TITLE: DETECTION OF MULTIDRUG EFFLUX SYSTEMS IN *STAPHYLOCOCCUS AUREUS* BY REAL-TIME FLUORIMETRY

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ABSTRACT:

The most common technique for phenotypic detection of multidrug efflux systems is the comparison of the minimum inhibitory concentration (MIC) in the absence and presence of an efflux pump inhibitor (EPI). However, this methodology restricts which efflux system families are tested due to the specificity of antimicrobials and EPIs and often does not give us clear results of pump efflux action, which may underestimate it. In this sense, the objective was to demonstrate the SEM activity through real-time fluorimetry of ethidium bromide with or without carbonyl cyanide m-chlorophenylhydrazone (CCCP) - a EPI that potentially acts on all *S. aureus* pump efflux families by decoupling the proton-motive force. We selected 34 multiresistant (resistance to at least three classes of antimicrobials) *Staphylococcus aureus* isolates from human and animal origin that had genes from different families of efflux systems, detected by PCR. According to the previously demonstrated resistance profile, 21 of these isolates had their ciprofloxacin MIC tested in the presence and absence of CCCP and 28 isolates were tested for tetracycline under the same conditions. To quantify fluorimetry, isolates were loaded with ethidium bromide in the presence of CCCP, then sedimented and resuspended in broth with or without CCCP, and fluorescence was monitored using a real-time PCR thermocycler. No isolate showed difference in the MIC of both ciprofloxacin and tetracycline, when in the presence of CCCP. In relation to the fluorescence emission, 19 (55.88%) isolates showed higher emission in the absence of CCCP, showing efflux system activity. The quantification of fluorescence emission can help the detection of efflux system, where this activity was not evidenced by the determination of MIC, contributing to clarify the role of these pumps as a mechanism of antimicrobial resistance.

Keywords: efflux pumps; antibiotic resistance, One Health, bacteria, phenotypic detection

Development Agency: Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior