Radiometric standardization and application of sublethal aPDT for the study of β-lactam resistance in Escherichia coli

DANIELLY CHRISTINE ADRIANI MAIA MOTA[1], LUISMAR BARBOSA DA CRUZ JUNIOR[1], DANIEL JOSE CHIANFRONE[1], VANDERLEI SALVADOR BAGNATO[1], KATE CRISTINA BLANCO[1]

[1.] UNIVERSITY OF SAO PAULO , SAO CARLOS INSTITUTE OF PHYSICS , SÃO CARLOS, BRASIL

β-lactam resistance mediated by β-lactamases in Gram-negative bacteria is a therapeutic challenge. Antimicrobial photodynamic inactivation (aPDT) combines a photosensitizer, light, and oxygen to generate reactive oxygen species increases the oxidative stress. In this sence, aPDT under sublethal conditions may provide an option to investigate resistance phenotypes. This study aimed to standardize sublethal methylene blue-mediated aPDT at 660 nm in susceptible and β-lactam-resistant *Escherichia coli* (*E. coli*) to evaluate photodynamic stress on TEM-1-mediated resistance. E. coli ATCC 25922 and a strain transformed with the pQE30 plasmid carrying the blaTEM-1 gene were used. Initially, minimum inhibitory concentrations (MICs) were determined for ampicillin, amoxicillin, ceftriaxone, gentamicin, and controls. Growth curves based on OD600 were also evaluated for inoculum standardization. Initially, the Biotable® 660 nm system was subjected to radiometric calibration in 24- well plates, with spatial irradiance mapping and interpolation using radial basis functions with a multiquadric kernel. The platform was modified by implementing three independent electrical circuits and an optical diffuser, reducing light dose heterogeneity between wells and within wells. The aPDT assays were then performed using methylene blue at concentrations of 5 to 20 μM and light doses of 10 to 20 J/cm². Light-only controls, photosensitizer-only controls, and methylene blue plus light treatments were evaluated. Bacterial survival was determined by colony-forming unit counts, expressed as CFU/mL. The ATCC 25922 strain showed a susceptibility profile compatible with quality control standards, whereas the pQE30 strain showed elevated MICs for amoxicillin and ampicillin, consistent with the presence of blaTEM-1 and the expected hydrolysis of penicillins. Growth curves were similar between the two strains, allowing controlled physiological comparisons. Radiometric calibration of the Biotable® system showed that the original configuration presented irradiance variation of approximately 21% between wells and 16% within the same well. After modification, these values were reduced to 9.99% and 0.87%, respectively. In the aPDT assays, the controls remained stable, whereas treated groups showed bacterial reduction dependent on the photodynamic condition. Reproducible sublethal conditions were established, with viability reductions of approximately 2 to 3 logs. Radiometric standardization was important to ensure that the biological differences observed were attributed to the photodynamic treatment rather than to heterogeneity in the irradiation platform. The selection of sublethal conditions allows further investigation of the effects of aPDT on the resistance phenotype and on TEM-1 β-lactamase without complete elimination of the bacterial population. Methylene blue-mediated aPDT at 660 nm, using a radiometrically calibrated platform, promoted controlled reduction in E. coli ATCC 25922 and in the resistant pQE30 strain. Lastly, the sublethal conditions established in this study assisted a better comprehension of the photodynamic stress and TEM-1 β-lactamase-associated resistance. These findings support aPDT as an approach to study

adjuvant strategies against bacterial resistance and the need for standardized instrumentation to reduce instrumental errors.

Agradecimentos: This study was financed in part by the Coordination for the Improvement of Higher Education Personnel, Brazil (CAPES), Finance Code 001, and was supported by the São Paulo Research Foundation (FAPESP), through Grants 2013/07276-1 (CePOF) and 2014/50857-8 (INCT).