AIMS Biophysics

AIMS Biophysics, 12(4): 572−585.
DOI: 10.3934/biophy.2025028
Received: 29 August 2025
Revised: 14 November 2025
Accepted: 28 November 2025
Published: 10 December 2025

http://www.aimspress.com/journal/biophysics

Research article

Synergistic antibacterial activity of combined blue and red light photodynamic therapy

Jaouhra Cherif[1,*], Anis Raddaoui[2], Nada Souissi[3] and Nakkach Mohamed[4,5]

[1] Laboratory of Biophysics and Medical Technologies, LR13ES07, Higher Institute of Medical Technologies of Tunis, University of Tunis El Manar, Tunis, Tunisia
[2] Laboratory Ward, National Bone Marrow Transplant Center, LR18ES39, Faculty of Medicine of Tunis, University of Tunis El Manar, Tunis, Tunisia
[3] Bacteriology Laboratory, Tunisian Institute of Veterinary Research, University of Tunis El Manar, Tunis, Tunisia
[4] Laboratoire de Spectroscopie Atomique, Moléculaire et Applications (LSAMA), Faculty of Science, Tunis El Manar University, Tunis, Tunisia
[5] Higher Institute of Medical Technologies of Tunis,Tunis El Manar University, Tunis, Tunisia

* **Correspondence:** Email: jaouhra.cherif@istmt.utm.tn; Tel: +21699326426.

Abstract: Due to increasing antibiotic resistance and a lack of new antibiotics, alternative treatments are urgently needed. This study investigates photodynamic therapy (PDT), which uses light-activated photosensitizers to produce reactive oxygen species that effectively inactivate bacteria. We evaluated the antibacterial efficacy of PDT against two pathogens that are resistant to current antibiotics, namely *Staphylococcus epidermidis* (Gram-positive) and *Acinetobacter baumannii* (Gram-negative), by testing various illumination protocols. The results showed that combining blue light (468 nm) and red light (632 nm) with methylene blue (MB) produced a synergistic effect in bacterial inactivation compared with protocols using either blue or red light individually in combination with methylene blue (MB). Specifically, after just 30 minutes of exposure, *S. epidermidis* showed a 3.3 log reduction (99.95%), while *A. baumannii* showed a 3.1 log reduction (99.92%) after 60 minutes. Overall, *S. epidermidis* was more sensitive to all tested protocols than *A. baumannii*. We also examined the effects of this protocol on antibiotic susceptibility. For most antibiotics tested, there was no change in the size of the inhibition zones. However, for linezolid, we observed a significant increase in the inhibition zone's diameter, indicating a possible enhanced susceptibility to this antibiotic.

Keywords: multidrug-resistant bacteria; blue light; red light; photoinactivation; photosensitizer; synergistic effect

1. Introduction

The increasing spread of multidrug-resistant (MDR) bacteria poses a critical threat to global public health by reversing decades of medical progress. Originally limited to hospitals, these bacteria are now causing more community infections, leading to increased illness and death. MDR infections also complicate many hospital stays, resulting in longer treatment times and higher costs [1]. This alarming trend is driven largely by the widespread misuse and overuse of antibiotics in both human healthcare and agriculture, which have created unprecedented selection pressures that have accelerated the emergence of resistance mechanisms. These include chromosomal mutations and plasmid-mediated gene transfer, which enable bacteria to rapidly adapt and proliferate in response to antibiotic exposure. The situation is further complicated by the limited number of novel antimicrobial agents currently in development, leaving few effective treatment options [2]. Beyond individual infections, the consequences of MDR bacteria compromise critical medical procedures, such as organ transplants and surgeries, by diminishing the effectiveness of prophylactic antibiotics [3]. This significantly increases the risks associated with these interventions.

In light of this escalating crisis, there is an urgent need for the rapid development of alternative therapeutic strategies that operate through nontraditional mechanisms of action. Photodynamic therapy (PDT) using light-based technologies has recently attracted increased attention as an innovative approach for combating bacterial infections and offer a potential alternative to traditional antibiotics [4,5].

Blue light in the 405−480 nm wavelength range has been shown to inactivate bacteria through interaction with endogenous photosensitizers, such as porphyrins and flavins, within bacterial cells. These molecules absorb specific wavelengths of light and become excited and subsequently interact with oxygen to produce reactive oxygen species (ROS) [6]. These ROS damage various intracellular components, leading to bacterial death [4,7]. Several in vitro studies support the potential of blue light for clinical application, particularly for treating superficial wound infections [8−10]. As a targeted antimicrobial approach, blue light therapy could help prevent the progression of localized infections to more severe systemic conditions. Moreover, the multi-target nature of antibacterial blue light therapy appears to hinder the development of microbial resistance [11,12], as pathogens cannot easily evolve simultaneous defenses against all the oxidative damage pathways induced by photodynamic action.

Red light therapy in the 600–660 nm range demonstrates versatile therapeutic applications. When used alone, it primarily functions as a biostimulant, enhancing wound healing through mitochondrial activation and anti-inflammatory effects [13,14], though with minimal direct antibacterial activity [10]. However, when combined with methylene blue as a photosensitizer, red light generates ROS that effectively destroy bacteria, including antibiotic-resistant pathogens, while preserving healthy tissues [5,15].

To the best of our knowledge, no studies have specifically investigated the combined effect of blue and red light. Therefore, the aim of this study was to explore the possible synergistic effects of blue (468 nm) and red (632 nm) when used together with methylene blue in inactivating bacteria. Additionally, we examined how this photodynamic treatment protocol influences the bacteria's

response to antibiotics. To evaluate this approach, we focused on two clinically relevant bacterial species, namely *Staphylococcus epidermidis* and *Acinetobacter baumannii*, both of which pose a significant therapeutic challenge as nosocomial pathogens of global concern. As a commensal member of the human skin microbiota, *S. epidermidis* can transition into an opportunistic pathogen, particularly in immunocompromised patients or those with medical devices, where it may cause serious infections [16]. *A. baumannii* exhibits remarkable environmental persistence and can cause diverse infections including pneumonia, bloodstream infections, and wound infections, particularly in intensive care settings [17]. Of particular concern is the frequent development of multidrug resistance in both strains, which significantly complicates treatment with conventional antibiotics [18].

2. Materials and methods

2.1. Bacterial strain isolation and preparation

This study involved two bacterial strains, *S. epidermidis* and *A. baumannii*, isolated from clinical samples of immunocompromised patients hospitalized at the National Bone Marrow Transplantation Center. Identification of the isolates was performed using conventional microbiological methods, as well as the *API 32 Staph* and *API 20NE* systems (bioMérieux). The isolates were stored in 10% glycerol at −70 °C for future use.

2.2. Photosensitizer

Methylene blue was used as the photosensitizer in this protocol. The stock solution was dissolved in phosphate-buffered saline (PBS) and subsequently sterilized by filtration through a polytetrafluoroethylene membrane. For the experiments, the final concentration of methylene blue in the bacterial suspension was adjusted to 10 μM, equivalent to approximately 3 μg/mL.

2.3. Light sources and PDT setup

To control the illumination conditions and expose the bacterial strains to blue and red light, either separately or in combination, an RGB (red, green, blue) light-emitting diode (LED) (5 mm, four pins) was employed. The three diodes are integrated within the same substrate, and each channel can be independently activated using an external switch. The emission spectrum of the device shows peaks at 468 nm (blue) and 632 nm (red) (Figure 1A). A matrix of 25 RGB LEDs arranged in a 5 × 5 configuration was used as the illumination source (Figure 1B). The system was powered by a DC supply, with the operating parameters set at 3 V and 10 mA per LED.

For the light exposure experiments, bacteria were freshly subcultured and grown for 24 hours. IN total, 3 mL of a bacteria suspension in a liquid culture medium at approximately 10^8 colony-forming units (CFU)/mL was transferred into 35-mm sterile petri dishes. The suspensions containing methylene blue were prepared by adding the photosensitizer to the bacterial suspensions 15 minutes prior to irradiation. During this incubation period, the samples were kept in the dark to prevent premature photoactivation. The experiment included four exposure modalities: (i) The bacterial suspension was exposed to blue light only (BL), (ii) exposed to red light only (RL); (iii) treated with methylene blue and exposed to red light (RL + MB); and (iv) treated with methylene blue and exposed to both blue

and red light simultaneously (BL + RL + MB). The final concentration of methylene blue in the bacterial suspension was adjusted to 10 μM, equivalent to approximately 3 μg/mL.

Each modality was tested with exposure times of 20, 30, and 60 minutes and the suspensions were exposed to light with the lid of the petri dish removed. The distance between the LEDs and the suspensions' surface was 5 cm. Temperature was monitored during the irradiation process using a digital thermometer placed at the same distance as the samples from the LED source. Measurements were taken throughout the exposure period, and only minimal variations were observed, with a maximum increase of 0.3 °C. This slight change is negligible and cannot affect bacterial viability. This stability is due to the fact that LED sources do not emit infrared radiation and therefore generate very little heat. Controls groups, with and without methylene blue, were maintained in the dark under the same environmental conditions but without irradiation. The control group containing methylene blue without light exposure was included to evaluate the effect of methylene blue alone.

This design allowed for the evaluation of the individual and combined effects of light wavelengths and methylene blue on bacterial viability under controlled and reproducible conditions.

2.4. Bacterial reduction after light exposure

Viable bacteria were quantified by the standard plate count method: After irradiation, serial dilutions of the bacterial suspensions were plated onto plate count agar (PCA) medium (Bio-Rad, France) and incubated at 37 °C for 24 hours. Colony-forming units per milliliter (CFU/mL) were calculated, based on colony counts and dilution factors. The resulting count was expressed as the log reduction.

2.5. Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed by the diffusion method on agar medium according to the French Society for Microbiology created an Antibiogram Committee (CA-SFM) standards: 0.5 McFarland standard bacterial suspensions were prepared and both exposed and unexposed to light. Three treatment groups were established: blue light alone, red light combined with methylene blue, and a combination of blue and red light with methylene blue, each applied for 60 minutes. After treatment, a sterile swab was used to evenly inoculate the entire surface of a Mueller–Hinton agar plate (Biokar, France) with the bacterial suspension. Antibiotic discs were then placed on the inoculated plates, which were incubated aerobically at 37 °C for 24 hours. Antibiotic susceptibility testing was performed using the following antibiotics (Bio-Rad, France): Tetracycline, gentamicin, penicillin, cefoxitin, sulfamethoxazole/trimethoprim, linezolid, ciprofloxacin, neomycin, ceftazidime, colistin, and cefepime. The diameter of the inhibition zones was measured manually in millimeters. The minimum inhibitory concentration (MIC) of linezolid was determined by the E-test (Biomerieux) for *S. epidermidis*. *Enterococcus faecalis* ATCC 29212 was included as a quality control for antimicrobial susceptibility testing. The results were interpreted according to the CA-SFM 2024 guidelines [19].

2.6. Statistical analysis

All data are presented as mean values ± standard deviations. Differences in bacterial log reductions were analyzed using one-way analysis of variance (ANOVA). For pairwise comparisons between modalities at each time point, a post hoc test was applied. Comparisons of the inhibition zones' diameters were performed using a two-tailed Student's *t*-test. Statistical analyses were conducted using SigmaPlot (Systat Software Inc., San Jose, CA, USA), and differences were considered statistically significant at $p < 0.05$.

The combined effect of the LB + LR + MB phototherapies was evaluated using the Bliss independence model [20,21], which calculates the Bliss-predicted response (E_{Bliss}) as follows:

$$E_{Bliss} = E_{LB} + E_{LR+MB} - E_{LB} \times E_{LR+MB}$$

Before applying the model, experimental log-reduction values were normalized to a 0−1 inhibition scale. The observed combination effect (E_{obs}) was compared with this predicted value E_{Bliss}, and the synergy index (SI) was calculated. The SI is widely used to assess combination effects according to the following criteria: A SI value equal to 1 indicates an additive effect, greater than 1 indicates synergy, and less than 1 indicates antagonism.

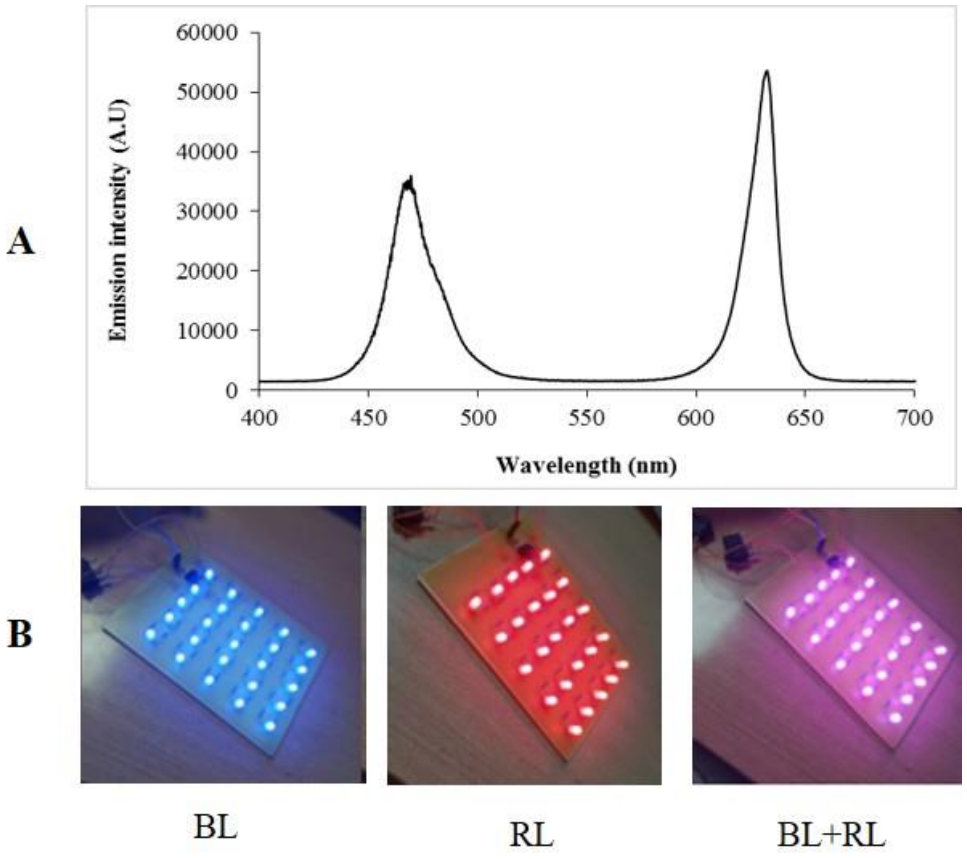

Figure 1. Emission spectrum of the RGB LEDs showing the two activated channels: Blue (peak at 468 nm) and red (peak at 632 nm) (A). Matrix configuration of 25 RGB LEDs arranged in a 5 × 5 array (B). BL, blue diode activated; RL, red diode activated; BL + RL: blue and red diodes activated simultaneously for the combined treatment.

3. Results

This study evaluated the antibacterial efficacy of PDT against two pathogenic bacteria, *S. epidermidis* (Gram-positive) and *A. baumannii* (Gram-negative), by testing various illumination protocols. We compared the antimicrobial activity of blue light and red light used alone or in combination with methylene blue, as well as a combined protocol using both lights with methylene blue (BL + RL + MB). The reduction in bacterial viability was measured for different exposure times. Figure 2 represents the results expressed as log-reductions. The statistical significance (p-values) highlights differences between treatment modalities.

Figure 2A illustrates the results obtained for *S. epidermidis* and shows that bacterial inactivation depends on both the treatment type and the exposure duration. Blue light alone begins to have a significant effect after 30 minutes of exposure ($p = 0.048$). This effect becomes more pronounced after 60 minutes, with a bacterial log-reduction of 1.74 ($p = 0.018$). In contrast, red light applied alone did not show any photoinhibitory effect. However, when combined with methylene blue, red light becomes effective as early as 20 minutes of exposure, resulting in a 1.10 log-reduction ($p = 0.012$). This effect intensifies over time, reaching a 3.61 log-reduction ($p < 0.001$). These results highlight the essential role of methylene blue as an external photosensitizer for red light to inactivate *S. epidermidis* effectively. Treatment with methylene blue alone resulted in a nonsignificant log-reduction of 0.18 ($p > 0.05$), demonstrating that red light activation is essential for its antibacterial effect. A low, nontoxic concentration of methylene blue was used, ensuring that the observed reduction was primarily due to photodynamic activity rather than chemical toxicity.

When blue and red light were combined with methylene blue (BL + RL + MB), a highly significant log-reduction of 3.30 ($p < 0.001$) was observed after just 30 minutes of exposure, reaching a log-reduction of 4.43 ($p < 0.001$) after 60 minutes. This reduction is much greater than that seen with blue light alone or with red light combined with methylene blue. The synergy of blue light, red light, and methylene blue was evaluated using the Bliss independence model. At 20 minutes, the combination showed slight antagonism (SI = 0.74), but at 30 minutes, a clear synergistic effect was observed (SI = 1.26), which remained, though less pronounced, at 60 minutes (SI = 1.13), indicating that the combination is more effective than the sum of the individual effects.

Concerning *A. baumannii*, our results show that this strain is not sensitive to blue light, except after 60 minutes of exposure, where a significant log-reduction of 0.91 was observed ($p = 0.043$) (Figure 2B). In contrast, exposure to red light alone did not produce any effect on the bacteria, similar to what was observed for *S. epidermidis*.

However, when red light was combined with the photosensitizer methylene blue (RL + MB), a notable effect appears as early as 30 minutes of exposure, with a bacterial log-reduction of 1.42 ($p = 0.023$). This effect becomes more pronounced, reaching a 2.1 log-reduction ($p = 0.001$) after 60 minutes of exposure. These results indicate that the RL + MB combination is more effective than blue light alone in inactivating *A. baumannii*. In contrast, methylene blue in the absence of light led to only a nonsignificant log-reduction of 0.13 ($p > 0.05$).

When blue and red light were combined with methylene blue (BL + RL + MB), a more pronounced effect was observed. Specifically, a significant log-reduction of 0.88 ($p = 0.032$) was detected after just 20 minutes of exposure, which increased to 3.1 log ($p = 0.004$) after 60 minutes, indicating substantial bacterial inactivation. The combination of BL and RL + MB showed synergistic effects at all time points (20, 30, and 60 minutes) with SI values of approximately 1.24, 1.12, and 1.30,

respectively. These results are consistent with those observed for the *S. epidermidis* strain and suggest that combining both blue and red light with methylene blue produces a synergistic effect, enhancing bacterial inactivation.

If we compare the two bacterial strains, there is a marked difference in their response to PDT. *S. epidermidis* shows increased sensitivity to this treatment compared with *A. baumannii.*

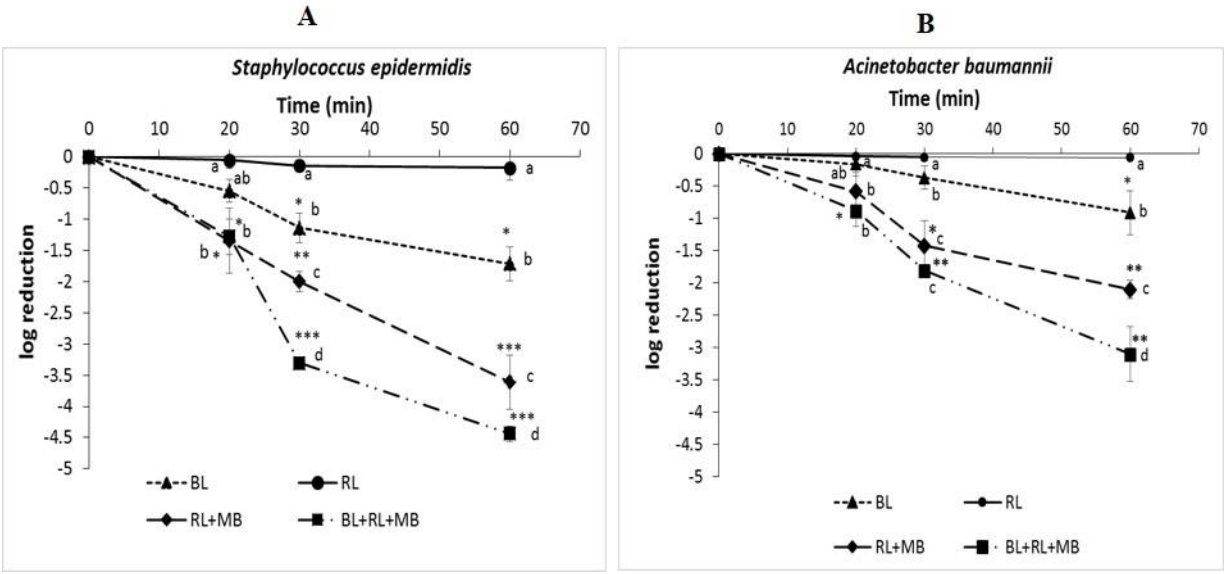

Figure 2. Photodynamic inactivation of *S. epidermidis* (A) and *A. baumannii* (B) using blue light (BL) and red light (RL) applied alone, in combination with methylene blue (MB), or both lights together with methylene blue (BL + RL + MB). Error bars represent the standard deviation of three independent experiments. Statistical significance at each exposure time is indicated by asterisks: $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) versus the unexposed control samples. Different lowercase letters indicate statistically significant differences between modalities at the same time point according to the post hoc test.

We also examined the effect of visible light on bacterial susceptibility to antibiotics. Tables 1 and 2 show the inhibition zones' diameters (in mm) for the antibiotics tested against *S. epidermidis* and *A. baumannii*, respectively.

The results from Table 1 indicate that the treatments with blue light, RL + MB, and BL + RL + MB had different effects on antibiotic efficacy. Notably, there was a significant increase in the inhibition zone's diameter for the antibiotic linezolid under blue light alone (+ 3.33 mm; $p = 0.022$), as well as when blue light was combined with red light and methylene blue (+ 2.33 mm; $p = 0.007$) in *S. epidermidis*. E-tests for this strain revealed that the MIC of linezolid in the control sample was 2 mg/L, whereas after exposure to BL or BL + RL + MB, the MIC decreased to 0.75 mg/L (data not shown). These findings suggest that blue light further enhances the sensitivity of *S. epidermidis* to linezolid.

A slight increase in the inhibition zone's diameter for trimethoprim-sulfamethoxazole was also observed under the combined treatment (BL + RL + MB), but this increase was not statistically significant ($p > 0.05$). No significant changes were noted for the other antibiotics tested.

Regarding the *A. baumannii* strain, the results presented in Table 2 revealed no statistically significant changes ($p > 0.05$) in antibiotic susceptibility following the various light treatments. These findings suggest that, under the tested conditions, exposure to blue light, red light combined with methylene blue, or the combination of all three, does not affect the antibiotic susceptibility of the *A. baumannii* strain.

Table 1. Antibiotic susceptibility of *S. epidermidis* in control and treated samples exposed to blue light (BL), red light combined with methylene blue (RL + MB), and the combination of all three (BL + RL + MB). The inhibition zones' diameters (mm) are presented as the mean ± SD, based on three independent measurements.

Antibiotic	Control	BL	RL + BM	BL + RL + BM
Tetracycline	28.33 ± 0.58	27.00 ± 1.00	28.00 ± 1.00	27.67 ± 0.58
Gentamicin	23.67 ± 0.58	23.00 ± 1.00	23.33 ± 1.15	22.67 ± 0.58
Penicillin	0	0	0	0
Cefoxitin	31.67 ± 0.58	31.00 ± 1.00	31.67 ± 0.58	32.00 ± 1.00
Sulfamethoxazole/ trimethoprim	29.00 ± 0.00	28.67 ± 1.15	30.00 ± 1.00	31.00 ± 1.00
Linezolid	31.33 ± 0.58	34.67 ± 1.16 (*)	32.00 ± 1.00	33.67 ± 0.58 (**)
Ciprofloxacin	16.33 ± 0.58	16.67 ± 1.16	18.33 ± 1.53	18.33 ± 1.53

*Significantly different from unexposed samples ($p < 0.05$). **Highly significantly different from unexposed samples ($p < 0.01$).

Table 2. Antibiotic susceptibility of *A. baumannii* in control and treated samples exposed to blue light (BL), red light combined with methylene blue (RL + MB), and the combination of all three (BL + RL + MB). The inhibition zones' diameters (mm) are presented as mean ± SD, based on three independent measurements.

Antibiotic	Control	BL	RL + BM	BL + RL + BM
Tetracycline	13.67 ± 0.58	13.00 ± 1.00	14.00 ± 0.00	14.33 ± 1.53
Neomycin	15.33 ± 1.16	15.67 ± 0.58	15.33 ± 0.58	15.00 ± 0.00
Ceftazidime	0	0	0	0
Ciprofloxacin	10 ± 1.00	10 ± 0.00	10.33 ± 0.58	10.00 ± 1.00
Colistin	12.00 ± 0.00	12 ± 0.00	12.67 ± 1.16	12.33 ± 0.58
Cefepime	0	0	0	0
Gentamicin	16.00 ± 0.00	15.67 ± 0.58	16.67 ± 0.58	16 ± 1.00
Sulfamethoxazole/trimethoprim	0	0	0	0

4. Discussion

In this study, we focused on the effect of PDT on bacterial inactivation by testing various illumination protocols on two multidrug-resistant strains, *S. epidermidis* and *A. baumannii*. We observed that the effectiveness of photodynamic inactivation depends not only on whether the bacteria are Gram-positive or Gram-negative but also on the specific combination of wavelength, photosensitizer, and exposure duration.

This study showed that *S. epidermidis* is significantly more sensitive to inactivation by blue light than *A. baumannii*. Indeed, blue light effectively inactivated the *S. epidermidis* strain after just 30 minutes of exposure, whereas a significant effect on *A. baumannii* was only observed after 60 minutes. These findings are consistent with those reported by Maclean et al. [22] and Murdoch et al. [23], who highlighted that Gram-positive bacteria are generally more susceptible to blue light than Gram-negative bacteria.

The bactericidal effect of blue light, within the wavelength range of 400–480 nm, is primarily due to the activation of endogenous photosensitizers such as protoporphyrin and coproporphyrin present inside bacterial cells [24,25]. When excited at the appropriate wavelength, these compounds react with oxygen to generate ROS, including hydrogen peroxide, superoxide radicals, hydroxyl radicals, and singlet oxygen ($^1O_2$) [7,26]. These ROS cause damage to essential bacterial components such as the membrane, cell wall, and DNA, ultimately leading to cell death.

The more pronounced inactivation of *S. epidermidis* by blue light compared with *A. baumannii* can likely be explained by the higher levels of coproporphyrin found in Gram-positive bacteria relative to Gram-negative bacteria [27,28]. This greater abundance of endogenous photosensitizers facilitates increased ROS production upon light exposure, enhancing bacterial susceptibility.

Regarding red light, the results showed that it did not induce inactivation of the two tested bacterial strains. These findings are consistent with several previous studies, which demonstrated that red light (620–1000 nm) does not have a significant bactericidal effect [10,29−31]. This outcome can be explained by the fact that endogenous photosensitizers are not sensitive to red light, particularly at the wavelength of 632 nm.

However, adding an external photosensitizer, such as methylene blue, makes red light highly effective in inactivating bacteria. Indeed, we observed a significant reduction in bacterial viability for both strains after 20 minutes of exposure for *S. epidermidis* and after 30 minutes for *A. baumannii*. After 60 minutes, the log-reduction exceeded 3 for *S. epidermidis* and 2 for *A. baumannii*. Methylene blue absorbs red light and, in the presence of oxygen, generates ROS that damage essential biological molecules, leading to bacterial inactivation [5]. *S. epidermidis* was more susceptible to antimicrobial PDT than *A. baumannii*. This difference in susceptibility is mainly explained by structural variations in their morphology, particularly differences in the thickness and composition of the cell wall, as well as the porosity of the bacterial membrane [15,32]. Gram-positive bacteria are generally more sensitive to antimicrobial PDT because their cell walls, characterized by a thick peptidoglycan layer and the absence of an outer membrane, are more permeable, facilitating easier diffusion of photosensitizers into the cell [33]. In contrast, Gram-negative bacteria possess an additional outer layer of lipopolysaccharides, which acts as a barrier and significantly limits photosensitizers' penetration, reducing treatment efficacy [34].

The most significant finding of this study was the synergistic effect observed when combining blue light and red light with methylene blue, which resulted in the fastest and most substantial bacterial

inactivation in both *S. epidermidis* and *Acinetobacter* species. Compared with using blue light alone or red light with methylene blue, this approach achieved significantly higher reductions in bacterial counts. The observed synergistic effect can be explained by the increase in membrane permeability induced by blue light, which facilitates the penetration of methylene blue into the cells, thereby enhancing its efficacy upon activation by red light. Several studies have documented that blue light exposure increases cellular membranes' permeability [35,36]. This phenomenon was further characterized by Chu et al. [37], who demonstrated that blue light treatment of *Cronobacter sakazakii* progressively elevated intracellular malondialdehyde concentrations, a biochemical marker confirming that oxidative stress mediates membrane damage and subsequent permeability changes. The underlying process involves blue light-mediated generation of diverse ROS. Through lipid peroxidation of membrane components, these ROS collectively compromise the membrane's integrity, ultimately enhancing permeability [36,38]. Blue light can induce additional cellular dysfunction by photo-exciting sensitive chromophores, resulting in effects such as the loss of efflux pumps and alterations in membrane potential and integrity [35]. Bowman et al. [39] also observed in methicillin-resistant *Staphylococcus aureus* that blue light exposure causes a rapid change in transmembrane potential within the first 5 minutes of irradiation, along with disruption of bacterial cells' membrane integrity. The synergistic effect observed with the simultaneous use of red and blue light can be explained by the complementary activation of the photosensitizers. Blue light stimulates internal photosensitizers, while red light activates methylene blue, an external photosensitizer. This dual activation leads to a significant production of ROS, which increases cellular damage and accelerates bacterial inactivation.

It was interesting to examine the effects of different antimicrobial PDT protocols on the antibiotic susceptibility of two bacterial strains in order to determine whether any of these protocols could enhance their susceptibility or, conversely, induce resistance. The results showed that only linezolid, tested on *S. epidermidis*, exhibited a significant increase in the diameter of the inhibition zone, making the bacteria more sensitive to this antibiotic. This effect was observed after treatment with blue light alone or in combination with red light. In contrast, red light alone had no impact on bacterial inhibition. A proposed hypothesis suggests that the stress induced by blue light could increase the expression of ribosome-dependent stress proteins (50S), the target of linezolid, thereby enhancing its action. These findings are consistent with a recent study conducted on *A. baumannii*, which demonstrated a synergistic effect between blue light and chloramphenicol, another antibiotic that inhibits protein synthesis [40].

5. Conclusions

In conclusion, our study demonstrates that the efficacy of antimicrobial PDT depends on both the targeted bacterial species and the applied protocol. To our knowledge, this is the first investigation of the combined application of blue and red light against multidrug-resistant bacteria, revealing a promising synergistic effect that enhances bacterial inactivation. These findings open new perspectives for the development of combined therapeutic strategies, offering an innovative and complementary alternative to conventional antimicrobial treatments. Although we did not assess the effect of MB PDT on mammalian cells in this study, future work will examine its safety using HaCaT cells and will also evaluate its activity against biofilms in vivo to better understand its clinical potential.

Use of generative AI tools declaration

The authors declare they have not used artificial intelligence (AI) tools in the creation of this article.

Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

JC designed and planned the study, set up the methodology, and performed the experiments. MN developed the light sources device. AR and NS provided resources and validation. JC, AR and MN wrote and reviewed the manuscript. All authors approved the final version.

Ethical approval

As the bacterial strains were analyzed anonymously, the study was exempted from Human Research Committee approval according to the regulations of the Local Medical Ethical Committee of the National Bone Marrow Transplant Center.

References

1. Cassini A, Högberg LD, Plachouras D, et al. (2019) Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet Infect Dis* 19: 56–66. https://doi.org/10.1016/S1473-3099(18)30605-4
2. van Duin D, Paterson DL (2020) Multidrug-resistant bacteria in the community: an update. *Infect Dis Clin North Am* 34: 709–722. https://doi.org/10.1016/j.idc.2020.08.002
3. Medina E, Pieper DH (2016) Tackling threats and future problems of multidrug-resistant bacteria. *Curr Top Microbiol Immunol* 398: 3–33. https://doi.org/10.1007/82_2016_492
4. Hoenes K, Bauer R, Meurle T, et al. (2021) Inactivation effect of violet and blue light on ESKAPE pathogens and closely related non-pathogenic bacterial species – A promising tool against antibiotic-sensitive and antibiotic-resistant microorganisms. *Front Microbiol* 11: 612367. https://doi.org/10.3389/fmicb.2020.612367
5. Willis JA, Cheburkanov V, Chen S, et al. (2022) Breaking down antibiotic resistance in methicillin-resistant *Staphylococcus aureus*: combining antimicrobial photodynamic and antibiotic treatments. *Proc Natl Acad Sci USA* 119: e2208378119. https://doi.org/10.1073/pnas.2208378119
6. Hessling M, Spellerberg B, Hoenes K (2017) Photoinactivation of bacteria by endogenous photosensitizers and exposure to visible light of different wavelengths – a review on existing data. *FEMS Microbiol Lett* 364: fnw270. https://doi.org/10.1093/femsle/fnw270

7. Huang S, Lin S, Qin H, et al. (2023) The parameters affecting antimicrobial efficiency of antimicrobial blue light therapy: a review and prospect. *Biomedicines* 11: 1197. https://doi.org/10.3390/biomedicines11041197
8. Wang Y, Wang Y, Wang Y, et al. (2017) Antimicrobial blue light inactivation of pathogenic microbes: state of the art. *Drug Resist Updat* 33–35: 1–22. https://doi.org/10.1016/j.drup.2017.10.002
9. Hoenes K, Wenzel U, Spellerberg B, et al. (2020) Photoinactivation sensitivity of *Staphylococcus carnosus* to visible-light irradiation as a function of wavelength. *Photochem Photobiol* 96: 156–169. https://doi.org/10.1111/php.13168
10. Galo IDC, Prado RP, Santos WGD (2021) Blue and red light photoemitters as approach to inhibit *Staphylococcus aureus* and *Pseudomonas aeruginosa* growth. *Braz J Biol* 82: e231742. https://doi.org/10.1590/1519-6984.231742
11. Leanse LG, Harrington OD, Fang Y, et al. (2018) Evaluating the potential for resistance development to antimicrobial blue light (at 405 nm) in Gram-negative bacteria: in vitro and in vivo studies. *Front Microbiol* 9: 2403. https://doi.org/10.3389/fmicb.2018.02403
12. Rapacka-Zdonczyk A, Wozniak A, Nakonieczna J, et al. (2021) Development of antimicrobial phototreatment tolerance: Why the methodology matters. *Int J Mol Sci* 22: 2224. https://doi.org/10.3390/ijms22042224
13. Hamblin MR (2017) Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophys* 4: 337–361. https://doi.org/10.3934/biophy.2017.3.337
14. Salman S, Guermonprez C, Peno-Mazzarino L, et al. (2023) Photobiomodulation controls keratinocytes inflammatory response through Nrf2 and reduces Langerhans cells activation. *Antioxidants* 12: 766. https://doi.org/10.3390/antiox12030766
15. Piksa M, Fortuna W, Lian C, et al. (2023) Treatment of antibiotic-resistant bacteria colonizing diabetic foot ulcers by OLED induced antimicrobial photodynamic therapy. *Sci Rep* 13: 14087. https://doi.org/10.1038/s41598-023-39363-4
16. Pérez-Laguna V, García-Luque I, Ballesta S, et al. (2021) Photodynamic therapy combined with antibiotics or antifungals against microorganisms that cause skin and soft tissue infections: a planktonic and biofilm approach to overcome resistances. *Pharmaceuticals (Basel)* 14: 603. https://doi.org/10.3390/ph14070603
17. da Fonseca AS, Mencalha AL, de Paoli F (2021) Antimicrobial photodynamic therapy against *Acinetobacter baumannii*. *Photodiagnosis Photodyn Ther* 35: 102430. https://doi.org/10.1016/j.pdpdt.2021.102430
18. Siciliano V, Passerotto RA, Chiuchiarelli M, et al. (2023) Difficult-to-treat pathogens: a review on the management of multidrug-resistant *Staphylococcus epidermidis*. *Life (Basel)* 13: 1126. https://doi.org/10.3390/life13051126
19. CA-SFM, Comité de l'Antibiogramme de la Société Française de Microbiologie, Paris, France, 2024. Available from: https://www.sfm-microbiologie.org/boutique/_comite-de-lantibiogramme-de-la-sfm-ca-sfm-v1-0-juin-2024/.
20. Bliss CI (1939) The toxicity of poisons applied jointly. *Ann Appl Biol* 26: 585–615. https://doi.org/10.1111/j.1744-7348.1939.tb06990.x
21. Goldoni M, Johansson C (2007) A mathematical approach to study combined effects of toxicants in vitro: evaluation of the Bliss independence criterion and the Loewe additivity model. *Toxicol In Vitro* 21: 759–769. https://doi.org/10.1016/j.tiv.2007.03.003

22. Maclean M, MacGregor SJ, Anderson JG, et al. (2009) Inactivation of bacterial pathogens following exposure to light from a 405-nanometer light-emitting diode array. *Appl Environ Microbiol* 75: 1932–1937. https://doi.org/10.1128/AEM.01892-08
23. Murdoch LE, McKenzie K, Maclean M, et al. (2013) Lethal effects of high-intensity violet 405-nm light on *Saccharomyces cerevisiae*, *Candida albicans*, and on dormant and germinating spores of *Aspergillus niger*. *Fungal Biol* 117: 519–527. https://doi.org/10.1016/j.funbio.2013.05.004
24. Choby JE, Skaar EP (2016) Heme synthesis and acquisition in bacterial pathogens. *J Mol Biol* 428: 3408–3428. https://doi.org/10.1016/j.jmb.2016.03.018
25. Dailey HA, Dailey TA, Gerdes S, et al. (2017) Prokaryotic heme biosynthesis: multiple pathways to a common essential product. *Microbiol Mol Biol Rev* 81: e00048−16. https://doi.org/10.1128/MMBR.00048-16
26. Hyun JE, Moon SK, Lee SY (2021) Antibacterial activity and mechanism of 460–470 nm light-emitting diodes against pathogenic bacteria and spoilage bacteria at different temperatures. *Food Control* 123: 107721. https://doi.org/10.1016/j.foodcont.2020.107721
27. Nitzan Y, Salmon-Divon M, Shporen E, et al. (2004) ALA induced photodynamic effects on Gram positive and negative bacteria. *Photochem Photobiol Sci* 3: 430–435. https://doi.org/10.1039/b315633h
28. Hadi J, Wu S, Brightwell G (2020) Antimicrobial blue light versus pathogenic bacteria: Mechanism, application in the food industry, hurdle technologies and potential resistance. *Foods* 9: 1895. https://doi.org/10.3390/foods9121895
29. Kim S, Kim J, Lim W, et al. (2013) In vitro bactericidal effects of 625, 525, and 425 nm wavelength (red, green, and blue) light-emitting diode irradiation. *Photomed Laser Surg* 31: 554–562. https://doi.org/10.1089/pho.2012.3343
30. Pereira PR, de Paula JB, Cielinski J, et al. (2014) Effects of low intensity laser in in vitro bacterial culture and in vivo infected wounds. *Rev Col Bras Cir* 41: 49–55. https://doi.org/10.1590/s0100-69912014000100010
31. Angarano V, Akkermans S, Smet C, et al. (2020) The potential of violet, blue, green and red light for the inactivation of *P. fluorescens* as planktonic cells, individual cells on a surface and biofilms. *Food Bioprocess Technol* 124: 184–195. https://doi.org/10.1016/j.fbp.2020.07.019
32. Martins Antunes de Melo WC, Celiešiūtė-Germanienė R, Šimonis P, et al. (2021) Antimicrobial photodynamic therapy (aPDT) for biofilm treatments. Possible synergy between aPDT and pulsed electric fields. *Virulence* 12: 2247–2272. https://doi.org/10.1080/21505594.2021.1960105
33. Vieira C, Gomes ATPC, Mesquita MQ, et al. (2018) An insight into the potentiation effect of potassium iodide on aPDT efficacy. *Front Microbiol* 9: 2665. https://doi.org/10.3389/fmicb.2018.02665
34. Minnock A, Vernon DI, Schofield J, et al. (2000) Mechanism of uptake of a cationic water-soluble pyridinium zinc phthalocyanine across the outer membrane of *Escherichia coli*. *Antimicrob Agents Chemother* 44: 522–527. https://doi.org/10.1128/AAC.44.3.522-527.2000
35. Kim MJ, Tang CH, Bang WS, et al. (2017) Antibacterial effect of 405 ± 5 nm light emitting diode illumination against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* on the surface of fresh-cut mango and its influence on fruit quality. *Int J Food Microbiol* 244: 82–89. https://doi.org/10.1016/j.ijfoodmicro.2016.12.023

36. Wu J, Chu Z, Ruan Z, et al. (2018) Changes of intracellular porphyrin, reactive oxygen species, and fatty acids profiles during inactivation of methicillin-resistant *Staphylococcus aureus* by antimicrobial blue light. *Front Physiol* 9: 1658. https://doi.org/10.3389/fphys.2018.01658
37. Chu Z, Hu X, Wang X, et al. (2019) Inactivation of *Cronobacter sakazakii* by blue light illumination and the resulting oxidative damage to fatty acids. *Can J Microbiol* 65: 922–929. https://doi.org/10.1139/cjm-2019-0054
38. Kruszewska-Naczk B, Grinholc M, Rapacka-Zdonczyk A (2024) Mimicking the effects of antimicrobial blue light: Exploring single stressors and their impact on microbial growth. *Antioxidants* 13: 1583. https://doi.org/10.3390/antiox13121583
39. Bowman C, Bumah VV, Niesman IR, et al. (2021) Structural membrane changes induced by pulsed blue light on methicillin-resistant *Staphylococcus aureus* (MRSA). *J Photochem Photobiol B* 216: 112150. https://doi.org/10.1016/j.jphotobiol.2021.112150
40. Leanse LG, Anjos CD, Kaler KR, et al. (2023) Blue light potentiates antibiotics in bacteria via parallel pathways of hydroxyl radical production and enhanced antibiotic uptake. *Adv Sci* 10: e2303731. https://doi.org/10.1002/advs.202303731

AIMS Press