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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 ($100 \times$ 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 10^{12} CFU/mL were exposed to ciprofloxacin under the same incubation conditions. At predetermined time points, samples were centrifuged, washed, serially diluted, and plated ($10 \times$ 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 $\mu\text{g/mL}$. 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,



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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