TITLE: ANALYSIS OF MUTATIONS IN TWO-COMPONENT REGULATORY SYSTEMS RELATED TO POLYMYXIN RESISTANCE IN CLINICAL MCR-1-PRODUCERS *Escherichia coli* ISOLATES.

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ABSTRACT:

Polymyxins are cationic peptides used as antibiotics against multiresistant pathogens, such as carbapenem-resistant bacteria. Its mechanism of action is related to the interaction with lipopolysaccharide (LPS) on the surface of Gram-negative bacteria. Modifications of LPS that decrease its negative net charge, such as of phosphoethanolamine (PEtN) or 4-amino-4-deoxy-L-arabinose (L-Ara4N), alter their affinity for polymyxins. Mutations in two-component systems (TCSs) PhoPQ and PmrAB that result in the activation and consequent overexpression of LPS modifying genes and the acquisition of the newly described plasmidial *mcr-1* gene may contribute to these modifications. In a screening of 189 carbapenem-resistant *E. coli* strains received by our laboratory from January 2013 to September 2016, three of them (CCBH20178, CCBH20180, CCBH20607) were positive for the *mcr-1* gene by standard PCR and colistin resistant with MIC of 4μg/mL (CCBH20178 and CCBH20607) or 8μg/mL (CCBH20180). The CCBH20178 and CCBH20607 strains were isolated from the same patient and genetically related (ST167). CCBH20180 belonged to ST354. The present study aimed to evaluate the influence of six regulatory genes involved in polymyxin resistance in the *mcr-1*-positives *E. coli*. Geneious 9.1.3 software and PROVEAN tool were used for comparative nucleotide analyzes of the regulatory genes: *mgrB*, *phoP*, *phoQ*, *pmrD*, *pmrA* and *pmrB*, using the previously obtained whole-genome sequencing of the 3 *mcr-1*-positive strains, and *Escherichia coli* ATCC25922 (GenBank: CP0099072.1) as a wild-type genome reference. Both ST167 isolates had the same 13 missense mutations: *pmrB* (n= 4), *pmrA* (n=3), *pmrD* (n=3), *phoP* (n=1), *phoQ* (n=1) and *mgrB* (n=1). For the CCBH20180 isolate, 12 missense mutations were observed: *pmrA* (n=3), *pmrB* (n=3), *pmrD* (n=3), *phoQ* (n=2) and *mgrB* (n=1). No *phoP* mutations were found for CCBH20180. According to the PROVEAN tool, none of the translational changes had deleterious effects on proteins, all of which were classified as neutral. In this way, this study demonstrates that although several mutations were found in the TCSs PhoPQ and MgrAB in all isolates, probably there was no relationship between these translational modifications and polymyxin resistance in these samples. This may justify the low MICs found for the three isolates. However emphasizes the importance of screening for the *mcr-1* gene under these conditions.

Keywords: Polymyxin resistance; regulatory systems; *Escherichia coli*; *mcr-1*.

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