

TITLE: ANALYSIS OF MUTATIONS IN TWO-COMPONENT SYSTEMS (TCSS) IN COLISTIN RESISTANT *Pseudomonas aeruginosa* ISOLATES

AUTHORS: SILVA, I.A.P¹; BARBOSA, B.G.V¹; CAVALCANTI, F.L.S⁴; MELO, B.S.T³; ALMEIDA, A.C.S²; MORAIS, M.M.C¹

INSTITUTIONS: ¹INSTITUTO DE CIÊNCIAS BIOLÓGICAS – ICB/UPE (RUA ARNÓBIO MARQUES, 310, CEP 50100-130, RECIFE – PE, BRAZIL); ²DEPARTAMENTO DE BIOLOGIA, UFRPE (RUA MANUEL DE MEDEIROS, 97, CEP 52171-900, RECIFE – PE, BRAZIL); ³INSTITUTO AGGEU MAGALHÃES (AV. MORAES REGO, S/N, CEP: 50670-420, RECIFE-PE, BRAZIL); ⁴CENTRO DE TECNOLOGIAS ESTRATÉGICAS DO NORDESTE (AV. PROF. LUÍS FREIRE, 1, CEP 50740-545, RECIFE-PE, BRAZIL)

ABSTRACT: In recent years many infections caused by colistin resistant *Pseudomonas aeruginosa* have been reported worldwide. The objective of this study was to characterize the genetic mechanisms involved in colistin resistance in three isolates from a public hospital in Recife, Brazil. For this purpose, whole genome sequencing (WGS) was performed by Illumina HiSeq 2000 platform. The obtained sequences were assembled in contigs form, using the software Velvet 1.2.10, and then aligned and compared with the sequences found in NCBI database. The analysis was done through BioEdit [™] in order to identify the presence of possible mutations in genes of the two component systems (TCSs) which are involved in resistance to colistin (*phoPQ*, *pmrAB*, *parRS*, *colRS* and *cprRS*). Sequences of *P. aeruginosa* PAO1 were used as reference. Molecular typing was performed by MLST. The results showed that the isolates belonged to distinct sequence types: ST 277 (PAE15), ST446 (PAE77) and ST 3079 (PAE 88). These STs have been reported in Brazil, but all have, so far, been described as susceptible to colistin, suggesting a big potential for any isolate to acquire resistance to colistin as a response to environmental pressure. TCSs sequence analysis showed interesting features. Despite to belong to distinct sequence types, common findings were present in the three isolates. Genes *phoP* and *phoQ* were conserved intact in the isolates. Moreover, all the isolates showed the same *pmrA* mutation, a 213T>G transversion resulting in a Leu71Arg exchange, and same *pmrB* mutations, the 4A>C, 16C>G transversions, resulting in Ser2Por and Ala4Thr, respectively, and a 1033T>C transition, resulting in Tyr345His. In addition, in *parS*, a 1193A>G transition led to a His398Arg exchange in the three genomes. In contrast, mutations in *cprS* gene were observed only in two isolates and no alterations were detected in *cprR*, *colR* and *colS* in any of the isolates. Taken together, the results suggest that the *phoP*, *phoQ*, *cprS*, *cprR*, *colR* and *colS* were not crucial to the colistin resistance observed. By contrast, all the isolates acquired the same mutations in genes *pmrA*, *pmrB* and *parS*, suggesting these mutations as important to develop colistin resistance in the *P. aeruginosa* isolates.

Keywords: mutations, two-component systems, colistin resistance

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