Genomic Islands in *Klebsiella pneumoniae* 13

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Abstract

Genomic Islands (GI) of *Klebsiella pneumoniae* include integrative and conjugative elements (ICEs), prophages, integrons, and transposons belonging to a group of genetic elements transferred horizontally and have integrated into the genome of *K. pneumoniae*. Integrative and conjugative elements of *K. pneumoniae* (ICE*Kp*) are flanked by direct repeats, encode the yersiniabactin (*ybt*) locus, a mobilization locus-type 4 secretion system (T4SS), and other variable regions based on which they are classified into 14 types (ICE*Kp*1–14). Their sizes range from 75–200 kb and their chromosomal insertion site is mostly one of the four tRNA-Asn sites. Each *K. pneumoniae* genome can harbor one to six prophages; accounting for 0.1–8% of the genome. The site of phage integration could be either the tRNA or ABC transporter permease SapC. Class I integrons are the most commonly found integrons in *K. pneumoniae*. They contain three essential components for the capture of external genes: an integrase, attI site, and an outwardly oriented promoter (Pc) that controls transcription of the captured genes. Conjugative transposons (CTn) in *K. pneumoniae* are associated with resistance (Tn916 and Tn6009) and hypervirulence (Tn6497).

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13.1 Introduction

Klebsiella spp. are non-motile, Gram-negative, encapsulated, bacteria found as commensals (on human mucosal surfaces) as well as in the environment. In the last two decades, a particular species (*Klebsiella pneumoniae*) has caused havocs by causing life-threatening diseases. Further, the situation has become uncontrollable as it is a frequent source of hospital-acquired pneumonia and the second most important cause of other nosocomial infections including urinary tract infections (Russo and Marr 2019). The virulence and antibiotic resistance of *K. pneumoniae* are the main factors leading to fatal outcomes. One of the major concerns for *K. pneumoniae* is that it is the reservoir of antimicrobial resistance (AMR) genes, and it efficiently spreads AMR in many other *Enterobacteriaceae* (Navon-Venezia et al. 2017). Continuous surveillance studies have indicated that resistance in *K. pneumoniae* has increased in the last few years and hence it contributes majorly to the burden of antibiotic resistance. It has been grouped as one of the ESKAPE pathogens and happens to be one of the critical priority pathogens listed by WHO (Mogasale et al. 2021). Though *K. pneumoniae's* capability to acquire genes (resistance and virulence) is marvelous, *Klebsiella* strains have so far shown a distinct demarcation of resistance (i.e., Carbapenem resistance *K. pneumoniae* [CRKP] strains) and virulence (hypervirulence *K. pneumoniae* [hvKP] strains). However, recent years have noticed a convergence (CR-hvKP strains) of these two kinds of traits and the situation seems threatening (Rodrigues et al. 2022; Lam et al. 2019; Yang et al. 2021). The worldwide occurrence of multidrug-resistant clinical strains is a result of the acquisition of AMR genes on mobile genetic elements (mostly plasmids) followed by the spread of these lineages. Horizontal gene transfer is the most important phenomenon that aids in the acquisition of AMR genes, and the emergence of multiple phenotypes is owed to the accumulation of gene arrays on plasmids, transposons, integrons, integrative and conjugative elements (ICEs), and prophages. Most of this mobilizable DNA when integrated into the bacterial genome is referred to as a genomic island (GI). A stretch of DNA on the bacterial genome having the following common features are GIs (Langille et al. 2010): (1) their size is between 10 and 200 kb; (2) their GC content and codon usage differ from the rest of the genome; (3) they are commonly incorporated at the tRNA genes (tDNAs); (4) the direct repeats that flank them, correspond to the 3′ portion of the tDNA; (5) they, by and large, have integrases that help in the island integration or excision; (6) few carry other mobility genes such as transposases or factors that contribute to conjugation; and (7) they normally carry genes conferring new metabolic proficiencies to the respective host.

K. pneumoniae GIs coding virulence and antibiotic resistance-related determinants are grouped under (1) Integrative conjugative elements (abbreviated as ICE*Kp*), (2) Prophages, (3) Conjugative transposons (CTs), and (4) Integrons. Though ICEs and prophages qualify to be GIs (according to their size range) here we are attempting to compile all information regarding all elements integrated into the genome of *K. pneumoniae*.

13.2 Integrative and Conjugative Elements—*Kp* (ICE*Kp*)

ICE*Kp* is a self-transmissible GI, and its excision occurs due to gene *xis*. An extrachromosomal circular intermediate is a prerequisite for mobilization to the recipient cells. The process requires integrase (*int*) and direct repeats (17 bp) at both ends. The virB1, mobB, and oriT are needed for mobilization. Integration occurs at attO sites present in four tRNA-Asn copies in the chromosome (Lin et al. 2008; Lery et al. 2014). *K. pneumoniae* chromosome region containing the tRNA-Asn sites with incorporated yersiniabactin ICE*Kp* elements is shown in Fig. 13.1. The hotspots for ICE*Kp* insertion are highlighted in the figure and occur inside four tRNA-Asn sites, which are denoted by green colored blocks. Coding sequences are represented by arrows, which are labeled with the gene symbol or the product.

In *K. pneumoniae*, ICE*Kp* mobilizes the yersiniabactin (*ybt*) locus, and its extensive genomic characterization using a large number of strains (n = 2499) identified 17 diverse *ybt* lineages and 14 ICE*Kp* structural variants (Lam et al. 2018). Each ICE*Kp* comprises (1) an integrase (P4-like); (2) the *ybt* locus (29 kb); (3) the oriT transfer origin (14 kb), virB-type4 secretion system (T4SS), and mobBC proteins (mobilization); and (4) genes at the right end (variable region) which were utilized to classify the ICE into 14 separate structures.

ICE*Kp* integration was identified at all four tRNA-Asn sites with varying frequencies. Sites 1, 3, and 4 showed 35.7, 44.7, and 19.5% integration, respectively, while site 2 had only one integration. Most *ybt* lineages had several ICE*Kp* integration sites, indicating that ICE*Kp* variations do not target particular tRNA-Asn copies. Yersiniabactin, along with other siderophores are important for bacterial virulence as

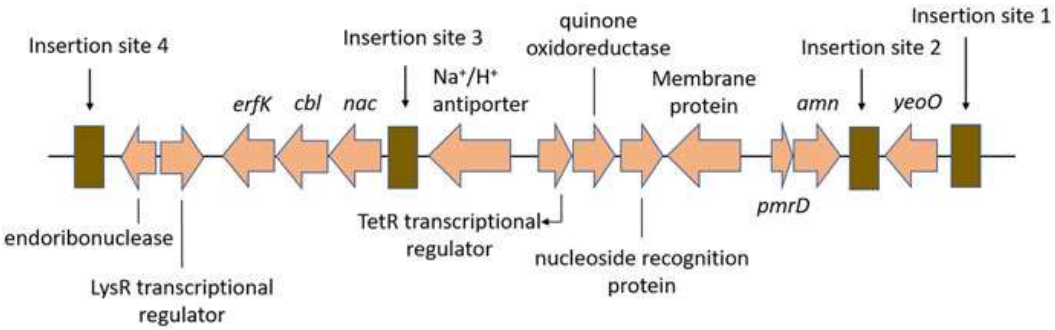

Fig. 13.1 *K. pneumoniae* chromosome region with tRNA-Asn insertion sites for yersiniabactin ICE*Kp*

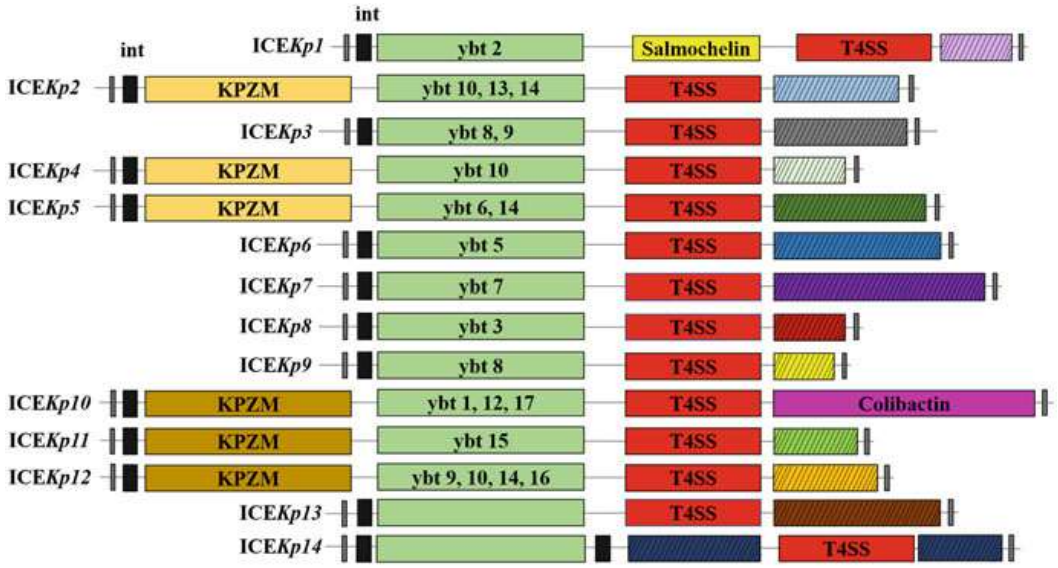

Fig. 13.2 Integrative and conjugative elements of *K. pneumoniae* (ICE*Kp*). Inverted repeats (gray boxes at ends), Integrase gene (black), Yersiniabactin synthesis locus *ybt* (light green, labeled with the most prevalent associated *ybt* lineage), immobilization module (red), Zn2+/Mn2+ module (brown: generally present; light brown: seldom present), diverse gene contents specific to each ICE*Kp* structure (presented in a unique colors with cross-line)

they scavenge iron from host proteins, thereby increasing the chance of survival within the host (Ramirez et al. 2014; Gorrie et al. 2017; Runcharoen et al. 2017). Yersiniabactin is present in approximately one-third of clinical strains, particularly with strains isolated from bacteremia and systemic infections (Lin et al. 2008; Holt et al. 2015). The siderophore enterobactin is produced by many clinical isolates of *K. pneumoniae*, but human lipocalin-2 (Lcn2) inhibits its scavenging mechanisms. Lcn2 binds to ferric and aferric enterobactin with high affinity (Goetz et al. 2002) following which an inflammatory response is induced (Bachman et al. 2009). Yersiniabactin functions importantly in invasive infections as it avoids Lcn2 binding and also avoids the inflammatory response. Thus, it enhances bacterial persistence in the host (Bachman et al. 2009, Bachman et al. 2011; Holden et al. 2016; Lawlor et al. 2007). The *ybt* locus was initially discovered in the Yersinia high pathogenicity island (HPI), and variations in additional *Enterobacteriaceae* species (Wami et al. 2021) are reported, along with *K. pneumoniae*, where *ybt* is found within ICE*Kp*. The very first reported ICE in *K. pneumoniae* was ICE*Kp*1 in 2008 (Lin et al. 2008) and with the comparison of a large number of sequence data 14 other variants have been reported (Lam et al. 2018). ICE*Kp* acquisition occurs in both cKp and hvKp strains of *K. pneumoniae* population. Figure 13.2 shows the diagrammatic representation of the 14 ICE*Kp* variants, classified as distinct structures (Lam et al. 2018).

The common elements of all ICE*Kp* (1–14) are the inverted repeats, the integrase, the bt locus, and the T4SS—mobilization module. In addition to these, ICE*Kp*s (2, 4, 5, 10, 11, 12) have the Zn^{2+}/Mn^{2+} metabolism module (KpZM); while the module is absent in the rest. The salmochelin (iro) locus is only present in ICE*Kp*1 and the

colibactin locus is only present in ICE*Kp*10. Colibactin is genotoxic and hybrid non-ribosomal peptide polyketide that not only crosslinks with DNA but also causes double-strand DNA breaks in host cells (Vizcaino and Crawford 2015). It was firstly discovered in *E. coli* (Nougayrède et al. 2006), but it is now found in 3.5–4% *K. pneumoniae* isolates (Putze et al. 2009; Lam et al. 2018) where it was shown to cause DNA breaks in HeLa cells (Putze et al. 2009). The absence of colibactin is related to the reduction in dissemination to the blood and organs, e.g., liver, spleen, and brain (Lu et al. 2017). Colibactin-positive *K. pneumoniae* is very widespread in Taiwan, where it is present in 17–25% of cases of non-abscess infections and is strongly linked to K1 strains (mainly ST23) (Huang et al. 2012; Dalmasso et al. 2015) Further, all ICE*Kp*'s have a variable region and some of these are hypothetical proteins (not mentioned in Fig. 13.2) whose functions are yet to be known. In the following section, a summary of predominantly found ICE*Kp* (1, 2, 3, 4, 5, 10, 12, and 14) is given.

13.2.1 ICE*Kp*1

The first ICE in *K. pneumoniae* (named ICE*Kp*1) was described by Lin et al. (2008). It is a 76-kb region in a hvKP strain NTUH-K2044 and harbors genes for the biosynthesis of siderophores; yersiniabactin and salmochelin. The unique genes in the variable region include a transporter protease, mucoid phenotype regulator, methyltransferase (Sam-dependent), three transposases, and two hypothetical proteins. The role of ICEKp1 in hvKp pathogenesis was shown in this study as it was found to be more prevalent in hvKp strains (38/42) than cKp strains (5/32). Along with the yersinia pathogenicity island, another region similar to the virulence plasmid pK2044 and genes homologous to salmochelin (iro) and the capsular polysaccharide regulator *rmpA* biosynthesis were also present. Later ICE*Kp*nRJF293, a highly syntenic ICE to ICE*Kp*1 was reported from a hvKp strain RJF293 belonging to ST374 and K2 serotypes (Wang et al. 2018). ICE*Kp*nRJF293 is a 56-kb region incorporated into a tRNA-Asn locus and also contained yersiniabactin gene cluster, a type IV secretion system but lacked salmochelin (iroBCDN) gene cluster. Remarkably, ICE*Kp*nRJF293 contains a unique 10 Kb region at the tRNA-distal end, which encodes a restriction modification system, an ABC transporter, two transposases, and one hypothetical protein (Shen et al. 2019). documented a sequence type 35 (ST35) hypervirulent *Klebsiella pneumoniae* strain (RJY9645) that produced NDM-5 and was isolated from the blood of a patient who underwent a liver transplant. Apart from ICE*Kp*1 (75.4 kb region), additional four chromosomally borne ICE variants were identified, including two type VI secretion system (T6SS) loci (23.1 and 27.1 kb) and two prophages (21.4 and 67 kb). The chromosomal integration of ICE*Kp*1 and the acquisition of the blaNDM-5-carrying plasmid may have contributed to the formation of CR-hvKp strain RJY9645. Though, subsequent reports documented that ICE*Kp*1 was not representative of ICE*Kp* homologs present in the majority of other hvKp strains, recent isolated

reports on the convergence of strains having both virulence and resistance are troublesome.

13.2.2 ICE*Kp*2

ICE*Kp*2, a member of the PAPI family, was reported in 2019 in a *K. pneumoniae* strain (HS11286) from China (Farzand et al. 2019). It was present along with ICE*Kp*1 in the same isolate. A 34-Kb Zn^{2+} and Mn^{2+} metabolism module abbreviated as KpZM was a part of the conserved region along with the *ybt* locus and T4SS locus. The variable region consisted of thymidylate synthase, adenylate kinase, TIR domain protein, and nine hypothetical proteins. In the same study, authors examined 1000 *Klebsiella* genomes and found that ICE*Kp*1 and ICE*Kp*2 are present individually and co-occurred (150 out of 1000 isolates). The occurrence was ICE*Kp*1 (500 out of 1000) and ICE*Kp*2 (300 out of 1000). The element was present in sequence types ST11, ST258, and ST512 of *Klebsiella pneumoniae* from the USA, the UK, and Asia. This was the first evidence of two integrative and conjugative elements interacting with one another. The study showed, that in an isolate with two elements (i.e., ICE*Kp*1 and ICE*Kp*2), ICE*Kp*2 clearly affected the mobility of plasmid positively driven by ICEKp1. It was proposed that Mob2ATPase of ICE*Kp*2 may be a factor for the conjugation of ICE*Kp*1.

13.2.3 ICE*Kp*3

The conserved region of ICE*Kp*3 contains the *ybt* locus and T4SS locus, while the variable region has genes for restriction endonuclease, phosphatase, reverse transcriptase, DDE endonuclease, and five hypothetical proteins. Shankar et al. (2020) reported the *ybt*9 locus located in ICE*Kp*3 in two MDR hypervirulent isolates of sequence type (ST23). In the global collection, isolates of lineage CG23-I are accompanied by *ybt*1 located on ICE*Kp*10 while other sub-lineages either lack ICE*Kp* or carry *ybt*8/9 on ICE*Kp*3. Moreover, the CG23-II isolates produced aerobactin and salmochelin but not colibactin. In a recent review, an elaborate summary of global incidence of hypervirulent and carbapenem-resistant *Klebsiella pneumoniae* showed that ICE*Kp*3 was predominantly found in strains from China, Singapore, and India while only two reports from UK and Canada were noted. According to a stool metagenomic analysis done by Molton et al. (2021), ICE*Kp*3 was found in 2 isolates (out of 24) with *ybt*9 lineage and one of these two isolates also had the clb3 gene.

13.2.4 ICE*Kp*4 and ICE*Kp*12

Apart from the common conserved regions (KpZM, *ybt*, T4SS), the variable region in ICE*Kp* 4 has the enzyme (transposase), a transporter (ABC), a restriction

endonuclease (Type I), a DNA methyltransferase and a hypothetical protein. In one of our studies, a pan drug-resistant strain (DJ) had a *ybt*10 placed on ICE*Kp*4 (Rodrigues et al. 2022). The phylogenetic origins of this strain were investigated within the global diversity of CG147 using publicly available genome sequences of isolates from 2002 to 2018 ($n = 217$). The three main branches of CG147 were ST147, ST273, and ST392. First, a group of 29 genomes emerged in the year 2007, that showed the presence of *ybt*16/ICE*Kp*12. Second, a group of 22 genomes appeared in the year 2009 having *ybt*10/ICE*Kp*4. Further, the *ybt*; ICE*Kp* was rarely detected among ST392 and ST273 genomes. Despite a high diversity of ICE observed among ST147 isolates, *ybt*16; ICE*Kp*12 and *ybt*10; ICE*Kp*4 were two predominant variants found in ST147 genomes and overall it was found in 53% of ST147 genomes. Recently, a CTX-M-15-producing *K. pneumoniae* (TIES-4900 strain) was isolated from an urban Brazilian river. TIES-4900 strain was of sequence type ST15, had a yersiniabactin locus on ICE*Kp*4, the K locus was KL24 (wzi-24), and had O1v1 locus (Cardoso et al. 2022). The authors validated the virulent behavior of TIES-4900 strain in the insect (*Galleria mellonella*) infection model and concluded that the convergence of resistome and virulome in the high-risk clone ST15 is a critical issue, which could be contributing to severe infections in humans, and persistence and adaptation to aquatic environments impacted by anthropogenic activities like hospital and urban discharges. In a recent study on 17 *K. pneumoniae* isolates from wild animals found that six isolates harbored 4 distinct *ybt* lineages (*ybt*1, *ybt*5, *ybt*9, and *ybt*16) harbored on different integrative conjugative elements (ICE*Kp* 1, 3, 6, and 12, respectively) (Chiaverini et al. 2022). ICE*Kp*1/3 was present in approximately 50% of clinical isolates studied in the UK and a global study (Farzand et al. 2019).

13.2.5 ICE*Kp*5

The variable region of ICE*Kp*5 has helicases, thiamine biosynthesis, 2 patatin-like phospholipases, and 6 hypothetical proteins. ICE*Kp*5 appears to be prevalent in Asia and Southeast Asia. To understand the genomic features of Kp ST231 lineage and compare our isolates M2 and M6 (collected from patients with Urine Infection in Gujarat, India) with the ST231 genomes worldwide, we performed comparative genomic analysis using $n = 95$ publicly available genomes of ST231 lineage, collected between 2010 and 2018. The *ybt*14; ICE*Kp*5 was the most prevalent (79.4%; 77/97) in ST231 lineage (Desai 2021). ICE*Kp*5 was recently found in nine XDR isolates collected from bloodstream infections belonging to ST2095–K64 serotype from South India (Shankar et al. 2020). All nine isolates had the ICE*Kp*5 integrated into the chromosome that carried yersiniabactin (*ybt14*).

13.2.6 ICE*Kp*10

ICE*Kp*10 possesses the bacterial genotoxin—colibactin (clb) cluster in addition to the rest of the elements. It was first described by Lai et al. (2014) as a 208-kb chromosomal region with ideal characteristics of a genomic island in *K. pneumoniae* 1084 strain. This 208 kb genomic island was named KPHPI208 (*Klebsiella pneumoniae* high pathogenicity island 208) which also composed 7 other genomic modules (GMs). GM1 contained genes ~100% identical to the pks colibactin gene cluster reported in *E. coli* IHE3034. The other modules were predicted to be having functions like integration, conjugation, yersiniabactin production, microcin production, and some unknown functions. Later, Struve et al. (2015) mapped the evolutionary profile of hypervirulent K1 isolates belonging to clonal complex 23 (CC23), and found ICE*Kp*s similar to 208 kb genomic island mentioned above. Homologs of ICE*Kp*1 were detected in 24 of CG23 isolates as well as in the ST260 CG23 hybrid strain. Though the yersiniabactin cluster was constant, the center region (containing salmochelin and *rmpA* genes) was missing in the ICE region of CC23-related isolates except for NTUH-K2044. Furthermore, in all CC23-related isolates the six ORFs in the third region of ICE*Kp*1 encoding hypothetical proteins were swapped by a 50-kb segment encoding the polyketide genotoxin colibactin. Hence, the ICE region of all CC23 isolates studied by Struve et al. (2015) resembled the ICE described in the Taiwanese ST23 liver abscess strain 1084 (Lai et al. 2014). It is also observed that in the 3 non-CG23 hvKp strains studied, ICE*Kp*10 was poorly conserved, with 2 of the 3 strains possessing only genes that encoded yersiniabactin. Such ICE's having the presence of colibactin along with yersiniabactin are now designated as ICE*Kp*10 (Lam et al. 2018). Their comparative analysis of CG23 genomes ($n = 97$) elucidated that the 81 members of sublineage CG23-I had acquired ICE*Kp*10, which contained genes that encode yersiniabactin and colibactin. This event was estimated to occur in the year 1928, which was followed by the global population expansion of CG23-I. In a recent study, nearly 375,000 bacterial genome sequences were screened to correlate the diversity and evolution of yersiniabactin and colibactin carrying ICEs (i.e., ICE*Kp*10 in case of *Kp*) (Wami et al. 2021). Interestingly, the colibactin-*ybt* carrying ICE was detected only in *E. coli, Klebsiella* species, and *Citrobacter koseri*. To find if the frequency of the colibactin gene cluster is constrained to particular lineages of *E. coli* and *Klebsiella* species, the sequence types of the corresponding *E. coli* and *Klebsiella* species isolates were also analyzed. The clb gene cluster was enriched in a relatively meager group of *E. coli* STs (12/11,537 STs), *K. aerogenes* STs (2/214 STs), and *K. pneumoniae* STs (6/5237 STs), respectively. In *K. pneumoniae*, all ST3 isolates were clb-positive, and over 75% of the examined ST23 and ST234 isolates had the colibactin gene cluster. However, a lower number of the *K. pneumoniae* isolates of sequence types (ST11, ST258, and ST48) had the clb gene cluster. Though the percentage of clinical isolates processing ICE*Kp*10 is minor, a recent study showed the presence of ICE*Kp*10 in a *K. pneumoniae* ST66/K2 strain isolated from a community-acquired infection (Rodrigues et al. 2020). The four *K. pneumoniae* isolates (from the same patient) exhibited a positive string test, i.e., a

hypermucoviscous phenotype and a susceptible antimicrobial profile. Phylogenetic analysis pointed out that the SB5881 strain was close to AJ210 strain (ST66/K2 serotype reported from Australia) which did not harbor ICE*Kp*. The authors describe a worrisome clinical presentation of a typical community-acquired invasive infection caused by *K. pneumoniae* strain that had spread to multiple organs. The dissemination was attributed to the high pathogenic potential due to the acquisition of virulence plasmids and the genomic island (ICE*Kp*10).

13.3 Prophages

Prophages are bacteriophages that have integrated into the bacterial chromosome, can enable horizontal gene transfer and contain important genetic information for the bacteria (Saltzman 2003). Prophages integrate into the bacterial genome and use host machinery for their replication. Genome analysis studies have emphasized on mosaicism in phage genomes suggesting presence of different regions corresponding to different evolutionary histories due to horizontal transfer of genes (Dion et al. 2020). The integration of prophages in the bacterial genome causes degradation of the phage genome or transposition of genes into the host which might lead to toxin production and antibiotic resistance hence making the bacteria more virulent and resistant. The presence of prophage also contributes to fitness and evolution of bacteria (Marques et al. 2021).

Several groups studying prophages have reported that all prophages isolated from chromosomal DNA of *K. pneumoniae* belong to four families of the order *Caudovirales* whose members are characterized by non-enveloped phages that are tailed and have icosahedral heads containing double-stranded DNA. The majority of phages found in *K. pneumoniae* belong to the family *Myoviridae* possessing straight, long contractile tail with a large variation in genome size ranging from 33 to 244 kb. Phages belonging to *Siphoviridae* and *Podoviridae* families have also been observed in *K. pneumoniae*. *Siphoviridae* phages are characterized by long, flexible, non-contractile tail with genome of about 50 kb while *Podoviridae* phages have short, non-contractile tails with genome varying between 40 and 42 kb (Marques et al. 2021). Most studies on prophages classified them as intact or complete phage and defective/incomplete/questionable using the tool PHASTER. Intact phages have a complete sequence of the reference phage and it indicates that integration has been recent (Marques et al. 2021). On the other hand, defective or questionable phage often lack essential phage function (Maxwell 2017) and are indicative of the integration of phage into the bacterial genome. A study has shown that the inductive frequency of AMR carrying phages decreased in presence of antibiotics and hence frequently phages become defective and are inherited in the bacterial genome (Bobay et al. 2014; Wendling et al. 2021).

Kondo et al. (2021) performed a comparative study between prophages from pathogens, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* spp. which are grouped under ESKAPE pathogens. The study involved analysis of

408 *K. pneumoniae* strains and other ESKAPE pathogens. The results reveal that 20.9% of total strains of *K. pneumoniae* encoded AMR genes with is the highest proportion in the ESKAPE pathogen group. On the contrary, only 1.2% and 0.3% proportion of prophages harbored virulence factor (VF) genes and both AMR and VF genes, respectively, which is the lowest among the ESKAPE population under study (Kondo et al. 2021). While *Klebsiella* prophages carried the AMR genes, these do not belong to the high-priority AMR genes (e.g., carbapenemases).

13.3.1 Integration of Phages in Genome

In lysogenic cycle, integration of phage in bacterial genome is an extremely crucial step. Previous records have shown that prophages integrate site specifically in the genome. It is observed that prophages encoding tyrosine integrase integrate adjacent to host tRNA, and one probable reason for this integration is the affinity of temperate phage toward palindromic sequences present near that region (Bobay et al. 2013).

In the study by Marques et al. (2021), they analyzed upstream and downstream regions of bacteriophage insertion site and found that maximum prophages integrated between genes clusters involved in metabolic pathways, transcriptional regulators transporters, tRNA genes, protein synthesis, transferases, recombinant proteins, membrane proteins, and ribosome biogenesis. Bleriot et al. (2020) and Baliga et al. (2021) have also obtained similar results. *K. pneumoniae* prophages and their site of integration with additional remarks are listed in Table 13.1. And Antimicrobial resistance, Virulence, and genes regarding phage defense associated with *K. pneumoniae* are listed in Table 13.2.

13.4 Integrons

Integrons can be defined as genetic systems of bacteria that detain and express gene cassettes. They usually have an *intI* gene that encodes an enzyme known as integrase and via site-specific recombination that catalyzes the excision or incorporation of gene cassettes, a site for recombination (attI), along with a promoter that controls inserted gene cassettes's expression (Mazel 2006) (Fig. 13.3).

IntI integrase amino acid sequences have been used to divide integrons into different "classes," with those harboring intI1 being classified as "class 1," intI2 as "class 2," intI3 as "class 3," and so on. IntI1, intI2, and intI3 are most often accompanied on mobile genetic elements, while intI4 and rests were discovered in association with chromosomal integrons (Deng et al. 2015). Integrons are assembly platforms that use site-specific recombination to include exogenous open reading frames (ORFs) and by assuring their correct expression alter them to functional genes. Three components have so far been discovered to be crucial for the capture of foreign genes in all integrons: an *intI* gene that encodes a tyrosine-recombinase integrase, a main recombination site (attI), and an outwardly oriented promoter (Pc) that controls transcription of the acquired genes (Hall and Collis 1995). Gene

Table 13.1 Site of integration of bacteriophage in the *K. pneumoniae* genome

Sr. no.	Phages	Site of integration	Note
1	ST405-OXA48phi1.2, ST15-VIM1phi2, ST437-OXA245phi4.1, ST101-KPC2phi6.1, ST147-VIM1phi7.2, ST405-OXA48phi1.3, ST11-VIM1phi8.1, ST101-KPC2phi6.2, ST13-OXA48phi12.1, ST512-KPC3phi13.1, ST13-OXA48phi12.2, ST512-KPC3phi13.6 ST258-KPC3phi16.1, ST13-OXA48phi12.5	Before or after intact Host tRNA	Commonly tRNA-arg is found before prophage
2	ST11-OXA245phi3.1, ST340-VIM1phi10.2, ST437-OXA245phi4.2, ST11-VIM1phi8.4, ST512-KPC3phi13.2, ST11-OXA48phi15.3, ST258-KPC3phi16.2	Between intact genes of TerT transcriptional regulator and transporter intact genes	Genes remained intact after phage integration
3	ST405-OXA48phi1.1, ST16-OXA48phi5.2, ST11-OXA245phi3.2, ST846-OXA48phi9.1	Adjacent to bacterial transcription regulator	ST16-OXA48phi5.2, ST846-OXA48phi9.1 Disruption of adjacent genes due to phage integration

(continued)

Table 13.1 (continued)

Sr. no.	Phages	Site of integration	Note
4	ST16-OXA48phi5.1, ST846-OXA48phi9.2, ST974-OXA48phi18, ST11-VIM1phi8.2,	Integration between *sapB* and *sapC* intact gene of *sapABCDEF* operon coding for ATP binding cassette (ABC transporter)	
5	ST16-OXA48phi5.3, ST340-VIM1phi10.1, ST11-VIM1phi8.3, ST11-OXA48phi15.1, ST512-KPC3phi13.5	Immediately after an intact Protease	
6	ST101-KPC2phi6.3, ST13-OXA48phi12.3, ST147-VIM1phi7.1, ST15-OXA48phi14, ST13-OXA48phi12.4	Next to gene coding for an unknown protein	Integration of ST15-OXA48phi14 phage caused truncation of gene
7	ST16-OXA48phi5.4	After a sensor domain-containing diguanylate cyclase	Disruption of adjacent genes due to phage integration

cassettes (Gc) typically contain a promoter less open reading frame (orf) and a recombination site attC (Also known as the element of 59-base) for integration. They can occur in the form of free circular molecules or as integrons (Hall et al. 1999). Integrons are highly mobile as they are placed on transposons, plasmids, and pathogenicity islands, allowing them to be transferred across bacteria. The nucleotide sequence of the integrase gene has classified integrons into five types (Guérin et al. 2011). The most common integrons are class 1, and are found in *K. pneumoniae* and other gram-negative clinical isolates (Lima et al. 2014).

13.4.1 Integrons Associated with Antibiotic Resistance

Two conserved segments, the 3′ conserved segment (3′ CS) and the 5′ conserved segment (5′ CS), together with internal gene cassettes (antimicrobial resistance

Table 13.2 Antimicrobial resistance, Virulence, and Phage defense genes associated with prophages in *K. pneumoniae*

Strain's accession number/name of prophage	Most common phage/closely related phage	Gene present in prophage/protein coded by prophage
AMR genes (Kondo et al. 2021)		
Kp-AP018748	Escher_RCS47	*$bla_{CTX-M-15-1}$, aac(6′)-Ib_1, blaTEM-1A_1, tet(D)_1, dfrA14_5, ant(3″)-Ia_1, qnrB1_1, aac(6′)-Ib-cr_1, $bla_{OXA-1_}$1, catB3_1*
Kp-CP008797	Entero_P1	*$bla_{TEM-105\_1}$, $bla_{TEM-105\_1}$, sul1_5, aadA2_1, aac(3)-Ib_1*
Kp-CP009876, Kp-CP015382	Entero_186	*bla_{KPC-2_1}*
Kp-CP011578	Entero_186	*$bla_{CTX-M-15\_1}$*
Kp-CP018140	Entero_mEp237	*aac(6′)-Ib-cr_1, bla_{OXA-1_1}, catB3_1, aac(3)-IIa_1*
Kp-CP018447, Kp-CP018450	Entero_P2	*oqxB_1, oqxA_1*
Kp-CP018816	Escher_HK639	*sul1_5, aadA2_1, aac(3)-Ib_1*
Kp-CP018883, Kp-CP018885, Kp-CP020071, Kp-CP020837, Kp-CP021539, Kp-CP043047	Entero_P1	*sul1_5, aadA2_1, aac(3)-Ib_1*
Kp-CP022023	Salmon_SJ46	*sul2_2, aadA2_1, dfrA12_8, ant (3″)-Ia_1*
Kp-CP022882, Kp-CP022997, Kp-CP023722, Kp-CP023933, Kp-CP023941, Kp-CP024191, Kp-CP024521, Kp-CP024528, Kp-CP024535, Kp-CP024570, Kp-CP024563, Kp-CP024556, Kp-CP024549, Kp-CP025951, Kp-CP026130, Kp-CP026132, Kp-CP026149, Kp-CP026145, Kp-CP026140, Kp-CP026136, Kp-CP027068, Kp-CP028548, Kp-CP028542, Kp-CP029384, Kp-CP031721, Kp-CP032163, Kp-CP032207, Kp-CP033954, Kp-CP034123, Kp-CP034415, Kp-CP036300, Kp-CP036365, Kp-CP036371, Kp-CP041373	Entero_phi80	*sul1_5, aadA2_1, aac(3)-Ib_1*
Kp-CP023949	Salmon_RE_2010	*mdf(A)_1*
Kp-CP025456	Entero_phi80 Salmon_Fels_2	*sul1_5, aadA2_1, aac(3)-Ib_1 oqxA_1*
Kp-CP025461, Kp-CP027146, Kp-CP028180	Escher_HK639	*sul1_5, aadA2_1, aac(3)-Ib_1*
Kp-CP026159, Kp-CP028787, Kp-CP037963, Kp-CP041099, Kp-CP043932, Kp-CP011624, Kp-CP013322	Entero_P4	*oqxB_1, oqxA_1*
Kp-CP026177	Entero_mEp235	*oqxB_1, oqxA_1*

(continued)

Table 13.2 (continued)

Strain's accession number/name of prophage	Most common phage/closely related phage	Gene present in prophage/protein coded by prophage
Kp-CP028583, Kp-CP033396	Entero_phi80 Salmon_Fels_2	*sul1_5, aadA2_1, aac(3)-Ib_1* *mdf(A)_1*
Kp-CP028797	Salmon_Fels_2 Entero_Tyrion	*oqxB_1, oqxA_1* *mdf(A)_1*
Kp-CP029738	Escher_RCS47	$bla_{\text{SHV-12\_1}}$
Kp-CP031800	Salmon_RE_2010	*mdf(A)_1*
Kp-CP033625	Salmon_Fels_2	*oqxB_1, oqxA_1*
Kp-CP034249	Escher_HK639	*sul1_5, aadA2_1, aac(3)-Ib_1*
Kp-CP034327	Salmon_Fels_2	*oqxA_1*
Kp-CP036305	Entero_phi80 Salmon_Fels_2	*sul1_5, aadA2_1, aac(3)-Ib_1* *oqxB_1, oqxA_1*
Kp-CP036320, Kp-CP036327	Salmon_RE_2010	*mdf(A)_1*
Kp-CP040533, Kp-CP040539, Kp-CP040545, Kp-CP033960	Entero_phi80 Salmon_Fels_2	*sul1_5, aadA2_1, aac(3)-Ib_1* *oqxB_1*
Kp-CP042481	Entero_P4	*oqxA_1*
Virulence genes (Bleriot et al. 2020)		
ST512-KPC3phi13.1		Invasion-associated protein B T4SS
ST258-KPC3phi16.1, ST512-KPC3phi13.6, ST437-OXA245phi4.1		Transferase-kinase
ST13-OXA48phi12.5, ST16-OXA48phi5.2, ST13-OXA48phi12.3, ST405-OXA48phi1.3, ST101-KPC2phi6.3, ST15-VIM1phi2.1, ST11-VIM1phi8.2		MarR family of transcriptional regulators
Genes regarding phage defense (Bleriot et al. 2020)		
ST405-OXA48phi1.2 ST16-OXA48phi5.3		RelBE-like TA proteins
ST11-VIM1phi8.3 ST846-OXA48phi9.2		HigBA-like TA modules
ST512-KPC3phi13.6 ST437-OXA245phi4.1		CRISPR-associated Endoribonuclease Cas2
ST846-OXA48phi9.2		Putative anti-CRISPR/ Cas9 protein, AcrIIC3-like
ST13-OXA48phi12.3		TerB protein from The operon *terZABCDEF*

genes), make up the class 1 integrons's structure (Lima et al. 2014). In *K. pneumoniae* Class 2 integrons are occasionally discovered (Odumosu et al. 2013). According to Firoozeh et al. (2019), the most common cassettes were 1000–1500 bp long *aadA1* and *dfrA1-sat1* cassette arrays. Meanwhile, class

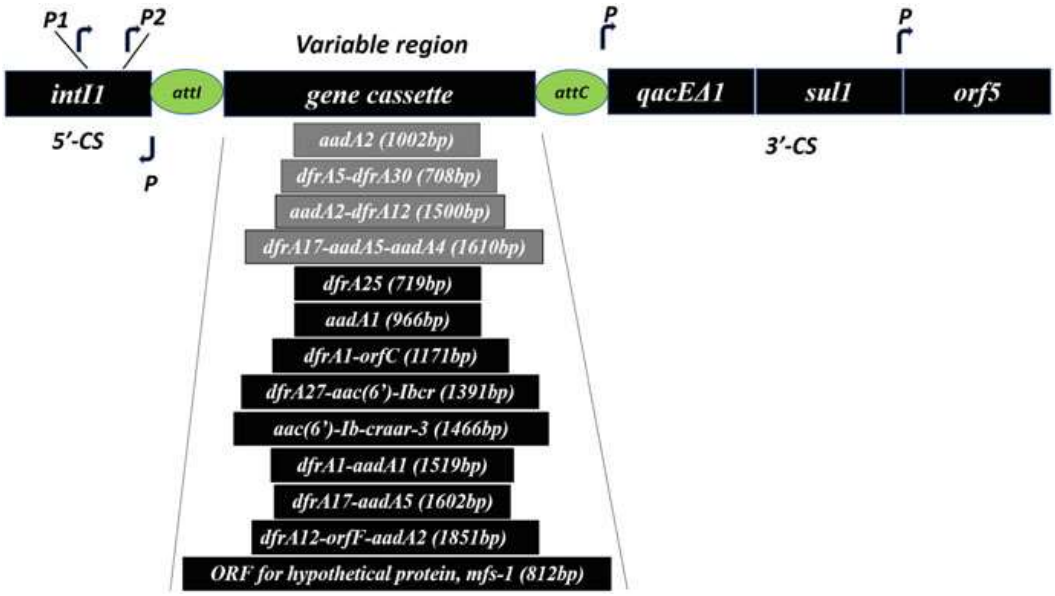

Fig. 13.3 A class 1 integron is represented in this diagram (Deng et al. 2015). P1 promoter for gene cassette transcription, P2 another promoter that is often inactive, an int gene for integrase, an attI1 integration site, moderately deleted gene *qacE* that encodes resistance against quaternary ammonium compounds (QACs), sulfonamide resistance gene *sulI*, orf5 uncharacterized function, P promoters for the *sulI and qacEΔ1* genes. An integrase recognizes the attC sequence on the gene cassette. Gene cassette which is a variable region of the class 1 integron. Some gene cassettes are mentioned below in gray (Firoozeh et al. 2019) and black (Li et al. 2013) color boxes

3 integrons have only been found in a few strains of *K. pneumoniae*. Correia et al. (2003) described a natural *K. pneumoniae* plasmid p22K9 that had a 2863-bp long class 3 integron that included an intI3 integrase gene, two (Pint and Pc) promoter areas, an attI3 recombination site, a cassette of $bla_{\text{GES-1}}$ gene, and a fused cassette of $bla_{\text{OXA-10}}$-type/*aac(6)-Ib* gene (Correia et al. 2003). Many different resistance gene cassettes are carried by class 1 integrons, the majority of which hold the *aadA* gene, which confers streptomycin/spectinomycin resistance. It has been shown that the distribution of class 1 integrons carrying different aadA alleles is widespread (Deng et al. 2015). In addition, the *dfrA* cassette arrays, which encode trimethoprim resistance, are typically seen in both class 1 and 2 integrons (Kiiru et al. 2013).

Firoozeh et al. (2019) studied clinical isolates of MDR *K. pneumoniae* (MDR*Kp*) (n = 150) from specimens such as urine, wounds, blood, respiratory tract samples, CSF, and catheters were used to isolate *K. pneumoniae* in Iran and identified class 1, 2, and 3 integrons. All of the MDRKp strains n = 150 (100%) had class 1 integrons and *K. pneumoniae* n = 55 (36.66%) had class 2 integrons. IntI-positive strains were used for sequencing indicated that the cassette arrays of class 1 integron included ten different array groups ranging from A to J, consisting of (1610 bp, 1500 bp, 1002 bp and 708 bp integrons) and gene cassettes were identified and shown in Fig. 13.3. Whereas, four separate groups of cassette array (1000 bp and 1500 bp integrons) were discovered, ranging from a to d in class 2 integron which harbored gene cassettes were as follows: (no cassette; *aadA1; dfrA1-sat1; aadA1, dfrA1-sat1*).

708 bp arrays were the most prevalent type identified in class 1 integrons, and the *dfrA5* & *dfrA30* gene cassettes, which contain dihydrofolate reductases enzymes, were identified. Class 1 integron-positive *K. pneumoniae* strains also have a high frequency of other *dfrA* gene variants, such as *dfrA12* and *dfrA17*. whereas the most common cassettes in class 2 integrons were 1000–1500 bp.

In another study by Li et al. (2013), they studied *K. pneumoniae* isolates ($n = 176$) of patients from tertiary care hospitals. The isolates found positive for class 1 integron contained ten different class 1 integron gene cassette arrays ranging between 700 bp and 1860 bp, which were classified as types I–X and shown in Fig. 13.3. There were no ESBL-expressing gene cassettes or proteins connected to carbapenem resistance detected. The majority of *K. pneumoniae* isolates contained a 1171-bp integron with the *dfrA1* and orfC genes (type I), which was the most prevalent integron gene cassette array seen. Additionally, compared to class 1 integron-negative isolates, class 1 integron-positive isolates showed resistance to a significantly greater number of drugs (Li et al. 2013). Class 1 integrons are highly prevalent in Gram-negative bacteria, and this association with the presence of MDR is significant (Wu et al. 2012; Li et al. 2013). Other investigations have found a high prevalence of integron-positive MDR*Kp* (Gruteke et al. 2003; Wu et al. 2012). Integrons may provide a selective advantage to strains residing in environments where selected pressures are induced by antibiotic abuse, such as hospitals, explaining the high occurrence of integrons in MDR strains.

In *Klebsiella* species, Salimizand et al. (2013) reported a *dfrA17* variation. The genes *dfrA17, dfrA12, dfrA1, dfrA25,* and *dfrA27* were found in class 1 integron cassette arrays in *K. pneumoniae* strains of intI1-positive in China (Li et al. 2013; Cao et al. 2014). The *dfrA17* and *dfrA12* variants have been detected in Gram-negative bacteria carrying class 1 integrons in the United States (Adams-Sapper et al. 2012), indicating that these variants are prevalent across class 1 integron cassettes around the world. Some *K. pneumoniae* strains can produce $bla_{\text{NDM-1}}$carbapenemase and have a class 1 integron with the following configuration in their genome and plasmid (Cortés-Ortíz et al. 2021).

ISMRK (imipenem-susceptible but meropenem-resistant *Klebsiella*) is a term used by Kayama et al. (2015) to describe isolates that were extended-spectrum beta-lactamase (ESBL) positive and displayed a contradictory pattern of resistance, being extremely resistant to nearly all antibiotics of beta-lactam except imipenem. The class 1 integron, In722, has a cassette containing the MBL gene $bla_{\text{IMP-6}}$, and pKPI-6, a 47-kb self-transmissible plasmid, which also had the ESBL gene $bla_{\text{CTX-M-2}}$ in ISMRK bacteria. Isolates of ISMRK have a phenotype called "stealth" that is undetectable with imipenem when IMP-6 (Shigemoto et al. 2012) and CTXM-2 are combined. In pKPI-6, there are three acquired extra DNA insertions that carry resistance genes: an integron region containing $bla_{\text{IMP-6}}$, a Tn1721 segment containing *tetA* and *tetR*, and a stability operon region harboring $bla_{\text{CTX-M-2}}$ (Fig. 13.4).

Kondo et al. (2021) discovered that the integron cassette array is similar to other AMR gene combinations seen in prophage area, and used the INTEGRALL database to investigate the integrons in these prophage regions. They discovered that certain *K. pneumoniae* prophage sites had integrase belonging to Class1 and cassette

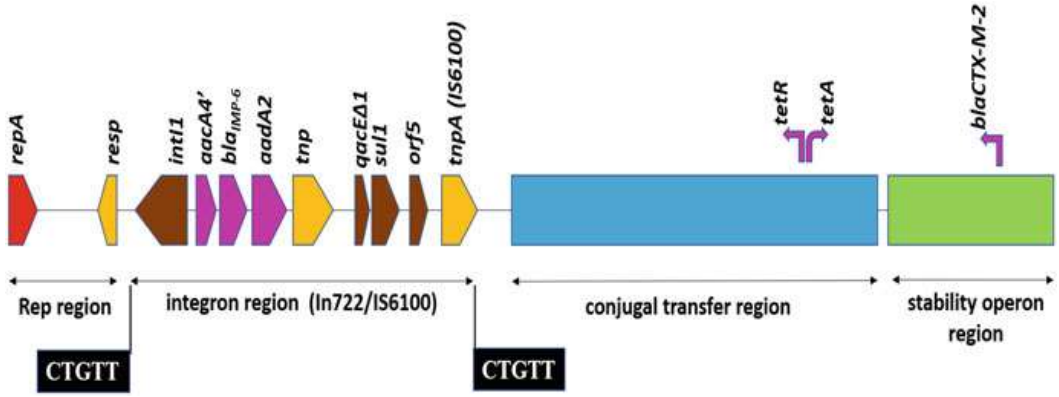

Fig. 13.4 Representative image of pKPI-6 plasmid (Kayama et al. 2015). The ORFs of the rep region and integron regions are symbolized by Pentagons, the genes that have been annotated are colored based on the expected gene function as follows: antimicrobial resistance genes, pink; conjugation genes, sky blue (in conjugation transfer regions); transposons, yellow; integrons, brown and plasmid maintenance genes, red

Table 13.3 List of different Integrons harboring AMR genes in cassette array in *Klebsiella pneumoniae*

Integron class	Integron number (In)	AMR genes in cassette array	Year	Source	Accession number
1	**In722**[a]	*aacA4'-3, bla*$_{\text{IMP-6}}$*, aadA2, sul1*	2012	Japan	AB616660
1	**In719**[a]	*sul1, aadA2, dfrA12D6*	2011	n.m.	CP003225
1	–	*dfrA17, aadA5*	2009	Russia	GQ896493
1	–	*aacA4*	2009	Russia	GQ924771
1	**In560**[a]	*dfrA30b*	2011	Libya	HE613853
1	**In578**[a]	*sul1, cmlA1l, aadA1e, ereC, arr-2*	2011	Kenya	JN157804
1	**In27**[a]	*dfrA12, aadA2, sul1*	2011	n.m.	JN233704
1	**In191**[a]	*dfrA14b*	2012	Czech Republic	JX424423
1	**In27**[b]**, In191**[b]	*bla*$_{\text{CTX-M-15\_1}}$*, aac(6′)-Ib_1, bla*$_{\text{TEM-1A\_1}}$*, tet(D)_1, dfrA14_5, ant(3″)-Ia_1, qnrB1_1, aac(6′)-Ib-cr_1, bla*$_{\text{OXA-1\_1}}$*, catB3_1*	2016	Thailand	AP018748
1	**In127**[b]	*bla*$_{\text{TEM-105\_1}}$*, bla*$_{\text{TEM-105\_1}}$*, sul1_5, aadA2_1, aac(3)-Ib_1*	2012	USA	CP008797
1	**In127**[b]**, In610**[b]	*sul1_5, aadA2_1, aac(3)-Ib_1*	2014	China	CP026130
1	**In1680**[b]**, In610**[b]	*sul1_5, aadA2_1, aac(3)-Ib_1*	2014	China	CP026145

Note—n.m – not mentioned, In[a] – From Moura et al. (2009), In[b] – From Kondo et al. (2021)

arrays for antimicrobial resistance (AMR) (Table 13.3). These distinctive areas including AMR genes cassette arrays were referred to as integron cassette arrays, i.e., integron-associated prophages. Additionally, they found that all phage regions

with an integron included three or greater than three AMR genes, but those lacking an integron contained less number of AMR genes. These results showed that compared to other groups, prophages carrying integrons had a significantly more number of AMR genes.

Integrons numbers (In) were defined based on an arrangement, and INTEGRALL database (http://integrall.bio.ua.pt/?list) (Moura et al. 2009) were retrieved for all the available integrons and their association with AMR in *Klebsiella pneumoniae* are mentioned in Table 13.3.

13.5 Conjugative Transposons

Vertical transmission of conjugative transposons (CTns) occurs through chromosomal replication and partitioning (Wright and Grossman 2016). It is challenging to determine the original host for any conjugative transposon since a species of bacterium that has been initially identified as containing a novel CTn may not be the species from which the CTn developed (Scott 2002). Conjugative transposons can move into a new host by transposition. They are capable of conjugative transfer into new hosts without being mediated by plasmids (Tomich et al. 1979). CTns are known for their heterogeneity in form and function, thereby conferring the adaptive features and evolution in *Klebsiella pneumoniae*. Conjugative transposons as well as other genomic Islands are integrated within the chromosome and are regarded as important as conjugative plasmids involved in the transfer of chromosomal-borne genes among diverse bacterial species (Scott 2002) using the self-encoded transmission machinery or the type IV secretion system (T4SS) that is conjugation machinery (Wozniak and Waldor 2010; Johnson and Grossman 2015).

Usually, the CTns identified in the environment often code for resistance to heavy metals, and aromatic compounds and also encode functions such as Nitrogen fixation; mobile catabolic genes encoding degradation of xenobiotic compounds.

13.5.1 Antibiotic Resistance

CTns, reportedly hosting cascades of genes encoding Antibiotic resistance have been detected in quite a lot of pathogenic strains of *K. pneumonia* (Soge et al. 2008; Roberts and Mullany 2011). CTns are known to encode essential functions that enhance the survival of bacteria under specific environmental conditions as seen in Antibiotic resistance. Many bacteria including *Klebsiella pneumoniae* can adapt to any environment either by introducing a compensatory mutation in genes or by conditioning the expression of the resistance genes. Here, we discuss the most commonly found CTns associated with resistance (Tn916 and Tn6009) and hypervirulence (Tn6497).

13.5.1.1 Tn916

Tn916 is a 16.4-kb broad-host-range conjugative transposon originally discovered in *Enterococcus faecalis* (Rice 1998). It confers resistance to tetracycline via *tet(M)*. This transposon has been detected in various bacteria including *K. pneumoniae* (Soge et al. 2008). It is a self-transmissible genomic island usually associated with the chromosome and also found on certain plasmids (Rice 1998). Two transposon-encoded proteins; Xis-Tn and Int-Tn are required for the excessive recombination. Although the latter alone is enough for integration (Storrs et al. 1991). In some cases, the active integrase of both the donor and the receiver is necessary for the conjugative transposition of Tn916 (Storrs et al. 1991).

13.5.1.2 Tn6009

This is a novel, 17.8 kb size, non-composite conjugative transposon which belongs to the Tn916 family. It contains a Tn916 element which is incorporated with a functional inorganic mercury resistance (*merA*) that sits upstream of the conjugation module (Roberts and Mullany 2011). The *mer* genes and the *tet(M)* genes are directly related, and 24 orfs of the Tn916 are linked to a distinct 37-bp sequence that comes before the *merA, merB,* and *merT*, among other *mer* genes. These features make it unique (Soge et al. 2008). The successful demonstration of the conjugative transfer of Tn6009 from *Klebsiella pneumoniae* to *Enterococcus faecalis* (Soge et al. 2008) subsequently conferred its resistance to mercury and tetracycline due to the actions of the *merA* and *tetM* genes, respectively.

13.5.1.3 Tn6497

A transposon called Tn6497 was discovered in the hypervirulent strain of *Klebsiella pneumoniae* 11492's high pathogenicity island (HPI). IS903D, the colibactin gene cluster (clbABHIJKLMNOPQ), and the yersiniabactin gene cluster are all present (fyuA, *ybt*ETU, irp1, irp2, *ybt*APQXS) (Shen et al. 2019).

13.6 Concluding Remarks

GIs contribute to the genomic plasticity of *K. pneumoniae*. ICE*Kp* acts as a reservoir for virulence genes and is more stably integrated compared to others. The polylysogenic property of *K. pneumoniae* helps many prophages to reside on one genome and is intimately associated with virulence, resistance, evolution, and fitness. Antibiotic resistance genes primarily accumulate due to integrons and transposons. There is fast information generated about the GIs from the whole genome sequencing data and much of the data is lying without experimental proof of concept. There is a need to deepen our understanding through functional analysis. Certain pressing questions to be addressed are (1) under which conditions do the ICEs express? (2) can antibiotics induce prophages? And (3) association between prophages and integrons residing in them. It is also necessary to understand the situations in which the horizontal transfer of GIs occurs. Future functional

translational studies should be designed to heighten our understanding of GIs in *K. pneumoniae*.

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