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version. Assessment of an experimental biofilm model to study persistence and antibiotic-induced killing of *Pseudomonas aeruginosa*. The spread of antibiotic resistance represents a threat to public health. A major focus in the scientific community has been to study bacterial colonization and biofilm formation. However, the majority of studies have been descriptive in nature, focusing on the physicochemical conditions of bacterial growth and survival. Here, a

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model for the study of biofilm formation in vitro, employing the antibiotic-killing properties of antimicrobial agents is presented. *Pseudomonas aeruginosa* PAO1 was chosen as the model organism for studying antibiotic-induced killing during biofilm formation. A biofilm formation protocol was established and the antimicrobial potency of ciprofloxacin, an inhibitor of DNA synthesis, and piperacillin, an inhibitor of cell wall biosynthesis, was determined. First, the growth and viability of planktonic cells were assessed using dynamic light scattering and plate counts, respectively. Next, the biofilm formation ability was assessed by confocal microscopy. No significant difference in bacterial growth was observed during biofilm development.

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Additionally, biofilm integrity was assessed by XTT assay. A 30-40% reduction in biofilm formation was observed following 48 hours of ciprofloxacin or piperacillin exposure. Next, an assay to assess the effect of ciprofloxacin and piperacillin on pre-formed biofilms was established. This involved the removal of pre-formed biofilms, incubation with antibiotics and a subsequent re-growth period without antibiotics. At the end of the resazurin assay, ciprofloxacin was 10- to 20-fold more potent than piperacillin. No significant difference in bacterial viability was observed following the 6d1f23a050

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