TITLE: INFLUENCE OF MULTIDRUG EFFLUX SYSTEM INHIBITOR ON BIOFILM FORMATION IN *ESCHERICHIA COLI*

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ABSTRACT:

Previous studies report that multidrug efflux systems contribute to biofilm formation in *Eshcerichia coli*. They also describe that deletions of efflux systems genes result in decreased planktonic growth, reduced biofilm formation and increased susceptibility to antimicrobials. Thus, the objective was to evaluate the formation of biofilms and planktonic growth in *Escherichia coli* isolates that contained efflux systems submitted to treatments with an efflux pump inhibitor (EPI). Thirty multiresistant *E. coli* isolates (resistance to at least three classes of antimicrobials) of human and animal origin that contained genes from different efflux system families demonstrated by PCR were selected. These isolates were cultivated in 96-well plates in Luria-Bertani broth (LB) (control); LB added with phenyl-arginine-β-naphthylamide (PAβN) – an EPI; LB added with ampicillin (AMP) at a sub-inhibitory dose, or even LB added with PAβN + AMP. All treatments were performed in duplicate. Each isolate was cultivated in three plates and these were incubated for 24 h at 37°C. One of the plates was incubated under shaking to determine the planktonic growth, by measuring the absorbance in a spectrophotometer (550 nm). The remaining plates were incubated without shaking; one for determination of biofilm biomass through crystal violet staining and the other for determination of biofilm viability through microdrop cultivation on LB agar after sonication of biofilms in recovery medium. Assessing the planktonic growth, the PAβN + AMP treatment had lower growth compared to the control (LB) ($p<0.05$), as well as AMP compared to the control ($p<0.05$). In all treatments, biofilms were viable. Assessing the biofilm biomass, the PAβN treatment formed a smaller biomass of biofilm compared to the control, AMP, and PAβN + AMP ($p<0.05$). The AMP treatment formed biofilm as well as control ($p>0.05$), although there was less planktonic growth, likewise PAβN+AMP. This means that proportionally these treatments formed more biofilm than the control. In turn, the PAβN treatment had the same planktonic growth as the control, but produced substantially less biofilm than the control. Thus, EPIs can be allies in controlling the formation of biofilms, even in multiresistant isolates.

Keywords: planktonic cells, bacteria, One Health, resistance

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