TITLE: ELUCIDATING THE POLYMYXIN RESISTANCE MECHANISM OF A KLEBSIELLA PNEUMONIAE ST 437, A KPC PRODUCER

AUTHORS: PANCOTTO, L.R.; ANTOCHEVIS, L.C.; ZAVASCKI, A.P.; MORALES, D.L.; BARTH, A.L.

INSTITUTION: [1]LABORATÓRIO DE PESQUISA EM RESISTÊNCIA BACTERIANA (LABRESIS), CENTRO DE PESQUISA EXPERIMENTAL (CPE), HOSPITAL DE CLÍNICAS DE PORTO ALEGRE (HCPA), (R. RAMIRO BARCELOS, 2350, SANTA CECILIA, CEP 90035-903, PORTO ALEGRE – RS, BRAZIL). [2]PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS (PPGCF), FACULDADE DE FARMÁCIA, UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL (UFRGS), (AV. IPIRANGA, 2752, SANTA CECÍLIA, PORTO ALEGRE - RS, BRAZIL). [3]PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS (PPGCM), FACULDADE DE MEDICINA, UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL (UFRGS), (AV.JERÔNIMO DE ORNELAS, 721, SANTANA, PORTO ALEGRE – RS, BRAZIL).

Polymyxins are the last resort for the treatment of infections caused by Carbapenem Resistant Enterobacteriaceae (CRE), in this context infections caused by CRE with decreased susceptibility to polymyxin (PR-CRE) are associated with high mortality rates. Resistance to polymyxins is usually due to chromosomal mutation which lead to lipid A modification. These modifications are carried out by the products of the *pmrHFIJKLM* operon that are positively regulated by the PhoQ/PhoP and PmrAB system which in its turn, is negatively regulated by the transmembrane protein MgrB. Point mutations as well as sequence insertions in those genes can lead to polymyxin resistance. This study aimed to evaluate, by Next Generation Sequencing, a *K. pneumoniae* KPC-producer clinical isolate which was resistant to polymyxins. The antimicrobial susceptibility profile was evaluated by broth microdilution. Pulsed Field Gel Electrophoresis (PFGE) was used to genotype the isolates. Eighteen clones were identified and representative isolates of each clone were evaluated for polymyxin susceptibility which ranged from 4µg/mL until ≥64µg/mL. The isolate *K. pneumoniae* 3800F was highly resistant to meropenem (≥256µg/mL) and resistant to polymyxin (16µg/mL) and was chosen to be submitted to WGS. The DNA was extracted with ReliaPrep™ gDNA Tissue Miniprep System (PROMEGA). The library was made with the Nextera® XT DNA Sample Preparation Kit (Illumina, San Diego, CA), followed by DNA quantification on TapeStation (Agilent) and sequencing in the MiSeq Platform (Illumina, San Diego, CA). The genome was trimmed with Trim Galore! and assembled with SPAdes Genome Assembler. After assembly the sequence was annotated on the Rast Server and detailed analyzes were performed in Geneious. The resulting genome was 5,506,348 bp and presented 57.3% GC content. The isolate belonged to the ST-437, according to *in silico* MLST. The KPC gene was located in an *Inc*N plasmid family. All genes involved in polymyxin resistance were analysed in detail and it was possible to identify an insertion in the *mgr*B gene. This insertion sequence belonged to an IS5-like element and is probably upregulating the PhoQ/PhoP system, resulting in polymyxin resistance. This ST is usually related to KPC producers and was observed in many countries such as Spain, China and Brazil, demonstrating that it is worldwide disseminated. The elucidation of the molecular mechanism involved in polymyxin resistance is of epidemiological interest.

Keywords: carbapenemases, colistin-resistance, *mgrB*

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