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1 LETTER TO THE EDITOR

2 **Small IncQ1 and Col-like Plasmids Harboring *bla*<sub>KPC-2</sub> and non-Tn4401 Elements**  
3 **(NTE<sub>KPC</sub>-IId) in High-Risk Lineages of *Klebsiella pneumoniae* CG258**

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20 **KEYWORDS:** Carbapenemase; KPC-2; CG258; Mobilome; Plasmidome, NTE<sub>KPC</sub>, Brazil

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

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

33 Interestingly, in KPN535 and KPC45, the *bla*<sub>KPC-2</sub> gene was found on small IncQ1 and  
34 Col-like (Col-KPC) plasmids named pKPN535a and pKPC45a, respectively (Fig. 1A and 1B).  
35 The pKPN535a plasmid is 14,873 bp in size, with G+C content of 54.6%, containing the *higA*  
36 antitoxin-encoding gene, genes encoding ParE/RelE-superfamily toxins, and the *aph(3')-Vla*  
37 aminoglycoside resistance gene. On the other hand, Col-KPC is 9,548 bp in size (with G+C  
38 content of 52.3%), sharing >90% identity with the Col (MGD2) plasmid (NC\_003789) (3), and  
39 carrying *relaxase* and *mobC* genes.

40 Both plasmids contain a variant of non-Tn4401 elements (NTE<sub>KPC</sub>), designated NTE<sub>KPC</sub>-  
41 IId, with the gene array *tnpR*- $\Delta$ *bla*<sub>TEM</sub>-*bla*<sub>KPC-2</sub>- $\Delta$ *ISKpn6*/*traN* (Fig. 1C). Interestingly, in the two  
42 plasmids, NTE<sub>KPC</sub>-IId elements were flanked by two identical 243-bp direct repeats, whereas  
43 pKPN535a carries a third 243-bp repeat downstream *repC*. NTE<sub>KPC</sub> have been separated in three  
44 groups according to the absence or presence of *bla*<sub>TEM</sub>, where the second group (NTE<sub>KPC</sub>-II)  
45 includes variants that have a truncated *bla*<sub>TEM</sub> gene (4, 5); whereas all NTE<sub>KPC</sub> structures  
46 described to date (including, NTE<sub>KPC</sub>-IId) contain genetic remnants of Tn4401, consistent with

47 their having evolved from Tn4401 by recombination and/or insertion of other smaller mobile  
48 genetic elements. By using NCBI blast against NR database, we noted that similar NTE<sub>KPC</sub>-IId  
49 structures (100% identity) have been recently identified in *Klebsiella aerogenes* from Brazil  
50 (GenBank accession numbers: MG786907, MH000708). Therefore, although no additional  
51 information is available, the possibility that *Enterobacteriales* carrying *bla*<sub>KPC-2</sub> on NTE<sub>KPC</sub>-IId  
52 elements have spread in Brazil and into other countries is deeply concerning. In fact, NTE<sub>KPC</sub>  
53 elements have been described in China, Argentina, Brazil and Russia (4-7). Therefore, the role of  
54 NTE<sub>KPC</sub> elements in global dissemination of *bla*<sub>KPC</sub> deserves additional investigation.

55

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

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

76

### 77 **Nucleotide sequence accession number**

78 The nucleotide sequence of pKPN535a and pKPC45a plasmids were deposited at GenBank  
79 under the accession numbers [MH595533](#) and [MH595534](#), respectively.

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139 **Figure legends**

140 **Fig. 1.** Genetic structures of the small (A) IncQ1 pKPN535a (MH595533) and (B) Col-KPC  
141 pKPC45a (MH595534) plasmids harboring the *bla*<sub>KPC-2</sub> gene and non-Tn4401 elements  
142 (NTE<sub>KPC</sub>-IId) identified in *K. pneumoniae* strains belonging to ST11 and ST340 (CG258),  
143 respectively. Protein coding sequences are represented by the arrows and labeled with gene name  
144 or product. In C, Alignment of Tn4401 and NTE<sub>KPC</sub> genetic elements harboring *bla*<sub>KPC</sub> genes  
145 identified in Brazil. NTE<sub>KPC</sub> genetic elements encompass NTE<sub>KPC</sub>-Ic associated with *bla*<sub>KPC-2</sub>  
146 carried by IncX3 plasmids (4), and the two NTE<sub>KPC</sub>-IId elements identified in this study. Based  
147 on the insertion of  $\Delta$ *bla*<sub>TEM</sub> upstream of the *bla*<sub>KPC</sub> gene, NTE<sub>KPC</sub> elements have been classified  
148 as NTE<sub>KPC</sub>-II, whereas NTE<sub>KPC</sub>-II variants are based on the differences of the length of  $\Delta$ *bla*<sub>TEM</sub>  
149 and deletions between  $\Delta$ *bla*<sub>TEM</sub> and *bla*<sub>KPC-2</sub> (4). In both plasmids, NTE<sub>KPC</sub>-IId elements were  
150 flanked by two identical 243-bp direct repeats [DR (open circles):  
151 AGGGGTCGTCTCAGAATTCGGAAAATAAAGCACGCTAGCGGTTGATCTGTCAGGTT  
152 GAAGCCTGAGAGGCCGAGCGCAGATCGTCAGAAAAGGCGAAAAACGATCCTAATCT  
153 GACGCAACATAGGTGGGGTGCCTGACGCCCGGTTGAGGCGTACTTCAACTGGACAC  
154 CATTCCAGAAAGACCAAGCATGGCATGGCCTGCCGCTGTCTTACCGTGCTTTATTTC  
155 CCGTTTTCTCTATCGACC]. Protein coding sequences are represented by the arrows and  
156 labeled with gene name or product. Light blue shading denotes shared regions of homology  
157 (>95%).

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