

TÍTULO: EFFECT OF PYOCYANIN ON COLIFORM BIOFILM FORMATION

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ABSTRACT:

Pseudomonas aeruginosa is a ubiquitous Gram-negative rod known for its metabolic versatility whose preferred habitat is freshwater. It synthesizes at least five different pigments, however the blue-greenish pyocyanin is the most important. Its antimicrobial property gives an advantage to *P. aeruginosa* when competing with a myriad of other microorganisms for nutrients and space, including enterobacteria. The aim of this work was to evaluate the effect of pyocyanin on the adhesion of four multidrug resistant enterobacteria (*Hafnia alvei* AV01, *Escherichia coli* AV12, *Citrobacter freundii* AV13 and *Enterobacter aerogenes* AV14). The minimum inhibitory concentration (MIC) of the pyocyanin on the strains was determined employing the microdilution technique at concentrations ranging from 0.38-0.0015 mM for 96h at 24h intervals at 37 °C. The viability of the enterobacteria cells was verified by the formation of turbidity, confirmed by the inoculation in nutrient broth. The biofilm adhesion assay was aseptically performed in plastic reactors filled with sterilized mineral water added with 0.5 g/L of yeast extract. A ceramic coupon measuring 1 cm² of area was immersed and the suspension of each coliform bacteria, standardized at approximately 10³ CFU/mL, was transferred to the reactors with and without pyocyanin at the subinhibitory concentration. At 24 and 48 h of static incubation at 37 °C, the coupon was aseptically removed and the cells developed on the surface were transferred to 0.85% NaCl solution by scraping and quantified by the pour plate technique. Only AV01 and AV14 strains were susceptible to pyocyanin at concentrations of 0.38 and 0.19. The adhesion of the cells on the coupon at subinhibitory concentrations was more impaired in AV01, which decrease ten fold of cell concentration compared to the control. These results suggest the interference of the pigment on the adhesion of the coliform bacteria.

Keywords: *Pseudomonas aeruginosa*, natural phenazines, coliform bacteria, biofilm

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