**ID TRABALHO:** 204/3068-0

**ÁREA DO TRABALHO:** PATOGENICIDADE BACTERIANA

**TÍTULO DO TRABALHO:** Avaliação Da Inativação Fotodinâmica Em Bacteria Persistentes A Ciprofloxacina

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**RESUMO:**

Microbial resistance represents a critical global health challenge, as emphasized by the World Health Organization (WHO). Persistence, a tolerance mechanism distinct from resistance, allows a subpopulation of bacteria to survive prolonged antibiotic exposure without harboring specific resistance genes. It is characterized by biphasic killing curves, with a rapid initial decline in susceptible cells followed by a slower death rate in tolerant cells. This behavior promotes survival across antibiotic classes and may facilitate the evolution of resistance. Photodynamic inactivation (PDI) emerges as a promising alternative treatment, relying on the generation of reactive oxygen species (ROS) via light-activated photosensitizers to induce oxidative damage in vital microbial components, effectively targeting persistent cells. The objective of this study was to characterize the persistent phenotype of Escherichia coli strains and to standardize PDI protocols for assessing phenotypic changes following ciprofloxacin exposure. The study employed Escherichia coli (ATCC 25922) as the model microorganism. The minimum inhibitory concentration (MIC) of ciprofloxacin was determined in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines, using serial dilutions in Mueller-Hinton broth distributed across 96-well plates. Bacterial viability was assessed using resazurin as a metabolic activity indicator. For the time-kill assay, an overnight culture was diluted (100 μL into 20 mL of Brain Heart Infusion broth) and incubated at 150 rpm and 37 °C for three hours. Upon reaching the mid-log phase, aliquots with a final concentration of 10⁸ CFU/mL were exposed to ciprofloxacin under the same incubation conditions. At predetermined time points, samples were centrifuged, washed, serially diluted, and plated (10 μL per dilution) on Petri dishes for colony-forming unit (CFU/mL) quantification. For the photodynamic inactivation (PDI) assay, a 20 mM stock solution of methylene blue was prepared in water and subsequently diluted to a final concentration of 10 µM. Samples were incubated with the photosensitizer for 15 minutes prior to irradiation with an LED-based device (Biotable®) at a wavelength of 660 nm, power output of 35 mW, and energy dose of 2.5 J/cm². The MIC of ciprofloxacin for the E. coli strain was established as 0.0019 µg/mL. To evaluate persistence, time-kill assays were conducted using a ciprofloxacin concentration 10 times higher than the MIC. The killing kinetics displayed a biphasic curve,

indicative of a population harboring persistent or heteroresistant subpopulations. To determine whether PDI influenced bacterial susceptibility, time-kill assays were also performed on survivors of the PDI treatment. The resulting killing curve exhibited gradual bacterial death, suggesting that PDI may suppress the emergence of persistence