TITLE: ASSOCIATION OF COLISTIN-RESISTANT KPC-KP CLONAL STRAINS WITH INFECTIONS AND COLONIZATIONS: INSIGHTS INTO ADAPTATIVE RESISTANCE, *mgrB* INACTIVATION AND BIOFILM PRODUCTION

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ABSTRACT

Today, carbapenemase-producing organisms are pandemic and a significant threat to public health. We investigated, by genomic analyses, the clonal relationship of colistin-susceptible and colistin-resistant KPC-producing *Klebsiella pneumoniae* (KPC-KP) strains causing infections or colonizations, and additionally, we evaluated adaptative resistance to colistin, *mgrB* gene inactivation, and biofilm production. Twenty-three KPC-KP strains were recovered from patients in an adult ICU (Intensive Care Unit) in a large hospital in Brazil. Seventy-five percent of isolates were genetically related by PFGE (Pulsed Field Gel Electrophoresis) and had a similarity higher than 80%. None presented the plasmid-mediated *mcr-1* and bla_{NDM} genes, confirmed by PCR (Polymerase Chain Reaction). The initial adhesion, biofilm formation, and biomass were examined by quantitative assays in replicates, in three independent experiments. Adaptive resistance was evaluated after serial daily passages of isolates in Mueller-Hinton plates containing increasing colistin concentrations. Minimum Inhibitory Concentrations (MICs) to colistin of colonies growing at the highest colistin concentration were determined after daily sub-cultured in antibiotic-free medium and after storage at −80°C. This study showed, through the whole-genome sequencing, the insertion of the $bla_{\text{CTX-M-15}}$ gene along with the insertion element ISE*cp1* into the *mgrB* gene in a highly resistant, strongly biofilm producer strain, recovered from an environment where it is common the high usage of antibiotics. Additionally, this study presents some findings: (i) we do highlight the potential of KPC-KP strains to acquire adaptive resistance to colistin on relatively short time, which may have implications for the evolution of patients, and we believe that resistance to this antibiotic should be monitored in local and global surveillance programs; (ii) we demonstrated that the majority of repeated colistin-resistant KPC-KP infections in adult were caused by the same strains that caused the previous infections/colonization even after a long time intervals (iii) this study illustrates the capacity of multiples clones to coexist in the same patient at the same period, becoming a constant reservoir of KPC-KP in the hospital environment.

Keywords: colistin-resistant *Klebsiella pneumoniae,* adaptative resistance, *mgrB* gene, biofilm.

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