebsiella* (Sherif et al., 2021; Wyres et al., 2020; Wyres et al., 2019; Wyres and Holt, 2018; Lam et al., 2018; Poirel et al., 2017; Bowers et al., 2015; Chen et al., 2014). Wyres and Holt had insightfully explained the movement of *Kp* strains and acquisition of plasmids (Wyres and Holt, 2018). They showed that *Kp* strains are capable of spreading between niches in the environment, animal and/or human hosts, and acquire AMR genes and/or plasmids via HGT; *Kp* had also acquired numerous genes from different donor species (figure 1.2).

1.3.1 Multilocus Sequence Typing (MLST)

The rapid emergence of multidrug-resistant (MDR) *Kp* is mainly driven by the successful combination of resistance plasmids dissemination and wide

geographic spread of MDR *Kp* sublineages, or clones (e.g., CG15, CG101, CG147, CG258, CG307) (Wyres et al., 2020). Sequence type (ST) of *Klebsiella* is determined based on multilocus sequence typing (MLST) scheme. MLST is a scheme for unambiguous identification of *Kp* isolates universally, where the

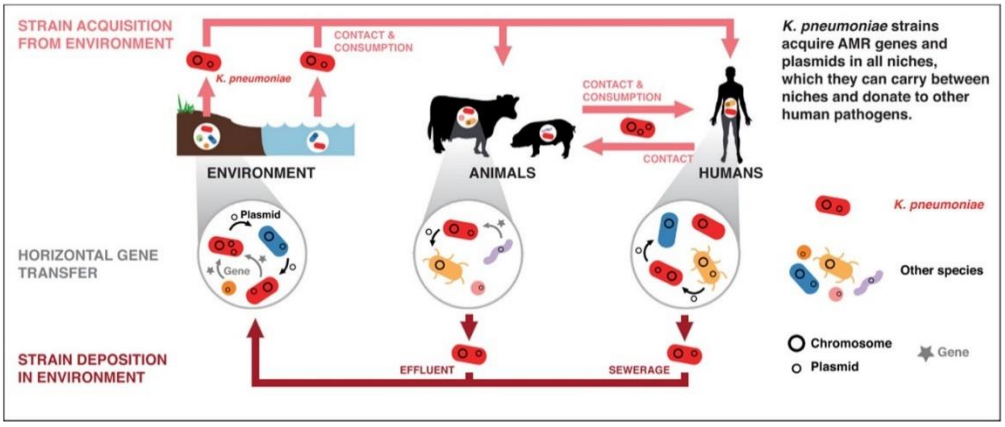

Figure 1.2 Acquisition of AMR genes and plasmids by *Kp* strains. *Kp* strains are capable of spreading between niches in the environment, animal and/or human hosts, and acquire AMR genes and/or plasmids. The spread or movement of strains between different niches occurred via contact or consumption of contaminated water sources or plant materials; spread from hosts back to the environment occurred via effluent or sewerage. Moreover, *Kp* strains can receive or donate plasmids via horizontal gene transfer (HGT) with a diverse array of donor species in any of these niches, this provides a pathway for transfer of resistance genes from environmental microbes to human pathogens (Source: Wyres and Holt, 2018).

sequences of seven housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpob,* and *tonB*) are considered to determine the MLST or ST of the isolate; the sequence types are further grouped by their similarity to a central allelic profile and form clonal groups (CG) (Diancourt et al., 2005); for an instance CG147 includes three STs namely, ST147, ST273 and ST392 (Lee et al., 2016). *Kp* strain belonging to certain STs such as ST147, ST231, ST258 are capable of successfully acquiring resistant plasmids and become clinically prevalent by clonal expansion and evolve as high-risk clone (figure 1.3) (Navon-Venezia et

al., 2017). Isolates belong to the same ST according to the multilocus sequence type (MLST) scheme, they may have been isolated in a different geographic

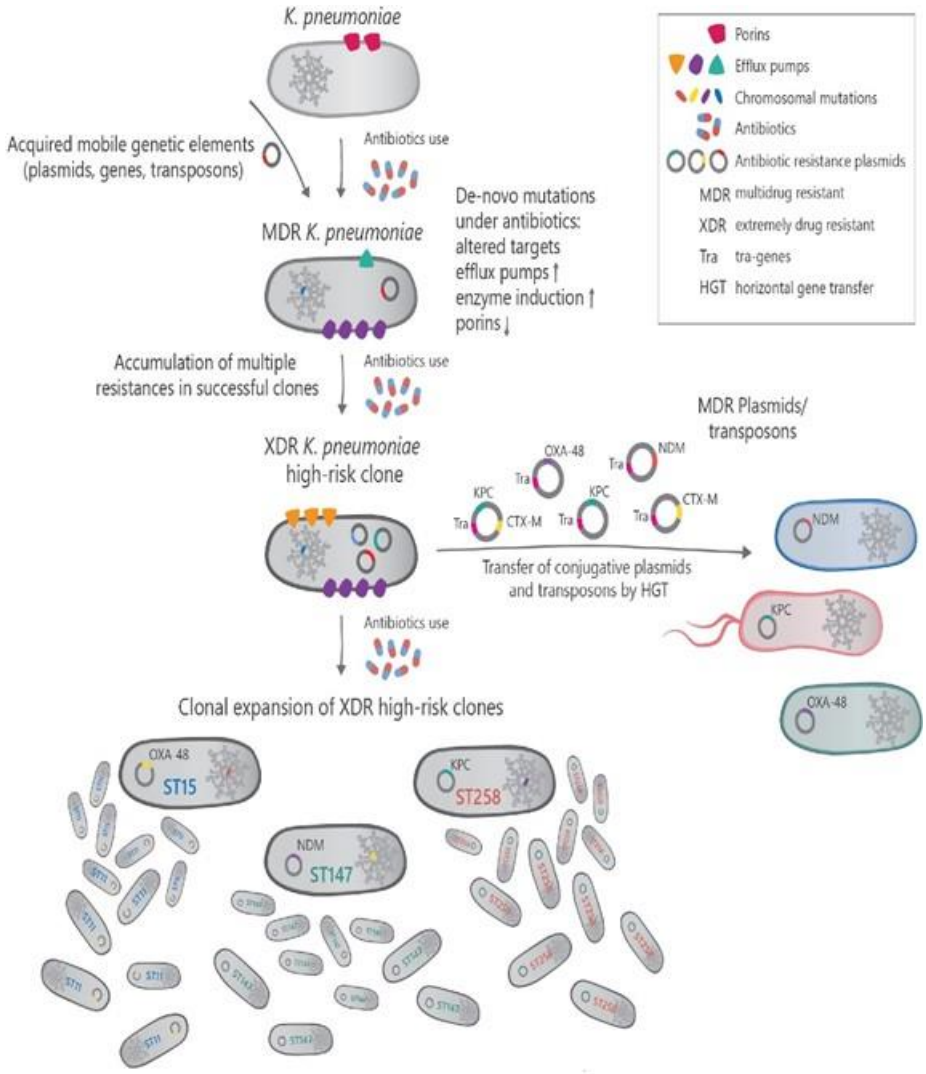

Figure 1.3 Emergence and spread of antibiotic resistance genes and high-risk clone of *K. pneumoniae*. *Kp* belong to certain STs (ST147, ST15, ST258) acquire resistance genes by means of mobile genetic elements (MGEs), becomes MDR or XDR and then becomes prevalent by clonal expansion. Emergence and spread of high-risk clones of resistant *Kp* is the result of successful combination of ST acquiring AMR genes (Navon-Venezia et al., 2017).

location, and time are called 'high-risk clones' (Woodford et al., 2011). Certain STs in *Kp* are considered more probable outbreak-causing agents as compared to others (Woodford et al., 2011; Chmelnitsky et al.2013). For instance, ST16,

ST20, ST48, ST307, and ST340, ST336 and ST395, although recognized as important international outbreaks clones (Navon-Venezia et al., 2017). These high-risk clones are often associated with various antimicrobial determinants. High-risk ST14, ST147, ST37 and ST101 tend to carry carbapenemases, whereas ST15 and ST17 harbour mostly ESBL genes; 40% of isolates belonging to ST15 and ST17 are CTX-M producers (Navon-Venezia et al., 2017). Further, a report from Taiwan suggested that the high-risk clones ST11, ST15, ST147 and ST37 all share the same *ompK36* allele (Yan et al., 2015). Wyres et al., reported that several globally distributed carbapenem-producing and ESBL-associated STs are present in South and Southeast (SE) Asia (Wyres et al., 2020). ST23 was significantly associated with SE Asia and ST14 and ST231 were both significantly associated with South Asia. In Indian isolates, OXA-232 variants of OXA-48-carbapenemase was found to be associated with ST231 (Shankar et al., 2019). Apart from clonal expansion, evolution of *Klebsiella* is also due to homologous recombination (Brisse et al., 2009). Initially, high-risk clones are usually associated with particular geographical locations, such as ST231 in India (Shankar et al., 2019; Wyres et al, 2020), ST147 in India and Greece (Peirano et al., 2020), ST258 in United States (Kitchel et al., 2009); ST307 in Italy (Bonura et al., 2015; Gona et al., 2014), Combodia (Ocampo et al., 2016), South Africa and United States (Long et al., 2017). ST307 and ST147 both were reported to be associated with various carbapenemases such as KPCs, NDMs, OXA-48-like, and VIMs (Peirano et al., 2020; Gona et al., 2014). The epidemic high-risk clones of certain STs then spread globally by means of international travel to endemic countries (Yong et al., 2009; Chen et al., 2017; Espenhain et al., 2018).

1.3.2 Mechanism/genes responsible for antibiotic resistance in *Klebsiella*

Two main types of antibiotic resistance mechanism are commonly observed in *Klebsiella*: 1) expression of ESBLs 2) Expression of carbapenemases. ESBLs exhibit resistance to broad range of β-lactams except carbapenems. Among others, *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{CTX-M}$ are important genes responsible for ESBL production in *Klebsiella* spp. In 2016 it was reported that ESBL-producing

strains caused 23% of nosocomial *Kp* infections, totalling 17,000 infections, and 1,100 deaths. Carbapenem-resistant *Kp* strains caused 11% of nosocomial infections, totalling 7,900 infections, and 520 deaths (CDC, 2017; Paczosa, and Mecsas, 2016). Carbapenemases provide resistance to almost all available ß -lactams, including the carbapenems (Lee et al., 2016; CDC., 2015; Chen et al., 2014). *bla*$_{NDM}$, *bla*$_{OXA}$ (Pitout et al., 2015), *bla*$_{KPC}$ (Yigit et al., 2001) are major genes responsible for carbapenemase production.

Resistance to beta lactams is mainly due to the presence of beta-lactamase enzymes which degrades beta-lactams. β-lactamases are the most abundant (Philippon et al., 2016) and important mode of resistance as there are total 2771 beta-lactamases detected so far (Bush., 2018). Serine β-lactamases alone contain more than 500 enzymes, including the most clinically significant ESBL variants, i.e., CTX-M-, TEM-, and SHV-type enzymes (Bush and Fisher, 2011). Further, each beta-lactamase contains numerous variants based on the changes in amino acids; for an instance, *bla*$_{OXA}$ alone contain n=95 and numerous variants (Evans, and Amyes, 2014). Each β-lactamase gene is classified in four classes, class A, B, C and D (figure 1.4).

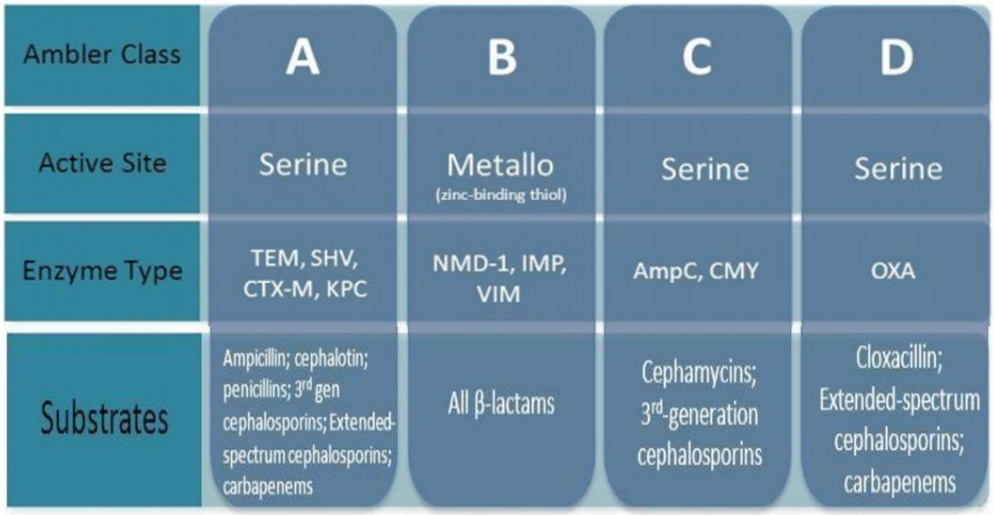

Figure 1.4 Classification of Beta-Lactamases. Beta-lactamases are classified into major four classes: A,B,C, and D. The above image shows active site, type of enzyme and substrates belonged to each class of beta-lactamase. (Source: Biosafety Risk Assessment; Department of Louisiana (Source: https://ldh.la.gov/assets/oph/Center-PHCH/Center-CH/infectious-epi/HAI/HAIworkshop2017/Fall2017/pdfs/Biosafety.pdf).

Class A, C and D are zinc independent Beta-lactamases, whereas, class B β-lactamases such as NDM-1, VIM, IMP are metallo-β-lactamases that require zinc or another heavy metal for catalysis (Jeon et al., 2015). Various class B and D carbapenemases were detected in hospital-acquired multi-resistant *Kp* infections (Nordmann et al., 2011a), whereas class C carbapenemases are not reported that commonly. Dissemination of major carbapenemase genes such as, *bla*$_{KPC}$, *bla*$_{OXA-48}$, and *bla*$_{NDM-1}$, are of particular concern. All three genes have been reported in diverse *Kp* lineages. The prevalence of *bla*$_{NDM-1}$ is worrisome because it can hydrolyse all β-lactams except aztreonam (Yong et al., 2009); but, the presence of ESBLs or AmpC β-lactamase hide this gap as they exhibit resistance to aztreonam (Molton et al. 2013). Presence of extra copies of carbapenemase genes were also reported to be responsible for higer resistance level (Chaves et al. 2001). *bla*$_{NDM-1}$ was first identified in ST14 isolate of UTI from a Swedish patient who travelled to India, New Delhi. (Yong et al., 2009). *bla*$_{OXA-48}$ recovered from Turkey in 2001 (Poirel et al., 2004). *bla*$_{KPC}$ was first recovered from United States in 1996 (Yigit et al., 2001).

Prevalence of carbapenemase genes varies geographically, for instances, *bla*$_{NDM-1}$ is endemic to Indian subcontinents including India, Pakistan, and Bangladesh (Nordmann et al., 2011b; Nordmann and Poirel, 2014). It was reported to be accounted for 75.22% of the carbapenemase-producing isolates in India (Kazi et al., 2015). *bla*$_{OXA-48}$ is most prevalent in Spain where prevalence of *bla*$_{KPC}$ is very low (2–3%; Oteo et al., 2013b; Palacios-Baena et al., 2016). On other hand, in USA *bla*$_{KPC}$ producing *Kp* is extremely prevalent (Rojas et al., 2013). In the Arabian Peninsula, *bla*$_{NDM-1}$ and *bla*$_{OXA-48-like}$ carbapenemases are more prevalent (Sonnevend et al., 2015b). The travel of patients for medical tourism and other international travels between countries might serve as a major factor in global dissemination of these endemic genes (Berrazeg et al., 2014). The carriage of resistance genes is also associated with clinically important high-risk sequence types; for instances, *bla*$_{NDM-1}$ with ST11 or ST147 (Lascols et al., 2013), *bla*$_{OXA-48-like}$ in ST11 (Oteo et al., 2013b), *bla*$_{KPC}$ with ST258 (Adler et al., 2014).

Regarding genes responsible to other antimicrobial categories, mutations in plasmid-mediated quinolone resistance (PMQR) genes (*qnrA*, *qnrB*) and

quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* genes are responsible for resistance to quinolones and fluoroquinolones (FQs) (Kotb et al., 2019; Mirzaii et al., 2018). Kotb et al., has demonstrated that FQs resistance is caused by interaction between PMQR genes and mutations in both *gyrA* and *parC* genes while quinolone resistance could result from mutation in one gene (Kotb et al., 2019). Several mechanisms and genes are involved in resistance to aminoglycosides like aminoglycoside-modifying enzymes (AMEs) such as AAC(3)-IIa, AAC(6′)-Ia, AAC(6′)-Ib C, AAC(3)-Ia C, etc are the most prevalent (Galani et al., 2019; Krause, et al., 2016). Another less common mechanism 16S rRNA methyltransferases (RMTs), which modify bacterial 16S rRNA, the molecular target of aminoglycosides and confer high-level resistance to all widely used aminoglycosides have been identified (Doi et al., 2016). armA, rmtA, rmtB, rmtC, rmtD, rmtE and npmA are reported so far in *Kp* (Yamane et al., 2004; Galimand et al., 2003). Few efflux pumps are also reported as an important mechanism of antimicrobial resistance. For instances, overproduction of efflux pumps like AcrAB and overexpression of RamA, a positive regulator of the AcrAB efflux system, are reported to be responsible for resistance to Tigecycline (Ruzin et al., 2005; Rosenblum et al., 2011; Sun et al., 2013). Similarly, mutation in *ramR* leads to overexpression of AcrAB efflux, results in resistance to tigecycline (Villa et al., 2014). *tetA*, *tetB* (Bokaeian et al., 2014) and class-1 integrons (Rezaee et al., 2012) are responsible for resistance to tetracyclines. Expression of *tetA* tightly regulates TetR, tetracycline-responsive repressor; TetR regulates TetA energy dependent efflux pump. Hence, mutation in tetA leads to activation TetA leading to resistance to tetracycline (Møller et al., 2016). Similarly, tetB also being an important tetracycline efflux protein, responsible for resistance against tetracyclines but not tigecyclines (https://card.mcmaster.ca/ontology/36305; CARD, Alcock et al. 2020). *strA* and *strB* are aminoglycoside phosphotransferase genes responsible for resistance to streptomycin (Massé et al., 2020; Kozak et al., 2009); *dfrA* encodes dihydrofolate reductase which is responsible for trimethoprim resistance (de Groot et al., 1996) and *ereA* encodes erythromycin esterase, responsible for resistance against erythromycin (Zhai et al., 2016; Arthur et al., 1990). *sul1, sul2* (encoding dihydropteroate synthases) and *dfr* (encoding dihydrofolafte reductase) have been reported to cause

resistance against trimethoprim/sulfamethoxazole in Gram-negative bacteria (Kor et al., 2013). *cat* genes encode chloramphenicol acetyltransferase and confer resistance to chloramphenicol (Roberts et al., 1980). *aadB* gene cassette is usually contained within class-1 integron and could result in resistance to aminoglycosides such as gentamycin, tobramycin and kanamycin (White et al., 2001; Jones et al., 2005). *aadA* is also associated with class-1 integrons and is responsible for resistance to streptomycin and spectinomycin (Sundström et al., 1988; Jones et al., 2005). *mphA* (Lalaoui et al., 2019; Noguchi et al., 1996) and *ermB* are phosphorylase and rRNA methylase genes (Robert et al., 1999), respectively and are responsible for macrolide resistance (Ojo et al.,2004).

Regarding resistance to the last-resort drug, colistin, MgrB is a small transmembrane protein (encoded by ***mgrB*** gene) that is produced upon activation of the PhoPQ signalling system, and acts as a negative regulator on this system (Lippa, and Goulian, 2009). Mutation in *mgrB* leads to upregulation of PhoPQ and PmrHFIJKLM, which results in alteration in LPS layer. This alteration in LPS limit the ability of cationic antibiotic like colistin to bind to the bacteria (Poirel et al., 2017) and hence, leads to resistance against colistin. Apart from *mgrB*, *mcr*-1 is also colistin-resistance gene, which encodes phosphoethanolamine (pEtN) transferase enzyme; this enzyme modifies the outer membrane lipopolysaccharides by decreasing the net negative charges (Karki et al., 2021; Liu et al., 2016). Several variants of *mcr* (*mcr-1* to *mcr-9*) are reported (Luo et al., 2020). A reduction in the proportion of colistin-resistant isolates harboring *mcr-1* from 2016 to 2017 is reported (Snyman et al., 2021). OqxAB efflux pump was shown to contribute to tigecycline resistance (Zhong et al., 2014). Many IncA/C plasmids with bla_{NDM-1} were reported to carry various antibiotic resistance genes including CMY-type β-lactamases, associated with broad- spectrum cephalosporin resistance; *qnrA*, associated with quinolone resistance (Pitout et al., 2015) and 16S *rRNA* methylases (RmtA, RmtC and RmtF), associated with aminoglycoside resistance (Lee et al., 2016). Alteration of porins are most common in carbapenemase-associated clones (Fajardo-Lubián et al., 2019. Specific mutations in the outer membrane porin OmpK36, particularly when coupled with loss of OmpK35, contribute substantially to the carbapenem-resistance problem in *Kp* (Wong et al., 2019;

Lunha et al., 2016). Porin defects can increase the MIC above the level conferred by acquired carbapenemase genes alone (Lunha et al., 2016).

Timeline of antimicrobial resistance (AMR) genes is shown in figure 1.5. All the genes shown did not originate in *Kp*, but they were first found in mobile form (i.e. within mobile genetic elements on plasmids) in *Kp* isolates. These mobile genes shown in figure 1.5 have been reported in *Kp* first and since been reported in clinically important Enterobacteriaceae and other Gram-negative bacteria (Wyres and Holt, 2018).

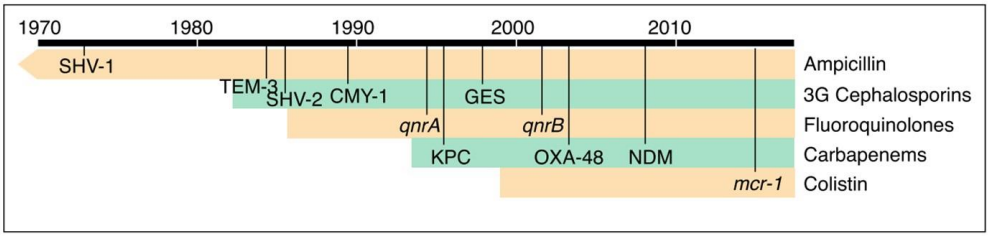

Figure 1.5 Timeline of antibiotic resistance genes first detected in *Kp*. Respective year indicates timeline for detection of the resistance genes (as labelled in timeline) in *Kp* for the first time in mobile form. Color-shaded area (yellow and green) indicates the period since which *Kp* isolates remained resistant to each drug class (*right-side*) (regardless of mechanism) (Source: Wyres and Holt, 2018).

Super resistome harbouring *Kp* was found to carry 4 different β-lactamases on multiple plasmids (*bla*$_{\text{NDM-1}}$, *bla*$_{\text{CMY-16}}$, and *qnrA* on one plasmid and *bla*$_{\text{OXA-48}}$ and *bla*$_{\text{CTX-M-15}}$, each on separate plasmids), along with porin deficiency, quinolone and additional antibiotic resistance genes (Seiffert et al., 2014). These super strains were reported to be resistant to all available categories of antimicrobials, and challenging to the clinical field (Munoz-Price et al., 2013; Nordmann and Poirel, 2014).

1.3.3 Mobile genetic elements involved in antibiotic resistance

Mobile genetic elements (MGEs) are segments of DNA that encode enzymes and other proteins that mediate the movement of DNA within genomes (intracellular mobility) or between bacterial cells (intercellular mobility) (Frost

et al., 2005). MGEs includes plasmids, transposons insertion sequences (IS) or insertion sequence elements (ISEs) and integrative conjugative elements (ICEs) can generate significant genotypic and phenotypic variation within bacterial populations, driving the emergence of niche- or host-adapted lineages or pathotypes (McNally et al., 2016). This pool of mobile genetic elements is called 'mobilome' (Frost et al., 2005). Transformation, conjugation and transduction and homologous recombination are the mechanisms of genetic transfer (Thomas, 2000).

Various MGEs are reported to be associated with dissemination of resistance genes over the years (Shankar et al., 2019; Hudson et al., 2014; Partridge et al., 2011). Each of the carbapenemase genes is associated with various MGEs that mobilises it between different plasmid backbones (which can then disseminate to other strains and species) and sometimes into the chromosome of *Kp* itself (Sheppard et al., 2016; Zowawi et al., 2015). Table 1 shows the list of plasmids and MGEs associated with carbapenemase genes. Complete or truncated ISAba1 is often observed upstream of *bla*$_{\text{NDM-1}}$, suggesting a role of IS in dissemination of *bla*$_{\text{NDM-1}}$ (Czobor et al., 2016; Nordman et al., 2011). *bla*$_{\text{NDM}}$ in *Kp* have always been reported to be carried by plasmids, for instances, *bla*$_{\text{NDM-1}}$ on IncFI (Zheng et al., 2016); *bla*$_{\text{NDM-5}}$ on IncX3 (Yuan et al., 2019); *bla*NDM-7 on IncA/C2 (Shankar et al., 2019) and IncX3 (Chen et al., 2020). Only Sakamoto et al., reported the presence of *bla*$_{\text{NDM-1}}$ on chromosome of *Kp* (Sakamoto et al., 2018). Other mobilization mechanisms such as mobilization of *bla*$_{\text{NDM-1}}$via IS26 or ISCR1 have also been reported (Doi et al., 2014; Chen et al., 2014). *bla*$_{\text{CTX-M-15}}$ is also reported to be mobilised by ISEcp1 (Coelho et al., 2010). *bla*$_{\text{CTX-M-15}}$ is most commonly observed to be associated with IncFII plasmids in *Kp* that also carry other antimicrobial resistance genes (Markovska et al.,2014; Stoesser et al., 2014; Dolejska et al., 2013). Regarding the association between MGEs and *bla*$_{\text{OXA}}$ genes, previously it is reported that *bla*$_{\text{OXA48-like}}$ genes are always carried by plasmids. Initially, the spread of *bla*$_{\text{OXA-48-like}}$ genes was mediated by IncL (Wang et al., 2014). Later, they have been reported among other plasmid types such as IncH, IncA/C, IncX3 and Col*KP*3 (Pitout et al., 2015; Ma et al., 2015; Mataseje et al., 2017., Lutgring et al., 2018). *bla*$_{\text{OXA-232}}$ is reported to be strongly associated with Col*KP*3 plasmid (Lutgring

et al., 2018). In *bla*$_{OXA48\text{-}like}$ carbapenemases are also known to be present on transposons Tn1999 and Tn1999.2; on Tn19999, it was observed to be flanked by IS1R (Beyrouthy et al., 2014; Cuzon et al., 2011). Association between *bla*$_{OXA232}$ and ISEcp1 is reported among isolates from France and Brunei belonging to ST14 and ST231 (Momin et al., 2017; Potron et al., 2013). Regarding Indian isolates, Shankar et al., reported that the North Indian isolates belonging to ST14 carried MGEs from IS5 and Tn3 families, while isolates from South India carried MGEs from IS1, IS5 and IS630 families (Shankar et al.,2019). Collectively, studies involving a comparative genomic analysis of MGEs demonstrated that homologous recombination at multicopy sequences (Partridge et al., 2011), site-specific recombination by resolvases (Sampei et al., 2010), switching of integron cassettes, and transpositions play a key role in the mosaic nature of *Klebsiella* plasmidome and resistome (gene pool of antibiotic resistance genes) (Hudson et al., 2014).

Table 1 Carbapenemase genes associated with plasmids and mobile genetic elements (MGE).

Carbapenemase genes	**Associated plasmid/MGE**	**References**
*bla*NDM-1	IncFI	Zheng et al., 2016
	IncA/C	Carattoli et al., 2012; Lascols et al., 2013
	IncH	Dolejska et al., 2013
	mobilisation by IS26 or ISCR1	Doi et al., 2014; Chen et al., 2014
	IS*Aba1*	Nordmann et al., 2011
	Chromosome	Sakamoto et al., 2018
*bla*NDM-5	IncX3	Yuan et al., 2019
	Chromosome	Rodrigues et al., 2021
*bla*CTX-M-15	IncFII	Markovska et al.,2014; Stoesser et al., 2014; Dolejska et al., 2013
	IncH	Vila et al., 2012
	mobilisation by ISEcp1	Coelho et al., 2010

*bla*OXA-48-like	IncL/M	Mataseje et al., 2017; Guo et al., 2016; Poirel et al., 2012
	IncA/C	Ma et al., 2015
	IncX3	Wang et al., 2014; Mataseje et al., 2017
*bla*OXA-232	Col*KP*3	Lutgring et al., 2018; Mataseje et al., 2017
	Tn1999	Beyrouthy et al., 2014; Aubert et al., 2006
	Tn1999.2	Cuzon et al., 2011
	ISEcp1	Momin et al., 2017; Potron et al., 2013
*bla*KPC-3	pKpQIL	Leavitt et al., 2010
	IncFIIK	Chen et al., 2013; Andrade et al., 2011; Tofteland et al., 2013
	Tn*4401*	Chen et al., 2014

1.4 Virulence genes

Virulence genes encoding basic virulence factors such as lipopolysaccharide, capsular polysaccharide, type-3 fimbriae (*mrkABCD* cluster) and enterobactin (*ent*) siderophore are common to all *Kp* and conserved in the chromosome as core genes (Holt et al., 2015). However, genes encoding additional siderophore system such as yersiniabactin (Ybt) (Holt et al., 2015; Lam et al., 2018; Lin et al., 2008), aerobactin (*iuc*) (Nassif et al., 1986) and salmochelin (*iro*) (Müller et al., 2009) are of particular concern. Yersiniabactin (*ybt*) and other siderophore systems are considered to be key bacterial virulence factors as they provide mechanisms for scavenging iron (an essential nutrient) from host transport proteins, thereby enhancing the ability of bacteria to survive and replicate within the host (Gorrie et al., 2017; Runcharoen et al., 2017; Ramirez et al., 2014;). Yersiniabactin is a high-virulence determinant, present in approximately a third of clinical isolates, and is significantly associated with strains isolated from

bacteremia and tissue-invasive infections like liver abscess, compared with those from non-invasive infections (Lin et al., 2008; Holt et al., 2015). Ybt biosynthesis is encoded by the *ybt* locus, which is typically located on a chromosomal ICE known as ICE*Kp,* of which there are at least 14 distinct variants called ybt;ICE*Kp* variants. Ybt was also reported to be present on plasmids (Russo et al., 2015 and 2018; Lam et al., 2018); In *Kp*, ICE is associated with synthesis and mobilization of ybt locus (Lin et al., 2008). *iuc* appears to play the most critical role in both *in vitro* and *in vivo* virulence (Russo et al., 2015) and serves as an important biomarker for identifying hypervirulent isolates (Russo et al., 2018). *rmpA* (regulator of mucoid phenotype) is a gene associated with synthesis of capsular polysaccharide (CPS). These are the major virulence genes associated with *Klebsiella* genomes. None of the virulence genes were reported to have a significant association with mortality; however, pathogenic *Kp* harboring single to multiple virulence genes can cause complicated infections (Remya et al., 2019).

Convergence of antimicrobial resistant genes and virulence genes is threatening as it's a dual-risk. Few studies have investigated the occurrence of convergence in *Kp* strains (Wyres et al., 2020; Bialek-Davenet et al., 2014; Holt et al., 2015). Wyres et al., reported that South and Southeast Asia could be the hub for convergent *Kp* isolates. Further, they reported that i*uc* + ESBL isolates were more common in South Asia, whereas, *iuc* + CP *Kp* were detected only in India (Wyres et al., 2020). Holt et al., observed that many isolates of the epidemic *bla*$_{KPC}$-producing ST258/ ST11 clonal complex (CC258) already have acquired yersiniabactin (Holt et al., 2015). Hypervirulent carbapenem-resistant *Kp* ST11 isolate in China was also reported to acquired portions of the hypervirulent virulence plasmid pLVPK, and also contained several virulence factors like *rmpA* (Gu et al., 2017). Recently, Yang et al reported that convergence of CRKP and hvKp occurred due to plasmid recombination and fusion processes. They reported that plasmids contain numerous transposable elements, which facilitate undergo frequent genetic transpositions; this results in plasmid fusion (Yang et al., 2021).

1.5 Virulence factors of *Klebsiella* spp.

Kp is an extremely resilient bacterium and its mechanism of following the model of "the best offence for a pathogen is a good defence" led it to be a successful pathogen (Paczosa, and Mecsas, 2016). This is explained by the ability of these bacteria to evade and survive, rather than actively suppress, many components of the immune system and grow at many sites in hosts. Hence, virulence factors of *Klebsiella* plays a crucial role in pathogenesis of *Klebsiella* spp. Virulence factors are the molecules produced by pathogens that contribute to the pathogenicity of the organism and enable them to achieve the colonization or helps in their survival inside the host (this includes attachment to cells). Capsule or capsular polysaccharides (CPS), exopolysaccharides (EPS), biofilm, siderophores and lipopolysaccharides (LPS) are major virulence factors of *Klebsiella* (Wang et al., 2020). Capsule are the outermost layer of the cell which is made up of acidic polysaccharides called capsular polysaccharides (Lawlor et al., 2006). Capsule play a role in resistance/inhibition of phagocytosis and protects bacteria from engulfment by phagocytic cells (Domenico et al., 1994). Capsule also protects bacteria from desiccation, and from phage predation (March et al., 2013). Exopolysaccharides are polymers linked to the cell surface via covalent bond to phospholipid or lipid A molecules (Wang, 2016). They are loosely bound to the outer cell surface and often sloughed off to form slime (Taylor and Roberts, 2005). EPS are important virulence factors as they play most prominent role against desiccation, phagocytosis, cell recognition, phage attack, antibiotics or toxic compounds and osmotic stress (Angelin and Kavitha, 2020). Biofilm is a web-like 3-Dimentional structure made up of bacterial cells, proteins, eDNA and exopolysaccharides (Donlan., 2002). Biofilm formation by *Klebsiella* is a critical problem in UTIs and other nosocomial infections (Wang et al., 2020; Piperaki et al., 2017). Siderophores are proteins with a high affinity for iron and during infection inside a host tissue, siderophores helps the bacteria in iron-acquisition and transport (Podschun et al., 1993). *Klebsiella* produce four types of siderophores: enterobactin, salmochelin, yersiniabactin, and aerobactin. LPS is an outer membrane and necessary component of outer leaflet of Gram-negative bacteria (Frirdich and Whitfield, 2005). LPS typically comprised of an

O-antigen, a core oligosaccharide, and lipid A (Cortés et al., 2002). LPS are usually potential activators of immune system, but *Kp* and *K. oxytoca* can modify its LPS during infections and O-antigen present in LPS layer inhibits subsequent activation of the complement pathway (Montminy et al., 2006).

1.5.1 Capsular polysaccharides

Polysaccharide capsule of *Klebsiella* is a major virulence determinant and epidemiological marker (Zang et al., 2020; Wyres et al., 2016; Hsu et al., 2011). Capsule is synthesized by *wzy* polymerization pathway (Rahn et al., 1999; Whitfield, 2006) and the associated genes are present in the capsule synthesis locus, which is known as **K-locus**. K-locus is 10-30 kbp long and includes a set of genes in terminal regions that encodes core machinery for capsular biosynthesis. Genes involved in *Klebsiella* capsule synthesis are *galF*, *wzi*, *wza*, *wzb*, *wzc*, *gnd* and *ugd* (Fevre et al., 2011; Pan et al., 2008 and 2015; Shu et al., 2009; Yu et al., 2006). The central region of capsular locus is highly variable, and it encodes the synthesis of capsule specific sugars, export proteins as well as the core assembly components *wzx* (flippase) and *wzy* (capsule repeat unit polymerase) (Pan et al., 2015). Each K-locus type of *Klebsiella* is designated with a unique number, which is called **KL-type**. *Kp* is also identified based on its K-type. **K-type** is determined by type of K-antigen present in capsular polysaccharide layer (Wyres et al., 2016; Choi et al., 2020). However, there are only 77 immunologically distinct K-types **(serotypes**) defined by serology (Edwards and Fife, 1952), further 10-70% of *Kp* isolates are serologically non-typeable (Jenney et al., 2006). Hence, the concept of identifying or designating KL-type was introduced using different sequencing techniques such as ***wzi* sequencing**, *wzy*-PCR based typing (Brisse et al., 2013; Pan et al., 2013), RFLP or C-typing (Brisse et al., 2004), *wzi* and *wzy* single gene typing (Bialek-Davenet et al., 2014; Bowers et al., 2016; Zhou et al., 2016) etc. Regardless of numerous methods some of the isolates still remained non-typeable. Hence, Wyres et al., developed the tool named Kaptive to identify the K-locus type of the isolate (Wyres et al., 2016). Distinct capsular types are reported to be associated with various invasive infections (Choi et al., 2020). For instances, K1, K2 and K5 are associated with liver abscess conditions, other tissue-

invasive diseases and enhanced pathogenicity (Liu et al., 2020; Shen et al., 2017); and K3 is restricted to the rare rhinoscleromatis lineage (ST67) (Brisse et al., 2009). K1 K2 serotype are also reported to cause upper and lower UTIs (Remya et al., 2018; Nagano et al., 2008; Podschun and Ullmann, 1998). Nagano et al., also demonstrated that serum killing resistance was significantly higher in *K. pneumoniae* K2 than in K1 (Nagano et al., 2008). K1 K2 serotypes were also reported to be associated with hypervirulence (Surgers et al., 2016; Fang et al., 2007; Fung et al., 2002).

1.5.1.1 Capsular polysaccharides and Phagocytosis

Phagocytosis is a process by which phagocytic cells of host immune system engulf pathogen. The bacterial capsule is the first line of defense for most bacterial pathogens. Capsular polysaccharides resist the binding with phagocytic cells, evade phagocytosis and opsonization by macrophages and neutrophils; thus, confers resistance from phagocytosis (Cress et al., 2014). Murphy et al., demonstrated the interaction between *Kp* isolate from bacteraemia and human serum and observed that the capsule of serum resistant *Kp* isolates provides defence mechanism against complement-mediated lysis (Murphy et al., 2012). Further, it was demonstrated that capsules possessing the Man-α-2-Man sequence exhibit a greater binding and susceptibility to phagocytic cells, this phenomenon is known as lectinophagocytosis. Thus, the capsule influence susceptibility to phagocytosis by direct binding to these cells; the composition of capsule influences opsonophagocytosis. (Clegg *et al*., 2016)

Other factors influencing the resistance against phagocytosis were also reported such as String phenotype (Shon et al., 2013; Yu *et al*, 2006) and K-type of *Klebsiella* isolates (yeh et al., 2007; Lin et al., 2004). **String phenotype** is defined as formation of a viscous strings of >5mm in length when a loop is used to stretch the colony on an agar plate (Shon et al., 2013). Lin et al., described that K1/K2 isolates of *Kp* exhibit more resistance to phagocytosis compared to nonK1/K2 isolates in case of liver abscess conditions (Lin et al., 2004). Yeh et al., also reported that K1/K2 capsular serotypes are major determinants of virulence of *Kp* rather than *magA* and *rmpA* (yeh et al., 2007). Recently,

Panjaitan et al., demonstrated an association of CPS production with two phosphoenolpyruvate carbohydrate phosphotransferase systems (PTSs) and showed that PTS plays a role in bacterial resistance to phagocytosis (Panjaitan et al., 2021). Recently few studies have discussed that CPS of *Kp* could be used as a potential target for vaccine development and other preventative treatment (Oloomi et al., 2020; Ravinder et al., 2020; Opoku-Temeng et al., 2019).

1.5.2 *rmpA*

Regulator of mucoid phenotype (*rmpA*) is reported to play a crucial role in the development of urinary tract infection and pneumonia (Ikeda et al., 2018). It is reported to be associated with capsular synthesis as expression of *rmpA* could lead to increased production of CPS. rmpA is a plasmid-borne transcriptional regulator (Hsu et al., 2011); DNA-binding domain in the C-terminal region of rmpA activates transcription of *cps* genes by binding to the promoter region of these genes (Yu et al., 2006). Certain environmental factors affect the expression of *rmpA*, for instances, an increased glucose concentration result in the upregulation of capsule production though rmpA, while relatively high extracellular iron concentrations result in the downregulation of capsule production (Russo and Marr, 2019). *rmpA* is also responsible for hypercapsule production, which led to hypervirulent (hv) strains of *Klebsiella* (Russo and Marr, 2019; Lai et al., 2003). It is reported that 55 to 100% of hv *Kp* strains express at least one copy of *rmpA* or *rmpA2* (homologous gene of *rmpA*), compared to 7 to 20% of non-hvKp strains (Li et al., 2014; Hsu et al., 2014). *rmpA* has been also reported to activate CPS biosynthesis in *Kp* CG43 via a regulator of capsule synthesis, *RcsB*-dependent manner. The expression of rmpA is regulated by the availability of iron and is negatively controlled by Fur (ferric uptake regulator) (Cheng et al., 2010). **Hypervirulent *K. pneumoniae* (hvKp)**, it has emerged as an important pathogen capable of causing community-acquired and HAIs than classical Kp (cKp); unlike cKp, hvKp often causes infection in otherwise healthy individuals (Pomakova et al., 2012; Fang et al., 2007; Lin et al., 1986). hvKp was first recognized as a cause of pyogenic liver abscesses in Asia (Lederman et al., 2005).

Collectively, the hypermucoviscous phenotype is reported to be resulted from increased capsule production that is regulated, at least in part, by *rmpA* (Chenge et al., 2010). String positive phenotype is reported to be an important trait of hypermucoviscosity (Fang et al., 2007). Further, this hypermucoviscous *Kp* is reported to be hypervirulent in nature (Chenge et al., 2010). Capsular serotypes K1 and K2 are known to be associated with hypervirulence (Fung et al., 2002; Lin et al., 2004). Hence, it's important to study the virulence factors like CPS, *rmpA* and string phenotype to determine their role in virulence as well as association between these virulence factors in clinical isolates of *Klebsiella spp*.

1.5.3 Exopolysaccharides (EPS)

Kp is known to produce copious amounts of EPS. EPS plays crucial role in biofilm formation (Ostapska et al., 2018; Maunders and Welch, 2017; Koo and Yamada, 2016; Whitchurch et al., 2002); it also helps in promoting maturation of biofilm (Balestrino et al., 2008). Benicassa et al., demonstrated that the presence of EPS inside biofilm matrix increased protection against antibiotic compared to planktonic stage. Matrix exopolysaccharides also interacts with protein and induce formation of α-helical conformation (Benicassa et al., 2016). Further, EPS mask the potential bacterial surface structures from host immune system and protects bacteria from immune evasion (Balestrino et al., 2008). EPS is formed by three pathways, namely, *wzy*/*wzx* dependent polymerization pathway, ATP-binding cassette (ABC) transporter-dependent pathway and synthase-dependent pathway (Schmid et al., 2015). EPS contains sugars as components such as L-fucose or uronic acids (Kumar et al., 2007). Some strains of *Kp* were also reported to have a rare sugar (rhamnose) as a component of EPS (Bellich et al., 2018). It is reported that the primary structures of EPS extracted from *Kp* strain *Kp*Ts113 have K24 CPS-repeating units and the *Kp* strain *Kp*Ts101 was identical to the O1 antigen present on LPS (Cescutti et al., 2016; Balestrino et al., 2008).

1.6 Biofilm

Biofilms are a major issue in healthcare and are reported to be involved in 65% of bacterial infections, allowing cells to persist and leading to increased antibiotic resistance (Muhsin et al., 2018; Høiby et al., 2011). The cycle of biofilm formation starts when planktonically grown bacteria come into contact with a surface, adhere, and grow inside a web like 3-Dimensional complex structure called biofilm matrix. Then, as biofilm becomes mature, the attached cells dispersed from the network of biofilm matrix and release by free-floating. These free-floating bacteria again colonize to the surface and forms biofilms (Vasudevan et al., 2014) (figure 1.6). Biofilm is an aggregate of microorganisms attached to an inert or living surface by a self-produced exo-polymeric matrix, which include polysaccharides, proteins, and extracellular DNA (eDNA) (Bandeira et al., 2014). eDNA release by autolysis of cells inside biofilm matrix and the released eDNA plays an important role in the development and structure stabilization of the biofilm as well as in gene transfer mechanisms (Montanaro et al., 2011). This genetic transfer is responsible for spreading of virulence and antibiotic resistance genes in circulating strains exposed to the selective pressure of medical treatment. eDNA and exopolysaccharides (EPS) also form complexes with by interacting with each other (Hu et al., 2012); and eDNA also crosslinks with proteins (Huseby et al., 2010; Domenech et al., 2013). This increases mechanical strength and adhesion capacity of the bacteria by acid-base interaction with the surfaces (Okshevsky et al., 2015). Moreover, polysaccharides, proteins, and DNA allow the initial steps in the colonization and temporary immobilization of bacterial cells to the surfaces (Flemming and Wingende, 2010; Jakubovics et al., 2013). Further, it is reported that addition of extracted DNA (either endogenous or exogenous DNA) to microtiter plate stimulated biofilm formation in *P. aeruginosa* and *Kp* (Oleiwi and Abid, 2017). During biofilm formation of biofilm bacteria communicate with one another, employing quorum sensing (Jamal et al., 2018). The most common organisms which commonly contaminate urinary catheter and develop biofilms are biofilm forming strains of *Escherichia coli, Pseudomonas aeruginosa, Enterococcus, Proteus mirabilis* and *Klebsiella pneumoniae* (Stickler, 2014). Lenchenko et al., reported that *Kp* has high adhesive activity and is a was strong biofilm producer

(Lenchenko et al., 2020). *Kp* can also form a mixed biofilm with *Proteus mirabilis*, *P. aeruginosa, Streptococcus* and *Candida albicans* (Maione et al., 2021; Juarez and Galván, 2018; Macleod et al 2007; Lee et al., 2014). Biofilms inhibit effective antibiotic penetration, reduce the bacterial growth rate, lead to the development of persister cells, and facilitate genetic exchange (Cala et al., 2015; Lewis, 2008). Hence, a detailed understanding of the biofilm may help in developing strategies to combat biofilm formation.

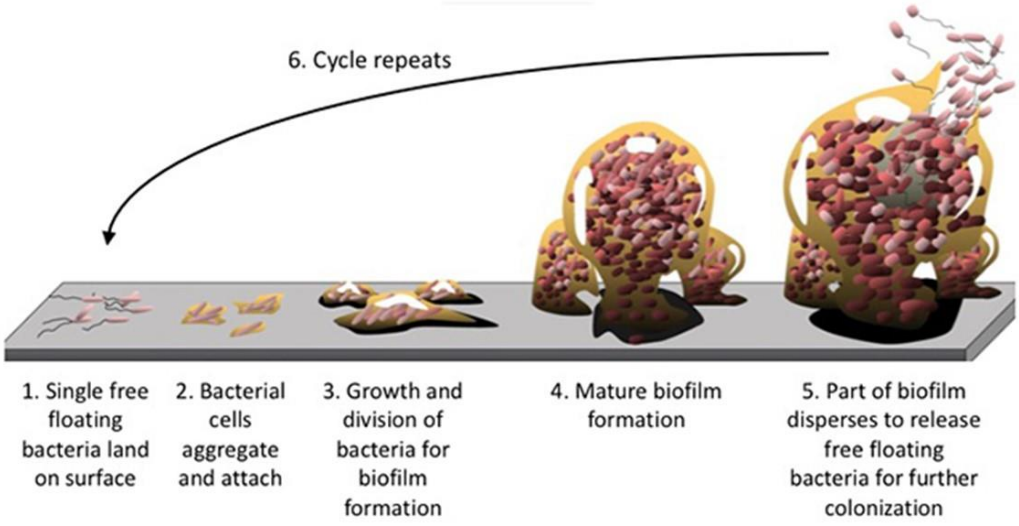

Figure 1.*6* Cycle of biofilm formation on abiotic surface. Major steps of biofilm formation are shown in the figure. Free-floating bacterial cells attach to the surface, more number of cells aggregate and promote cell division and growth of the cells. Biofilm becomes mature and then part of biofilm (away from the surface) detaches, freely-float. These free-floating bacterial cells dispersed form the biofilm will attach again to a different surface and repeat the cycle of biofilm formation (Source: https://www.zmescience.com/science/what-are-biofilms).

Catheter-associated urinary tract infections (CAUTIs) by *Kp* represent one of the most common hospital-acquired infections (HAIs) leading to increased patient morbidity (Liu et al., 2020; Ushieet al., 2020; Nicolle et al., 2014), and biofilms play a central role in pathogenesis of CAUTIS (Lenchenko et al., 2020; Trautner et al., 2004). The catheterized urinary tract provides ideal conditions for the development of enormous biofilm populations (Wang et al., 2020; Stickler, 2008). Bacterial biofilm formation on the interior and exterior surfaces

of the catheter has been identified as the most important cause of CAUTIs and *Kp* is a frequent cause of CAUTIs (Ong et al., 2008). There are three types of catheters mainly used among others, latex based catheters, silicone catheters and silicone coated latex catheters. It is reported that biofilm formation was decreased on silicone and polyurethane catheters compared to latex catheters (Verma et al., 2016; Hawser and Douglas, 1994).

Recently, several studies report the association of antibiotic resistance with biofilm formation in clinical isolates of *Klebsiella* spp. (Shadkam et al., 2021; Ostria-Hernandez, et al., 2018; Vuotto et al., 2017; De Campos et al., 2016). Other virulence factors such as fimbriae and siderophores were also reported to affect the process of biofilm formation. Fimbriae plays a role in adhesion to the surface and biofilm formation by *Klebsiella* (El-Domany et al., 2020; Schroll et al., 2010). There are reports suggesting neither type of fimbria is expressed during planktonic stage of *Kp*; however, type 3 fimbriae are expressed during biofilm formation on catheters, while the expression of type 1 fimbriae is controversial (Stahlhut et al., 2012; Schroll et al., 2010). In case of liver abscess conditions, it was observed that iron in the environment can promote growth, biofilm formation and hence, lower expression of siderophores enhance virulence of *K. pneumoniae* in case of pyogenic liver abscess (PLA) (Chen et al., 2020). Many recent reports on *Kp* biofilms have studied bacteriophage as an effective therapy against biofilm formation as some phages degrade capsular and EPS matrix polymers (Cano et al., 2020; Santiago et al., 2020; Lewis et al., 2020).