TITLE: SMALL ENIGMATIC INCQ1 PLASMIDS HARBORING *bla*$_{KPC-2}$ IN NON-CLONAL *Klebsiella pneumoniae* CG258 LINEAGES

AUTHORS: CAMPOS, P.A.[1]; FUGA, B.[1]; CERDEIRA, L.T.[2]; FERREIRA, M.L.[1]; DIAS, V.L.[1]; MACHADO, L.G.[1]; ROSSI, I.[1]; LINCOPAN, N.[2]; GONTIJO-FILHO, P.P.[1]; RIBAS, R.M.[1]

INSTITUTIONS: 1. LABORATÓRIO DE MICROBIOLOGIA MOLECULAR, INSTITUTO DE CIÊNCIAS BIOMÉDICAS, UNIVERSIDADE FEDERAL DE UBERLÂNDIA, BRASIL. 2. LABORATÓRIO DE RESISTÊNCIA BACTERIANA E ALTERNATIVAS TERAPÊUTICAS, UNIVERSIDADE DE SÃO PAULO, BRASIL.

ABSTRACT:

Although increasing, our understanding of resistance spread in KPC remains relatively limited, as methods for tracking mobile resistance genes through plasmids are scarce. This study reported two small plasmids isolated from two carbapenem-resistant clonally unrelated *K. pneumoniae* strains (B29/ST340 and KPC05/ST11), carrying the *bla*$_{KPC-2}$ gene, belonging to the clonal group (CG) 258, recovered in 2010 and 2014, from urine and rectal swab cultures from hospitalized patients in southeastern and northeastern Brazil, respectively. Interestingly, these strains harbor mobilizable IncQ1 plasmids (pB29 and pKPC05 plasmids, at 10,796-bp and 10,784-bp, respectively), much smaller than the sizes reported in the literature. The total genomic DNA of both *K. pneumoniae* strains (pB29 depth of 25x; pKPC05 depth of 80x) was sequenced using an Illumina NextSeq 500 paired-end reads (150-bp). The *de novo* assemblies were performed using the Unicycler (v0.4.0) software, while the contigs were curated using the Geneious (R9) and and Bandage programs, being possible to close the plasmids. IncQ1 differentiation was assessed by comparisons with the pKPN535a plasmid genome (MH595533.1), using Mauve Alignment. This study showed that the *yddM* (*higA*), *higB* and *rop1* genes were absent from both plasmids assessed herein. The first two are involved in toxin and antitoxin systems, and *rop1* gene in the regulation of replication of the plasmid; while maintaining the *aphA(3')-VIa*, *bla*$_{TEM}$ and *bla*$_{KPC-2}$ genes. To the best of our knowledge, this is the first study to indicate the possible loss of these genes in a small *K. pneumoniae* plasmids. Comparing the plasmids, pKPN535a isolated in 2016 contains more than four thousand base pairs than those reported in the present study. The temporal evidences demonstrate that the plasmids reported in this work are older (2010 and 2014), and that over time were disseminated and acquired toxin-antitoxin (TA) system (2016). In summary, this study reports the identification and complete sequence of two small plasmids, pKPC05 (MK330868) and pB29 (MK330869), which may be capable of spreading the *bla*$_{KPC-2}$ gene among high-risk *K. pneumoniae* CG258 lineages. The potential of these small plasmids in transferring antibiotic resistance genes in *K. pneumoniae* should not be overlooked, as they may have a significant impact on the evolution of this microorganism in different ecosystems and should not escape consideration simply because of their small size.

Keywords: Brazil, Carbapenemase, KPC-2, Mobilome, Plasmidome

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