TITLE: TRANSCRIPTOME PROFILING ANALYSIS OF *KLEBSIELLA PNEUMONIAE* PLASMID GENES IN RESPONSE TO POLYMYXIN B

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Abstract

Increasing antibiotic resistance in Gram-negative bacteria, particularly in *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, presents a global medical challenge. Colistin and polymyxin B are increasingly used as the last-resort therapeutic options for treatment of infections caused by multidrug-resistant (MDR) Gram-negative bacteria. However, alarming resistance rates to polymyxins have recently been reported among carbapenem-resistant *K. pneumoniae*.

The main objective was to study the activation of other antibiotic resistance mechanisms triggered by exposure to polymyxin B (PB) during *K. pneumoniae* cell growth. Thus, for carrying out it, we analyzed the transcriptome profiling of plasmid genes encoded by *Klebsiella pneumonia* subsp. *pneumoniae* Kp13 subcultured under different stress conditions in the presence of PB. For comparative reasons, we also analyzed all expressed genes carried by plasmids of *K. pneumoniae* subsp. *pneumoniae* MGH 78578 that was subcultured under the same conditions.

The bacterial growth conditions were: (i) absence of PB (control); (ii) presence of PB (CLSI condition; 25 µg/mL CaCl2 and 10,5 µg/mL MgCl2, pH 7,0); (iii) high Ca2+ concentration plus PB (Ca condition; 75 µg/mL CaCl2 and 10,5 µg/mL MgCl2, pH 7,0); (iv) absence of Mg2+ plus PB (Mg condition; 25 µg/mL CaCl2, pH 7,0); (v) high Fe3+ concentration plus PB (Fe condition; 25 µg/mL CaCl2; 10,5 µg/mL MgCl2 and 75 µg/mL FeSO4, pH 7,0) and (vi) low pH plus PB (pH condition; 25 µg/mL CaCl2 and 10,5 µg/mL MgCl2, pH 5,8). For each condition, two biological replicates were sequenced. RNA-seq was performed using 454 Life Science/Roche Genome FLX sequencer. Bioinformatics tools, such as Bowtie, edgeR package and Venn diagram were used for data analysis.

Overall, some plasmid genes of Kp13 or MGH 78578 were transcribed in all PB conditions. A Venn diagram considering all intersections among each stress conditions was performed for each analyzed bacterium. Using this approach we identified several plasmid genes in both bacterial strains related to the MDR phenotype, which were common or not to all tested conditions. The most up-regulated genes were associated with beta-lactam antibiotic resistance. This observation was positively linked to the susceptibility testing results, which had been assessed prior to the RNA-seq. Our results constitute a starting point for future studies in elucidating the complex network involved in resistance to polymyxins among Klebsiella strains.

Key-words: *Klebsiella pneumonia* subsp *pneumoniae*, plasmid genes, RNA-seq, MDR.

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