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Carbapenemase-producing *Klebsiella pneumoniae:* molecular and genetic decoding

Liang Chen[1], **Barun Mathema**[1,2], **Kalyan D. Chavda**[1], **Frank R. DeLeo**[3], **Robert A. Bonomo**[4,5], and **Barry N. Kreiswirth**[1]

[1]Public Research Institute TB Center, New Jersey Medical School, Rutgers University, Newark, NJ 07103

[2]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032

[3]Laboratory of Human Bacterial Pathogenesis, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT 59840

[4]Research Service, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio 44106

[5]Departments of Medicine, Pharmacology, Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, OH 44106

Abstract

Klebsiella pneumoniae carbapenemases (KPCs) were first identified in 1996 in the USA. Since then, regional outbreaks of KPC-producing *K. pneumoniae* have occurred in the USA, and have spread internationally. Dissemination of bla_{KPC} involves both horizontal transfer of bla_{KPC} genes and plasmids, and clonal spread. Of epidemiological significance, the international spread of KPC-producing *K. pneumoniae* is primarily associated with a single multilocus sequence type (ST), ST258, and its related variants. However, the molecular factors contributing to the success of ST258 largely remain unclear. Here, we review the recent progresses in understanding KPC-producing *K. pneumoniae* that is contributing to our knowledge of plasmid and genome composition and structure among the KPC epidemic clone, and identify possible factors that influence its epidemiological success.

Keywords

Klebsiella pneumoniae carbapenemase; carbapenem-resistant; ST258

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Corresponding author: Kreiswirth, B.N. (kreiswba@njms.rutgers.edu).

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Epidemiology and impact of *Klebsiella pneumoniae* carbapenemases

Carbapenem-resistant Enterobacteriaceae (CRE), have recently emerged as the major class of bacterial pathogens that pose a significant threat to global public health, to high risk patients undergoing life threatening procedures, and to vulnerable patients in long-term care facilities (www.cdc.gov/drugresistance/threat-report-2013/) [1, 2]. It is possible that no infectious agent since the introduction of HIV has threatened our last line therapies more than these pathogens.

Resistance to carbapenems involves multiple mechanisms, including alterations in outer membrane permeability mediated by the loss of porins, upregulation of efflux systems along with hyperproduction of AmpC β-lactamases or extended-spectrum β-lactamases (ESBLs), or more commonly, the production of carbapenemases. Currently, *Klebsiella pneumoniae* carbapenemase (KPC) is the most clinically significant serine carbapenemase in the United States and its rapid international spread has become a noted public health threat globally [3, 4].

KPC emerged in the late 1990s and was identified in a *K. pneumoniae* isolate in North Carolina, USA [5]. To date, 22 different KPC enzyme variants have been identified (http://www.lahey.org/Studies/). KPC β-lactamases can hydrolyze all β-lactams, including carbapenems, cephalosporins, cephamycins, monobactams, and clavulanic acid [5, 6]. KPCs have been found in many Gram-negative species, including both Enterobacteriaceae and non-fermenters (e.g. *Pseudomonas aeruginosa* and *Acinetobacter baumannii*), with *K. pneumoniae* the most predominate species. KPCs are frequently found in *K. pneumoniae* associated with nosocomial infections, such as urinary tract infections, septicemia, pneumonia, and intra-abdominal infections, but are not common in community-acquired infections.

Since its emergence, CREs containing bla_{KPCs} have spread in the Northeastern USA and caused several outbreaks in New York and New Jersey hospitals. In the middle 2000s, these microbes spread from the Northeastern USA to several other countries, including Israel, Greece and Columbia, presumably associated with the travel of patients between advanced care institutions. KPC-producing bacteria are considered to be endemic in certain parts of the world, including the Northeastern USA, Argentina, Brazil, Colombia, Eastern China, Greece, Israel, Italy, Poland and Puerto Rico [4, 7]. The clinical and molecular epidemiology of KPC has been detailed in recent reviews and is not further addressed in this review [3, 4, 7, 8].

Transmission of the KPC gene, bla_{KPC}, can be mediated by different molecular mechanisms, from mobility of small genetic elements (e.g. Tn*4401* transposon) to horizontal transfer of plasmids and via clonal spread [9]. Interestingly, similar to the epidemiological success of CTX-M-producing *Escherichia coli* ST131, the international spread of KPC-producing *K. pneumoniae* (KPC-Kp) has been linked to a major multilocus sequence type (MLST or ST), namely ST258, and its related variants [10]. ST258 has been reported in more than twenty-five countries from four continents, including the majority of the KPC epidemic countries mentioned previously. To illustrate, ST258 is responsible for >77% of

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the USA outbreaks and 90% of all KPC-Kp infections in Israel [11, 12]. The factors contributing to the epidemiologic success of ST258 remain unknown; however, chromosomal or plasmid factors, beyond antibiotic resistance, may increase the strain's fitness and provide an advantage that underlies its prevalence [13, 14]. Identification of these factors is an important step toward understanding the molecular epidemiology of KPC-Kp and will likely contribute to the development of effective measures for infection control and prevention.

Population structure of KPC-Kp strains

Several molecular methods have been used for tracking and characterization of *K. pneumoniae* isolates; including repetitive sequence-based PCR (rep-PCR), pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing (MLST), with MLST the most common technique. *K. pneumoniae* MLST is based on genetic variation in seven housekeeping genes (*rpoB, gapA, mdh, pgi, phoE, infB*, and *tonB*) that together, provide a relative genetic profile (ST, strain type) among different isolates [15]. In practice, a different allelic number is assigned to each distinct sequence within each locus, and the ST is created by linking the seven different allelic numbers in a standard order. These ST data can be further defined by eBURST (http://eburst.mlst.net/) which groups closely related strains (clonal complexes, CCs), and identifies the founding genotype of each group [16].

As of April 1, 2014, a total of 1536 STs have been deposited in the *K. pneumoniae* MLST online database (http://www.pasteur.fr/mlst). The population structure of *K. pneumoniae* is illustrated in Figure 1A. Using the most stringent criteria, where all members assigned to the same group share identical alleles at 6 of the 7 loci with at least one other member of the group, 136 CCs and 528 singletons (single STs that do not correspond to any CCs) were identified with a central CC comprising of 504 STs (32.8% of all STs). However, it is suggested that the accuracy of the eBURST grouping is questionable if the proportion of STs in a single CC exceeds 25% of all STs for predicting ancestor-descendant links since unrelated groups of STs may join into the same eBURST group [17]. In addition, the presence of a single large heterogeneous and straggly CC also suggests the likelihood of high rate of homologous recombination and DNA transfer between related and unrelated STs, instead of diversification from a single common ancestor [17].

In an attempt to provide an easily defined epidemiologically meaningful phylogenetic structure in *K. pneumoniae*, Breurec *et al*. proposed to subdivide CCs into clonal groups (CGs), where the most prevalent ST would be the central to the CG and include both single-locus variants (SLVs) and their SLVs [18]. The CGs are named according to the central (main) ST. For example, ST258 is the central ST for CG258, ST512 is a SLV when compared to ST258, ST650 is a SLV compared to ST512, and ST650 is still within the CG258 phylogenetic lineage [18] (Figure 1B). Using this approach, Breurec *et al*. revealed that two major CGs, CG15 (ST15 and ST14) and CG258 (ST340 and ST11), define the predominant international *K. pneumoniae* clones resistant to third-generation cephalosporins in five African and two Vietnamese cities [18]. A similar population study by Baraniak *et al*. revealed four major CGs that are responsible for ESBL-producing *K. pneumoniae* colonizing patients and the genetic diversity in population structures was geographically

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linked: CG17 in France, CG101 in Italy, CG15 in Spain, and CC147 in Israel [19]. MLST analysis of ESBL isolates have shown that the spread of ESBL-producing *K. pneumoniae* is largely multi-clonal, in contrast, the international spread of KPC-Kp is limited to specific clones, at least for now.

To date, KPC has been found in more than 115 different STs (~ 7.5 % of all STs), showing a broad heterogeneous distribution (Figure 1A). Nevertheless, the vast majority of KPC-Kp isolates worldwide belong to CG258 and the two predominant sequence types are ST258 (ST allele profile, 3-3-1-1-1-1-79) and ST11 (3-3-1-1-1-1-4). Secondary clones include ST340 (3-3-1-1-1-1-18), ST437 (3-3-1-1-1-1-31), and ST512 (54-3-1-1-1-1-79). ST258 prevails mainly in North America, Latin America, and several countries in Europe, while ST11 is the major KPC-Kp ST in Asia and Latin America [4, 20–22]. ST512 has been discovered in Colombia, Italy and Israel; and ST340 was mainly reported from Brazil and Greece [4]. In contrast, the spread of other non-CG258 KPC-Kp STs are largely limited to certain geographic regions. For example, the recently emerged multi-drug resistant ST442 isolates (*e.g* strain Kp13) have only been described in Southern Brazil [23].

CG258 is a large group, containing 43 different STs. Among them, sixteen STs (ST258, 379, 418, 512, 554, 650, 744, 745, 868, 1084, 1199, 1406, 1458, 1461, 1481, and 1519) carry a unique MLST *tonB* allele, tonB79, which is primarily found in ST258 and its SLV and double locus variant (DLV). According to the Breurec *et al*. nomenclature, we propose to group strains with the tonB79 allele in CG258 and refer to them as the CG258-tonB79 cluster in order to subdivide CG258. Phylogenetically, members of tonB79-CG258 (*e.g.* ST258, 379, 418 and 512) appear to be more closely related than other members in CG258 (*e.g.* ST11) [24, 25]. This cluster of STs can be easily identified by a real-time PCR targeting of two single nucleotide polymorphisms (SNPs) [26] or more conventionally, by direct sequencing of the *tonB* allele.

Comparative *K. pneumoniae* genomics

To date (April 1, 2014), thirteen *K. pneumoniae* genomes have been completely sequenced (ftp://ftp.ncbi.nih.gov/genomes/Bacteria/). A multiple genome alignment obtained using Mauve (http://gel.ahabs.wisc.edu/mauve/) is illustrated in Figure 2. In addition, more than 350 *K. pneumoniae* draft genomes have been sequenced by next-generation sequencing (http://www.ncbi.nlm.nih.gov/genome/genomes/815).

Unlike its closely related species, such as *Salmonella enterica* and *Escherichia coli*, *K. pneumoniae* appears to be characterized by a low degree of nucleotide divergence among orthologous genes [23, 27] and as shown in the Mauve plots, the gene synteny among the chromosomes is conserved (Figure 2). A previous comparative genomics analysis of six *K. pneumoniae* genomes (including both chromosome and plasmids) identified 3,631 proteins in common that accounted for 65 to 75% of the total number of predicted protein-coding genes for any one of the genomes [28]. However, if only the chromosome bearing genes were compared, the *K. pneumoniae* genomes are more conserved. For example, comparison of *K. pneumoniae* strain HS11286 chromosome with four other *K. pneumoniae* chromosomes (MGH 78578, NTUH-K2044, Kp342, and KCTC2242) identified only 422

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unique genes, accounting for 8% of a total of 5,316 genes [29]. This finding is consistent with the observation that the diversity in *K. pneumoniae* genomes is primarily due to the mobile genes that move frequently by horizontal transfer, including plasmids, phages, integrative and conjugative elements (ICEs), and insertion elements (ISs).

DeLeo *et al*. recently sequenced to closure two ST258 genomes (NJST258_1 and NJST258_2), and compared them with eight other completed genomes in the public databases [24]. The *K. pneumoniae* genomes have similar chromosomal lengths of ~5.3 Mbp, but vary significantly in the number of mobile genetic elements (MGEs), including plasmids, prophages, ICEs, and IS elements [24]. Notably, the chromosome-borne large MGE structures are similar between ST258 (NJST258_1 and NJST258_2) and ST11 (JM45 and HS11286) genomes. A comparative genomic study further suggests ST258 is a hybrid strain — 80% of the genome originated from ST11-like strains and 20% from ST442-like strains [25], similar to the hybrid pandemic methicillin-resistant *S. aureus* ST239 strains [30].

Brisse *et al*. suggested that the evolution of *K. pneumoniae* is mainly driven by homologous recombination, in contrast to the accumulation of mutations [27]. An example that supports this notion is that the same K type-associated capsular polysaccharide (CPS) synthesis operon is frequently found among unrelated STs, the likely result of horizontal transfer of the *cps* operon between different STs [27]. Using genomic comparisons based upon high resolution restriction mapping as well as *in silico*-generated restriction maps of six *K. pneumoniae* genomes, Ramirez *et al*. identified a ~160 kb highly heterogeneous region (based on the genome of MGH 78578), designated as a ‘high heterogeneity zone (HHZ)’ in the *K. pneumoniae* chromosome [31]. The HHZ consists of several ‘hot spot’ recombination regions, including the above mentioned *cps* operon and the high-pathogenicity genetic island, ICEKp1 (in NTUH-K2044) [31].

In an effort to decipher the molecular evolution of epidemic KPC-Kp ST258 strains, DeLeo *et al*. sequenced 83 CG258-tonB79 cluster isolates (including ST258, 379, 418 and 512) recovered from patients at diverse geographic locations[24]. These genomes were compared to the two closed ST258 scaffolds [24]. The 83 queried isolates differed from NJST258_1 on average by 350 SNPs (range, 116—784 SNPs) in the core genome, further supporting the idea that CG258-tonB79 strains are closely related. Phylogenetic analysis of the SNPs revealed that ST258 can be segregated into two distinct genetic clades (clade I and II) [24]. Notably, genetic differentiation between the two clades is largely due to a ~215 kb region of divergence that includes genes involved in *cps* region, and overlaps with the above mentioned HHZ region identified in other *K. pneumoniae* genomes [31]. Moreover, two distinct *cps* operons were identified in ST258 clades (ST258 *cps*1 in clade 1 and *cps*2 in clade 2). Similar findings were reported in independent and contemporary studies conducted by van Duin *et al*. [32], using rep-PCR and epidemiological analysis of a KPC surveillance network, and by Wright *et al*. [33], who examined the population structure of KPC bearing *K. pneumoniae* strains from the Great Lakes region. Furthermore, ST258 clade I strains may have evolved from a clade II strain as a result of *cps* region replacement [25]. Therefore, horizontal transfer of the *cps* region appears to be a key element driving the molecular diversification in *K. pneumoniae* strains.

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bla$_{KPC}$-bearing genetic elements

The original source of *bla*$_{KPC}$ remains unknown, but it is likely that this resistance gene was acquired from an ancestral chromosome of an environmental organism. β-lactamases existed long before the antibiotic era [34]. For example, recent metagenomic analyses of rigorously authenticated ancient DNA from 30,000-year-old Beringian permafrost sediments identified the presence of genes encoding resistance to β-lactams [35]. Fevre *et al.* estimated that *bla*$_{OXY}$, the β-lactamase gene in *Klebsiella oxytoca*, originated as early as 100 million years ago [36]. Therefore, it is plausible that *bla*$_{KPC}$ may have had an ancient origin associated with an environmental organism, and that its present success is the consequence of its capacity for horizontal transfer, the dramatic and man-made increase in antibiotic selection pressure, and the ability for Enterobacteriaceae to readily accept foreign DNA.

The most common *bla*$_{KPC}$-containing mobile element is a Tn*3*-based transposon, Tn*4401* [37]. Tn*4401* is 10 kb in length, delimited by two 39-bp imperfect inverted repeat (IR) sequences, and harbors *bla*$_{KPC}$, a Tn*3* transposase gene (*tnpA*), a Tn*3* resolvase gene (*tnpR*), and two insertion sequences, IS*Kpn6* and IS*Kpn7* [37] (Figure 3). Tn*4401* is commonly flanked by a 5-bp target site duplication (TSD), as a result of its integration. Tn*4401* is believed to originate from the Tn*3*-based *tnpA* and *tnpR* insertion upstream of *bla*$_{KPC}$, followed by the integration of IS*Kpn6* and IS*Kpn7* downstream and upstream of *bla*$_{KPC}$, respectively [37]. Two sets of IRs and TSDs are adjacent to IS*Kpn6* and IS*Kpn7*, suggesting the recent insertion of both ISs in the backbone of Tn*4401* [37]. Five Tn*4401* isoforms (a–e) have been identified, differing by 68- to 255-bp deletions upstream of *bla*$_{KPC}$ (a, −99 bp; b, no deletion; c, −215 bp; d, −68 bp; e, −255 bp) [38]. Cuzon *et al*. subsequently showed that Tn*4401* is a highly active transposon capable of transposition with a 5-bp TSD and without target site specificity in an *in vitro* model [39]. However, one common hot-spot for Tn*4401* is the transposon Tn*1331*, creating a hybrid transposon structure that has been observed on plasmids of different backgrounds; notably, IncN, IncI2 and IncFIA plasmids [40–42]. Tn*1331* carries Tn*3*-like transposase and resolvase genes (*tnpA* and *tnpR*); aminoglycoside modifying enzyme genes, *aac(6')-Ib* and *aadA1*; and β-lactamase genes, *bla*$_{OXA-9}$ and *bla*$_{TEM-1}$ [43]. Even without understanding whether Tn*4401* has repeatedly inserted at the same location in Tn*1331* or whether the hybrid transposon has jumped onto different plasmids, the association of *bla*$_{KPC}$ with other antibiotic resistance determinants provides a very simple scenario for a carbapenemase to spread as a hitchhiker gene, and most alarmingly, in the absence of carbapenem selection.

Moreover, different Tn*4401* isoforms appear to be associated with different *bla*$_{KPC}$-harboring plasmids. For example, the *bla*$_{KPC-3}$-harboring IncFII$_{K2}$ plasmid pKpQIL is associated with Tn*4401a* [44–46], while the *bla*$_{KPC-3}$-bearing IncI2 plasmid pBK15692 carries Tn*4401b* [40]. In addition, the recently reported *bla*$_{KPC-3}$-harboring IncFIA plasmids pBK30661 and pBK30683 are associated with Tn*4401d* [47]. The association of *bla*$_{KPC}$ variants with specific Tn*4401* isoforms can be used as a genetic marker to distinguish different KPC plasmids.

bla$_{KPC}$ has also been found in other non-Tn*4401* mobile elements from isolates in China, Argentina and other regions, as well as in other non-*K. pneumoniae* species [48–50]. To

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simplify the nomenclature of these novel elements, we propose to name them as *bla*$_{KPC}$-bearing non-Tn*4401* elements (NTE$_{KPC}$). As shown in the alignment in Figure 3, seven *bla*$_{KPC}$ elements that contain genetic remnants of Tn*4401* have been characterized and catalogued on the basis of the genes adjacent to *bla*$_{KPC}$. Partial IS*Kpn6* genes, located downstream of *bla*$_{KPC}$, are identical in elements subgrouped as types I and II and intact in Tn*4401*; more importantly, IS*Kpn6* associated left IR (IRL) is intact among NTE$_{KPC}$-I, -II and Tn*4401*, suggesting NTE$_{KPC}$-I and -II may evolve from Tn*4401* by genetic recombination. It is noteworthy that NTE$_{KPC}$s are primarily found in non-ST258 *K. pneumoniae* or other non-*K. pneumoniae* species; whereas, *bla*$_{KPC}$ in epidemic ST258 *K. pneumoniae* strains is exclusively carried on Tn*4401*.

bla$_{KPC}$-harboring plasmids

bla$_{KPC}$ is typically plasmid-borne, and is carried on plasmids of different incompatibility (Inc) groups, including IncFII, FIA, I2, A/C, N, X, R, P, U, W, L/M and ColE [40, 42, 44, 50–55]. Unlike other carbapenemase genes, *bla*$_{KPC}$ is present mainly in plasmids in Enterobacteriaceae. However, two separate reports identified *bla*$_{KPC}$ in the *P. aeruginosa* chromosome; evidence that the gene can transpose from a plasmid and integrate into the host genome [56, 57].

Currently (April 1, 2014), more than 40 *bla*$_{KPC}$-harboring plasmids have been completely sequenced; the majority of these plasmids are from *K. pneumoniae* (Table 1). These *bla*$_{KPC}$-containing plasmids often contain several genes that encode resistance to other antimicrobial agents, such as the aminoglycosides, quinolones, trimethoprim, sulphonamides and tetracyclines. These findings amplify the complexity of controlling the spread of these plasmids, as co-selection leads to the transmission of multidrug resistance among members of the Enterobacteriaceae.

bla$_{KPC}$-harboring plasmids of different Inc groups, *e.g.* IncFII$_{K1}$, FII$_{K2}$, FIA, I2, X, A/C, R and ColE1, are also identified in epidemic ST258 isolates. The epidemiology associated with *bla*$_{KPC}$ plasmids indicates that certain incompatibility groups harboring Tn*4401* are more predominant [44–46, 58–60]. The IncFII plasmids are one salient example. They are commonly low copy number, harbor multiple replicons, and are widely distributed in different species of Enterobacteriaceae [61]. This finding is similar to the worldwide dissemination of *bla*$_{CTX-M-15}$, which is largely associated with *E. coli* ST131 and harbored on multidrug-resistant IncFII plasmids [61, 62]. pKpQIL was the first KPC-encoding plasmid described for ST258. It is an IncFII$_{K2}$ group plasmid containing Tn*4401a*; it was initially identified in 2006 in a *K. pneumoniae* ST258 strain from Israel, and then believed to have spread to Poland, Italy, Colombia, United Kingdom and other countries [44–46, 58–60]. However, pKpQIL-like plasmids spread in the New York and New Jersey area as early as 2003, and a PCR screening of 284 clinical *K. pneumoniae* isolates identified 35.6% as harboring pKpQIL-like plasmids in nine out of ten surveyed hospitals [45]. This study documented the wide dissemination of pKpQIL in this endemic region [45]. Further support for this observation is the finding that an IncF$_{IIK5}$ plasmid, pKp048, harboring a *bla*$_{KPC}$ element variant, is widely disseminated in China and associated with ST11 strains [48, 63].

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pBK15692 is the second predominant bla_{KPC} plasmid found among six New York City and New Jersey hospitals. This plasmid is an IncI2 bla_{KPC-3}-harboring plasmid that was identified in 23% of 256 KPC-bearing *K. pneumoniae* isolates [40]. In addition, novel bla_{KPC-3}-harboring IncFIA plasmids, pBK30661 and pBK30683, were identified in 20% of 491 *K. pneumoniae* isolates collected between 2002 to 2012 in ten New York City and New Jersey hospitals [47]. Although the spread of pBK15692 and pBK30683 in other geographical region remains unknown, these mobile genetic elements have successfully transferred to different *K. pneumoniae* genetic backgrounds and into different species, and we assume that their successful transmission is the result of strong antibiotic selection [40, 47].

The genetic structures of six bla_{KPC}-harboring plasmids from different incompatibility groups are shown in Figure 4. One common structure shared by these plasmids that is that they all carry a *tra* operon, which encodes the plasmid conjugation machinery that facilitates the spread of plasmids and resistance to other strains and species. Clearly, the successful epidemiology associated with plasmids that are able to conjugate and harbor selectable resistance genes is evidence that both factors are important for their dissemination.

Interestingly, there appears to be an association between different plasmid Inc groups and the genome clades in CG258 strains. In a recent genomics study, the pBK15692 (KPC-3)-associated IncI2 plasmids, and pBK30661/30683 (KPC-3)-associated IncFIA plasmids are found exclusively in clade II of CG258 strains [24]. In contrast, the pKpQIL-associated $IncFII_{K2}$ plasmids were found in both clade I and clade II [24], whereas clade I strains mainly carry bla_{KPC-2} and clade II strains primarily harbor bla_{KPC-3} [24, 64]. These findings clearly suggest that multiple plasmid acquisitions have occurred among strains that are catalogued collectively as the epidemic ST258 clone. These findings also indicate that convergent evolution has occurred within the ST258 lineage, and that the natural selection of different CG258 clade backgrounds with bla_{KPC}-carrying mobile elements or plasmids has given rise to predominant clones. The evolutionary fine-tuning of these associations may help to maintain or increase bacterial fitness of these epidemic clone, as demonstrated previously in CTX-M-producing *E. coli* ST131 strains [65].

Understanding the success of epidemic ST258

The spread and success of KPC-producing CRE strains is multifactorial, as bla_{KPC} is on a promiscuous transposon, Tn*4401*, and this transposon has jumped to numerous plasmids that are commonly conjugative. These plasmids have spread to different Enterobacteriaceae species and have found a highly compatible host in the *K. pneumoniae* ST258 background [10].

The molecular epidemiology of KPC-producing strains indicates that *K. pneumoniae* is the predominant species, suggesting a unique fitness and selective advantage beyond resistance. The finding that conjugative transfer of bla_{KPC}-carrying plasmids was successful within species of *Klebsiella*, but not among other Enterobacteriaceae, could explain the observed epidemiology [66]. Given the success of the *K. pneumoniae* ST258 lineage worldwide (i.e., it is widely disseminated), one could speculate that the fitness of this clone and/or the

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conjugative efficiency of the *bla*$_{KPC}$-harboring plasmids in this genetic background. Identifying the factors contributing to the epidemic success of ST258 remains an important public health question.

One could hypothesize that the success of KPC-Kp ST258 may also be associated with unique virulence traits, or their expression, that facilitates the ability to cause disease and spread. However, this argument is challenged by the recent study that showed ST258 to be virtually avirulent in immunocompetent and neutropenic animal models, highly susceptible to serum killing, and rapidly undergoing phagocytosis *in vitro* [67]. Genetic analysis by PCR amplification of targeted genes revealed that ST258 strains lack well-characterized *K. pneumoniae* virulence factors, including K1, K2, and K5 capsular antigen genes, the aerobactin genes, and regulator of mucoid phenotype gene *rmpA* [67]. The observation that epidemic ST258 strains are not highly virulent in animal models leads to the speculation that its successful spread is due largely to a combination of the genetic background being compatible with plasmids enhanced harboring Tn*4401*, and that this 'fitness' plus its multidrug resistance phenotype provides an advantage.

Undoubtedly, the multi-resistant phenotype of ST258 strains allows them to survive the barrage of antibiotics used in the treatment of hospital infections. Nevertheless, this cannot adequately explain the success of this clone. As described above, *bla*$_{KPC}$ was identified in more than 100 different STs, including STs that are distinct to ST258, but none of them have spread so widely. Meanwhile, other carbapenemase-producing *K. pneumoniae* strains, including those that carry *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$ and *bla*$_{OXA-48}$, are frequently identified, but none has disseminated to the extent of *K. pneumoniae* ST258. This leads us to conclude that, in addition to antibiotic resistance, other ST258 unique genetic factors, either on the chromosome or on specific plasmids, must contribute to the success and rapid spread of this clone.

The two closed ST258 genomes carry seven to eight prophages and two ICEs, and most of these mobile genetic elements are also present in ST11 strains [24]. ICEKp258.1 (harbored by both ST11 and ST258 strains) carries a type IV secretion system, which could potentially promote the transfer of genetic elements such as plasmids [68]. Meanwhile, ICEKp258.2, which is unique to ST258 strains [25], harbors a type IV pilus gene cluster that may facilitate adherence to living and nonliving surfaces, *e.g*. the gut of humans or the environment, as well as increase the uptake and exchange of DNA (e.g., plasmids) [69]. Moreover, ICEKp258.2 harbors a type III restriction-modification system that could serve as a 'host specificity' system that only allows the exchange of certain compatible plasmids [70]. These unique genetic factors may potentially contribute to the dissemination of ST258.

In addition to these attributes, a recent fitness study suggests other host-associated factors may contribute to epidemic success of the ST258 lineage. Benenson *et al*. compared the fitness of two distinct *K. pneumoniae* strains (KP314 and KP154) that harbor the same KPC plasmids (pKpQIL) [71]. KP314 is an ST321 isolate, while KP154 is classified as ST512, a member of CG258-tonB79 cluster [71]. In an *in vitro* model, KP314 (ST321) had a fitness advantage over KP154 (ST512), whereas in the clinical setting KP154 was more successful than KP314. This finding suggests that there are likely host-related factors that explain the

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discrepancy between the *in vitro* study and the epidemiological observation for these two related CG258 strains [71].

As described above, the ST258 'strain' is comprised of at least two distinct lineages or clades rather than a single clone. The two clades are differentiated largely by a ~215 Kb region that encodes capsule polysaccharide biosynthesis machinery (*cps1* and *cps2*). Meanwhile, the three closed ST11 genome strains carry three distinct *cps* regions (Figure 2). The *cps* locus is one of the primary determinants of antigenicity associated with *K. pneumoniae*, and capsule switching is a species-specific mechanism used by the microbe to escape the host immune response. DNA exchange in-and-around the *cps* regions may be an important mechanism used by *K. pneumoniae* to rapidly diversify and evolve [72]. Thus, chromosomal recombination is likely the major contributing factor to the global success of ST258, ST11 and other strains above and beyond antibiotic resistance.

Concluding remarks

K. pneumoniae was described more than one century ago, and it remains one of the most common pathogens causing healthcare-associated infections. In spite of the long history and considerable worldwide dissemination of *K. pneumoniae* infections, the population genetics and genomics have not attracted much attention until now. As a consequence of its continual increasing resistance to antibiotics, first by acquisition of ESBLs and now carbapenemases, there is compelling need to understand the plasmid and chromosome architecture of this pathogen. The studies reviewed here highlight recent advances that aim to address these key issues using novel approaches, *e.g.* comparative genomics, for exploring the determinants that contribute to the success of specific clones and circulating plasmids. Undoubtedly, understanding the molecular evolution of successful KPC-Kp lineages as well as their associated plasmids will lead to improved tracking of resistance, and thus control of the spread of carbapenem resistance (Box 1). Similar to other serious infections that have challenged humanity, KPC and other carbapenemase producing-Gram negative pathogens are changing the global face of resistance.

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Box 1. Outstanding questions

- Do bla_{KPC}-harboring plasmids have unique compatibility with *K. pneumoniae* ST258 or confer a fitness advantage that accounts for the global predominance of this resistant lineage?
- Is fitness of ST258 strains – which are hybrid descendants of ST11 and ST442 strains – enhanced compared to either of these two parental strains?
- Considering that *cps* region undergoes rapid change or exchange, is it reasonable to develop a polysaccharide vaccine for prevention and/or treatment of KPC-Kp infections?
- What is the mechanism underlying recombination of the *cps* locus in *K. pneumoniae*?
- Do specific chromosomal and/or plasmid factors explain why some bla_{KPC}-harboring plasmids are more frequently observed in certain *K. pneumoniae* genetic backgrounds?
- Do bla_{KPC}-harboring plasmids contribute to the overall fitness or virulence in *K. pneumoniae*?

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Highlight

1. Active spread of *Klebsiella pneumoniae* carbapenemases (KPCs) occurs through transposons, plasmids, and epidemic clones.
2. Certain KPC-producing epidemic clones have spread globally.
3. Numerous factors are responsible for the spread and success of epidemic KPC clones.

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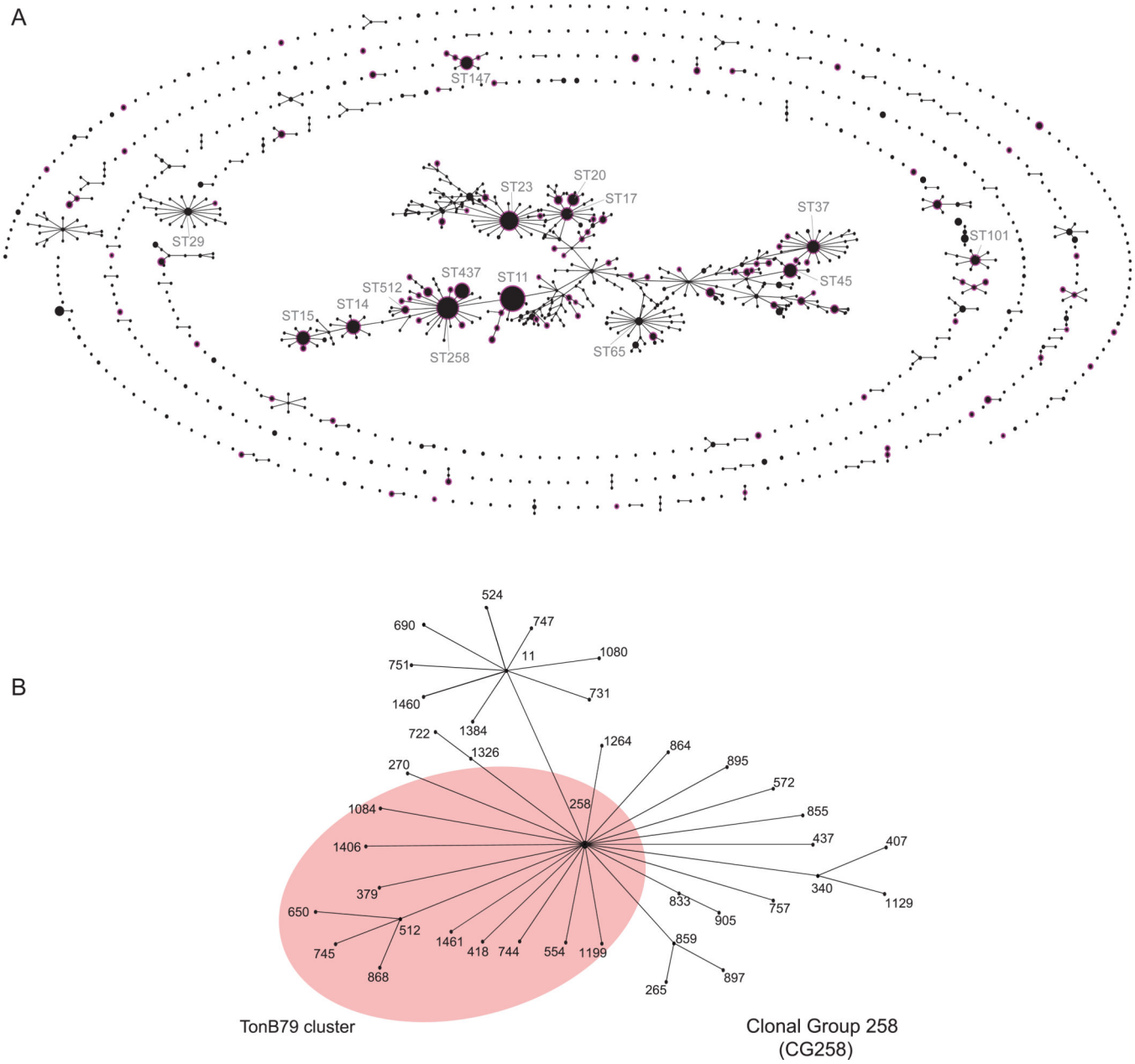

Figure 1.

(A). Population structure of KPC-Kp. The figure represents the population structure of the *K. pneumoniae* MLST database (http://www.pasteur.fr/mlst) as of April 1, 2014, depicted graphically by eBURST v.3 (http://eburst.mlst.net), and shown in the context of all of the 1,536 STs from 1,924 isolates. KPC-Kp STs are highlighted by a pink halo. (B). Population structure of CG258. The pink shading highlights the STs of CG258-tonB79 cluster.

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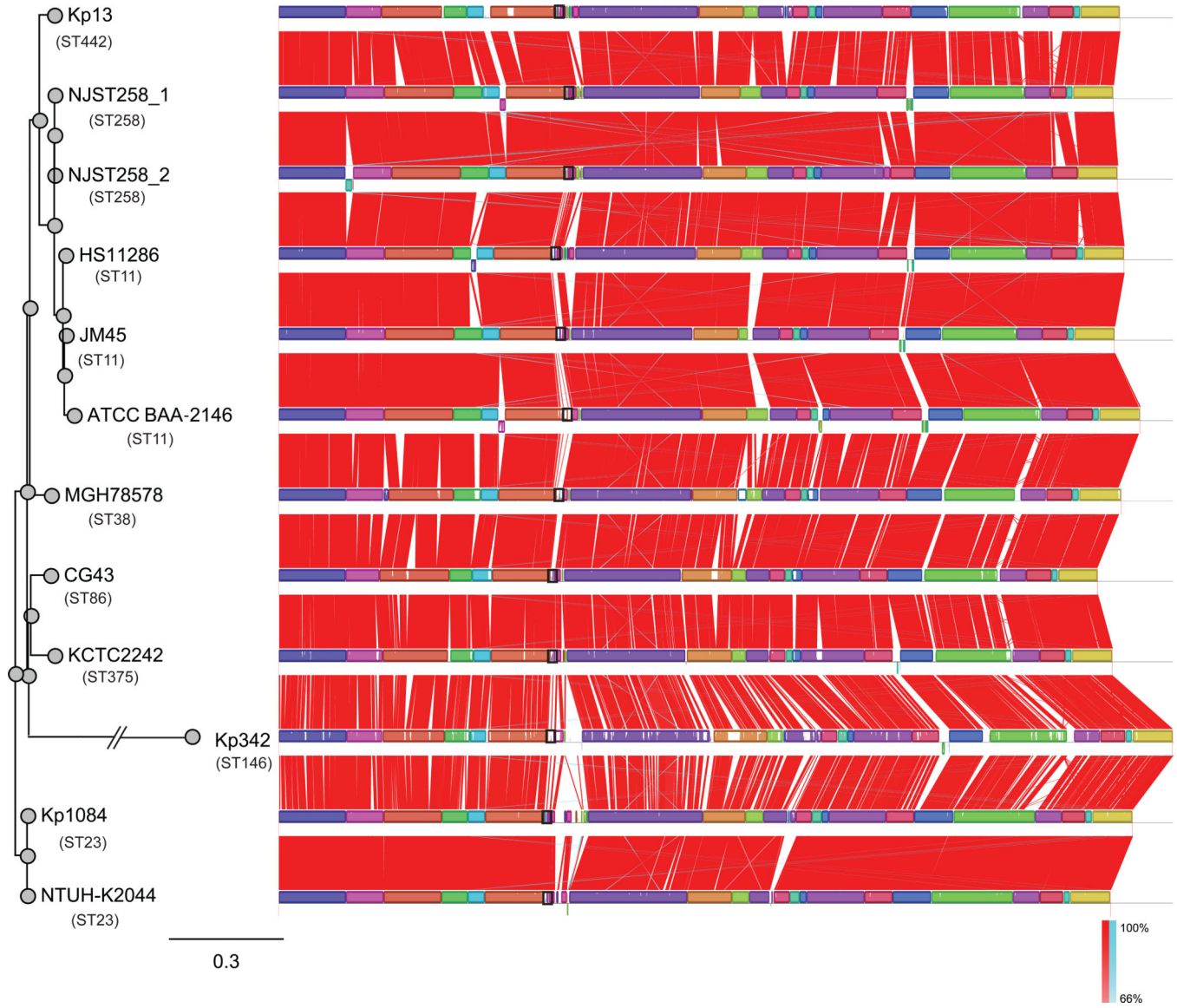

Figure 2.

Mauve plots of thirteen completely sequenced *K. pneumoniae* genomes. Boxes with identical colors represent local colinear blocks (LCB), indicating homologous DNA regions shared by two or more chromosomes without sequence rearrangements. LCBs indicated below the horizontal black line represent reverse complements of the reference LCB. Red (forward) and blue (reverse) shading denotes shared regions of homology, and the black box line illustrates the *cps* region in each genome. ST258 isolates (NJST258_1 and _2; CP006923 and CP006918) [24], ST11 isolate ATCC BAA-2146 (CP006659) [73], ST38 isolate MGH 78578 (CP000647) and one ST146 isolate (Kp 342) (CP000964) [74] were from United States; ST11 isolates HS11286 (CP003200) [29] and JM45 (CP006656) were from mainland China; two ST23 K1 isolates (NTUH-K2044 and Kp 1084; AP006725 and CP003785) and one ST86 K2 isolate (CP006648) were from were Taiwan [75, 76]; and ST375 isolate (KCTC 2242; CP002910) was from Korea [77], and one ST442 isolate

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(Kp13; CP003999) is from Brazil [23]. The left panel is the maximum likelihood tree generated using the SNPs extracted by Mauve.

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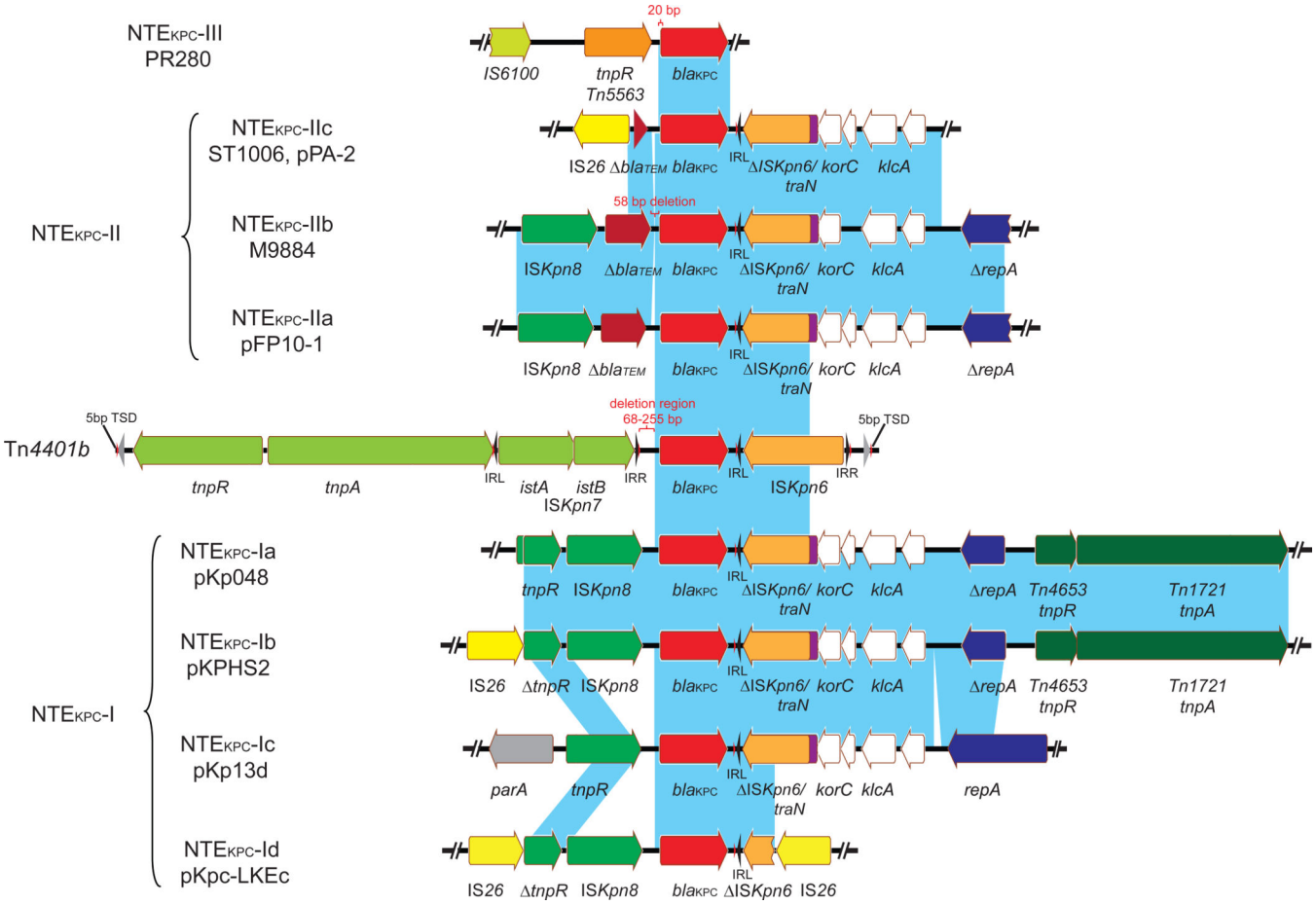

Figure 3.

bla$_{KPC}$-harboring genetic elements (Tn*4401* and NTE$_{KPC}$). Based on the insertion sequence upstream of *bla*$_{KPC}$, NTE$_{KPC}$ can be divided into three groups: NTE$_{KPC}$-I, no insertion [48]; NTE$_{KPC}$-II, insertion of Δ*bla*$_{TEM}$ [48]; and NTE$_{KPC}$-III, insertion of Tn*5563*/IS*6100* [78]. NTE$_{KPC}$-I can be further classified as -Ia (prototype, pKp048) [48], -Ib (pKPHS2) [29], -Ic (pKp13d) [23] and -Id (pKPC-LKEc) [79] based on the insertion sites of upstream and/or downstream of IS*26* and the presence of IS*Kpn8*. NTE$_{KPC}$-II can be subgrouped as -IIa (pFP10-1, and *bla*$_{KPC}$-harboring plasmids from strain M9196 and M11180) [49, 80], -IIb (from strain M9884 and M9988) [49], and -IIc (pPA-2) [50], based on the differences of the length of Δ*bla*$_{TEM}$ and the deletions. Light-blue shading denotes shared regions of homology.

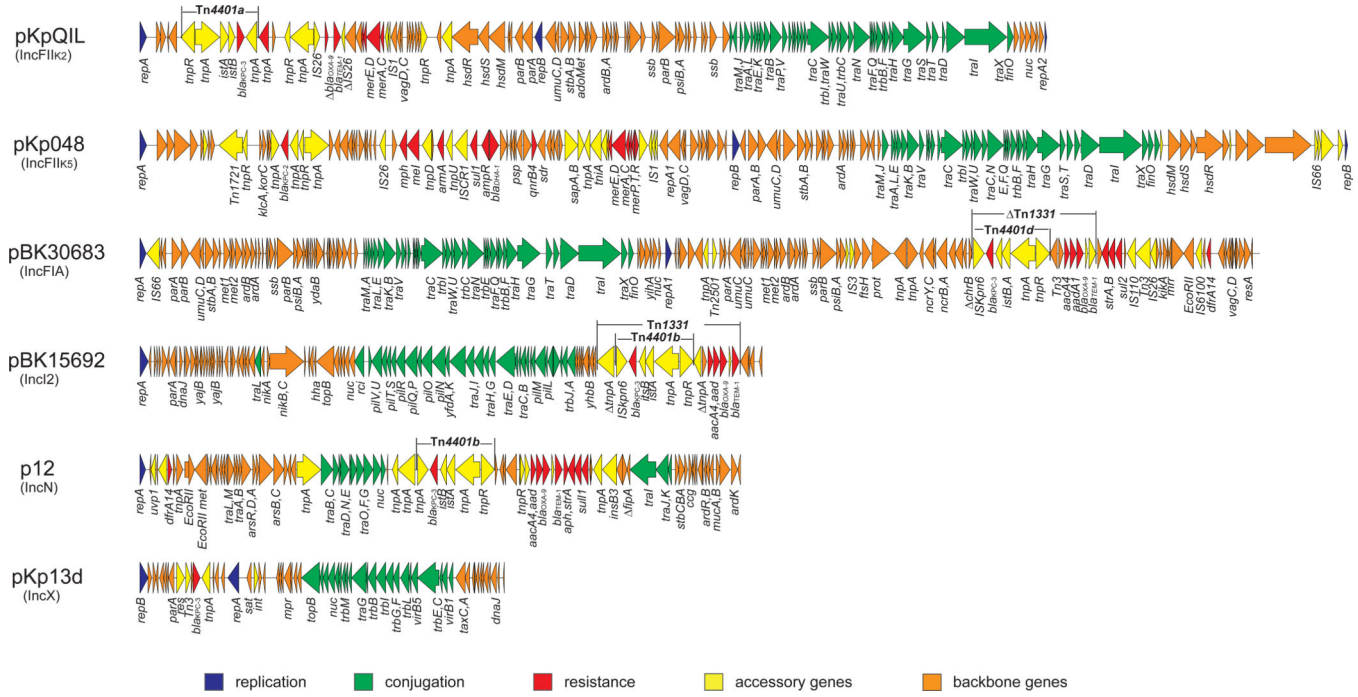

Figure 4.

Structures of *bla*$_{KPC}$-harboring plasmids: p12 (IncN), pKpQIL (IncFII$_{K2}$), pKp048 (IncFII$_{K5}$), pBK15692 (IncI2), pBK30683 (IncFIA), and pKp13d (IncX3).

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Table 1

Completely sequenced *bla*$_{KPC}$-bearing plasmids in *K. pneumoniae*

Plasmid	Accession	ST	Year	Location	Length (bp)	GC (%)	Inc	KPC	KPC element
pKP1433	JX397875	340	2009–2010	Greece	55,417	50.8	*a*	KPC-2	Tn*4401b*
pKpQIL-LS6	JX442975	258	2011	Italy	78,227	54.2	*b*	KPC-3	Tn*4401a*
p15S	FJ223606	-	2005	New York City	23,753	57.0	ColE1	KPC-2	Tn*4401a*
pNJST258C2	CP006919	258	2010	New Jersey	25,284	56.7	ColE1	KPC-3	Tn*4401b*
pBK30661	KF954759	258	2010	New Jersey	73,635	53.9	FIA	KPC-3	Tn*4401d*
pNJST258N2	CP006926	258	2010	New Jersey	73,636	53.9	FIA	KPC-3	Tn*4401d*
pBK30683	KF954760	963	2010	New Jersey	139,941	54.0	FIA	KPC-3	Tn*4401d*
pKPC-LK30	KC405622	11	2012	Taiwan	86,518	56.0	FIIK	KPC-2	NTE$_{KPC}$-Ib
pBK32179	JX430448	258	2010	New York City	165,295	52.7	FIIK1	KPC-2	Tn*4401a*
p1-JM45	CP006657	11	2010	China	317,154	53.0	FIIK1	KPC-2	NTE$_{KPC}$-Ia
pKPN101-IT	JX283456	101	2011	Italy	107,748	52.7	FIIK1, R	KPC-2	Tn*4401a*
pSLMT	HQ589350	-	-	-	21,138	56.3	FIIK2	KPC-2	Tn*4401a*
pKP1780-kpc	KF874497	-	-	Greece	113,622	53.9	FIIK2	KPC-2	Tn*4401a*
pKpQIL	GU595196	258	2006	Israel	113,637	53.9	FIIK2	KPC-3	Tn*4401a*
pKP1504-kpc	KF874496	-	-	Greece	113,640	53.9	FIIK2	KPC-2	Tn*4401a*
pKP3913-kpc	KF874499	-	-	Greece	113,640	53.9	FIIK2	KPC-2	Tn*4401a*
pKpQIL-IT	JN233705	258	2010	Italy	115,300	53.9	FIIK2	KPC-3	Tn*4401a*
pKP1870-kpc	KF874498	-	-	Greece	116,047	54.0	FIIK2	KPC-2	Tn*4401a*
pKPHS2	CP003224	-	-	China	111,195	53.3	FIIK2, R	KPC-2	NTE$_{KPC}$-Ib
pKP048	FJ628167	-	2006–2007	China	151,188	51.3	FIIK5, R	KPC-2	NTE$_{KPC}$-Ia
pBK15692	KC845573	258	2005	New Jersey	77,801	45.4	I2	KPC-3	Tn*4401b*
pKPC_FCF13/05	CP004366	-	2005	Brazil	53,081	52.5	N	KPC-2	Tn*4401b*^{c}
pKPC_FCF/3SP	CP004367	-	2009	Brazil	54,605	52.9	N	KPC-2	Tn*4401b*
pKo6	KC958437	-	-	China	65,549	51.0	N	KPC-2	NTE$_{KPC}$-Ic
p9	FJ223607	-	2005	New York City	70,655	54.3	N	KPC-2	Tn*4401b*
p12	FJ223605	-	2005	New York City	75,617	52.8	N	KPC-3	Tn*4401b*
pBK31551	JX193301	834	2005	New Jersey	83,712	53.5	N	KPC-4	Tn*4401b*
pHS062105-3	KF623109	-	2006	China	42,848	49.7	P	KPC-2	NTE$_{KPC}$-Ia

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Plasmid	Accession	ST	Year	Location	Length (bp)	GC (%)	Inc	KPC	KPC element
pKPC-NY79	JX104759	258	2011	New York City	42,447	48.5	X3	KPC-2	Tn*4401a*
pKP13d	CP003997	442	2009	Southern Brazil	45,574	46.0	X3	KPC-2	NTE_{KPC}-Ic
pKpS90	JX461340	258	2009	France	53,286	49.6	X3	KPC-2	Tn*4401a*
pBK31567	JX193302	429	2006	New Jersey	47,387	49.0	X5	KPC-5	Tn*4401b*

[a], IncN plasmid backbone, but lack the IncN *repA* replicon.

[b], IncFIIK2 plasmids are pKpQIL-like, but lack the IncFII *repA* gene.

[c], 256 bp insertion in *tnpA* in Tn*4401*.

Abbreviation: -, data not available.

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