TITLE: DISTINCT SEQUENCE TYPES OF *Klebsiella pneumoniae* CARRYING MUTATIONS OF PhoPQ AND PmrAB SYSTEMS ISOLATED FROM A PATIENT UNDER TREATMENT WITH POLYMYXIN

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ABSTRACT:

Polymyxin B and colistin are considered one of the last therapeutic options for infections caused by KPC-producing enterobacteria. However, their increased use led to the emergence of resistance. In this study, we described two isolates of colistin-resistant *Klebsiella pneumoniae* (Pol 18 and Pol 19) obtained from surgical wound samples of the same patient from a public hospital in Recife, with eight days isolation interval. Bacterial identification and antimicrobial susceptibility tests were performed by Vitek® automated system. Specific PCRs followed by sequencing were performed for detection of bla_{KPC}, bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{SHV}, *mcr-1* genes and for investigation of mutations in the two-component systems genes (*pmrA, pmrB, mgrB, phoP* and *phoQ*) in *K. pneumoniae*. Sequence type was determined by Multi Locus Sequence Typing (MLST). Both isolates showed multidrug resistance (MDR) phenotype, presenting resistance to most of the antimicrobials tested, including colistin. The results showed that Pol 18 belongs to ST11, whereas Pol 19 belongs to ST340 both STs prevalent in Brazil and commonly related to the dissemination of carbapenem resistance. The wide resistance profile observed can be justified by the presence of the $bla_{KPC\text{-}2}$, $bla_{TEM\text{-}like}$, $bla_{CTX\text{-}M\text{-}like}$ and $bla_{SHV\text{-}like}$ beta-lactamase genes in both isolates. None of the isolates had the *mcr-1* gene, so the colistin resistance was associated to the mutations in the PmrAB and PhoPQ systems. The analysis of *pmrA* gene of Pol 18 showed one nucleotide deletion (ΔT634) causing a frameshift mutation which may result in protein alteration. In Pol 19, the sequencing data of *pmrA* were inconclusive and are being repeated. For *pmrB* gene, Pol 18 showed one nucleotide insertion (Gins461_462) which also cause a frameshift mutation and Pol 19 showed two missense mutations (A418G; C766G), leading to amino acid alterations (T140A; R256G). The *phoP* gene in Pol 18 isolate showed one missense mutation (G571T), whereas no mutations where observed in this gene for Pol 19. For the *phoQ* gene both isolates showed a frameshift mutation caused by one nucleotide deletion at the same position (ΔA4). The *mgrB* gene was conserved in both isolates. Our results evidenced the acquisition of independent colistin resistance through mutations in the genes of the two-component systems in two distinct KPC-producing *K. pneumoniae* isolates from the same patient highlighting the great relevance of selective pressure in the emergence of colistin resistance.

Keywords: Colistin, Mutations, Resistance

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