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Complete Sequence of a bla_{KPC-2}-Harboring $IncFII_{K1}$ Plasmid from a *Klebsiella pneumoniae* Sequence Type 258 Strain

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We report the nucleotide sequence of a novel bla_{KPC-2}-harboring $IncFII_{K1}$ plasmid, pBK32179, isolated from a carbapenem-resistant *Klebsiella pneumoniae* ST258 strain from a New York City patient. pBK32179 is 165 kb long, consists of a large backbone of pKPN3-like plasmid, and carries an 18.5-kb bla_{KPC-2}-containing element that is highly similar to plasmid pKpQIL. pBK32179-like plasmids were identified in 8.3% of strains in a collection of 96 *K. pneumoniae* isolates from hospitals in the New York City area.

Carbapenem-resistant *Enterobacteriaceae*, especially *Klebsiella pneumoniae*, are associated with increased mortality and morbidity (1), posing a significant threat for both community and hospitalized patients. Currently, *K. pneumoniae* carbapenemase (KPC) is the most prevalent carbapenemase in the United States (2). A single KPC-producing *K. pneumoniae* clone, sequence type 258 (ST258), which initially emerged in the United States (3), has now successfully spread to a number of different geographic regions (2). Controlling the dissemination of carbapenem resistance is problematic, as the KPC-encoding gene, bla_{KPC}, is located on Tn*4401* or Tn*4401*-like transposons and carried on variable transferable plasmids, thereby facilitating the inter- and intraspecies dissemination of resistance (4–6).

Different incompatibility (Inc) replicon groups of bla_{KPC}-harboring plasmids have been reported, including IncFII, IncL/M, IncN, IncR, IncX, and ColE1 groups (5, 7–11). Among them, IncFII plasmids have been widely distributed in various species of *Enterobacteriaceae*, and they harbor a number of resistance determinants, including extended-spectrum β-lactamases (ESBLs) (e.g., $bla_{CTX-M-15}$) and plasmid-mediated AmpCs (bla_{CMY} and bla_{DHA}), as well as quinolone and aminoglycoside resistance genes (12, 13). Several IncFII KPC-encoding plasmids have been completely sequenced and characterized (12, 14–17). The first *K. pneumoniae* ST258-associated plasmid, pKpQIL, a 113-kb plasmid belonging to the $IncFII_{K2}$ group by replicon sequence typing (RST) (12), has spread in the United States, Israel, Poland, and Italy (14, 18–21). Other IncFII plasmids, including pKP48 (151 kb, $IncFII_{K5}$) (15) and pKPHS2 (111 kb, $IncFII_{K2}$) (16), have been described in strains from China, where the bla_{KPC} gene was carried on a distinct Tn*4401*-like element (6) and mainly associated with *K. pneumoniae* ST11 strains (single-locus variant of ST258). Another 21,138-bp $IncFII_{K2}$ plasmid, p39SLMT (GenBank accession no. HQ589350), was also deposited in GenBank (www.ncbi.nlm.nih.gov). Recently, an $IncFII_{K1}$ plasmid, pKPN101-IT (108 kb), was described from one *K. pneumoniae* ST101 strain in Italy (17). The frequent identification of KPC-encoding IncFII plasmids suggests that IncFII plasmids may be the primary vehicle for the dissemination of KPC. Here, we report a novel 165-kb bla_{KPC-2}-harboring $IncFII_{K1}$ plasmid isolated from a strain of the epidemic *K. pneumoniae* clone ST258.

As part of a regional surveillance program established in 2010, we determined the genetic characteristics of a *K. pneumoniae* isolate, BK32179, that was recovered from a urine culture in a patient with a urinary tract infection (UTI) in a New York City hospital. Multilocus sequence typing (MLST) confirmed that the isolate belonged to the epidemic *K. pneumoniae* ST258 clone (22). PCR amplification of β-lactamase genes and sequencing of the amplicons demonstrated the presence of bla_{KPC-2}, bla_{TEM-1}, and bla_{SHV-11} (23–25). Plasmid DNA was extracted from strain BK32179 using a Qiagen plasmid maxi kit (Qiagen, Valencia, CA), followed by electroporation into *Escherichia coli* DH10B using a Gene Pulser II instrument (Bio-Rad Laboratories). Transformants were selected on Luria-Bertani (LB) agar plates containing 100 μg/ml ampicillin and then screened by multiplex real-time PCR for the presence of the bla_{KPC} gene (23). bla_{KPC} PCR-positive transformants were examined by S1-pulsed-field gel electrophoresis (S1-PFGE) analysis, and a transformant with a single plasmid was selected and subjected to susceptibility testing. MICs 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 (26, 27).

K. pneumoniae BK32179 exhibited resistance to imipenem (MIC, 8 μg/ml), ertapenem (MIC, >4 μg/ml), meropenem (MIC, >8 μg/ml), doripenem (MIC, >2 μg/ml), cefotaxime (MIC, 32 μg/ml), ceftazidime (MIC, >16 μg/ml), and aztreonam (MIC, >16 μg/ml) but was susceptible to aminoglycosides (amikacin

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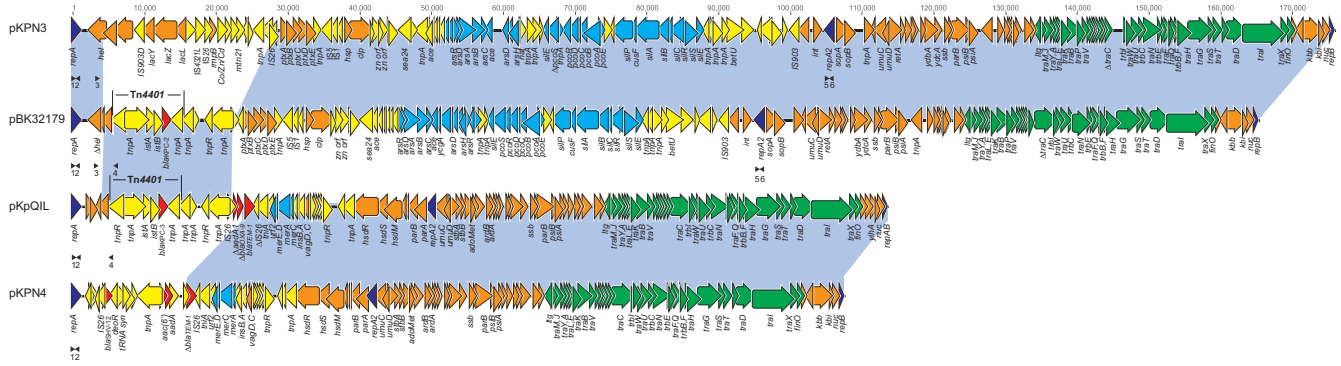

FIG 1 Comparative analysis of IncFII$_K$ plasmids pKPN3 (CP000648), pKPN4 (CP000649), pKpQIL (GU595196), and pBK32179 (JX430448). Light blue shading denotes shared regions of homology (>99.5% nucleotide similarity). Open reading frames (ORFs) are indicated by arrows and colored based on predicted gene function. Orange arrows indicate plasmid scaffold regions. The genes associated with the *tra* locus are indicated by green arrows, and replication-associated genes are denoted as dark blue arrows. Antimicrobial resistance genes are indicated by red arrows, while copper, mercury, and silver resistance genes are shown by light blue arrows. Other genes in the accessory region are indicated by yellow arrows. Small black arrowheads beneath the plasmids indicate the locations of primers used for PCR screening of pBK32179-like plasmids. Primer sequences used for PCRs were as follows: 1, repA-F (CTTCACGTCCCGTTTTGATT), 2, repA-R (CGCTTCAGCGCTTCTTTATC) (657 bp); 3, hel-F (ATGGTGGGGGAAAAATTCAT), 4, 4401-R (GTCACGTCAAGCGAGGAGTC) (1,996 bp); 5, repA2-F (ACGTTAAGATCACCGGTTCG), and 6, repA2-R (TAGGCTGACTGCACCAGATG) (271 bp). pBK32179-like plasmids are positive by targets of primer sets 1/2, 3/4, and 5/6, while pKpQIL-, pKPN4-, and pKPN3-like plasmids are positive only by PCRs of primer sets 1/2 and/or 5/6 but not set 3/4.

and gentamicin), tetracyclines (doxycycline and minocycline), and fluoroquinolones (ciprofloxacin and levofloxacin), as well as colistin (MIC, 0.5 μg/ml) and polymyxin B (MIC, 0.5 μg/ml). The *E. coli* DH10B transformant showed an antimicrobial resistance profile similar to that of the parental strain but had lower MICs against imipenem (MIC, 2 μg/ml), ertapenem (MIC, 1 μg/ml), meropenem (MIC, 2 μg/ml), and doripenem (MIC, 1 μg/ml).

Plasmid replicon typing (28) and replicon sequence typing (RST) (12) of the *E. coli* DH10B transformant indicated that the *bla*$_{KPC-2}$ gene was carried by an IncFII$_{K1}$ plasmid of approximately 165 kb (estimated by S1-PFGE), which is distinct from previously sequenced KPC-bearing IncFII plasmids. This plasmid was therefore selected for complete plasmid genome sequencing. The *bla*$_{KPC-2}$-harboring plasmid was extracted from an *E. coli* DH10B transformant, and the plasmid genome was sequenced by the Roche 454 GS-FLX platform. Sequencing reads were assembled into consensus *de novo* assembly contigs using the Roche Genome Sequencer FLX software GS Assembler. Sequence gaps on the plasmid were closed by PCR and standard Sanger sequencing; putative open reading frames (ORFs) were predicted and annotated using the RAST (rast.nmpdr.org) server (29).

Plasmid pBK32179 was determined to be 165,295 bp in size, with an average GC content of 52.7%, and harbored 165 predicted ORFs (Fig. 1). The overall structure of pBK32179 is highly similar (87% query coverage and 100% maximum nucleotide identity by Blast) to that of the plasmid pKPN3 (GenBank accession no. CP000648), harbored by the multidrug-resistant *K. pneumoniae* strain MGH 78578 (ATCC 700721) which was isolated from Massachusetts in 1994 (genome.wustl.edu/genomes/view/klebsiella_pneumoniae/). The region in common between pBK32179 and pKPN3 exhibited >99.5% nucleotide similarity and contained genes responsible for plasmid replication, maintenance, transmission, and metal (arsenic, copper, and silver) resistance (Fig. 1).

An 18,545-bp region of pBK32179 (from nucleotide 3,686 to 22,230), encompassing the *bla*$_{KPC-2}$-bearing Tn*4401a*, which is not found in pKPN3, showed >99.99% identity with the plasmid pKpQIL harbored by an ST258 *K. pneumoniae* strain isolated in Israel (30). The only difference between the two plasmids is that pBK32179 harbors *bla*$_{KPC-2}$ and pKpQIL harbors *bla*$_{KPC-3}$. Together, these findings suggest that plasmid pBK32179 may have been created by a Tn*4401*-carrying element from a pKpQIL-like plasmid displacing the region between ATP-dependent helicase gene *hel* and IS*26* on a pKPN3-like plasmid (Fig. 1). The helicase gene *hel*, located downstream of IncFII$_{K1}$ *repA*, was truncated from the 5′ end as a result of the Tn*4401*-carrying element displacement, lending further credence to the idea that pBK32179 acquired this element from a pKpQIL-like plasmid. Notably, pKpQIL has the same scaffold as pKPN4 (30) (Fig. 1), a second plasmid harbored by *K. pneumoniae* strain MGH 78578, demonstrating that both pKpQIL- and pBK32179-like plasmids (pKPN4 and pKPN3 backbone plasmids) can coexist within the same isolate even though they are from the same incompatibility group. Similarly, a recent study from Italy reported the coexistence of plasmid pKpQIL-IT (pKpQIL-like) and pKPN-IT (pKPN3- and pBK32179-like) in the same *K. pneumoniae* ST258 isolate (14). This finding supports our observation that one IncFII$_K$ plasmid may acquire a *bla*$_{KPC}$-carrying Tn*4401* element through horizontal transfer from another IncFII$_K$ plasmid with a different backbone in the same strain.

To determine the prevalence of the pBK32179-like plasmids among KPC-positive *K. pneumoniae* isolates in our surveillance study, we developed three PCR screening strategies targeting the aforementioned *hel* gene and Tn*4401* junction, IncFII$_K$ *repA*, and the second IncFIB-like *repA2* (Fig. 1). The analysis revealed that plasmids like pKpQIL, pKPN3, and pKPN4 are positive for one or two targets (IncFII$_K$ *repA* and/or *repA2*), while pBK32179-like plasmids are positive for all three targets (Fig. 1).

Using this approach, we tested 96 *K. pneumoniae* clinical isolates from our previous study that were collected from five hospitals in New Jersey and New York between 2010 and 2011 (24). The sequence type and *bla*$_{KPC}$ variant were previously characterized (24). The PCR analysis revealed that 75 (78.1%) and 79 (82.2%) of the isolates were positive using primers targeting *repA* and *repA2*, respectively, while 70 (72.9%) were positive with both *repA* primers. Eight (8.3%) were positive for all three targets, covering isolates from four of five hospitals in New Jersey or New York.

pBK32179-like plasmids of these eight isolates were successfully transferred to *E. coli* J53 by conjugation (31). Restriction enzyme EcoRV digestion of these eight plasmids from their *E. coli* J53 transformants revealed profiles similar to that of the test plasmid, pBK32179, in support of the high degree of homology of these pBK32179-like plasmids. Consistent with the genotype of strain BK32179, all eight isolates carry bla_{KPC-2} and belong to ST258, indicating that the spread of pBK32179-like plasmids is associated with the dissemination of the epidemic ST258 clone.

In summary, this study described the complete sequence of a novel bla_{KPC-2}-harboring $IncFII_{K1}$ plasmid. The comparative DNA analysis among related plasmids provides evidence to suggest that the rapid evolution of carbapenem resistance can occur by horizontal transfer of carbapenem resistance between plasmids of the same Inc group. This study contributes to the understanding of genetic characteristics of KPC-encoding plasmids and provides useful information for differentiating and tracking the prevalent $IncFII_K$ KPC-encoding plasmids.

Nucleotide sequence accession number. The complete nucleotide sequence of pBK32179 was deposited in GenBank under accession no. JX430448.

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