TITLE: CHARACTERIZATION AND COMPARATIVE ANALYSIS OF THE GENETIC CONTEXT OF *RMT*B IN ISOLATES OF *K. PNEUMONIAE* AND *E. COLI* FROM CLINICAL SAMPLES OF THE SAME PATIENT.

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ABSTRACT:

The emergence of Gram-negative bacilli (GNBs) presenting multiple drug resistance (MDR) has become a global concern. Aminoglycosides (AGs) are one of the last therapeutic options for the treatment of severe infections caused by enteric and non-fermenting GNBs. Resistance to AGs can be caused by several intrinsic and acquired mechanisms. Of increasing importance is the emergence of 16S rRNA methyltransferases (RMTases), which confer high-level resistance to AGs currently in clinical use. To date, 10 types of RMTases was described: RmtA to RmtH and NpmA. Dissemination of RmtB, KPC and CTX-M co-producing-*Klebsiella pneumoniae* in hospitals of São Paulo State have already been reported. The aim of this study was to characterize the *rmt*B genetic context in *K. pneumoniae* (598/14) and *Escherichia coli* (865/14) from clinical samples of the same patient. GNB isolates that showed total resistance to AGs in disk-diffusion tests were selected from a Culture Colection, of the Adolfo Lutz Institute, a Public Health Laboratory, in the period from 2012 to 2016. Isolates with MICs ≥ 256 mg/L for amikacin were submitted to PCR for the resistance genes *rmt*ases, bla_{KPC} and $bla_{CTX\text{-}M}$. Two RmtB-producing isolates (K.p 598/14 and E.c 865/14) were obtained from clinical samples of the same patient and were selected for complete genome sequencing and comparative analysis of *rmtB* genetic context. Genomic DNA was extracted using Pure Link Genomic DNA Mini Kit (Invitrogen). Sequencing was performed by the NextSeq Platform (Illumina) and *de novo* assembly was generated by SPADES. *rmtB* genetic context annotation was obtained by RAST. *rmt*B gene context in both strains showed that *rmtB* and a copy of $bla_{TEM\text{-}1b}$ are flanked by two copies of IS*CR2* (second copy truncated) as part of a transposon Tn*2*. The genetic context of rmtB in *K. pneumoniae* and *E. coli*, isolated from a clinical sample of the same patient showed 100% similarity, demonstrating capacity of inter-species dissemination of *rmt*B, suggesting horizontal transmission of this resistance gene. Plasmids harboring *rmt*B generally also carry other resistance genes encoding beta-lactamases. $bla_{TEM\text{-}1}$ is often related to the *rmt*B genetic context generally located upstream of *rmt*B in Tn*2* and Tn*3* transposons. The presence of IS*CR2* in the *rmt*B genetic context suggests that such insertion sequence plays an important role in the mobilization of 16S-RMTases as RmtB.

Keywords: 16S-RMTaes; RmtB; genetic context;

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