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Molecular Survey of the Dissemination of Two *bla*$_{KPC}$-Harboring IncFIA Plasmids in New Jersey and New York Hospitals

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***Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* strains have spread worldwide and become a major threat in health care facilities. Transmission of *bla*$_{KPC}$, the plasmid-borne KPC gene, can be mediated by clonal spread and horizontal transfer. Here, we report the complete nucleotide sequences of two novel *bla*$_{KPC-3}$-harboring IncFIA plasmids, pBK30661 and pBK30683. pBK30661 is 74 kb in length, with a mosaic plasmid structure; it exhibits homologies to several other plasmids but lacks the plasmid transfer operon (*tra*) and the origin of transfer (*oriT*) that are required for plasmid transfer. pBK30683 is a conjugative plasmid with a cointegrated plasmid structure, comprising a 72-kb element that highly resembles pBK30661 (>99.9% nucleotide identities) and an extra 68-kb element that harbors *tra* and *oriT*. A PCR scheme was designed to detect the distribution of *bla*$_{KPC}$-harboring IncFIA (pBK30661-like and pBK30683-like) plasmids in a collection of clinical *Enterobacteriaceae* isolates from 10 hospitals in New Jersey and New York. KPC-harboring IncFIA plasmids were found in 20% of 491 *K. pneumoniae* isolates, and all carried *bla*$_{KPC-3}$. pBK30661-like plasmids were identified mainly in the epidemic sequence type 258 (ST258) *K. pneumoniae* clone, while pBK30683-like plasmids were widely distributed in ST258 and other *K. pneumoniae* sequence types and among non-*K. pneumoniae Enterobacteriaceae* species. This suggests that both clonal spread and horizontal plasmid transfer contributed to the dissemination of *bla*$_{KPC}$-harboring IncFIA plasmids in our area. Further studies are needed to understand the distribution of this plasmid group in other health care regions and to decipher the origins of pBK30661-like and pBK30683-like plasmids.**

Carbapenem-resistant *Enterobacteriaceae* (CRE), especially *Klebsiella pneumoniae*, are a major threat in health care facilities, both in the United States and worldwide (1). According to a recent CDC report, 3.9% of short-stay hospitals and 17.8% of long-term acute-care facilities documented at least one carbapenem-resistant infection (2). Accordingly, the prevalence of carbapenem-resistant *Klebsiella* species increased from 1.6% in 2001 to 10.4% in 2011 (2). Notably, a single enzyme, namely, *K. pneumoniae* carbapenemase (KPC), is responsible for a majority of the cases of carbapenem resistance in the United States.

The KPC gene, *bla*$_{KPC}$, is commonly carried on numerous transferable plasmids, thereby facilitating its interspecies and intraspecies dissemination (3–5). Presently, *bla*$_{KPC}$ is identified on plasmids of different incompatibility (Inc) replicon groups, including IncFII, IncI2, IncL/M, IncN, IncA/C, IncR, IncX, and ColE1 (4, 6–9). Among them, certain plasmids appear to be epidemic, contributing significantly to the spread of KPC. For example, the first *K. pneumoniae* sequence type 258 (ST258)-associated plasmid, pKpQIL, which was originally found in Israel, has spread to several countries, including Italy, Poland, the United Kingdom, Columbia, Canada, and the United States (1, 7, 10, 11). An IncI2 plasmid, pBK15692, has spread widely in New Jersey and New York hospitals, accounting for 23% of a collection of 256 KPC-bearing *K. pneumoniae* isolates collected in 2007 to 2012 (8). Here, we report the complete sequences of two novel *bla*$_{KPC}$-harboring IncFIA plasmids, pBK30661 and pBK30683. Additionally, a PCR-based approach was developed to explore their prevalence among a collection of KPC-positive *K. pneumoniae* isolates from hospitals in New York and New Jersey.

MATERIALS AND METHODS

Bacterial strains. Two *K. pneumoniae* isolates, BK30661 and BK30683, were identified in a retrospective study of carbapenem-resistant *K. pneumoniae* isolates from New Jersey and New York hospitals. They were isolated from urine samples obtained from patients with urinary tract infections, in two separate New Jersey hospitals, in June 2010. A total of 491 *K. pneumoniae* unique clinical isolates, collected from 10 hospitals in New Jersey and New York in 2001 to 2012, were included to determine the distribution of pBK30661-like and pBK30683-like plasmids, using a PCR approach (described below). In addition, 52 KPC-producing non-*K. pneumoniae Enterobacteriaceae* isolates, including 33 *Enterobacter* isolates, 15 *Escherichia coli* isolates, and 4 *Citrobacter* isolates, collected from four of the 10 hospitals between 2009 and 2012 were subjected to PCR screening.

Characterization of strains and manipulation of plasmids. Isolates BK30661 and BK30683 were initially screened with a multiplex real-time PCR for the presence of *bla*$_{KPC}$ (12), and its sequence type (ST) was de-

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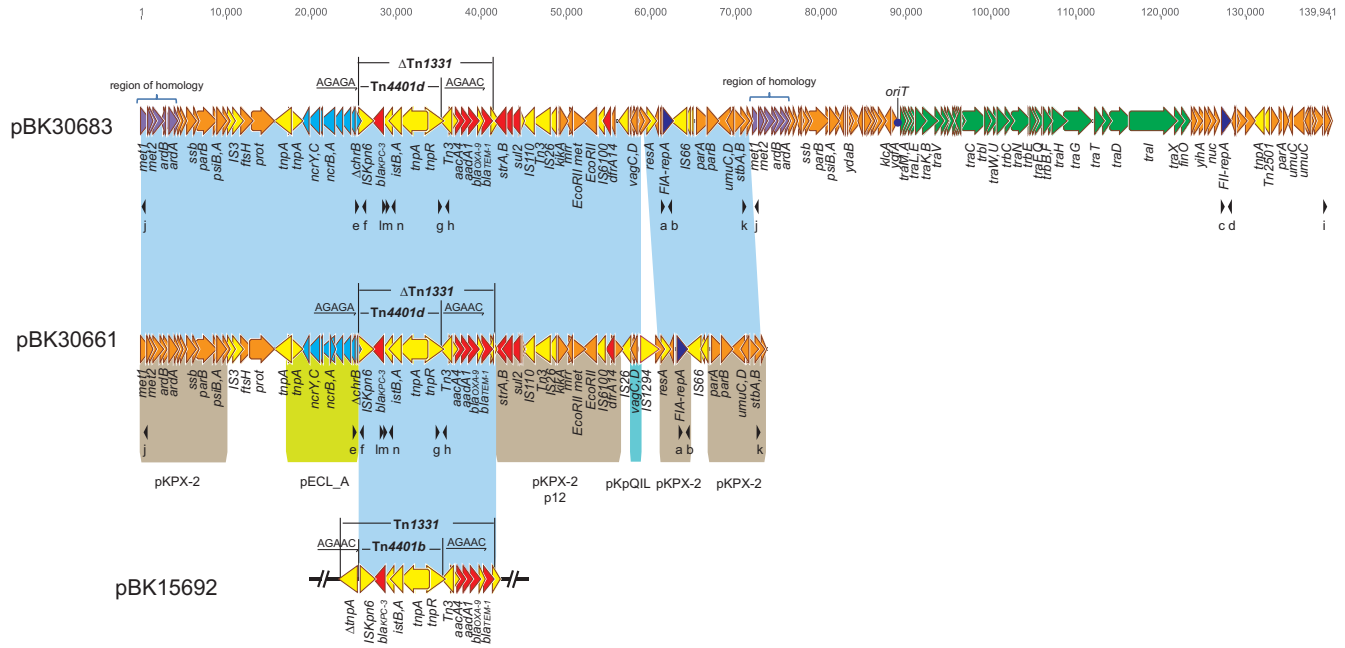

FIG 1 Plasmid structures of pBK30661 (GenBank accession no. KF954759), pBK30683 (GenBank accession no. KF954760), and the Tn*4401*/Tn*1331* nested transposon in pBK15692 (GenBank accession no. KC845573). Light blue shading, shared regions of homology among pBK30661, pBK30683, and pBK15692. Open reading frames (ORFs) are portrayed by arrows and colored based on predicted gene function. Orange arrows, plasmid scaffold regions; green arrows, genes associated with the *tra* locus; dark-blue arrows, replication-associated genes; red arrows, antimicrobial resistance genes; light-blue arrows, nickel resistance-associated genes; yellow arrows, accessory genes; purple arrows, genes located in the 4-kb repeated homologous regions in pBK30683. The 5-bp Tn*4401* adjacent sequences are underlined. Small black arrowheads beneath the plasmids indicate the locations of primers used for PCR screening. The primer sequences are shown in Table 1.

termined by multilocus sequence typing (MLST) (13). Plasmid DNA in strains BK30683 and BK30661 was extracted and electroporated into *Escherichia coli* DH10B (Invitrogen) by using the method described previously (6). *E. coli* DH10B transformants were selected on lysogeny broth (LB) agar plates containing 100 μg/ml ampicillin, and the presence of bla_{KPC} genes was confirmed by multiplex real-time PCR (12). The plasmid size of the transformants was estimated by S1 nuclease digestion of plasmid DNA, followed by pulsed-field gel electrophoresis (PFGE) using a Bio-Rad CHEF-DR III variable-angle system (14). Transformants with single plasmids were then selected and subjected to susceptibility testing and sequencing.

MICs for the two parental isolates and their *E. coli* DH10B transformants were determined by broth microdilution in cation-adjusted Mueller-Hinton broth (MHB), using Sensititre GNX2F panels (Thermo Fisher Scientific, Waltham, MA), according to Clinical and Laboratory Standards Institute methods and interpretations (15, 16). Transferability of bla_{KPC}-bearing plasmids of BK30661 and BK30683 was examined by conjugation experiments using the *E. coli* J53Az^R strain as the recipient, as described previously (17).

Plasmid sequencing and bioinformatics. Plasmid DNA from the *E. coli* DH10B transformants of BK30661 and BK30683 was extracted using a Qiagen plasmid maxikit (Qiagen, Valencia, CA). The plasmid DNA was sequenced using a Roche 454 GS-FLX system. Plasmid sequence assembly, closure, and annotation were performed as described elsewhere (6).

PCR screening for pBK30661-like and pBK30683-like plasmids. A PCR scheme with four duplex PCRs was designed to detect pBK30661-like and pBK30683-like plasmids (Fig. 1). Duplex I (PCR-1 and PCR-2) was designed to target the IncFIA plasmid-specific replication gene *repA* (in both pBK30661 and pBK30683) and the second IncFII replication gene *repA* in pBK30683. Duplex II (PCR-3 and PCR-4) was designed to target the upstream and downstream junctions between Tn*4401* and the neighboring regions of the plasmid. Duplex III (PCR-5 and PCR-6) was designed to detect both junctions of the 68-kb integrated fragment in pBK30683. Duplex IV (PCR-7 and PCR-8) was used to detect the pBK30661- and pBK30683-associated Tn*4401d* isoform. pBK30661-like plasmids are positive with PCR-1, PCR-3, PCR-4, PCR-6, PCR-7, and PCR-8, while pBK30683-like plasmids are positive with all eight PCRs. The primer sequences are shown in Table 1, and the primer locations are illustrated in Fig. 1. The PCR cycling conditions were as follows: an initial denaturation step of 95°C for 4 min, 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min, and a final extension step of 72°C for 7 min. DNA samples from strains BK30661, BK30683, and *E. coli* DH10B were used as positive and negative controls in each PCR run.

Currently, there are at least 5 defined Tn*4401* isoforms (isoforms a to e) that differ by deletions found upstream of bla_{KPC} (isoform a, 99-bp deletion; isoform b, no deletion; isoform c, 215-bp deletion; isoform d, 68-bp deletion; isoform e, 255-bp deletion), as well as some other forms with other deletions or insertions (3, 9, 18, 19). The pBK30661- and pBK30683-associated Tn*4401d* isoform in this study refers to the variant with a 68-bp deletion upstream of bla_{KPC}. Different Tn*4401* isoforms are distinguished by a PCR spanning the upstream IS*Kpn7* *istB* and bla_{KPC} regions (20). We introduced a new reverse primer specific for the Tn*4401b* and Tn*4401d* isoforms into the previously published PCR method (Fig. 1 and Table 1), in order to resolve Tn*4401a* and Tn*4401d* isoforms (31-bp difference) by agarose gel electrophoresis. The *K. pneumoniae* ST258 and bla_{KPC} variants of all isolates were characterized by two multiplex real-time PCR methods developed by our laboratory (12, 21).

Nucleotide sequence accession numbers. The complete nucleotide sequences of pBK30661 and pBK30683 were deposited in GenBank with accession numbers KF954759 and KF954760, respectively.

RESULTS

Microbiological characterization of isolates BK30661 and BK30683. MLST analysis revealed that BK30661 belongs to the epidemic *K. pneumoniae* ST258 clone (allelic profile 3-3-1-1-1-1-

TABLE 1 Oligonucleotide primers used in this study

PCR	Order[a]	Primer	Sequence (5′ to 3′)	Length (bp)	Target
Duplex I					
PCR-1	a	IA-1f	GCCGTCCTTTCTGTGACAAATCA		IncFIA *repA* of pBK30661 and pBK30683
	b	IA-1r	GGATGGACTGTGGGCACGTT	516	
PCR-2	c	IA-2f	CCGTTTCTGTGTCATTTGCTCCT		Second IncFII *repA* in pBK30683
	d	IA-2r	CTTATAGTGAGACGGCCGGAACC	250	
Duplex II					
PCR-3	e	IA-3f	ATACCGGTGCCGCCATGCTGCG		Tn*4401* upstream junction between *chrB* gene and IS*Kpn6* in both pBK30661 and pBK30683
	f	IA-3r	TCGTCATGCCGCGGACCACCCC	213	
PCR-4	g	IA-4f	CCGGCATCACCGGCCCTCACCT		Tn*4401* downstream junction between Tn*4401 tnpR* gene and neighboring Tn*3 tnpA* gene in both pBK30661 and pBK30683
	h	IA-4r	ACACTCCCGGCTGTGCGCCTGA	515	
Duplex III					
PCR-5	i	IA-5f	CGATGACGTGGAGAGCAGTA	534	Region between putative cytoplasmic protein gene and adenine-specific methyltransferase gene (*met1*) in pBK30683
	j	IA-56r	TCCCGAGAATGAATCTGGAC		
PCR-6	k	IA-6f	CGTGCATTCGGTGACTAAAA	768	Region between hypothetical protein gene and adenine-specific methyltransferase gene (*met1*) in both pBK30661 and pBK30683
Duplex IV[b]					
PCR-7	l	4401v-r (3781L)	CACAGCGGCAGCAAGAAAGC	635	Tn*4401d* isoform in pBK30661 and pBK30683
PCR-8	m	4401v-r1	GCAAGCCGCTCCCTCTCCAG	314	
	n	4401v-f (3098U)	TGACCCTGAGCGGCGAAAGC		

[a] The primer order is shown in Fig. 1.
[b] The sizes are variable for different Tn*4401* isoforms, as follows: isoform b, 703 bp and 382 bp; isoform a, 604 bp; isoform c, 487 bp; isoform d, 635 bp and 314 bp; isoform e, 448 bp. Primers 4401v-r (3781L) and 4401v-f (3098U) were published previously (20).

79), while BK30683 belongs to ST963 (allelic profile 2-9-2-1-13-1-25), which is genetically distinct from ST258. Multiplex real-time PCR assays for bla_{KPC} variants showed that both isolates carried bla_{KPC-3}. Susceptibility testing showed that both BK30661 and BK30683 were resistant to imipenem (MIC, >8 μg/ml), ertapenem (MIC, ≥4 μg/ml), meropenem (MIC, ≥8 μg/ml), doripenem (MIC, ≥4 μg/ml), cefepime (MIC, ≥32 μg/ml), cefotaxime (MIC, ≥32 μg/ml), ceftazidime (MIC, >16 μg/ml), aztreonam (MIC, >16 μg/ml), ticarcillin-clavulanate (MIC, ≥128 and ≥2 μg/ml), piperacillin-tazobactam (MIC, ≥128 and ≥4 μg/ml), levofloxacin (MIC, >8 μg/ml), ciprofloxacin (MIC, ≥4 μg/ml), tobramycin (MIC, ≥16 μg/ml), and co-trimoxazole (MIC, ≥4 and ≥76 μg/ml). BK30661 was also resistant to gentamicin (MIC, ≥16 μg/ml), amikacin (MIC, ≥64 μg/ml), and colistin (MIC, 4 μg/ml).

With selection on ampicillin-containing agar (100 μg/ml), we were successful in transferring carbapenem resistance from *K. pneumoniae* BK30683 to *E. coli* J53 by conjugation and to *E. coli* DH10B by electroporation. In contrast, multiple attempts to transfer the bla_{KPC}-bearing plasmid from BK30661 by conjugation were not successful; transfer was achieved only by electroporation. Representative *E. coli* DH10B transformants of BK30661 and BK30683 displayed resistance profiles similar to those of the parent strains but were susceptible to ciprofloxacin (MIC, ≤0.25 μg/ml), levofloxacin (MIC, ≤1 μg/ml), and colistin (MIC, 0.5 μg/ml).

Structure of bla_{KPC-3}-harboring plasmids pBK30661 and pBK30683. pBK30661 is 73,635 bp in length, with an average G+C content of 53.9%, and harbors 84 predicted open reading frames (ORFs) (Fig. 1). It carries a single replication gene, *repA*, with 99.7% nucleotide similarity to the replication gene of plasmid pKPX-2, which was identified from a multidrug-resistant *K. pneumoniae* strain in Taiwan (22). Plasmid sequence queries against the plasmid MLST databases (http://pubmlst.org/plasmid) showed that the replication region of pBK30661 was close to IncFIA allele 8, with 94.0% nucleotide identity, identifying pBK30661 as a member of the IncFIA plasmid group.

pBK30661 has a mosaic plasmid structure, with regions homologous to several other plasmids (pKPX-2, pECL_A, pBK15692, p12, and pKpQIL) (Fig. 1). The plasmid backbone genes are separated by several insertion sequence (IS) elements (IS*3*, IS*26*, IS*1294*, and IS*66*), suggesting that the IS elements play important roles in the architecture of the pBK30661 genome. Full plasmid BLAST queries against NCBI GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) showed that pBK30661 has 55% query coverage and overall 97% nucleotide identity to pKPX-2 (Fig. 1A). Notably, the plasmid transfer operon (*tra*) and the origin-of-transfer site (*oriT*) regions were absent in the pBK30661 genome.

pBK30661 harbors nine antimicrobial resistance determinants, including bla_{KPC-3}, bla_{TEM-1}, bla_{OXA-9} (β-lactam resistance), *aacA4*, *aadA1*, *strA*, *strB* (aminoglycoside resistance), *sul2* (sulfonamide resistance), and *dfrA14* (trimethoprim resistance), and a nickel resistance operon (Fig. 1A). The bla_{KPC-3} gene is carried on a truncated Tn*4401*/Tn*1331* nested transposon, closely resembling the nested transposon in the IncI2 plasmid pBK15692 we described recently (8). The main difference is that bla_{KPC-3} is carried by a Tn*4401d* variant with a 68-bp deletion located upstream of the bla_{KPC} gene in pBK30661, while it is carried by a

Tn*4401b* variant without deletions upstream of the bla_{KPC} gene in pBK15692. Tn*4401* is approximately 10 kb in size, delimited by two 39-bp imperfect inverted repeat (IR) sequences, and usually is associated with 5-bp target site duplications (TSDs) at both ends, adjacent to the IR sequences, as a result of integration (3, 23). In this study, Tn*4401d* was integrated into the Tn*1331 tnpA* gene at the same site in pBK30661 as in pBK15692, generating a 5-bp target sequence (AGAAC) (Fig. 1). However, the upstream region of Tn*1331* (Δ*tnpA*) in pBK30661 was truncated by an 8-kb nickel resistance operon (*nic* operon), which showed 98% nucleotide similarity to that of plasmid pECL_A from *Enterobacter cloacae* subsp. *cloacae* strain ATCC 13047 (24). The insertion of the *nic* operon also deleted the corresponding 5-bp target sequence (AG AAC) of Tn*4401d* in pBK30661, leaving a 5-bp unique sequence (AGAGA) adjacent to the upstream IR sequence of Tn*4401d* (Fig. 1). To our knowledge, this is the first completely characterized plasmid showing deletion of the Tn*4401*-associated TSD.

pBK30683 is 139,941 bp in length, with an average G+C content of 54.0%, and has 181 predicted ORFs (Fig. 1). The upstream 72-kb sequence in pBK30683 is almost identical to that in pBK30661, sharing >99.9% nucleotide similarity, except that pBK30661 harbors an extra IS*1294* (Fig. 1). pBK30683 carries an identical IncFIA *repA* gene, the truncated Tn*1331*/Tn*4401d* nested transposon, and the *nic* operon, in comparison with pBK30661. Interestingly, pBK30683 carries an extra 68-kb sequence that is unrelated to pBK30661, with the full machinery for genes that are responsible for plasmid replication, stability, and conjugation. As an example, pBK30683 harbors a second set of genes for plasmid replication (a novel IncFII *repA*), partition (*parA* and *parB*), SOS inhibition (*psiA* and *psiB*), antirestriction (*ardA* and *ardB*), and error-prone repair (*umuC* and *umuD*) (Fig. 1). Notably, pBK30683 carries a 35-kb *tra* operon that contributes to plasmid conjugation, similar to the conjugative region of several other IncFII plasmids (e.g., pKPN3 and pKPN4), with more than 97% nucleotide similarities. The *tra* operon-associated *oriT* sites were identified upstream of the *traM* gene (Fig. 1). Inspection of the junctions between the 72-kb pBK30661 homologous region and the remaining 68-kb sequence in pBK30683 revealed a 4-kb region of homology (>97% nucleotide similarity), containing the adenine-specific methyltransferase genes (*met1* and *met2*) and plasmid antirestriction genes (*ardA* and *ardB*) (Fig. 1).

Prevalence and dissemination of pBK30661-like and pBK30683-like plasmids. As part of an ongoing surveillance project, hospitals in New York and New Jersey routinely submit carbapenem-resistant and -susceptible *Enterobacteriaceae* isolates to our laboratory for genotyping. A total of 491 KPC-producing *K. pneumoniae* clinical isolates collected between 2001 and 2012 from 10 hospitals in our area were evaluated by PCR for the presence of pBK30661-like and pBK30683-like plasmid markers. Among them, 228 (46.4%) were bla_{KPC-2} positive, while 263 (53.6%) were bla_{KPC-3} positive; 411 (83.7%) belonged to ST258 (Table 2). Among these 491 isolates, 79 (16.1%) contained pBK30683-like plasmids and 18 (3.7%) harbored pBK30661-like plasmids. Interestingly, pBK30683-like and pBK30661-like plasmids were found to be exclusively associated with KPC-3-positive isolates, accounting for 30.0% and 6.8%, respectively, of the 263 KPC-3-producing isolates. Among the 79 pBK30683-like plasmids, 55 (69.6%) were in ST258 isolates, while 24 (30.4%) were in non-ST258 isolates. In contrast, all 18 pBK30661-like plasmids were in ST258 isolates.

Of 52 non-*K. pneumoniae* KPC-carrying *Enterobacteriaceae* isolates collected from four of the 10 hospitals, 22 were found to carry pBK30683-like plasmids (17 *Enterobacter* isolates, 4 *E. coli* isolates, and 1 *Citrobacter* isolate), and they all harbored bla_{KPC-3}. Surprisingly, two non-*K. pneumoniae* KPC-carrying *Enterobacteriaceae* isolates (1 *E. cloacae* isolate and 1 *E. coli* isolate) were found to harbor pBK30661-like plasmids (Table 2).

TABLE 2 Distributions of pBK30683-like and pBK30661-like plasmids among clinical KPC-producing *Enterobacteriaceae* isolates collected between 2001 and 2012 from 10 hospitals in the New York-New Jersey region

Isolate type	Distribution (*n* [%]) of:		Total distribution (*n*)
	pBK30683-like plasmid	pBK30661-like plasmid	
K. pneumoniae			
KPC-2 vs KPC-3			
KPC-2	0 (0)	0 (0)	228
KPC-3	79 (30.0)	18 (6.8)	263
ST258 vs non-ST258			
Non-ST258	24 (30.0)	0 (0)	80
ST258	55 (13.4)	18 (4.4)	411
Non-*K. pneumoniae*, KPC-2 vs KPC-3			
KPC-2	0 (0)	0 (0)	10
KPC-3	22 (52.4)	2 (4.8)	42

DISCUSSION

Transmission of antibiotic resistance genes, e.g., 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 clonal spread (25). The complexity of different transmission modes challenges our ability to identify, to track, and to control the spread of carbapenem-resistant *Enterobacteriaceae* strains as they become a global problem (26).

Since its initial emergence in 1996, KPC has spread in the United States and across the world, predominantly via a single *K. pneumoniae* sequence type, ST258, suggesting that the clonal spread of a single strain underlies the worldwide dissemination of KPC (1). An example of this is in a study from the Clinical Center of the U.S. National Institutes of Health (NIH) in 2011; an outbreak of drug-resistant ST258 *Klebsiella pneumoniae* infected 18 patients, causing the death of 6 of them, presumably due to the same strain introduced by an index patient (27). Other mechanisms, including horizontal transfer of bla_{KPC}-bearing plasmids and Tn*4401* transposons, have also been documented (28). Plasmid conjugation is dependent on the presence of *oriT* and the *tra* operon (29). Conjugation is initiated by the activity of the relaxase enzyme, which creates a nick in one of the strands of the conjugative plasmid at the *oriT* site. Consequently, both *oriT* and an intact *tra* operon are required for plasmid conjugation.

In this study, we sequenced to closure two bla_{KPC-3}-bearing plasmids. The first one, pBK30661, which could not be transferred in conjugation experiments, lacks both the *tra* operon and the *oriT* site. In contrast, plasmid pBK30683, harboring the transfer-associated *tra* operon and the *oriT* site, was successfully transferred by conjugation. Interestingly, a comparison between these two plasmids revealed that they share a nearly identical 72-kb region, encompassing the full length of pBK30661. In this case, pBK30683 appears to be a cointegrate of pBK30661 with an extra 68-kb genetic element.

The origins of pBK30661 and pBK30683 remain unknown. One possible explanation is that pBK30683 originated from the cointegration of pBK30661 and a second 68-kb plasmid or genetic element. This 68-kb element found in pBK30683 harbors a complete set of genes for plasmid replication, stability, and conjugation, and we presume it is from an independent plasmid that likely coexisted with pBK30661 in the same isolate. Notably, this element is flanked by a highly similar homologous 4-kb region, and it is possible that cointegration was achieved through homologous recombination among the large reiterated sequences. The 68-kb element carries the *tra* and *oriT* regions that are necessary for plasmid conjugation. This novel cointegrated plasmid, pBK30683, therefore acquired the ability to transfer by conjugation, facilitating dissemination of carbapenem resistance among different strains and species. Plasmid cointegration is not rare in Gram-negative or Gram-positive bacteria and has been associated with the spread of antimicrobial resistance genes, including genes for β-lactamases (30, 31). For example, Lin et al. recently reported the cointegration of a nonconjugative $bla_{CTX\text{-}M\text{-}17}$-bearing plasmid, pIP843, with an ~73-kb conjugative plasmid that may be responsible for the spread of CTX-M-17 (31).

An alternative hypothesis is that pBK30661 was formed by excision from pBK30683 through homologous recombination at the repeat regions. This argument appears to be supported by the evidence that pBK30661-like plasmids were found in other non-*K. pneumoniae Enterobacteriaceae*, including *E. cloacae* and *E. coli* (Table 2). As we described above, pBK30661 lacks the *oriT* site and the *tra* operon, disabling its capacity for lateral transfer or mobilization. In this case, the spread of pBK30661-like plasmids should be primarily through clonal spread of pBK30661-bearing ST258 strains. However, we also found two isolates, one *E. cloacae* and one *E. coli*, carrying pBK30661-like plasmids. A plausible explanation is that the pBK30661-like plasmids in *E. cloacae* and *E. coli* were transferred by a pBK30683-like plasmid, followed by excision of the 68-kb *tra*-harboring element. Routine sequencing and comparative analysis of resistance plasmids will ultimately increase our database of plasmid architectures and sequences and contribute to a better understanding of how plasmids such as pBK30661 and pBK30683 arose.

In this study, $bla_{KPC\text{-}3}$-bearing IncFIA plasmids (including both pBK30661-like and pBK30683-like plasmids) were identified in 19.8% of KPC-producing *K. pneumoniae* isolates from 10 hospitals, suggesting the widespread nature of this group of plasmids in our region. In *K. pneumoniae*, pBK30661-like plasmids are found only in ST258, indicating their clonal dissemination with ST258 isolates. In contrast, pBK30683-like plasmids have spread beyond the *K. pneumoniae* ST258 clone into other STs as well as into other species, presumably due to the transconjugal ability encoded by the *tra* operon. The finding of pBK30683-like plasmids in other STs and other species from the same hospitals suggested that interstrain and intraspecies transfers contribute significantly to the spread of this plasmid group.

In conclusion, this report presents the first complete sequences of two $bla_{KPC\text{-}3}$-harboring IncFIA plasmids, pBK30661 and pBK30683, in KPC-bearing strains. A screening study from 10 New Jersey and New York hospitals reveals that KPC-producing IncFIA (pBK30661-like and pBK30683-like) plasmids are spread widely in the New York-New Jersey region. The architecture of the plasmids described in this study also highlights the plasticity of KPC-carrying extrachromosomal elements. Further studies are required to determine the origins of the pBK30661 and pBK30683 plasmids and the distributions of this plasmid group in other geographic areas, in order to understand their contributions to international KPC epidemiology.

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B.N.K. discloses that he holds two patents that focus on using DNA sequencing to identify bacterial pathogens.

The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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