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Cocarriage of Distinct bla_{KPC-2} and bla_{OXA-48} Plasmids in a Single Sequence Type 11 Carbapenem-Resistant *Klebsiella pneumoniae* Isolate

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The endemic spread of carbapenemase-producing *Klebsiella pneumoniae* in Taiwan has become problematic (1). Besides the predominance of *Klebsiella pneumoniae* carbapenemase (KPC)-2, oxacillinase (OXA)-48 emerged at the end of 2013 (2) and has become the second most prevalent (1). Previously, we demonstrated that KPC-2 and OXA-48 accounted for 36.2% and 12.6% of carbapenem-resistant *K. pneumoniae* isolates, respectively, and most of them belonged to sequence type (ST) 11 (3).

KPC160111, a KPC-2 ST11 isolate, was isolated from the urine culture of an 81-year-old female patient who suffered from urinary tract infection in November 2014. Besides KPC-2, this strain was found to coproduce OXA-48 and exhibited extensive resistance to almost all antimicrobials (Table 1). Considering the clinical significance of coproduction of KPC-2 and OXA-48 in a single *K. pneumoniae* ST11 strain, here we report the in-depth characterization of KPC160111.

Whole-genome sequencing data (see the methods in the supplemental material) demonstrated that KPC160111 contained a 5.8-Mb genome, including a 5.43-Mb chromosome and six different plasmids. Ten β-lactamase-encoding genes (*bla*) and 21 antimicrobial resistance (AMR) genes were identified (Table 2). The KPC160111 chromosome carried three intrinsic AMR genes, bla_{SHV-11}, *oqxAB*, and *fosA* (Fig. 1A). Based on analysis with Kaptive (4), the type of capsular polysaccharide (CPS) biosynthesis loci (KL-type) and the type of lipopolysaccharide (LPS) biosynthesis loci (OL-type) of KPC160111 were determined as KL47/OL101 (see the methods in the supplemental material), which were closely related to two ST11 KPC-2 strains, GD4 (NZ_CP025951.1) (5) and SCKP020143 (NZ_CP028548.1).

KPC160111 carried bla_{KPC-2} and bla_{OXA-48} by two distinct plasmids, named pKPC_L111 and pOXA48-L111, respectively. pKPC_L111 (Fig. 1B), a 60,307-bp IncR plasmid, captured bla_{KPC-2} by a Tn*1722*-based transposon unit with the core structure of IS*Kpn27*-bla_{KPC-2}-ΔIS*Kpn6* as pKPC-LK30 (6) and acquired an additional IS*26*-based insertion of an IS*Ecp1*-$bla_{CTX-M-65}$-Δ*orf477* cassette downstream of bla_{KPC-2} (see Fig. S1). pOXA48-L111 (Fig. 1C) was a conjugative IncL plasmid (65,500 bp) and was transferred

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TABLE 1 Antimicrobial susceptibility test results for KPC160111

Antimicrobial	MIC (μg/ml)[a]	Interpretation[b]
Aminoglycosides		
Amikacin	>1,024	R
Gentamicin	>1,024	R
Beta-lactams		
Ampicillin	>1,024	R
Cefazolin	>1,024	R
Cefepime	>1,024	R
Ceftazidime	64	R
Ceftriaxone	>1,024	R
Ertapenem	256	R
Imipenem	>1,024	R
Meropenem	256	R
Piperacillin-tazobactam	>1,024/256	R
Ampicillin-sulbactam	>1,024/512	R
Ceftazidime-avibactam	2/1	S
Fluoroquinolones		
Ciprofloxacin	128	R
Levofloxacin	64	R
Fosfomycin		
Fosfomycin	>1,024	R
Sulfonamide		
Trimethoprim-sulfamethoxazole	>56/1,064	R
Polymyxin		
Colistin	0.5	S

[a]Antimicrobial susceptibility testing was performed with standard broth microdilution method and interpreted based on the criteria from the Clinical and Laboratory Standards Institute guidelines (see Table 2A in M100-ED29).
[b]R, resistant; S, susceptible.

from KPC160111 to *Escherichia coli* J53-2 with an average frequency of 5.9×10^{-8} under standard laboratory conditions (see the methods in the supplemental material). pOXA48-L111 had bla_{OXA-48} as the only AMR gene enclosed on a Tn*1999.2* composite transposon, similar to most of the bla_{OXA-48} carrying plasmids (7), and harbored an additional 1,911-bp fragment encoding RetA, a group II intron reverse transcriptase, upstream of the *mucAB* (see Fig. S2). Because KPC160111 was an ST11_KL47/OL101 strain related to other KPC-2 *K. pneumoniae* (3), the acquisition of pOXA48-L111, which brought the second carbapenemase OXA-48 into an already KPC-2 background, was suggested as a likely scenario for the emergence of this *K. pneumoniae* that carried both bla_{KPC-2} and bla_{OXA-48}. Besides the carriage of the KPC-2 and OXA-48 plasmids, KPC160111 harbored a novel IncC plasmid (194,181 bp), which exhibited hybrid type 1 and type 2 IncC backbone features (8), and had 4 mosaic cassettes holding a total of 25 AMR genes (see Fig. S3).

TABLE 2 Genomic features of *K. pneumoniae* KPC160111

Structure	Length (bp)	GC (%)	Antimicrobial resistance genes	Replicon type	Accession no.
Chromosome	5,430,212	57.4	bla_{SHV-11}, *fosA*, *oqxAB*		CP029689
Plasmid					
pKPC-L111	60,312	55.4	bla_{KPC-2}, $bla_{CTX-M-65}$	IncR	CP030134
pOXA48-L111	65,500	51.4	bla_{OXA-48}	IncL	CP030135
pIncAC2-L111	194,181	54.6	*aacA4*, *aph(3′)-Ia*, *aadA1*, bla_{OXA-10}, bla_{DHA-1}, *aac(6′)-Ib-cr*, *catB3*, *cmlA1*, *arr2*, *sul1*, *dfrA14*, *aph(3′′)-Ib*, *aph(6)-Id*, *aac(3)-IId*, *aadA2*, *rmtB*, bla_{TEM-1B}, bla_{TEM-2}, *erm*, *sul2*, *sul1*, *tet*(G), *dfrA12*, bla_{CMY-2}, $bla_{CTX-M-14}$	IncC	CP030132
pIncFII-L111	39,248	54.4	Not detected	IncFII	CP030133
p10K-L111	10,060	55.1	Not detected	ColRNAI	CP030130
p5.6K-L111	5,596	55.1	Not detected	ColRNAI	CP030131

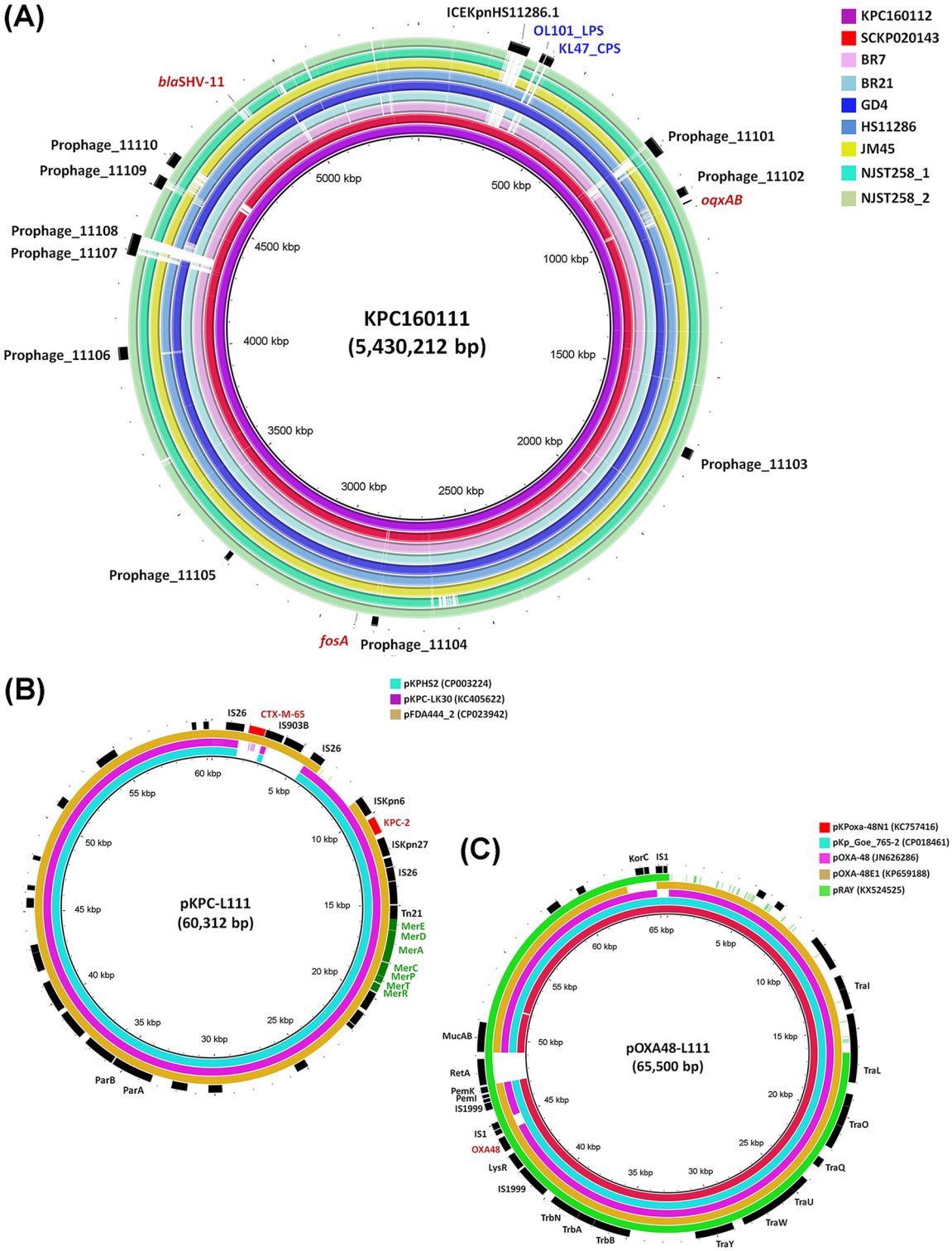

FIG 1 (A) Alignment of the KPC160111 chromosome and chromosomes of representative KPC-producing CG258 strains, including SCKP020143 (NZ_CP028548.1; ST11, KL47), GD4 (NZ_CP025951.1; ST11, KL47), JM45 (NC_022082.1; ST11, KL125), HS11286 (CP003200.1; ST11, KL103), BR7 (CP018883.1; ST437), BR21 (NZ_CP018885.1; ST437), NJST258.1 (NZ_CP006923.1; ST258), and NJST258.2 (NZ_CP006918.1; ST258). Notable features, including prophages (11101 to 11110), integrated conjugative element ICE*Kpn*HS11286.1, the CPS biosynthesis region for KL-47, the LPS biosynthesis region for OL101, and AMR genes, *oqxAB*, *fosA*, and bla_{SHV11}, are indicated in the outer ring. Circular maps were generated using the BLAST Ring Image Generator (BRIG). (B) The bla_{KPC-2} and $bla_{CTX-M-65}$-cocarrying plasmid pKPC-L111. Alignment of pKPC-L111 with three closely related plasmids, pKPHS2 (CP003224), pKPC-LK30 (KC405622), and pFDA444_2 (CP023942), generated using BRIG. Genes associated with resistance to antimicrobials and heavy metals are highlighted in red and green, respectively. (C) The bla_{OXA-48} plasmid pOXA48-L111. Alignment of pOXA48-L111 with the related plasmids, pKPoxa-48N1 (KC757416), pKp_Goe_765-2 (CP018461), pOXA-48 (JN626286), pOXA-48E1 (KP659188), and pRAY (KX524525), generated using BRIG. The bla_{OXA-48} gene is indicated in red.

To the best of our knowledge, this is the first report on the complete genome sequence of KPC-2-and OXA-48-coproducing *K. pneumoniae*. The uncommon cocarriage of genes encoding different classes of carbapenemases rendered KPC160111 extremely highly resistant to carbapenems (Table 1). Not restricted in our hospital, a total of 50 *K. pneumoniae* isolates coharboring bla_{KPC-2} and bla_{OXA-48} were also isolated from a regional hospital located in central Taiwan (9). For the better control of the endemic spread of carbapenemase-producing *K. pneumoniae*, horizontal spread of bla_{OXA-48} plasmids among KPC-2 strains needs to be actively monitored. The molecular mechanism underlying the superior capability of acquisition and maintenance of foreign DNA in ST11 *K. pneumoniae* deserves further studies.

This study was approved by the CSMUH Institute Review Board (IRB CS15022 and CS16104). Informed consent was obtained from all patients enrolled in this study. Patients were excluded if they were <18 years of age. All methods were carried out in accordance with relevant guidelines and regulations.

Data availability. The genome sequence of *K. pneumoniae* KPC160111 has been deposited at NCBI under the BioSample number SAMN09279554 and the GenBank accession numbers CP029689 (KPC160111 chromosome), CP030134 (pKPC-L111), CP030135 (pOXA48-L111), CP030132 (pIncAC2-L111), CP030133 (pIncFII-L111), CP030130 (p10K-L111), and CP030131 (p5.6K-L111).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02282-18.

SUPPLEMENTAL FILE 1, PDF file, 1.4 MB.

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M.-C.L., C.-S.C., and Y.-C.L. conceived and designed the experiments; Y.-C.W., M.-C.L., and H.-L.T. collected clinical samples; M.-C.L., Y.-C.W., H.-L.T., Y.-T.C., and Y.-C.L. performed the experiments; H.-L.T., C.-S.C., Y.-T.C., and Y.-C.L. analyzed the data; H.-L.T. and Y.-C.L. prepared the figures and tables; M.-K.C. and Y-.C.L. wrote the manuscript; all authors read and approved the manuscript.

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