LETTER TO THE EDITOR

Small IncQ1 and Col-like Plasmids Harboring *bla*$_{KPC-2}$ and non-Tn*4401* Elements (NTE$_{KPC}$-IId) in High-Risk Lineages of *Klebsiella pneumoniae* CG258

Louise T. Cerdeira,[a] Margaret M. C. Lam,[b] Kelly L. Wyres,[b] Ryan R. Wick,[b] Louise M. Judd,[b] Ralf Lopes,[c] Rosineide M. Ribas,[d] Marcia M. Morais,[e] Kathryn E. Holt,[b,f] Nilton Lincopan[a,c]

[a]Department of Clinical Analysis, School of Pharmacy, Universidade de São Paulo, São Paulo, Brazil

[b]Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Australia

[c]Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

[d]Institute of Biomedical Sciences, Federal University of Uberlandia, Uberlandia, Minas Gerais, Brazil

[e]Institute of Biological Sciences, University of Pernambuco, Recife, Brazil

[f]London School of Hygiene and Tropical Medicine, London, United Kingdom

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Address correspondence to Nilton Lincopan, lincopan@usp.br

A retrospective genomic study led to the identification of two carbapenem-resistant *K. pneumoniae* isolates (KPN535 and KPC45) carrying bla_{KPC-2} genes on non-conjugative plasmids. These isolates were recovered in 2011 and 2015, from rectal swab cultures of inpatients from two hospitals in Brazil, and belonged to the hospital-associated lineages ST340 and ST11 (CG258).

For both *K. pneumoniae* strains, total genomic DNA was extracted and sequenced using long-read (PromethION, Oxford Nanopore) and short-read (NextSeq, Illumina) sequencing technologies, with further hybrid *de novo* assembly using Unicycler (v0.4.0), which resolved complete circularized sequences of chromosome and plasmids (1, 2).

Interestingly, in KPN535 and KPC45, the bla_{KPC-2} gene was found on small IncQ1 and Col-like (Col-KPC) plasmids named pKPN535a and pKPC45a, respectively (Fig. 1A and 1B). The pKPN535a plasmid is 14,873 bp in size, with G+C content of 54.6%, containing the *higA* antitoxin-encoding gene, genes encoding ParE/RelE-superfamily toxins, and the *aph(3')-Vla* aminoglycoside resistance gene. On the other hand, Col-KPC is 9,548 bp in size (with G+C content of 52.3%), sharing >90% identity with the Col (MGD2) plasmid (NC_003789) (3), and carrying *relaxase* and *mobC* genes.

Both plasmids contain a variant of non-Tn*4401* elements (NTE_{KPC}), designated NTE_{KPC}-IId, with the gene array *tnpR*-Δbla_{TEM}-bla_{KPC-2}-ΔIS*Kpn6*/*traN* (Fig. 1C). Interestingly, in the two plasmids, NTE_{KPC}-IId elements were flanked by two identical 243-bp direct repeats, whereas pKPN535a carries a third 243-bp repeat downstream *repC*. NTE_{KPC} have been separated in three groups according to the absence or presence of bla_{TEM}, where the second group (NTE_{KPC}-II) includes variants that have a truncated bla_{TEM} gene (4, 5); whereas all NTE_{KPC} structures described to date (including, NTE_{KPC}-IId) contain genetic remnants of Tn*4401*, consistent with

their having evolved from Tn*4401* by recombination and/or insertion of other smaller mobile genetic elements. By using NCBI blast against NR database, we noted that similar NTE_{KPC}-IId structures (100% identity) have been recently identified in *Klebsiella aerogenes* from Brazil (GenBank accession numbers: MG786907, MH000708). Therefore, although no additional information is available, the possibility that *Enterobacterales* carrying bla_{KPC-2} on NTE_{KPC}-IId elements have spread in Brazil and into other countries is deeply concerning. In fact, NTE_{KPC} elements have been described in China, Argentina, Brazil and Russia (4-7). Therefore, the role of NTE_{KPC} elements in global dissemination of bla_{KPC} deserves additional investigation.

Plasmids have played a key role in the horizontal spread of antibiotic resistance genes, promoting the survival and selection of clonal lineages among clinically significant pathogens (8). IncQ plasmids are of particular interest as they are highly mobilizable, being stably maintained and transferred among a wide range of Gram-negative bacteria (9, 10). On the other hand, Col-like plasmids are mobilizable vectors that have been increasingly reported as antibiotic resistance carriers, in members of the Enterobacteriaceae family, being postulated as versatile gene capture platforms (11). These novel groups of IncQ1 and Col-KPC plasmids, identified in this study, might have originated through independent recombination events between NTE_{KPC}-IId and a recipient IncQ1 or Col-type plasmid backbone, which is consistent with independent recombination events generating the variability among members of this group of plasmids (10, 12). Interestingly, large direct repeats could flank genomic rearrangements between NTE_{KPC} elements and small mobilizable plasmids. In fact, recent studies have reported the presence of these small plasmids in KPC-2-producing *Pseudomonas aeruginosa* and *Escherichia coli*, and BKC-positive *Klebsiella pneumoniae* isolates (12-15).

In summary, in this study we report the identification and complete sequence of two plasmids, pKPN535a (MH595533) and pKPC45a (MH595534), which represent new groups of small IncQ1 and Col-KPC vectors conferring carbapenem resistance in high-risk lineages of *K. pneumoniae* CG258, representing a novel mechanism for dissemination of carbapenem resistance that may carry lower fitness costs and could potentially result in increased persistence and wider dissemination.

Nucleotide sequence accession number

The nucleotide sequence of pKPN535a and pKPC45a plasmids were deposited at GenBank under the accession numbers MH595533 and MH595534, respectively.

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Figure legends

Fig. 1. Genetic structures of the small (A) IncQ1 pKPN535a (MH595533) and (B) Col-KPC pKPC45a (MH595534) plasmids harboring the *bla*$_{KPC-2}$ gene and non-Tn*4401* elements (NTE$_{KPC}$-IId) identified in *K. pneumoniae* strains belonging to ST11 and ST340 (CG258), respectively. Protein coding sequences are represented by the arrows and labeled with gene name or product. In C, Alignment of Tn*4401* and NTE$_{KPC}$ genetic elements harboring *bla*$_{KPC}$ genes identified in Brazil. NTE$_{KPC}$ genetic elements encompass NTE$_{KPC}$-Ic associated with *bla*$_{KPC-2}$ carried by IncX3 plasmids (4), and the two NTE$_{KPC}$-IId elements identified in this study. Based on the insertion of Δ*bla*$_{TEM}$ upstream of the *bla*$_{KPC}$ gene, NTE$_{KPC}$ elements have been classified as NTE$_{KPC}$-II, whereas NTE$_{KPC}$-II variants are based on the differences of the length of Δ*bla*$_{TEM}$ and deletions between Δ*bla*$_{TEM}$ and *bla*$_{KPC-2}$ (4). In both plasmids, NTE$_{KPC}$-IId elements were flanked by two identical 243-bp direct repeats [DR (open circles): AGGGGTCGTCTCAGAATTCGGAAAATAAAGCACGCTAGCGGTTGATCTGTCAGGTT GAAGCCTGAGAGGCCGAGCGCAGATCGTCAGAAAAGGCGAAAAACGATCCTAATCT GACGCAACATAGGTGGGGTGCCTGACGCCCGGTTGAGGCGTACTTCAACTGGACAC CATTCCAGAAAGACCAAGCATGGCATGGCCTGCCGCTGTCTTACCGTGCTTTATTTC CCGTTTTCTCTATCGACC]. Protein coding sequences are represented by the arrows and labeled with gene name or product. Light blue shading denotes shared regions of homology (>95%).

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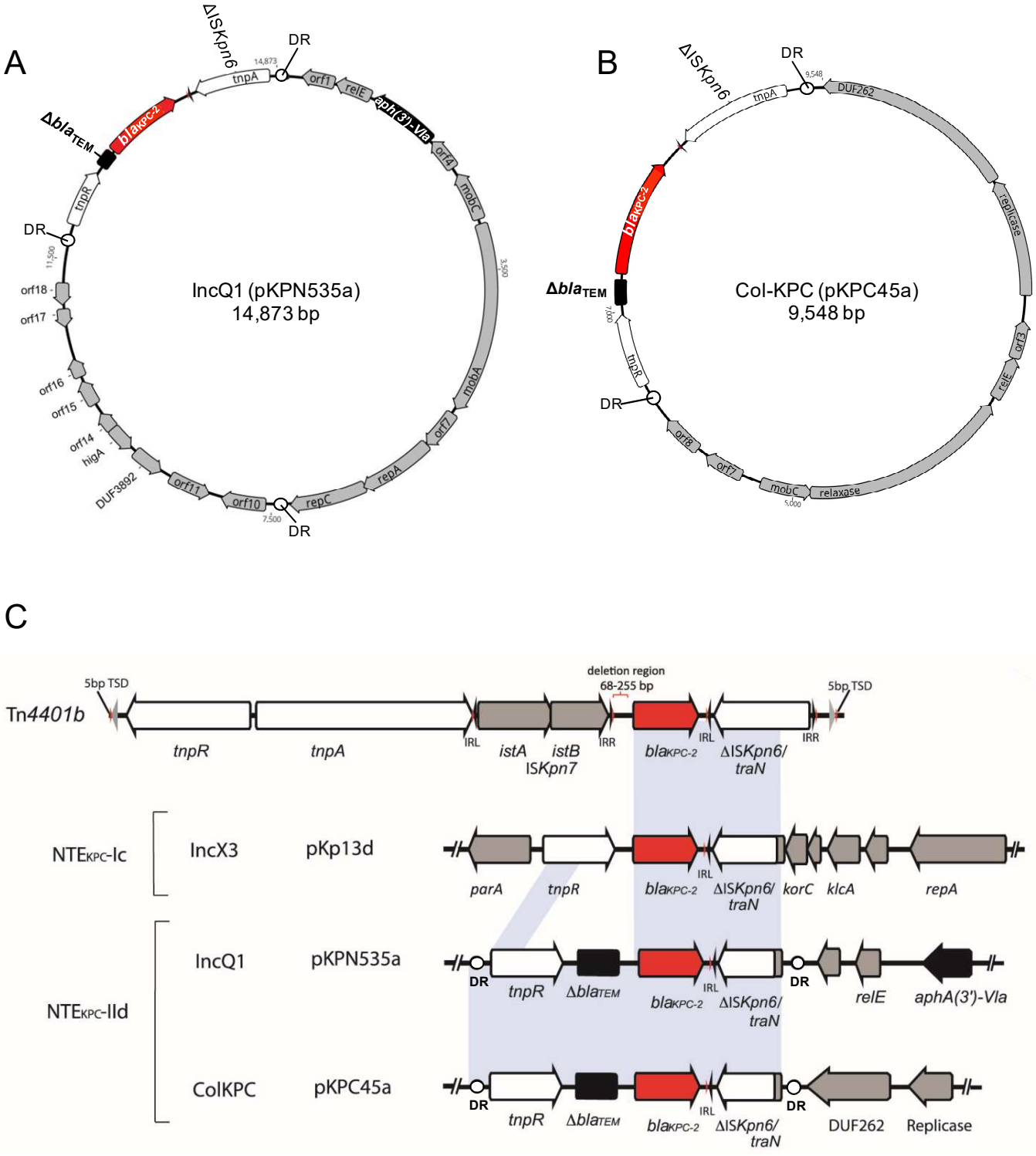
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ΔISKpn6
DR
tnpA
orf1
relE
aph(3')-VIa
orf4
mobC
mobA
orf7
repA
repC
orf10
orf11
DUF3892
higA
orf14
orf15
orf16
orf17
orf18
tnpR
ΔblaTEM
blaKPC-2
IncQ1 (pKPN535a)
14,873 bp
B
ΔISKpn6
DR
tnpA
DUF262
replicase
orf3
relE
orf4
orf7
mobC
relaxase
tnpR
ΔblaTEM
blaKPC-2
Col-KPC (pKPC45a)
9,548 bp
C
Tn4401b
5bp TSD
deletion region
68-255 bp
tnpR
tnpA
IRL
istA
istB
ISKpn7
IRR
blaKPC-2
ΔISKpn6/
traN
NTEKPC-Ic
IncX3
pKp13d
parA
korC
klcA
repA
NTEKPC-IId
IncQ1
pKPN535a
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relE
aphA(3')-VIa
ColKPC
pKPC45a
DUF262
Replicase