AAC Journals.ASM.org

Complete Sequence of a Conjugative IncN Plasmid Harboring bla_{KPC-2}, bla_{SHV-12}, and *qnrS1* from an *Escherichia coli* Sequence Type 648 Strain

Jun-Jie Li,[a,b] Chang-Seop Lee,[a,c] Ji-Fang Sheng,[b] Yohei Doi[a]

Division of Infectious Diseases, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA[a]; State Key Laboratory for Diagnosis and Treatment of Infectious Disease, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, People's Republic of China[b]; Department of Internal Medicine and Research Institute of Clinical Medicine, Chonbuk National University Medical School and Hospital, Jeonju, Republic of Korea[c]

We sequenced a novel conjugative bla_{KPC-2}-harboring IncN plasmid, pYD626E, from an *Escherichia coli* sequence type 648 strain previously identified in Pittsburgh, Pennsylvania. pYD626E was 72,800 bp long and carried four β-lactamase genes, bla_{KPC-2}, bla_{SHV-12}, bla_{LAP-1}, and bla_{TEM-1}. In addition, it harbored *qnrS1* (fluoroquinolone resistance) and *dfrA14* (trimethoprim resistance). The plasmid profile and clinical history supported the *in vivo* transfer of this plasmid between *Klebsiella pneumoniae* and *Escherichia coli*.

The rapid spread of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* has become a major public health threat (1). The gene encoding KPC, bla_{KPC}, is usually located on a plasmid and may transfer to other *Enterobacteriaceae* species. While KPC-producing *Escherichia coli* is still rare compared with KPC-producing *K. pneumoniae*, reports have emerged on *E. coli* strains acquiring bla_{KPC}-carrying plasmids and becoming resistant to carbapenems (2–5). We previously reported a series of patients who had KPC-producing *E. coli*, and many of these patients also had a second species that produced KPC at approximately the same time (6). One of the patients in this report (patient 13) had KPC-2-producing *E. coli* (strain YD626E) and *K. pneumoniae* (strain YD626K) in the same bronchoalveolar lavage specimen. He was treated for ventilator-associated pneumonia with colistin but did not survive the hospitalization. The *E. coli* strain was unusual in that it also produced SHV-12, an extended-spectrum β-lactamase (ESBL) commonly seen in *K. pneumoniae* isolates. The plasmid in the *E. coli* transformant from this *E. coli* strain (pYD626E), obtained by electroporation, carried bla_{KPC-2} and bla_{SHV-12}. We therefore conducted whole-plasmid sequencing to further characterize this unique plasmid.

The plasmid was extracted from the *E. coli* TOP10 transformant using the Qiagen plasmid maxi kit (Qiagen, Valencia, CA). Sequencing was performed on the PacBio RS II single-molecule real-time (SMRT) sequencing instrument (Pacific Biosciences, Menlo Park, CA) at the Yale Center for Genome Analysis. The average sequencing coverage was approximately 1,300× across the plasmid. The first-pass reads were assembled *de novo* using the hierarchical genome assembly process (HGAP) with the default settings of the SMRT Analysis v2.1 software package (Pacific Biosciences) (7). The single contig that represented the plasmid of interest was circularized and used as the reference for reassembling the first-pass reads within the default parameters of the consensus tool Quiver v1, also available in SMRT Analysis v2.1. This process was repeated until an internal consensus accuracy rate of 100% was achieved against the latest contig. No gap filling was required. The plasmid sequence was initially annotated with RAST (http://rast.nmpdr.org), and then it was further curated manually.

pYD626E is 72,800 bp long and has an IncN replicon with a GC content of 53.2%. It harbors 83 predicted open reading frames (ORFs) and is composed of a 39,230-bp core region and three distinct acquired regions (7,764 bp, 3,483 bp, and 22,323 bp) (Fig. 1). The core region includes the genes responsible for plasmid replication, horizontal transfer, and stability and maintenance functions, and it defines the plasmid backbone (8). pYD626E carries a core region that is similar to those of many other multidrug resistance IncN plasmids that have been identified in *K. pneumoniae* and *E. coli* from different countries, such as pBK31551, pECN580, pKo6, p9, p12, and pKOX105 (GenBank accession no. JX193301, KF914891, KC958437, FJ223607, FJ223605, and HM126016, respectively), and to the IncN prototype plasmid pR46 (AY046276), underscoring the high degree of plasticity of IncN plasmids and their ability to widely disseminate.

Similar to other IncN plasmids, the core region in pYD626E encodes the IncN replicon, comprising the replication initiation protein gene *repA* and two modules involved in the conjugative transfer of plasmids (*traGFOENDCBAML* and *traIJK*). The first module is located downstream of the *fipA* (fertility inhibition protein, truncated) gene and, in addition to the *tra* genes, contains other backbone genes, including *nuc* (DNA degradation), *eex* (exclusion of DNA entry), *korA* and *korB* (regulation of transfer and replication), *kikA* (killing of *Klebsiella oxytoca*), and the EcoRII and EcoRII methyltransferase genes (possibly mediating lysis of cells which have lost the plasmid) (9). However, the first module in pYD626E is in an orientation inverse to that in most other IncN plasmids, except for the bla_{VIM-1}-carrying plasmid pKOX105, which was found in a *Klebsiella oxytoca* strain isolated in Italy (GenBank accession no. HM126016) (10). The second module is located next to the second truncated copy of *fipA*, but it is upstream of the gene instead of downstream. Along with the *tra*

Received 13 June 2014 Returned for modification 9 July 2014
Accepted 23 August 2014
Published ahead of print 2 September 2014
Address correspondence to Yohei Doi, yod4@pitt.edu.

doi:10.1128/AAC.03632-14

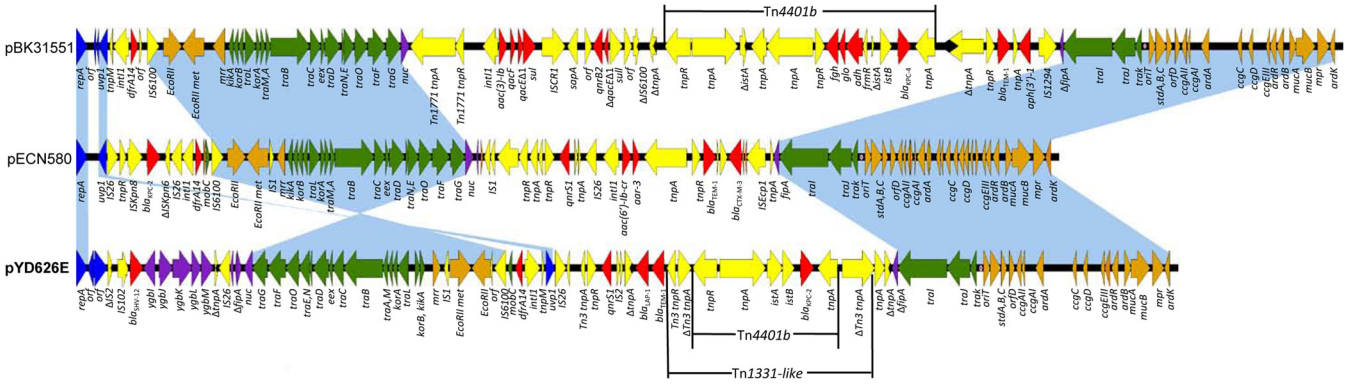

FIG 1 Comparative analysis of IncN plasmid pYD626E (GenBank accession no. KJ933392) with two other bla_{KPC}-carrying IncN plasmids, pBK31551 (JX193301) and pECN580 (KF914891). Light-blue shading indicates shared backbone regions with a high degree of homology. ORFs are portrayed by arrows and are colored according to their putative functions. Dark-blue arrows indicate replication-associated genes. Green arrows indicate genes that are associated with plasmid conjugal transfers, and brown arrows indicate genes that are involved in plasmid stability. Red and yellow arrows indicate antimicrobial resistance genes and accessory genes of mobile elements, respectively. Dark-purple arrows indicate other backbone genes and inserted foreign genes. Bold type indicates the plasmid sequenced in this study (pYD626E).

genes, this second module carries the *stbABC* operon, which guarantees plasmid stability during conjugation, and it carries the *ardAB* and *ardR* systems, which regulate antirestriction functions. Furthermore, like most but not all other IncN plasmids, pYD626E possesses the *ccgC* and *ccgD* genes, which encode products that protect plasmid DNA from type I restriction enzymes (10, 11).

pYD626E has three distinct acquired regions that harbor a variety of antimicrobial resistance genes. The 3,483-bp resistance region is located upstream of the *uvp1* gene next to the first core region, a previously described hot spot for integration within IncN plasmids (8), and this region has a class 1 integron. This integron harbors a trimethoprim resistance gene cassette, *dfrA14*, and has its 3′-conserved end truncated by the insertion of an IS*6100* element, as has been observed in other bla_{KPC}-carrying IncN plasmids (8, 10, 11). The 7,764-bp resistance region is located between the genes *repA* and *fipA* and is bracketed by the insertion elements IS*102* and IS*26*, respectively. It carries bla_{SHV-12} and an incomplete operon involved in sugar metabolism (*ygbI*, *ygbJ*, *ygbK*, *ygbL*, and truncated *ygbM*). The bla_{SHV-12} gene together with the incomplete sugar metabolism operon exhibits 99% identity to fragments of several *K. pneumoniae* chromosomes, such as MGH 78578, KCTC 2242, and KPR0928 (GenBank accession no. CP000647, CP002910, and CP008831, respectively), and bla_{SHV}-harboring plasmids, such as pTC2, pKEC-a3c, and p1658/97 (GenBank accession no. NC_019375.1, CP007558.1, and NC_004998.1, respectively). This shared identity supports the hypothesis that bla_{SHV} originated from the *K. pneumoniae* chromosome (12). The 22,323-bp region located between *uvp1* and the second copy of *fipA* is the primary cluster of resistance genes in pYD626E. It carries the β-lactamase genes bla_{KPC-2}, bla_{TEM-1}, and bla_{LAP-1} and the quinolone resistance gene *qnrS1*. *qnrS1* is bracketed by a Tn*3*-like transposase gene and a truncated IS*2* element. The *qnrS1*-containing region exhibits a high degree of identity (>99%) with several plasmids identified in *Salmonella enterica* serovar Typhimurium strains (GenBank accession no. HE652087, JN393220, EU715253, and AM746977). Between the regions containing *qnrS1* and the bla_{KPC-2}-carrying Tn*4401* transposon reside the β-lactamase genes bla_{LAP-1} and bla_{TEM-1} in tandem. The bla_{KPC-2} gene in pYD626E is carried on a typical Tn*4401b* transposon. However, the Tn*4401b* transposon was inserted into the transposase gene of Tn*1331*, generating a 5-bp target duplication sequence (AGAAC) and forming a nested transposon (Tn*1331*-like). The nested Tn*1331*-like-Tn*4401* transposon in pYD626E is highly similar to the Tn*1331*-Tn*4401* transposon of the bla_{KPC-3}-carrying plasmid pBK15692 (GenBank accession no. KC845573), which was previously identified in a *K. pneumoniae* strain from New York (13). However, the aminoglycoside-modifying enzyme genes *aac(6′)-Ib* and *aadA1* and the β-lactamase genes bla_{OXA-9} and bla_{TEM-1} were missing in pYD626E, as were the inverted repeats (IRs). This indicates that the nested transposon in pYD626E might have originated from that of pBK15692 but that gene rearrangements occurred afterward and resulted in the loss of some resistance genes and IRs.

Next, we compared the bla_{KPC-2}-carrying plasmids from YD626E (pYD626E) and YD626K (pYD626K). pYD626E and pYD626K rendered comparable susceptibility profiles in the *E. coli* TOP10 transformants (Table 1). The two plasmids were conjugated to *E. coli* strain J53Azi by broth mating at a relatively high frequency (approximately 1×10^{-4}/donor). PCR and subsequent sequencing were performed to screen resistance genes on pYD626K. In addition to bla_{KPC-2}, pYD626K harbored all other resistance genes present on pYD626E, including bla_{SHV-12}, bla_{TEM-1}, bla_{LAP-1}, *dfrA14*, and *qnrS1*. In addition, pYD626K fell into the same incompatibility group as the IncN plasmid pYD626E (14). Plasmid fingerprint analysis showed highly related but not identical restriction patterns between pYD626E and pYD626K (Fig. 2). These findings suggest that *E. coli* YD626E likely acquired the KPC-encoding plasmid from *K. pneumoniae* YD626K in this patient or vice versa. In either case, the IncN plasmid appears to have undergone minor structural rearrangements upon interspecies transfer that did not affect the complement of resistance genes.

Here, we present the complete sequence of a unique bla_{KPC-2}-harboring plasmid from an *E. coli* sequence type 648 (ST648) strain. Its structure was characterized by the incorporation of a bla_{SHV-12}-containing region similar to certain *K. pneumoniae* chromosomes and a *qnrS1*-containing region similar to several *S. enterica* plasmids into the IncN backbone. The coexistence of bla_{KPC} and bla_{SHV} on a single plasmid is rare and has been reported in only an $IncFII_K$ plasmid (coharboring bla_{KPC-2} and bla_{SHV-11}

TABLE 1 MICs for *E. coli* YD626E, *K. pneumoniae* YD626K, and the corresponding bla_{KPC}-positive *E. coli* TOP10 transformants

Strain	MIC (μg/ml) of[a]:									
	IPM	ETP	LVX	GEN	AMK	TET	SXT	NAL	CST	TGC
E. coli YD626E	3	6	>32	1	2	8	1	>256	0.094	0.38
E. coli TOP10 (pYD626E)[b]	0.75	0.19	0.38	0.38	1	2	1.5	3	0.047	0.25
K. pneumoniae YD626K	4	8	>32	0.75	3	6	0.75	>256	0.094	0.5
E. coli TOP10 (pYD626K)[c]	1	0.19	0.25	0.38	1	2	1	4	0.047	0.25

[a] MICs were determined by Etest. IPM, imipenem; ETP, ertapenem; LVX, levofloxacin; GEN, gentamicin; AMK, amikacin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; NAL, nalidixic acid; CST, colistin; TGC, tigecycline.
[b] *E. coli* TOP10 transformant of YD626E.
[c] *E. coli* TOP10 transformant of YD626K.

[encoding a non-ESBL resistance pattern]) and an IncX plasmid (coharboring bla_{KPC-2} and bla_{SHV-12}), both of which were from *K. pneumoniae* isolates (15, 16). In addition, ST648 has been associated with various β-lactamases, including ESBLs, NDM, and KPCs (6, 17), and is a predominant multidrug-resistant clone observed worldwide in humans, companion animals, livestock, and wild birds (18–20). The emergence of the conjugative plasmid encoding KPCs, ESBLs, and *qnr* genes in *E. coli* ST648 highlights the potential of bla_{KPC} to effectively spread to high-risk clones utilizing multidrug resistance plasmids as the vehicles.

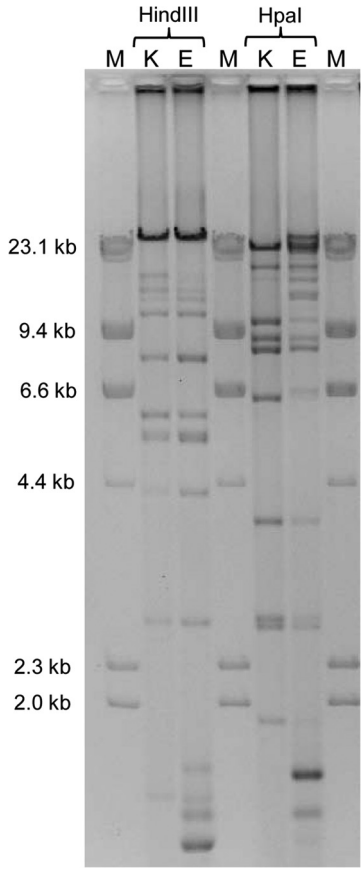

FIG 2 Plasmid profiles of bla_{KPC}-carrying plasmids of YD626E (pYD626E) and YD626K (pYD626K) using the restriction enzymes HindIII and HpaI. M, marker; K, plasmid pYD626K; E, plasmid pYD626E.

Plasmid sequence accession number. The plasmid sequence reported in this article has been submitted to GenBank under accession no. KJ933392.

ACKNOWLEDGMENT

This work was supported by National Institutes of Health research grants R21AI107302 and R01AI104895 (to Y.D.).

REFERENCES

1. **Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, Cornaglia G, Garau J, Gniadkowski M, Hayden MK, Kumarasamy K, Livermore DM, Maya JJ, Nordmann P, Patel JB, Paterson DL, Pitout J, Villegas MV, Wang H, Woodford N, Quinn JP.** 2013. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. Lancet Infect. Dis. **13:**785–796. http://dx.doi.org/10.1016/S1473-3099(13)70190-7.
2. **Cai JC, Zhang R, Hu YY, Zhou HW, Chen GX.** 2014. Emergence of *Escherichia coli* sequence type 131 isolates producing KPC-2 carbapenemase in China. Antimicrob. Agents Chemother. **58:**1146–1152. http://dx.doi.org/10.1128/AAC.00912-13.
3. **Mavroidi A, Miriagou V, Malli E, Stefos A, Dalekos GN, Tzouvelekis LS, Petinaki E.** 2012. Emergence of *Escherichia coli* sequence type 410 (ST410) with KPC-2 β-lactamase. Int. J. Antimicrob. Agents **39:**247–250. http://dx.doi.org/10.1016/j.ijantimicag.2011.11.003.
4. **Robledo IE, Aquino EE, Vazquez GJ.** 2011. Detection of the KPC gene in *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* during a PCR-based nosocomial surveillance study in Puerto Rico. Antimicrob. Agents Chemother. **55:**2968–2970. http://dx.doi.org/10.1128/AAC.01633-10.
5. **Goren MG, Navon-Venezia S, Chmelnitsky I, Carmeli Y.** 2010. Carbapenem-resistant KPC-2-producing *Escherichia coli* in a Tel Aviv medical center, 2005 to 2008. Antimicrob. Agents Chemother. **54:**2687–2691. http://dx.doi.org/10.1128/AAC.01359-09.
6. **Kim YA, Qureshi ZA, Adams-Haduch JM, Park YS, Shutt KA, Doi Y.** 2012. Features of infections due to *Klebsiella pneumoniae* carbapenemase-producing *Escherichia coli*: emergence of sequence type 131. Clin. Infect. Dis. **55:**224–231. http://dx.doi.org/10.1093/cid/cis387.
7. **Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J.** 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat. Methods **10:**563–569. http://dx.doi.org/10.1038/nmeth.2474.
8. **Carattoli A, Aschbacher R, March A, Larcher C, Livermore DM, Woodford N.** 2010. Complete nucleotide sequence of the IncN plasmid pKOX105 encoding VIM-1, QnrS1 and SHV-12 proteins in *Enterobacteriaceae* from Bolzano, Italy compared with IncN plasmids encoding KPC enzymes in the USA. J. Antimicrob. Chemother. **65:**2070–2075. http://dx.doi.org/10.1093/jac/dkq269.
9. **Bhagwat AS, Johnson B, Weule K, Roberts RJ.** 1990. Primary sequence of the *Eco*RII endonuclease and properties of its fusions with β-galactosidase. J. Biol. Chem. **265:**767–773.
10. **Chen L, Chavda KD, Fraimow HS, Mediavilla JR, Melano RG, Jacobs**

MR, Bonomo RA, Kreiswirth BN. 2013. Complete nucleotide sequences of bla_{KPC-4}- and bla_{KPC-5}-harboring IncN and IncX plasmids from *Klebsiella pneumoniae* strains isolated in New Jersey. Antimicrob. Agents Chemother. **57:**269–276. http://dx.doi.org/10.1128/AAC.01648-12.

11. **Chen L, Hu H, Chavda KD, Zhao S, Liu R, Liang H, Zhang W, Wang X, Jacobs MR, Bonomo RA, Kreiswirth BN.** 2014. Complete sequence of a KPC-producing IncN multidrug-resistant plasmid from an epidemic *Escherichia coli* sequence type 131 strain in China. Antimicrob. Agents Chemother. **58:**2422–2425. http://dx.doi.org/10.1128/AAC.02587-13.
12. **Drieux L, Decre D, Frangeul L, Arlet G, Jarlier V, Sougakoff W.** 2013. Complete nucleotide sequence of the large conjugative pTC2 multireplicon plasmid encoding the VIM-1 metallo-β-lactamase. J. Antimicrob. Chemother. **68:**97–100. http://dx.doi.org/10.1093/jac/dks367.
13. **Chen L, Chavda KD, Al Laham N, Melano RG, Jacobs MR, Bonomo RA, Kreiswirth BN.** 2013. Complete nucleotide sequence of a bla_{KPC}-harboring IncI2 plasmid and its dissemination in New Jersey and New York hospitals. Antimicrob. Agents Chemother. **57:**5019–5025. http://dx.doi.org/10.1128/AAC.01397-13.
14. **Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63:**219–228. http://dx.doi.org/10.1016/j.mimet.2005.03.018.
15. **Chen YT, Lin JC, Fung CP, Lu PL, Chuang YC, Wu TL, Siu LK.** 2014. KPC-2-encoding plasmids from *Escherichia coli* and *Klebsiella pneumoniae* in Taiwan. J. Antimicrob. Chemother. **69:**628–631. http://dx.doi.org/10.1093/jac/dkt409.
16. **Kassis-Chikhani N, Frangeul L, Drieux L, Sengelin C, Jarlier V, Brisse S, Arlet G, Decre D.** 2013. Complete nucleotide sequence of the first KPC-2- and SHV-12-encoding IncX plasmid, pKpS90, from *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **57:**618–620. http://dx.doi.org/10.1128/AAC.01712-12.
17. **Mushtaq S, Irfan S, Sarma JB, Doumith M, Pike R, Pitout J, Livermore DM, Woodford N.** 2011. Phylogenetic diversity of *Escherichia coli* strains producing NDM-type carbapenemases. J. Antimicrob. Chemother. **66:**2002–2005. http://dx.doi.org/10.1093/jac/dkr226.
18. **Guenther S, Grobbel M, Beutlich J, Bethe A, Friedrich ND, Goedecke A, Lubke-Becker A, Guerra B, Wieler LH, Ewers C.** 2010. CTX-M-15-type extended-spectrum β-lactamases-producing *Escherichia coli* from wild birds in Germany. Environ. Microbiol. Rep. **2:**641–645. http://dx.doi.org/10.1111/j.1758-2229.2010.00148.x.
19. **Ewers C, Bethe A, Stamm I, Grobbel M, Kopp PA, Guerra B, Stubbe M, Doi Y, Zong Z, Kola A, Schaufler K, Semmler T, Fruth A, Wieler LH, Guenther S.** 2014. CTX-M-15-D-ST648 *Escherichia coli* from companion animals and horses: another pandemic clone combining multiresistance and extraintestinal virulence? J. Antimicrob. Chemother. **69:**1224-1230. http://dx.doi.org/10.1093/jac/dkt516.
20. **Cortés P, Blanc V, Mora A, Dahbi G, Blanco JE, Blanco M, Lopez C, Andreu A, Navarro F, Alonso MP, Bou G, Blanco J, Llagostera M.** 2010. Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. Appl. Environ. Microbiol. **76:**2799–2805. http://dx.doi.org/10.1128/AEM.02421-09.