INACTIVATION OF *Staphylococcus aureus* BY PHOTOBIOMODULATION WITH BLUE AND GREEN LIGHT

Leticia Aparecida Margotto Schneider[1], Larissa Cristina Oaks de Souza Brum[1], Carlos Franz Benz[2], Fernanda Cristina de Abreu Quintela Castro[3], Rafael Mazioli Barcelos[4],Clairton Marcolongo-Pereira[5], Christiane Pavani[6], Orlando Chiarelli-Neto[7]

[1]Graduada em Odontologia pelo Centro Universitário do Espírito Santo - UNESC, [2]Graduação em Farmácia pela Faculdade de Farmácia e Bioquímica do Espirito Santo. Atualmente é proprietário e diretor técnico do Laboratório de Análises Clínicas Santa Maria Ltda.[3]Doutora em Pediatria e Saúde da Criança - PUC/RS, 2019), Mestre em Saúde Coletiva - UFES, Especialização em Atenção Primária à Saúde - UNESC, Especialização em Gestão do Trabalho e Educação na Saúde (ENSP/RJ, 2012), Especialização em Fisioterapia Neuro-funcional (UCB/RJ, 2008), com Graduação em fisioterapia- UNESC. Atualmente é Docente do Centro Universitário do Espírito Santo-UNESC.[4]Graduação em Ciências Biológicas Bacharelado pela Universidade Federal de Ouro Preto-UFOP, licenciado em Biologia pelo Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo - IFES, mestrado em Bioquímica Agrícola e doutorado em Bioquímica Aplicada, titulações estas realizadas na Universidade Federal de Viçosa - UFV. Atualmente é professor e pesquisador no Centro Universitário do Espírito Santo - UNESC.[5]Graduação em Administração pela Fundação de Assistência e Educação – FAESA, Medicina Veterinária pela Universidade Vila Velha, residência em Medicina Veterinária pela Universidade de Vila Velha, mestrado em Sanidade Animal pela Universidade Federal de Pelotas, doutorado em Sanidade Animal pela Universidade Federal de Pelotas, estágio pós doutoral pela Universidade Federal de Pelotas e pela Universidade Federal do Rio Grande do Sul. Atualmente é professor do Centro Universitário do Espírito Santo - UNESC.[6]Programa de Pós-Graduação em Medicina Fotônica, Universidade Nove de Julho (UNINOVE). [7]Licenciatura em Química pelo Instituto Federal do Espírito Santo - IFES, graduação (2007) e mestrado (2009) em Bioquímica pela Universidade Federal de Viçosa-UFV e doutorado em Ciências - Bioquímica (2014) pelo Instituto de Química da Universidade de São Paulo (IQUSP). Atualmente, é Professor Titular do Centro Universitário do Espírito Santo (UNESC). Membro Docente permanente do PPGCS e do Grupo de Pesquisa Interdisciplinar em Ciências da Saúde (GPICS) do UNESC (ES).

ABSTRACT

The emergence of antibiotic-resistant bacterial strains, such as methicillin-resistant Staphylococcus aureus *(MRSA), has driven the search for alternative antimicrobial therapies. Light-based therapies have demonstrated bactericidal effects, which are influenced by wavelength, dosage, and bacterial density. This study aimed to develop a protocol for inactivating* Staphylococcus aureus *using blue and green light while preserving host cell integrity. The bactericidal effects of blue (465 nm) and green (525 nm) light were tested in vitro on* Staphylococcus aureus *(ATCC 29213) cultured for 24 hours and diluted to concentrations of 0.75 × 10⁸ CFU/mL and 0.375 × 10⁸ CFU/mL. Blue light at 60 J/cm² was applied three times within 24 hours, whereas green light at 100 J/cm² was applied once. Both wavelengths effectively reduced bacterial colonies, with the most significant reductions observed in the lower-density colonies (0.375 × 10⁸ CFU/mL), achieving a 95% reduction with blue light and 78% with green light. In higher-density*

Autor correspondente: ochiarelli@unesc.br

colonies (0.75 × 10⁸ CFU/mL), blue light resulted in a 22% reduction, while green light produced a 47% reduction. Although complete eradication was not achieved, the significant reductions, particularly at lower bacterial densities, suggest that photobiomodulation with blue or green light, when used in combination with antibiotics, could be a promising strategy for reducing Staphylococcus aureus *populations.*

Keywords: *Staphylococcus aureus, Antibiotic-resistant bacteria, Photobiomodulation, Visible light.*

1 INTRODUCTION

Antimicrobial resistance is a global public health challenge that complicates infection treatment, prolongs hospital stays, and increases mortality (Tomb *et al*., 2017). A study conducted at Hospital São José (HSJ) in Espírito Santo, Brazil, revealed high antibiotic resistance in the region (Stinghel *et al*., 2022). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major concern due to its resistance, adaptability, and ease of spread in hospitals (Enright *et al*., 2002). *Staphylococcus aureus* is a gram-positive coccus commonly found on the skin and nasal cavities but can also colonize other body sites (Abegg; Silva, 2011; Campos *et al*., 2023). It is a high-priority pathogen for the World Health Organization due to antibiotic resistance (WHO, 2017). This bacterium can cause a wide range of infections, from minor skin conditions to severe diseases such as pneumonia, meningitis, and sepsis (Santos *et al*., 2007). Vulnerable groups, such as diabetics and immunosuppressed individuals, are at higher risk (Campos *et al*., 2023; Sørensen *et al*., 2016). Additionally, *S. aureus* is associated with nosocomial pneumonia, with dental biofilm and saliva serving as vectors (Zuanazzi *et al*., 2010).

Given the rise of resistance, alternative therapies like photodynamic therapy (PDT) and photobiomodulation have gained attention. PDT uses a photosensitizer and visible light at specific wavelengths for antimicrobial action (Kharkwal *et al*., 2011). However, challenges include difficulty delivering photosensitizers into bacterial cells and the risk of damaging healthy tissue (Wainwright, 1998; Dai *et al*., 2012). In contrast, photobiomodulation, using therapeutic light such as LASER and LED, offers antimicrobial effects without the need for exogenous photosensitizers (Anders et al., 2019; Dai et al., 2012). Visible light at specific wavelengths has been shown to suppress bacterial growth

without damaging human cells (Moreiras et al., 2021). However, the efficacy of visible light varies across studies due to differences in protocols (Dai *et al*., 2012; Kim *et al*., 2013). This study aims to evaluate whether blue and green light can inhibit *S. aureus* growth in vitro at doses that are safe for human cells, addressing the limited research on these specific dosages.

2 MATERIALS AND METHODS

Staphylococcus aureus (ATCC 29213) was cultured on blood agar for 24 hours in an incubator at 37°C at the Santa Maria Laboratory (LAC), a co-participant in the research. The seeding technique used was the spread plate method. Bacterial turbidity in the culture tubes was measured using the VITEK® 2 DENSICHEK® instrument (bioMérieux, Marcy-l'Etoile, France), calibrated to the McFarland 0.5 standard, which corresponds to 1.5 x 10^8 colony-forming units (CFU)/mL. From this, two dilutions were prepared: one at ½ concentration and the other at ¼ concentration, resulting in final bacterial densities of 0.75 x 10^8 CFU/mL and 0.375 x 10^8 CFU/mL, respectively (Figure 1).

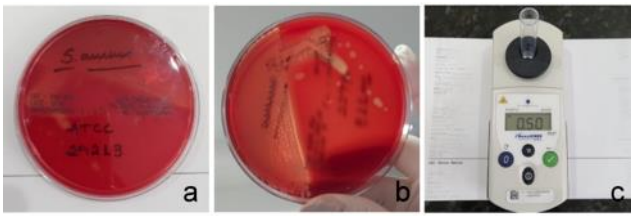

Figure 1. *Staphylococcus aureus* cultured on blood agar: (a) before incubation and (b) after 24 hours of incubation at 37°C; (c) VITEK® 2 DENSICHEK®: Instrument used for turbidity assessment in the tubes.
Source: The Authors

The dilutions were divided into three groups: a control group, a blue light irradiation group (Challenge A), and a green light irradiation group (Challenge B). The control group was kept in the dark under identical nutritional, temperature, and humidity conditions. The experimental groups were subjected to visible light (VL) irradiation using the Biolambda irradiation system (São Paulo, Brazil) with a Handheld High Power LED Module (blue light at 465 nm and green light at 525 nm). Irradiation was conducted at ambient temperature

(25°C). Cultures, initially stored at 8°C, were removed from refrigeration only for the duration of the experiment and were returned to 8°C immediately afterward (Figure 2).

In Challenge A, two tubes with bacterial densities of 0.75 x 10^8 CFU/mL and 0.375 x 10^8 CFU/mL were irradiated with blue light for 49 minutes and 47 seconds, three times within 24 hours. The second irradiation was performed 10 hours after the first, and the third 8 hours after the second. The dose used was 60 J/cm² with a power of 20.09 mW/cm².

In Challenge B, two tubes with bacterial densities of 0.75 x 10^8 CFU/mL and 0.375 x 10^8 CFU/mL were irradiated once with green light at a dose of 100 J/cm² and a power of 8.50 mW/cm² for 3 hours, 15 minutes, and 58 seconds.

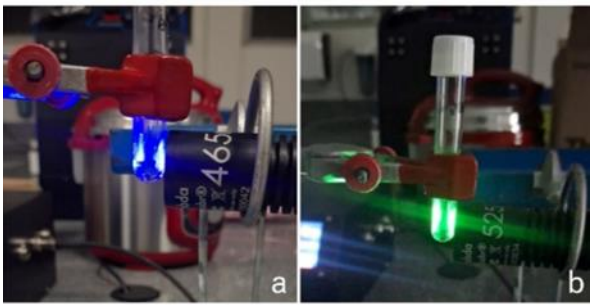

Figure 2. *Staphylococcus aureus* being irradiated with (a) blue light (465 nm) and (b) green light (525 nm) using Biolambda irradiation system (São Paulo, Brazil) with the LED Saber Handheld High Power LED Module. The experiment was conducted in the dark at an ambient temperature of 25°C.
Source: The Authors

All figures included in this article were created by the authors. Statistical analysis and graphing were performed using Origin 7.0 using one-way ANOVA followed by Tukey's post-hoc test revealed significant differences ($p < 0.05$) between the control and treatment group. All colony count was performed in triplicate.

3 RESULTS

All colonies showed a reduction in bacterial count following irradiation, regardless of the wavelength used, compared to the control (Figure 3a).

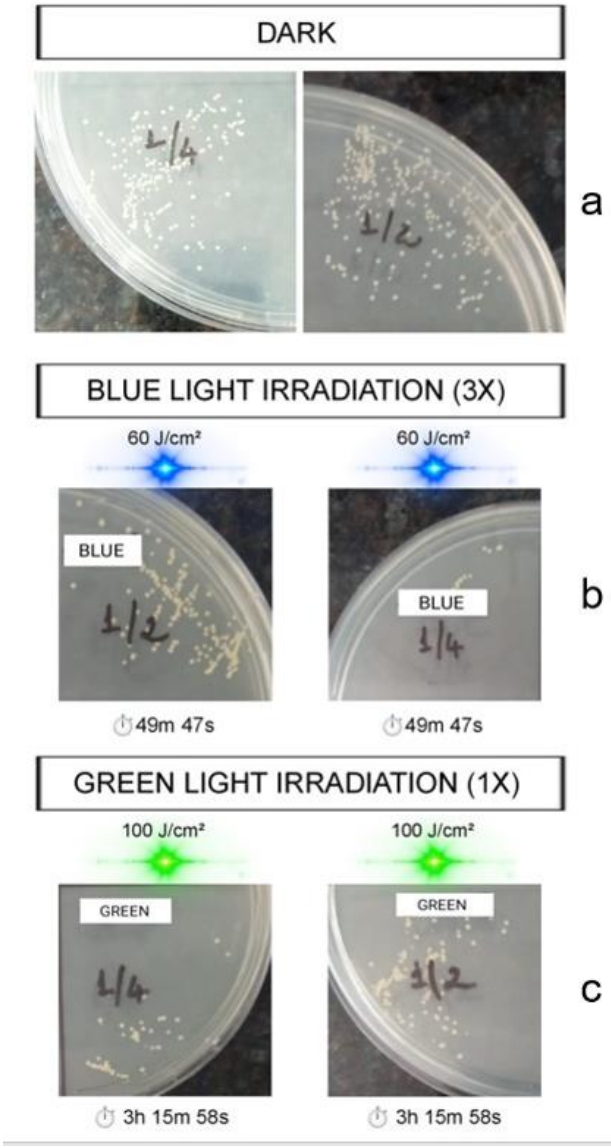

Figure 3. a: Control groups (kept in dark) with colony densities of 0.75 x 10^8 CFU/mL (½) and 0.375 x 10^8 CFU/mL (¼). b: Colonies at 0.75 x 10^8 CFU/mL (½) exposed to three doses of blue light irradiation (60 J/cm² each), each lasting 49 minutes and 47 seconds (Challenge A), and colonies at 0.375 × 10^8 CFU/mL (¼) under the same treatment. c: Colonies at 0.75 x 10^8 CFU/mL (½) exposed to a single dose of green light irradiation (100 J/cm²) for 3 hours, 15 minutes, and 58 seconds (Challenge B), and colonies at 0.375 × 10^8 CFU/mL (¼) receiving the same treatment.
Source: The Authors

The most significant reduction was observed in the lower-density colony (0.375 x 10^8 CFU/mL) irradiated with blue light, which achieved a 95% decrease in bacterial count (Table 1). The lower-density colony irradiated with green light exhibited a 78% reduction (Table 1).

In contrast, for higher-density colonies (0.75 × 10^8 CFU/mL), green light was more effective, achieving a 46.5% reduction, whereas blue light led to only a 22% decrease (Table 1). Therefore, while blue light was more effective for reducing bacterial counts in lower-density colonies, green light proved more efficient for higher-density colonies. Despite the superior effect of blue light on less dense colonies, green light also demonstrated significant antimicrobial activity against the denser ones (Figure 3).

Table 1. Percentage reduction in bacterial colony counts after exposure to blue and green light, based on control groups with densities of ½ (0.75 × 10^8 CFU/mL) and ¼ (0.375 × 10^8 CFU/mL).

Groups	Reduction of *S. aureus* after irradiation (%)
Dark 1/2	--
Dark 1/4	--
Blue light 1/2	22%
Green light 1/2	47%
Blue light 1/4	95%
Green light 1/4	78%

Source: The Authors

The highest reduction was observed in the ¼ density group (0.375 × 10^8 CFU/mL) irradiated with blue light, followed by the ¼ density group exposed to green light. These less dense colonies showed greater reduction, with approximately 95% of bacteria eliminated by blue light and 78% by green light. In contrast, denser colonies (½) were less affected, with blue light achieving only a 22% reduction and green light reaching 47%.

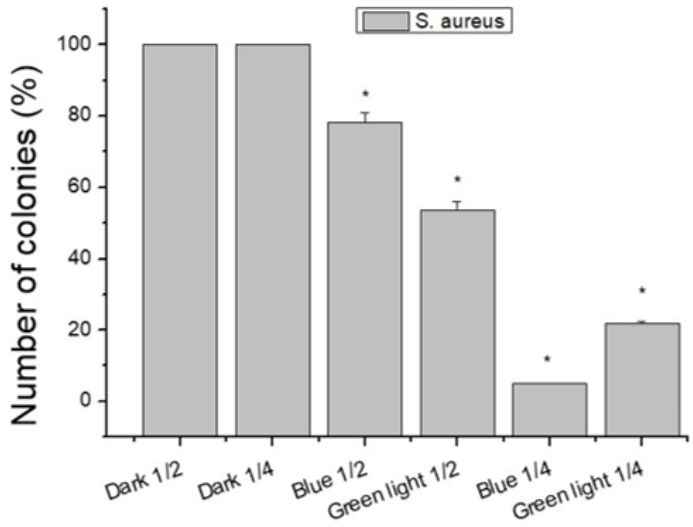

Figure 4. Percentage of remaining colonies after irradiation with blue and green light, with colony counts performed in triplicate. Control groups represent 100% of colony concentration after dilution, at densities of ½ (0.75 x 108 CFU/mL) and ¼ (0.375 x 108 CFU/mL). Statistical analysis was performed using Origin 7.0 software with statistical significance set at $p < 0.05$ (*).
Source: The Authors

The graph in Figure 4 shows that bacterial colonies at a higher concentration (0.75 × 10^8 CFU/mL – Blue ½) exposed to blue light irradiation exhibited a survival rate of 78%, indicating that a significant number of colonies were able to resist the treatment. However, when cultured at a lower concentration (0.375 × 10^8 CFU/mL – Blue ¼), blue light was significantly more effective, with only 5% of the colonies remaining, representing the most substantial reduction observed among all experimental groups.

In contrast, green light irradiation produced a different pattern. For colonies at a higher concentration (0.75 × 10^8 CFU/mL – Green ½), 53% survived, a lower survival rate than that of the Blue ½ group, suggesting that green light was more effective than blue light in high-density conditions. At the lower concentration (0.375 × 10^8 CFU/mL – Green ¼), survival decreased to 22%, demonstrating a greater reduction compared to the high-density group, although not as pronounced as the reduction observed in the Blue ¼ group (Figure 4). These findings confirm the robustness of the blue light treatment in low-density *Staphylococcus aureus* populations. The low variability reinforces the reliability of the observed 95% bacterial reduction.

4 DISCUSSION

Visible light (VL) therapy has gained attention for its ability to selectively destroy pathogens with a low risk of inducing resistance (Dos Anjos *et al*., 2023). VL, a low-energy electromagnetic radiation, generates Reactive Oxygen Species (ROS), such as singlet oxygen, which can damage microorganisms (Tonolli *et al*., 2021; Dai *et al*., 2012). In *Staphylococcus aureus*, blue light (aBL) has been shown to target intracellular porphyrins, with coproporphyrin being the most abundant, absorbing blue light (Fyrestam, 2018; Dos Anjos et al., 2023). While ROS generation can damage DNA, proteins, and lipids, tolerance to aBL may also depend on other factors, such as carotenoid pigment staphyloxanthin, which protects bacteria from ROS (Leanse et al., 2020; Dos Anjos et al., 2023).

Studies have shown that blue light is effective at inactivating MRSA in animal models without causing significant damage to human cells (Dai et al., 2013). *In vitro*, blue light at doses of 60 J/cm² showed minimal cell damage to human fibroblasts, but higher doses reduced viability (Bumah *et al*., 2021; Tonolli *et al*., 2023). Green light has also demonstrated bactericidal effects on *S. aureus*, with reductions in bacterial viability ranging from 10-90% depending on exposure time and dose (Kim *et al*., 2013). Our study observed a 46.5% reduction in higher-density colonies and 78% in lower-density colonies at 100 J/cm² of green light, a dose shown to have minimal effects on human cell proliferation (Tonolli *et al*., 2023).

The therapy was more effective in lower-density bacterial colonies, which aligns with findings from other studies (Dai et al., 2012; Wang et al., 2016). One of the study's limitations was the limited penetration of light in denser biofilms, which explains the lower bacterial reduction observed in these colonies compared to the less dense ones. Therefore, there is a need to develop devices capable of reaching deeper sites (Wang et al., 2016).

Despite promising results, further research is needed to explore potential recurrence or the use of light therapy as an adjunct to antibiotics. Combining light therapy with topical antibiotics could help prevent bacterial regrowth (Dai *et al*., 2013).

5 CONCLUSION

The bactericidal effects of blue and green light vary depending on wavelength, bacterial colony density, power, and dosage. Photobiomodulation using blue and green light was effective across all bacterial densities tested. Notably, blue light irradiation led to a 95% reduction in less dense colonies. However, the green light was more effective in denser colonies, reducing the number of colonies by nearly half, indicating its potential for treating bacterial infections.

Our study successfully reduced bacterial numbers using doses considered safe for human cells, suggesting that this therapy is viable for application on skin or mucosal surfaces. Thus, further research is needed to explore the possibility of achieving 100% suppression of bacterial growth with safe and effective doses against antibiotic-resistant bacteria.

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