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Complete Nucleotide Sequence of a *bla*$_{KPC}$-Harboring IncI2 Plasmid and Its Dissemination 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 significant public health threat. *bla*$_{KPC}$, the plasmid-borne KPC gene, was frequently identified on numerous transferable plasmids in different incompatibility replicon groups. Here we report the complete nucleotide sequence of a novel *bla*$_{KPC-3}$-harboring IncI2 plasmid, pBK15692, isolated from a multidrug-resistant *K. pneumoniae* ST258 strain isolated from a New Jersey hospital in 2005. pBK15692 is 78 kb in length and carries a backbone that is similar to those of other IncI2 plasmids (pR721, pChi7122-3, pHN1122-1, and pSH146-65), including the genes encoding type IV pili and shufflon regions. Comparative genomics analysis of IncI2 plasmids reveals that they possess a conserved plasmid backbone but are divergent with respect to the integration sites of resistance genes. In pBK15692, the *bla*$_{KPC-3}$-harboring Tn*4401* was inserted into a Tn*1331* element and formed a nested transposon. A PCR scheme was designed to detect the prevalence of IncI2 and pBK15692-like plasmids from a collection of clinical strains from six New Jersey and New York hospitals isolated between 2007 and 2011. IncI2 plasmids were found in 46.2% isolates from 318 clinical *K. pneumoniae* strains. Notably, 59 pBK15692-like plasmids (23%) have been identified in 256 KPC-bearing *K. pneumoniae* strains, and all carried KPC-3 and belong to the epidemic ST258 clone. Our study revealed that the prevalence of IncI2 plasmids has been considerably underestimated. Further studies are needed to understand the distribution of this plasmid group in other health care regions and decipher the association between IncI2 plasmids and *bla*$_{KPC-3}$-bearing ST258 strains.**

Since the initial report in 2001, *Klebsiella pneumoniae* strains producing *K. pneumoniae* carbapenemase (KPC) have spread worldwide and emerged as a significant public health threat. The KPC gene, *bla*$_{KPC}$, is located on Tn*3*-like transposon Tn*4401* and is commonly carried on numerous transferable plasmids, thereby facilitating its inter- and intraspecies dissemination (1–3). Presently, *bla*$_{KPC}$ is identified on plasmids that are distinguished on the basis of their different incompatibility (Inc) replicon groups, including IncFII, IncL/M, IncN, IncA/C, IncR, IncX, and ColE1 (2, 4, 5). Nevertheless, Tn*4401* and the *bla*$_{KPC}$ gene have not been found on IncI plasmids, although IncI plasmids are associated with multidrug-resistant *Enterobacteriaceae* worldwide and known to harbor numerous β-lactmase genes, including *bla*$_{CMY}$, *bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{TEM}$, and *bla*$_{VIM}$ (6, 7).

Originally, IncI plasmids were identified on the basis of their susceptibility to the similar filamentous bacteriophages If1 (8) and PR64FS (9). The phages recognize type IV pili, which are expressed from the *pil* gene cluster harbored on IncI plasmids. In addition to the *pil* genes, IncI plasmids are also characterized by containing the shufflon region that is involved in changing the C-terminal segment of the PilV protein, and in determining the recipient specificity in liquid mating (10). IncI plasmids, mostly IncI1 but also IncI2, have been associated with the spread of several antimicrobial resistance genes in humans, livestock, and wild animals (6, 11–14).

Currently, four IncI2 plasmids, from different bacterial species and hosts, have been completely sequenced and their sequences have been deposited in GenBank, including the plasmid pR721, from a trimethoprim-resistant clinical *Escherichia coli* strain identified in the early 1970s (15); pChi7211-3, from an avian pathogenic *E. coli* (APEC) strain, χ7122, isolated from a diseased turkey (16); pSH146-65, from a *Salmonella enterica* serovar Heidelberg strain isolated from porcine diagnostic specimen in 2002 (17); and pHN1122-1, from an *E. coli* strain isolated from dog feces (GenBank accession no. JN797501). Here we report the complete sequence of the first *bla*$_{KPC}$-harboring IncI2 plasmid (pBK15692) isolated from a strain of the epidemic *K. pneumoniae* clone ST258 in New Jersey. A survey of this plasmid among a collection of KPC-positive and -negative *K. pneumoniae* strains from New York and New Jersey hospitals surprisingly uncovered its significant prevalence and its association with *bla*$_{KPC-3}$-harboring ST258 strains.

MATERIALS AND METHODS

Bacterial strains. A multidrug-resistant *K. pneumoniae* strain, BK15692, was identified from a retrospective study of carbapenem-resistant *K. pneumoniae* from New Jersey and New York hospitals. BK15692 was isolated from a northern New Jersey hospital in 2005, but the patient's demographic information, underlying disease, and site of isolation were not recorded at that time. Three hundred eighteen *K. pneumoniae* unique

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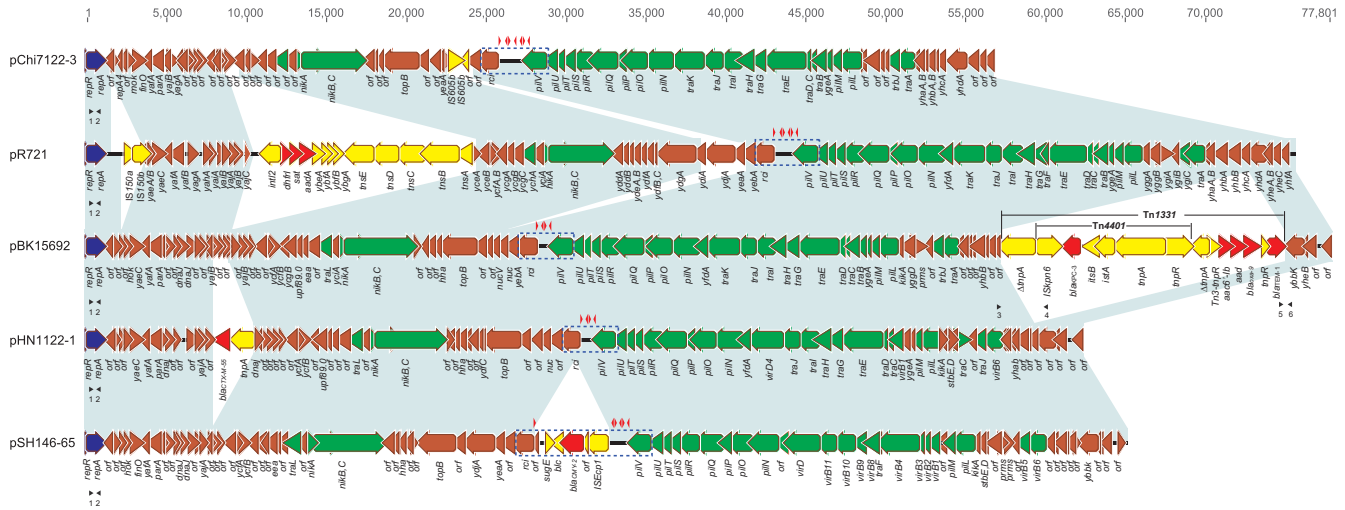

FIG 1 Comparative analysis of IncI2 plasmids pChi7122-3 (FR851304), pR721 (AP002527), pBK15692 (KC845573), pHN1122-1 (JN797501), and pSH146-65 (JN983044). Light blue shading denotes shared regions of homology. ORFs are represented by arrows and colored based on predicted gene function. Orange arrows indicate plasmid scaffold regions. The genes associated with plasmid transfer, including *tra*, *pil*, and *nikABC* loci, are indicated by green arrows, and replication-associated genes are represented as dark blue arrows. Antimicrobial resistance genes are indicated by red arrows, while other genes in the accessory region are indicated by yellow arrows. The shufflon regions, including their associated *rci* and *pilV* genes, are displayed by blue dashed box, while the red arrowheads above the shufflon regions indicate the locations and directions of the shufflon-associated 19-bp repeats. Small black arrowheads beneath the plasmids indicate the locations of primers used for PCR screening of pBK15692-like plasmids. Primers used for PCRs were as follows: 1 (I2-repR-F, TTACAGTGCAAGCTAAGTGCAG), 2 (I2-repA-R, GATTCACGGTCCCATATCGT) (615 bp), 3 (15692-F1, TTTAATGATTTGCTCA TTCGTGA), 4 (15692-R1, GCCTCAGATAGATGCGGTAGC) (2,337 bp), 5 (15692-F2, AGCCCTCCCGTATCGTAGTT), and 6 (15692-R2, GAAGGCAGAA GGGGAGAAAC) (611 bp).

clinical isolates collected from six hospitals in the New Jersey and New York area were included to check the prevalence of IncI2 and pBK15692-like plasmids, using a PCR approach (described below). An additional 19 non-*K. pneumoniae Enterobacteriaceae* KPC-positive isolates, including 11 *Enterobacter* species, 6 *E. coli*, and 2 *Citrobacter freundii* isolates collected from two of the six hospitals between 2009 and 2011, were also subject to the PCR screening.

Characterization of strain BK15692 and manipulation of plasmids. Strain BK15692 was initially screened by a multiplex real-time PCR for *K. pneumoniae* ST258 clone identification and the presence of bla_{KPC} (18), and the sequence type (ST) was further confirmed by multilocus sequence typing (MLST) (19). β-Lactam (bla_{CTX-M}, bla_{SHV}, bla_{TEM}, bla_{GES}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}, bla_{ACT-1}, bla_{ACC}, bla_{BIL-1}, bla_{CMY}, bla_{DHA}, bla_{FOX}, bla_{LAT}, bla_{MIR-1}, and bla_{MOX}), aminoglycoside (*aadA1*, *aadA2*, *aadB*, *aadA5*, *strA*, *strB*, *aphA1*, *aphA2*, *aphA6*, *aacC1*, *aacC2*, *aacC4*, *aacA4*, *armA*, *rmtC*, and *rmtB*), and fluoroquinolone (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *oqxA*, and *oqxB*) resistance genes were investigated by PCR using methods described elsewhere (20–24). Plasmid incompatibility groups were determined using the PCR methods described previously (25, 26). Specifically, 18 replicon types (FIA, FIB, FIC, HI1, HI2, I1-Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA) were screened by the methods of Carattoli et al. (25), and an additional 3 types (ColE, R, and U) were investigated by the method of García-Fernández et al. (26).

Plasmid DNA in strain BK15692 was extracted using a Qiagen plasmid maxikit (Qiagen, Valencia, CA), followed by electroporation into *E. coli* DH10B (Invitrogen) using a Gene Pulser II instrument (Bio-Rad Laboratories). *E. coli* DH10B transformants were selected on Luria-Bertani (LB) agar plates containing 0.5 μg/ml of imipenem and then confirmed by multiplex real-time PCR for the presence of bla_{KPC} genes (27). Transferability of bla_{KPC}-bearing plasmid was examined by conjugation experiments using BK15692 as the donor and *E. coli* J53 Az^r as the recipient as described previously (28). MICs of isolate BK15692 and its *E. coli* DH10B transformant 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 (29, 30).

Plasmid sequencing and bioinformatics. Plasmid DNA from the *E. coli* DH10B transformant was extracted as described above using a Qiagen plasmid maxikit. The plasmid DNA was sequenced using a Roche 454 GS-FLX system. Sequencing reads were *de novo* assembled into contigs using the Roche Genome Sequencer FLX software GSA assembler, version 2.5.3. Gaps between contigs were closed by PCR with standard Sanger sequencing. Open reading frames (ORFs) were predicted and annotated using the RAST (http://rast.nmpdr.org) server (31).

PCR screening for pBK15692-like plasmids. Based on the complete sequence of pBK15692, a PCR scheme, including three individual reactions, was designed to detect pBK15692-like plasmids (Fig. 1). PCR-1 was designed to target the IncI2 plasmid-specific replication genes *repA* and *repR*. PCR-2 and PCR-3 were designed to target the junction between Tn*4401* (IS*Kpn6*) and the upstream IncI2 plasmid backbone and the junction between Tn*1331* (bla_{TEM-1}) and the downstream IncI2 plasmid backbone, respectively. PCR-1 was able to identify the presence of IncI2 plasmids, and a combination of PCR-1 to -3 was able to detect pBK15692-like plasmids. The primer sequences and locations are illustrated in Fig. 1. The PCR cycling conditions were as follows: an initial denaturation step of 95°C for 4 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 2 min (PCR-2) or 45 s (PCR-1 and PCR-3) and a final extension step of 72°C for 7 min. The *K. pneumoniae* ST258 sequence type and bla_{KPC} variants of all strains were characterized by two multiplex real-time PCR methods developed by our lab (18, 27). DNAs from strain BK15692 and *E. coli* DH10B were used as positive and negative controls in each PCR run.

Statistical analysis. The statistical significance of differences in the distributions of IncI2 and pBK15692-like plasmids were determined using the chi-square test (Prism, GraphPad, San Diego, CA). Differences were considered statistically significant at a P value of ≤ 0.05.

Nucleotide sequence accession number. The complete nucleotide sequence of pBK15692 was deposited in GenBank under accession no. KC845573.

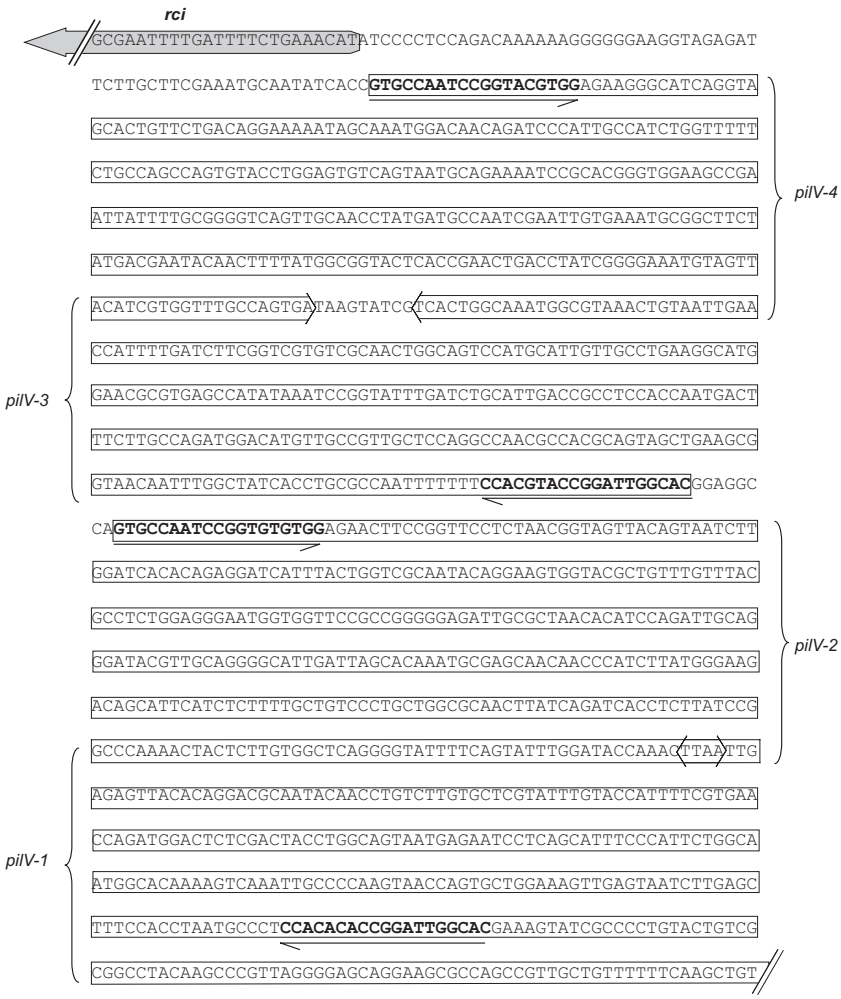

FIG 2 DNA sequences of shufflon region in pBK15692. Four 19-bp repeat sequences are in bold, and the directions are shown by the arrow beneath the repeats. The upstream *rci* gene and four forms of *pilV* genes (from the 19-bp repeat to the stop codon) are shown in boxed arrows. The predicted *pilV-1* to *-4* genes are 1,245, 1,377, 1,287, and 1,317 bp, respectively. Corresponding PilV-1 to -4 proteins are predicted to be 414, 459, 428, and 438 amino acids, sharing identical N-terminal 345 amino acids but distinct C-terminal sequences.

RESULTS

Microbiological and genetic characterization of strain BK15692. Susceptibility testing showed that BK15692 was resistant to imipenem (MIC = 8 μg/ml), ertapenem (≥4 μg/ml), meropenem (≥8 μg/ml), doripenem (≥4 μg/ml), cefepime (≥32 μg/ml), cefotaxime (≥32 μg/ml), ceftazidime (≥16 μg/ml), aztreonam (≥16 μg/ml), levofloxacin (≥8 μg/ml), ciprofloxacin (≥4 μg/ml), and co-trimoxazole (≥4/76 μg/ml) and was intermediate to amikacin (32 μg/ml) and gentamicin (8 μg/ml)). In contrast, BK15692 was susceptible to tetracyclines (doxycycline and minocycline), colistin (1 μg/ml), tigecycline (1 μg/ml), and polymyxin B (1 μg/ml). MLST PCR amplification and sequencing of antimicrobial resistance genes revealed that this clinical isolate belonged to the epidemic *K. pneumoniae* ST258 clone and harbored the β-lactamase genes bla_{KPC-3}, $bla_{CTX-M-15}$, bla_{OXA-9}, bla_{TEM-1}, and bla_{SHV-11}; the aminoglycoside-modifying enzyme genes *aadA1*, *aadA2*, *aphA1*, and *aac(6′)-Ib (aacA4)*; and quinolone resistance genes *oqxA* and *oqxB*. PCR-based replicon typing (PBRT) revealed that BK15692 contained IncFII and colE1 plasmids.

With selection on imipenen-containing agar (0.5 μg/ml), we were successful in transferring carbapenem resistance from *K. pneumoniae* BK15692 to *E. coli* J53 by conjugation and into *E. coli* DH10B by electroporation. A representative *E. coli* DH10B transformant displayed antimicrobial susceptibility profiles similar to those of the parent strain but was susceptible to ciprofloxacin and levofloxacin and was less resistant to ertapenem (2 μg/ml), imipenem (2 μg/ml), meropenem (1 μg/ml), and doripenem (1 μg/ml). Surprisingly, PBRT of the transformant was negative, raising the possibility that the bla_{KPC-3} gene was on a plasmid with an uncommon incompatibility type. Consequently, plasmid pBK15692 from the *E. coli* DH10B transformant was sequenced.

Structure of bla_{KPC-3}-harboring IncI2 plasmid pBK15692. pBK15692 is an IncI2 plasmid 77,801 bp in length with an average G+C content of 45.4%, which is lower than the G+C content of *K. pneumoniae* genomes (~57.5%), and harbored 105 predicted ORFs (Fig. 1). Three additional PilV proteins with different C-terminal segments could be created by shufflon multiple inversions as demonstrated in other IncI1 or I2 plasmids (10) (Fig. 2). Thus, 108 different proteins are encoded on plasmid pBK15692. The overall structure of pBK15692 is highly similar to that of the other

four completely sequenced IncI2 plasmids, with 73%, 68%, 65%, and 64% query coverage and a maximum of 100% nucleotide identity to plasmids pHN1122-1, pSH146-65, pR721, and pChi7211-3 by Blast. All IncI2 plasmids shared a common backbone set of sequences that are responsible for plasmid replication, maintenance, and transfer (Fig. 1).

The replication region (1,066 bp) of pBK15692, including the replication initiation protein gene *repA* and replication regulatory protein gene *repR*, share >96.5% nucleotide similarities with other four IncI2 plasmids (data not shown). We tentatively assigned the downstream locus of the replication region as the stability region because of the presence of several genes presumably required for plasmid maintenance and stability. For example, the gene *yaeC* encodes a protein that belongs to the FinO protein family and may function as a conjugal transfer repressor; *yafA* encodes a ribbon-helix-helix CopG family protein, presumably involving control of plasmid copy number (32); *parA* encodes the protein for plasmid partition; and *topB* encodes DNA topoisomerase III, which regulates the overwinding or underwinding of DNA. Juxtaposed to the stability region is the transfer region, which is organized into different gene clusters, including the *nikABC* gene operon for conjugative DNA processing, *tra* operon for general conjugation and regulation, and *pil* gene cluster for type IV pilus biogenesis. The replication, stability, and transfer gene clusters among the five characterized IncI2 plasmids have the same syntenic order (Fig. 1).

The characteristic shufflon region of IncI plasmids is also conserved in all IncI2 plasmids. The shufflons are located between the shufflon-specific recombinase gene, *rci*, and one of the pilus genes, *pilV* (Fig. 1). Shufflons are a multiple inversion system, originally described for IncI1 plasmid R64 (33) and later also found in IncI2 plasmids (34). The R64 shufflon consists of four DNA segments designated A, B, C, and D (33). They are flanked and separated by seven 19-bp repeat sequences oriented in either direction. Site-specific recombination (mediated by the above-described *rci* gene product) between any of two inverted repeats results in the inversion of DNA segments either independently or in groups. In plasmid pBK15692, four repeat sequences have been identified; therefore, four different PilV proteins can be presumably created, with the same PilV N-terminal sequences but with variable C-terminal sequences (Fig. 2). Similar to pBK15692, pHN1122-1 also harbors four repeats, and the other three IncI2 plasmids carry either six (pR721 and pSH146-65) or seven (pChi7122-3) repeats (Fig. 1). Interestingly, the shufflon region in pSH146-65 was disrupted by the insertion of a 4.7-kb transposon-like element (IS*Ecp1*-bla_{CMY-2}-*blc-sugE*), which may affect the function of shufflon, as the recombinase gene *rci* is distant from the gene *pilV*.

The major differences between pBK15692 and other IncI2 plasmids are found in the number of acquired genes. An 18-kb acquired resistance gene region is identified downstream of *tra* and *pil* operon in pBK15692, located between an unknown *orf* and the gene *ybbk* (Fig. 1), and includes β-lactamase genes bla_{KPC-3}, bla_{OXA-9}, and bla_{TEM-1} and aminoglycoside resistance genes *aac(6′)-Ib* and *aadA1*. The G+C content of this region is 56.6%, significantly higher than that of the rest of the plasmid (42.1%), suggesting that this region was acquired recently. Inspection of this region reveals that the bla_{KPC-3}-harboring Tn*4401b* element is inserted into the transposase gene (*tnpA*) of Tn*1331*, generating a 5-bp duplication of the target sequence (AGAAC) (Fig. 1) and forming a nested transposon.

TABLE 1 Distributions of IncI2 and pBK15692-like plasmids in different groups

Enzyme or clone	IncI2 plasmids, *n* (%)	pBK15692-like plasmids, *n* (%)	Total (*n*)
KPC vs non-KPC			
non-KPC	19 (30.6)	0 (0)	62
KPC	128 (50.0)[a]	59 (23.0)[a]	256
KPC-2 vs KPC-3			
KPC-2	3 (3.1)	0 (0)	96
KPC-3	125 (78.1)[b]	59 (36.9)[b]	160
ST258 vs non-ST258			
non-ST258	17 (29.8)	0 (0)	57
ST258	130 (49.8)[c]	59 (22.6)[c]	261

[a] $P < 0.01$ compared with non-KPC strains.
[b] $P < 0.01$ compared with KPC-2-bearing strains.
[c] $P < 0.01$ compared with non-ST258 strains.

A total of six unique integration sites for acquired regions were identified among the five IncI2 plasmids, including the insertion of the IS*Ecp1*-bla_{CMY-2}-*blc-sugE* element within the shufflon region in pSH146-65 (described above) (Fig. 1). Except for pChi7122-3, the remaining four completely sequenced IncI2 plasmids harbor at least one resistance gene: pBK15692 contains bla_{KPC-3}, bla_{TEM-1}, bla_{OXA-9}, *aadA1*, and *aac(6′)-Ib*; pR721 harbors *dfrA1* (trimethoprim resistance), *sat1* (streptothricin resistance), and *aadA1*; $bla_{CTX-M-55}$ (β-lactam resistance) is identified on pHN1122-1; and bla_{CMY-2} (β-lactam resistance) is found on pSH146-65.

Prevalence and dissemination of IncI2 and pBK15692-like plasmids. As part of an ongoing surveillance project, hospitals in New York and New Jersey routinely submit carbapenem-resistant and -susceptible *Enterobacteriaceae* to our laboratory for genotyping. A total of 318 clinical *K. pneumoniae* isolates from 6 hospitals were evaluated by PCR for the presence of IncI2 and pBK15692-like plasmid markers. Among them, 256 were bla_{KPC} positive (96 bla_{KPC-2} and 160 bla_{KPC-3}), while 62 were bla_{KPC} negative; 261 (82%) belonged to the ST258 clone (Table 1).

Surprisingly, this uncommon plasmid was found to be highly prevalent and a major vector for Tn*4401*. Among these 318 isolates, 46.2% ($n = 147$) were positive for IncI2 *rep* PCR (PCR-1), including both bla_{KPC}-positive and -negative strains. However, the prevalence of IncI2 plasmids in KPC-bearing isolates (50.0%) was significantly higher than that in non-KPC isolates (30.6%) ($P < 0.01$). Interestingly, IncI2 plasmids were found to be substantially associated with KPC-3-positive isolates: 78.1% of KPC-3 isolates carried IncI2 plasmids, in contrast to 3.1% for KPC-2 isolates. Among 256 bla_{KPC}-positive isolates, 59 (23.0%) pBK15692-like plasmids were identified (positive for PCR-1 to -3), covering isolates from all six hospitals. All 59 isolates are exclusively ST258 and carry bla_{KPC-3}, accounting for 36.9% of the total 160 bla_{KPC-3}-positive isolates in this study.

Among 19 non-*K. pneumoniae* KPC-carrying *Enterobacteriaceae* isolates, 12 were found to carry IncI2 plasmids (9 *Enterobacter* spp. and 3 *E. coli* isolates). pBK15692-like plasmids were identified in all nine *Enterobacter* isolates (positive for PCR-1 to -3), and they all harbor bla_{KPC-3}. The three *E. coli* strains were negative for pBK15692-like plasmids.

DISCUSSION

The rapid global dissemination of KPC-producing *K. pneumoniae* strains has been largely associated with the epidemic ST258 clone, although KPC enzymes have been detected in a number of other *K. pneumoniae* sequence types (18, 35–37). One possible explanation for the "epidemiological success of ST258" could be attributed to chromosomal and/or specific plasmids factors that contribute to the compatibility or fitness of this strain (38). Clearly, unraveling these factors will play a key role in understanding the current epidemiology associated with carbapenem-resistant ST258 *K. pneumoniae* and potentially lead to measures that may assist with infection control and prevention. A number of ST258-associated KPC-producing plasmids have been characterized. One example is the $IncFII_{K2}$ plasmid pKpQIL, which was originally reported from ST258 strains from Israel and has now been detected in the United States, Poland, Italy, and other regions (39–43). Interspecies transfer of pKpQIL has also been documented (44). Other completely characterized ST258 associated bla_{KPC}-harboring plasmids include IncX3 plasmid pKpS90 and $IncFII_{K1}$ plasmid pBK32179 (45, 46).

To our knowledge, IncI2 plasmids have never been reported for ST258 strains or been associated with carbapenem-resistant genes. This study yielded the first description of the presence and complete sequence of bla_{KPC}-bearing IncI2 plasmids in *K. pneumoniae* and the first observation that this resistant plasmid is relatively common in our region. Until now, the prevalence of IncI2 plasmids in ST258 was unappreciated, primarily because this plasmid is not included in the PCR-based replicon typing (PBRT) panel (25, 26). This limitation of the current replicon typing method needs to be expanded as more plasmids are described and more replication proteins are identified.

Our results show that among 318 clinical isolates of *K. pneumoniae* tested, IncI2 plasmids were found in 147 (46%) of them (Table 1). Having uncovered the successful spread of IncI2 plasmids raises the question of whether their unique plasmid structure could contribute to their dissemination. Genetically, IncI plasmids have the *pil* gene cluster, encoding type IV pili, and they encode a specific shufflon (clustered inversion) region that functions as a biological switch to select the C-terminal segment of the PilV protein (encoded by one of the *pil* genes, *pilV*). As an example, the IncI1 plasmid pR64 shufflon determines the recipient specificity in liquid mating experiments by selecting the C-terminal segments of PilV proteins, and DNA rearrangement of the shufflon in the *pilV* gene can alter this specificity to mate with different recipients (10). The type IV pili are also a potential virulence factor, and the association of virulence and resistance determinants may favor the positive selection of plasmids belonging to the IncI family (6).

IncI2 plasmids maintain both the *pil* gene cluster and shufflon regulation, and presumably they contribute to their ability to transfer into different strains and species. Our finding that IncI2 plasmids are found in different *K. pneumoniae* sequence types and in other *Enterobacteriaceae* species suggests that this plasmid is widely disseminated. In addition, IncI2 plasmid pChi7122-3 showed the properties of acid resistance and enhanced biofilm production, which could be related not only to *tra* operon expression but also to type IV fimbriae (47). Acid resistance and plasmid-driven biofilms in IncI2 plasmids could be essential for bacterial survival by enhancing persistence in acidic environments (e.g., human stomach) (47).

IncI2 plasmids share similar core genes essential for their replication, stability, and transferability, but their acquired regions are otherwise plastic. This is in contrast to IncN, IncX, and IncI1 plasmids, in which acquired genes share similar integration sites (4, 7, 48). As shown in Fig. 1, IncI2 plasmids have variable integration sites, which may facilitate their ability to rapidly adapt and acquire additional resistance genes to survive in an antibiotic-rich environment.

In pBK15692, the bla_{KPC}-bearing Tn*4401b* transposon was inserted into another transposon, Tn*1331*. 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} (49). The insertion of Tn*4401* within Tn*1331* disrupts the transposase gene, which presumably deactivates its mobility. In this case, it is most likely that Tn*4401* was inserted after the integration of Tn*1331* in pBK15692, instead of transferring as a nested transposon. Interestingly, a similar Tn*4401*/Tn*1331* nested transposon was previously described for plasmid pLRM24 from *K. pneumoniae* strain VA367, but this plasmid is different from pBK15692 in that it also harbors a *qnrB19*-harboring Tn*5387* element that inserted in the bla_{OXA-9} gene (50). However, it is not clear if pLRM24 was the same plasmid as pBK15692 or if the same insertion of Tn*4401* into Tn*1331* happened on multiple occasions.

In this study, pBK15692-like plasmids were identified in 23% of KPC-positive *K. pneumoniae* isolates from all six hospitals, suggesting the widespread nature of this single plasmid in our region. Moreover, pBK15692 was exclusively associated with KPC-3-bearing ST258 isolates in *K. pneumoniae*, indicating clonal dissemination with ST258 isolates. Remarkably, the finding of pBK15692-like plasmids in *Enterobacter* spp. from the same hospitals also suggested that the interspecies transfer could contribute to the spread of this plasmid.

In conclusion, this report presents the first complete sequence of a bla_{KPC}-harboring IncI2 plasmid, pBK15692, and the finding that Tn*4401* inserted into Tn*1331*, similar to the structure found in plasmid pLRM24. Comparative genomic analysis of IncI2 plasmids reveals that they possess highly conserved plasmid backbones but are quite divergent with respect to the integration sites of resistance genes. A screening study from six New Jersey and New York hospitals reveals that IncI2 and pBK15692-like plasmids are widely spread in the New York-New Jersey region and are significantly associated with KPC-3-harboring ST258 strains. Further studies are required to determine the distributions of this plasmid in other geographic areas and to understand its contribution to KPC epidemiology and virulence.

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