Supplemental Materials

Methods

Whole-genome sequencing and assembly. Genomic DNA was extracted from KPC160111 using Qiagen DNase Blood & Tissue Kit (Qiagen, Hilden, Germany). Genomic libraries were prepared with an approximately 250-bp insert size using NextEra XT Library Prep Kit (Illumina, San Diego, CA., USA) and sequenced with the Illumina MiSeq platform using paired-end 500 cycles protocol. The adapter sequences, ambiguous reads, and low-quality sequences were removed using Trimmomatic. Qualified reads were *de novo* assembled with Velvet Assembler into Contigs. Simultaneously, KPC160111 was subjected to sequencing with the PacBio RSII single-molecule real-time (SMRT) platform. PacBio long reads were assembled de novo using HGAP V3 and polished with Quiver. Illumina short reads were then mapped to the polished long-read contigs for minor error correction using Pilon 1.21. SPAdes Genome Assembler was also used for the combination of Illumina short reads and PacBio long reads. The final whole genome sequence of KPC160111 was validated and trimmed into a circular chromosome and plasmids using CLC Genome Finishing Module 1.8 (Qiagen, Hilden, Germany).

Whole-genome profiling. The complete sequences of KPC160111 chromosome and plasmids were annotated by the NCBI Prokaryotic Genome Automatic Annotation Pipeline and were subjected to genome profiling. Acquired AMR genes were identified with ResFinder 3.0 (1), and plasmid incompatibility (Inc.) groups were assessed with PlasmidFinder 1.3 database (2) from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). Insertion sequences (ISs) and prophage regions were identified using ISfinder (https://www-is.biotoul.fr/index.php) and PHAST (3), respectively. Sequence types (STs) were determined using the *K. pneumoniae* MLST scheme established in the Kp BIGSdb (Institute Pasteur *Klebsiella pneumoniae* BIGSdb) (4). The type of CPS biosynthesis loci (KL-type) and the type of LPS biosynthesis loci (OL-type) were analyzed using Kaptive (5) (http://kaptive.holtlab.net/).

Conjugation experiments. The horizontal transfer efficiency of pOXA48-L111 was examined by *in vitro* conjugation using KPC160111 as a donor and the rifampin-resistant strain *Escherichia coli* J53-2 as a recipient. Briefly, 1 ml overnight cultures of KPC160111 and J53-2 were thoroughly washed to remove the antibiotics, resuspended in 1 ml of sterile saline, mixed in a 1: 5 donor-to-recipient ratio, platted

onto nonselective solid LB plates, and incubated at 30℃ for 8 hours. One ml of sterile saline was used to remove the conjugation mix from the LB plates. Transconjugants were then selected by plating 100 μl of serial dilutions onto M9 plates supplemented with imipenem (Imp; 10 μg/ml) to measure the number of donors (KPC160111), or onto LB plates containing rifampicin (Rif; 100 μg/ml) and imipenem (Imp; 1 μg/ml) to quantify the number of transconjugants. Conjugation frequencies were calculated as the ratio of transconjugants (Imp^R Rif^R CFU on LB)/KPC160111 (Imp^R CFU on M9). The conjugation experiment was repeated three times. The presence of the pOXA48_L111 plasmid was confirmed by PCR analysis of six transconjugant colonies per experiment.

References

1. **Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV.** 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother **67:**2640-2644.
2. **Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Moller Aarestrup F, Hasman H.** 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother **58:**3895-3903.
3. **Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS.** 2011. PHAST: A Fast Phage Search Tool. Nucleic Acids Research **39:**W347-W352.
4. **Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S.** 2005. Multilocus sequence typing of Klebsiella pneumoniae nosocomial isolates. J Clin Microbiol **43:**4178-4182.
5. **Wyres KL, Wick RR, Gorrie C, Jenney A, Follador R, Thomson NR, Holt KE.** 2016. Identification of Klebsiella capsule synthesis loci from whole genome data. Microb Genom **2:**e000102.

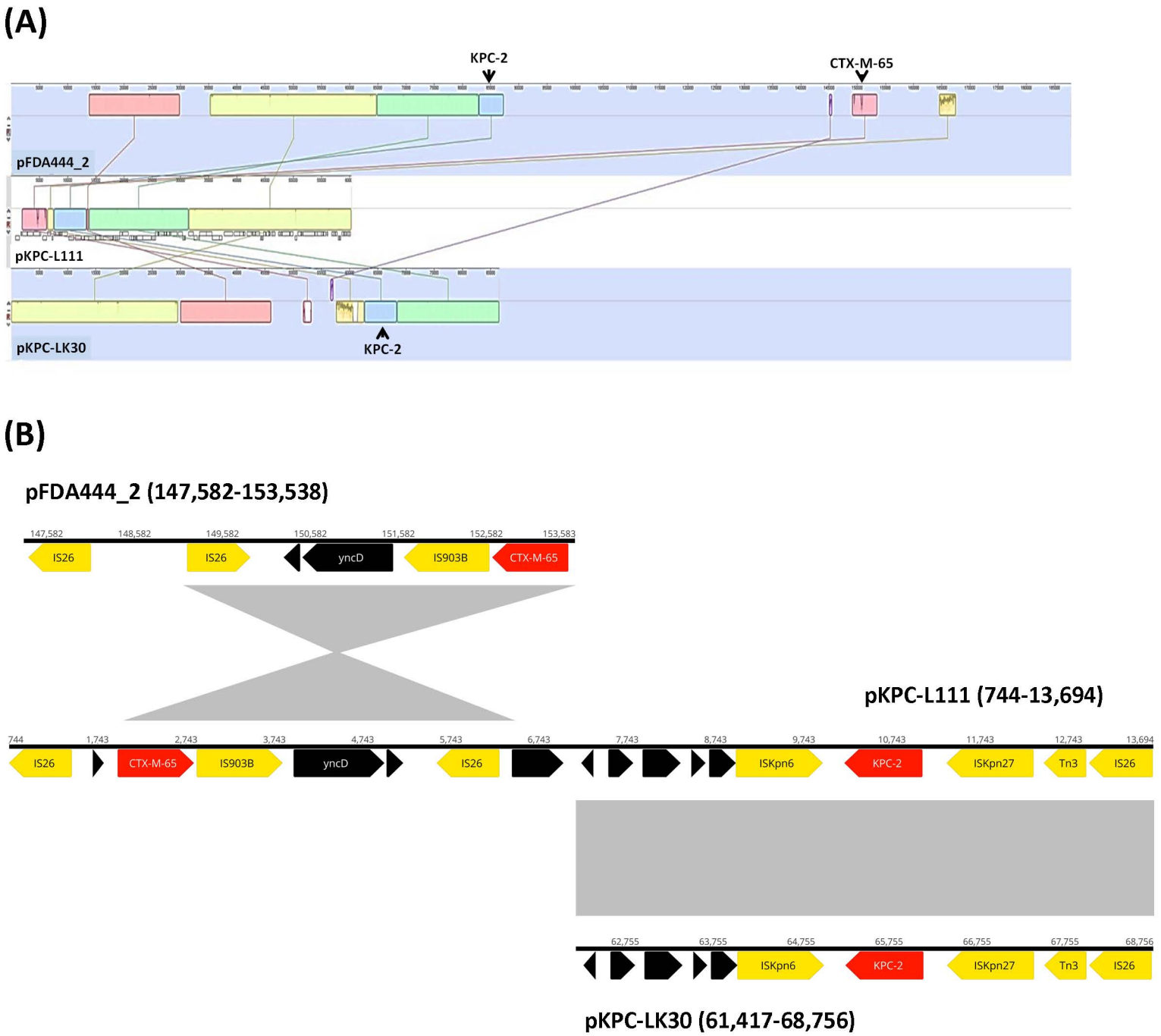


Fig. S1. The *bla*$_{KPC-2}$ and *bla*$_{CTX-M-65}$ co-carrying plasmid pKPC-L111. **(A)** Alignment of pKPC-L111 with pKPC-LK30 (KC405622) and pFDA444_2 (CP023942), generated using progressiveMAUVE. Colored rectangles represent locally collinear blocks (LCBs), regions of homology without rearrangement across the aligned sequences, connected by matching colored diagonal lines. The LCBs containing *bla*$_{KPC-2}$ and *bla*$_{CTX-M-65}$ are respectively indicated by an arrowhead. **(B)** Comparison of the 13-kb region of pKPC-L111 with similar regions of pKPC-LK30 and pFDA444_2. AMR genes, IS elements, and other protein-encoding genes, are shown in red, yellow, and black, respectively. Grey-shaded connections indicate high sequence identity (>99.9%).

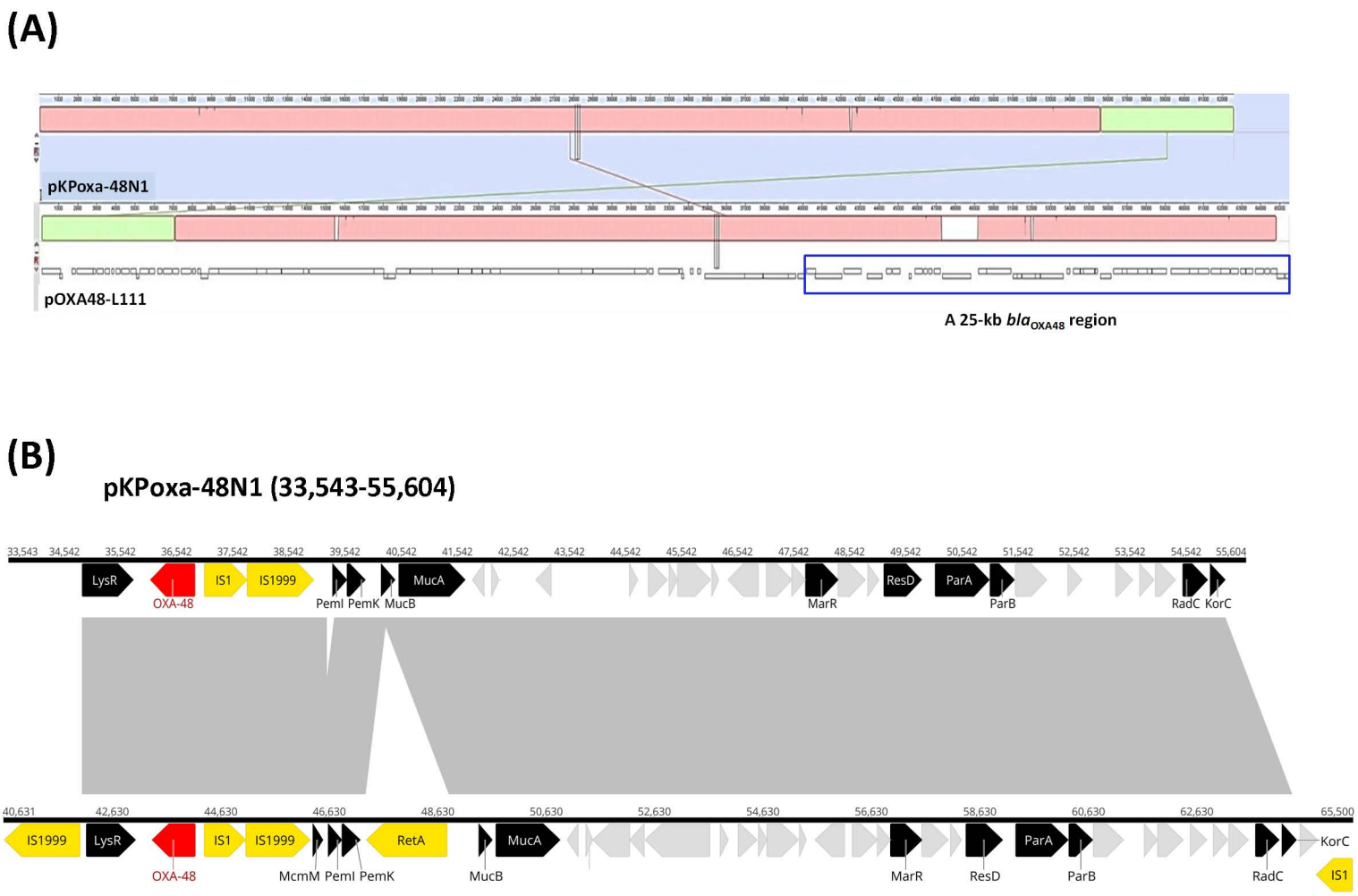
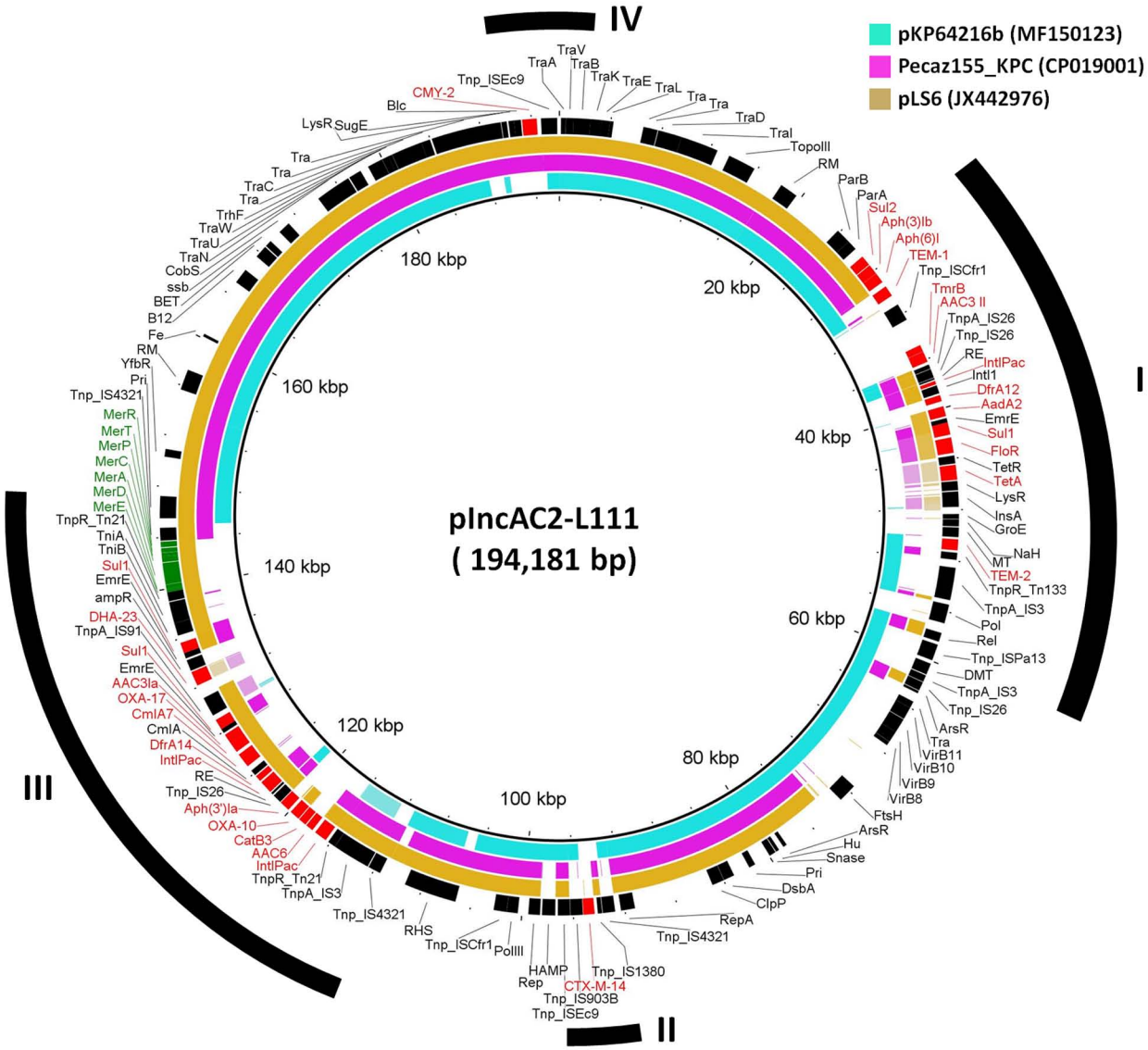


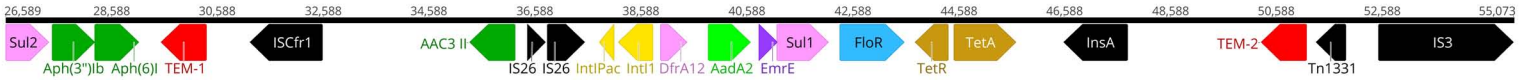
Figure S2. The *bla*$_{\text{OXA-48}}$ plasmid pOXA48-L111. **(A)** Alignment of pOXA48-L111 with pKPoxa-48N1 (KC757416) generated with progressiveMAUVE. Colored rectangles represent locally collinear blocks (LCBs), regions of homology without rearrangement across the aligned sequences, connected by matching colored diagonal lines. **(B)** Comparison of the 25-kb *bla*$_{\text{OXA-48}}$-containing region of pKPC-L111, highlighted in the blue box of (A), with the counterpart of pKPoxa-48N1. The *bla*$_{\text{OXA-48}}$ gene, IS elements, hypothetical, and other protein-encoding genes, are shown in red, yellow, grey, and black, respectively. Grey-shaded connections indicate high sequence identity (>99.9%).

(A)

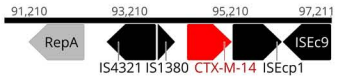


(B)

Region I: a 35,895-bp mosaic region composed with partial class I integron and IS*3* flanked by IS*26*



Region II: a conserved CTX-M-14 context (IS*Ecp1*-*bla*$_{CTX-M-14}$-ΔIS*1380*-IS*4321*)



Region III: a 33,293-bp class I integron region flanked by IS*4321*

Region IV: a conserved CMY context (IS*Ec9*-*bla*$_{CMY-2}$-*blc*-*sugE*)

Figure S3. The multidrug-resistance plasmid pIncAC2-L111. **(A)** Alignment of pIncAC2-L111 with the closely-related plasmids, pKP64216b (MF150123), Pecaz155_KPC (CP019001), and pLS6 (JX442976), generated using the BLAST Ring Image Generator (BRIG). Red denotes the AMR genes. Four AMR cassettes (I-IV) are indicated as black in the outermost ring. **(B)** The detailed information regarding the AMR cassette I, II, III, and IV. Black, yellow, and grey denote IS and class I integron related elements, and other protein-encoding genes, respectively. AMR genes of different families are shown in different colors: Red for β-lactams, green for aminoglycosides, blue for chloramphenicol and rifampicin, pink for sulphonamides and trimethoprim, light brown for tetracycline, and purple for macrolides.