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# Impact of methylene blue in complex with coherent and incoherent radiation on biofilms of *Staphylococcus aureus* and *Candida albicans*

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The formation of microbial biofilms is one of the mechanisms underlying the acquisition of antimicrobial resistance, which is associated, in particular, with the presence of an extracellular matrix and reduced metabolic activity of the cells. In addition, the development of chronic infections of bacterial and fungal etiology is closely associated with biofilm formation. Consequently, the investigation of non-pharmacological approaches targeting biofilm formation and established biofilms of opportunistic microorganisms is of particular relevance. The combined effect of a photosensitizer – a 0.1% aqueous solution of methylene blue – and coherent (low-intensity laser radiation) as well as non-coherent (polarized non-coherent low-intensity radiation) red-spectrum light on biofilms of clinical isolates of *Staphylococcus aureus* and *Candida albicans*, as well as reference test strains *S. aureus* ATCC 25923 and *C. albicans* ATCC 10231, was investigated. It was established that the use of antimicrobial photodynamic therapy leads to a significant reduction in the density of 5-day-old biofilms of the studied microbial strains, which is manifested by a decrease in the growth intensity of biofilm scrapings on solid nutrient media by an average of 88.2-94.7% compared with the control. A 20-minute exposure to methylene blue led to a reduction in biofilm density by 38.6-45.7% compared with the control group. At the same time, low-intensity laser radiation and polarized non-coherent low-intensity red-spectrum radiation with a power density of 40 mW/cm² did not produce a statistically significant effect on the density of the formed biofilms. Ten-minute irradiation of the microbial inoculum of the reference test strains *S. aureus* ATCC 25923 and *C. albicans* ATCC 10231 with PILER radiation showed a stimulatory effect, manifested by an increase in the biofilm density of these strains by 24.7–30.5%. When comparing the effects of low-intensity laser radiation and polarized non-coherent radiation on the biofilms of the studied strains, their similarity was noted both when used alone and when combined with a photosensitizer. Given the pronounced antibiofilm effect, the use of antimicrobial photodynamic therapy may be recommended as part of combination treatment for infections caused by *S. aureus* and *C. albicans*, with the aim of disrupting microbial biofilms and reducing the pharmacological burden on the body.

*Keywords*: antimicrobial photodynamic therapy; low-power laser radiation; polarized incoherent low-energy radiation; antimicrobial resistance.

## Introduction

The examination and observation of aggregated microbes' complexes surrounded by extracellular matrix attached to the surfaces have been known since the classical works of Leeuwenhoek and Pasteur. In medicine however the connection between chronic infection and microbial biofilms was established in early 1970-s during routine microscopic examination of Gram-stained sputum smears from cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection (Høiby, 2017; Shamim et al., 2023). According to Costerton's definition, biofilm is an aggregate of microbial cells surrounded by a self-produced polymer matrix (Costerton et al., 1999). Biofilms are now recognized as the predominant mode of microbial growth in both natural and clinical environments and are implicated in the pathogenesis of the majority of chronic and recurrent infectious diseases (Hall-Stoodley et al., 2004; Koo et al., 2017).

A key characteristic of microbial biofilms is their markedly increased tolerance to antimicrobial agents and host immune defense mechanisms compared to planktonic cells. This phenomenon is associated with limited penetration of antimicrobial compounds through the extracellular polymeric matrix, altered metabolic activity of biofilm-associated cells, the presence of persister populations, and enhanced horizontal gene transfer (Davies & Davies, 2010; Høiby et al., 2017). As a result, biofilm-associated infections are difficult to eradicate using conventional antibiotic therapy and often lead to chronic inflammation, treatment failure, and recurrence of disease. These features make biofilms a major contributor to the global problem of antimicrobial resistance (AMR), which is currently recognized as one of the most serious threats to public health worldwide (Watkins et al., 2016; Murugaiyan et al., 2022). Opportunistic microorganisms such as *Staphylococcus aureus* and *Candida albicans* are among the most clinically relevant biofilm-forming pathogens. *S. aureus* is a leading cause of purulent-inflammatory infections, including periodontal, skin, soft tissue, and implant-associated infections, and demonstrates a remarkable ability to rapidly acquire resistance to multiple classes of antibiotics (Otto et al., 2014; Foster et al., 2017). *C. albicans* is the most common fungal pathogen in humans and readily forms biofilms on mucosal surfaces and medical devices, frequently participating in mixed bacterial-fungal biofilms that exhibit enhanced resistance and pathogenicity (Azeredo et al., 2017; Koo et al., 2017). The coexistence of bacterial and fungal species within biofilms further complicates antimicrobial therapy and underscores the need for alternative treatment strategies.

Given the limited effectiveness of conventional antimicrobial therapy against biofilm-associated infections, increasing attention has been directed toward non-drug physical methods of microbial inactivation. Among these, low-intensity light-based approaches, including laser radiation and polarized incoherent low-energy radiation (PILER), have been widely applied in various fields of medicine due to their minimal invasiveness and low incidence of adverse effects (Mansouri et al., 2020, Pantyo et al., 2020). Low-level laser radiation has been shown to influence cellular metabolism, proliferation, and membrane permeability, thereby modulating the susceptibility of microorganisms to antimicrobial agents (Mussttaf et al., 2019; Songca & Adjei, 2022).

A particularly promising application of low-intensity radiation is its use in antimicrobial photodynamic therapy (aPDT), which combines light exposure with photosensitizing agents to induce oxidative damage in microbial cells and biofilm structures. Methylene blue is one of the most extensively studied photosensitizers for antimicrobial photodynamic therapy owing to its favorable photochemical properties, low toxicity, and absorption in the red spectrum (Kwiatkowski et al., 2018). Previous studies have demonstrated that the combined application of methylene blue and red-spectrum low-intensity radiation significantly enhances antimicrobial and antibiofilm effects compared to either factor alone (Rolim et al., 2012; Pantyo et al., 2025). However, data regarding the comparative effects of coherent and incoherent radiation sources, including laser and PILER light, on mature microbial biofilms remain limited.

Therefore, the aim of this study was to investigate the impact of methylene blue in combination with coherent (laser) and incoherent (PILER) radiation on the biofilms of *Staphylococcus aureus* and *Candida albicans*.

## Material and methods

Objects of study were clinical isolates of *Staphylococcus aureus* (n = 5) and *Candida albicans* (n = 5) as well as collection test-strains *S. aureus* ATCC 25923 and *C. albicans* ATCC 10231 (Selectrol®, UK). Clinical strains were collected from periodontal pockets of the patients with chronic generalized periodontitis who were undergoing treatment in Uzhhorod University Dental Clinic. Identification of clinical isolates was carried out using generally accepted methods (Franco-Duarte et al., 2019) with the application of bacterioscopic and bacteriological techniques. The primary inoculation of pathological material from periodontal pockets was performed on blood agar. To isolate pure culture of *S. aureus*, mannitol-salt agar (Sanimed-M, Ukraine) was used. For the collection and isolation of *C. albicans* pure culture, Sabouraud agar with chloramphenicol (Sanimed-M, Ukraine) was applied. For the final identification of pure cultures of microorganisms, a biochemical method using test systems STAPHYtest 16 and CANDIDAtest 21 ("PLIVA-Lachema a.s.", Czech Republic) was employed.

To determine the combined effect of the photosensitizer 0.1% aqueous methylene blue solution, as well as coherent and incoherent radiation, on formed biofilms, the microtiter plate method was used (Djordjevic et al., 2002, Azeredo et al., 2017). For this purpose, 24-hour agar cultures of the studied microorganisms were prepared and standardized using a densitometer (DEN-1, Biosan, Latvia, 2016) to an optical turbidity of 0.5 according to the McFarland scale. Next, a 10 µL volume of the microbial inoculum was added to the wells of 96-well microtiter plates (C bottom, middle binding, detachable, sterile, DNase and RNase free), into which 190 µL of sterile nutrient broth had been previously dispensed. The plates were incubated in a thermostat for 5 days at a temperature of 37 °C.

All studied microorganisms were divided into several groups. The first (control) group consisted of 5-day-old microbial biofilms. To investigate the effect of coherent and incoherent low-intensity radiation on biofilm formation, the inoculum of microorganisms of group 2 was irradiated with low-intensity laser radiation (group 2A) and PILER (group 2B) immediately before being added to the wells of the microtiter plates. In both cases, duration of light application was 10 min. Formed biofilms of microorganisms in group 3 were exposed to 20-minute sessions of low-intensity laser radiation (group 3A) and PILER (group 3B). A 0.1% aqueous solution of methylene blue was added to the wells containing biofilms of microorganisms in group 4, incubated in the dark for 20 minutes, after which the photosensitizer was removed and the wells were rinsed with distilled water. Methylene blue was added to the wells containing biofilms of microorganisms in group 5 (in the same manner as for group 4), followed by irradiation with low-intensity laser radiation (group 5A) and PILER (group 5B) with the duration of 20 minutes.

After removal of planktonic forms and washing of the wells, the density of the formed biofilms of microorganisms in groups 1–3 was determined as follows. A volume of 200 µL of crystal violet was added to each well for 10 minutes, the wells were rinsed with distilled water, and ethyl alcohol was then added for 45 minutes. The optical density of the biofilms was measured using an ELx800 microplate reader (BioTek, USA) at wavelengths of 630 nm and 492 nm. Sterile meat peptone broth, added to the wells of the microtiter plates at a volume of 200 µL, served as the control for biofilm formation. Since the addition of methylene blue to the wells containing biofilms of microorganisms in groups 4 and 5 increased their optical density, the following method was used to determine biofilm density of all 5 groups. A volume of 200 µL of sterile distilled water was added to the wells containing microbial biofilms. The biofilms were then carefully scraped from the bottom of the wells using pipette tips and serially diluted in Eppendorf tubes to obtain tenfold dilutions ranging from 1/10 to $1/10^4$. Subsequently, 10 µL of the different dilutions were inoculated onto Petri dishes containing mannitol salt agar for *S. aureus* and Sabouraud agar supplemented with chloramphenicol for *C. albicans*. The cultures were incubated in a thermostat at 37 °C for 24 hours (24-48 hours for *C. albicans*), after which the growth intensity of microorganisms from different groups was compared.

Irradiation of microbial biofilms with PILER using a red-light filter was performed from a distance of 5 cm from the wells of the microtiter plates (Fig. 1, left). Irradiation of biofilms with red-spectrum low-intensity laser radiation was carried out from a distance of 50 cm using a scanning pattern described as a "circle converging to a point" (Fig. 1, right). The source of PILER radiation with the wavelength 570–660 nm and the power density 40 mW/cm$^2$ was the Bioptron "MedAll" device (Bioptron light therapy system by Zepter Group, Switzerland, 2017). Low-intensity laser radiation with the wavelength 660 nm and the power density 40 mW/cm$^2$ was generated using a scanning two-channel device "Medik 2K" (manufactured by "Photonika Plus", Cherkasy, Ukraine, 2010).

**Fig. 1.** Irradiation of microbial biofilm by PILER (on the left) and laser radiation (on the right)

The obtained data on the number of microbial colonies in different groups on Petri dishes were statistically analyzed to determine the arithmetic mean (x) and standard deviation (SD) of the samples. The significance of differences between the studied groups of microorganisms was assessed using ANOVA (analysis of variance) with the Tukey post test. Differences were considered statistically significant at $P < 0.05$. To ensure statistical significance, all experiments involving the collection test strains were conducted in five replicates, corresponding to the number of clinical isolates of the species studied.

## Results

It was established that all studied clinical isolates and collection test strains of *S. aureus* and *C. albicans* were capable of forming biofilms, as evidenced by significantly higher optical density values in microorganisms of groups 1–3 compared with the biofilm formation control – sterile nutrient broth (Table 1). The results of optical density measurements of the formed 5-day-old biofilms showed no statistically significant differences in their density between the control groups and those exposed to irradiation, either at the stage of microbial inoculum during biofilm formation or after biofilms had already formed. In addition, no statistically significant differences were observed in the optical density of biofilms between subgroups 2A and 2B, as well as between subgroups 3A and 3B (P > 0.05). At the same time, a statistically significant difference was observed when comparing the optical density of biofilms in groups 2 and 3 (P < 0.05). An exception was observed for biofilms formed by clinical isolates of *S. aureus*: the differences in biofilm density between groups 2B and 3A (P = 0.09), as well as between groups 2B and 3B (P = 0.07), were not statistically significant.

**Table 1**
Impact of low power radiation on the optical density of microbial biofilms (x ± SD; n = 5)

| Species of microorganisms | Control of biofilm formation (sterile broth) | Group 1 (control) | Group 2 A (irradiation of inoculum with laser) | Group 2 B (irradiation of inoculum with PILER) | Group 3 A (irradiation of biofilm with laser) | Group 3 B (irradiation of biofilm with PILER) |
|---|---|---|---|---|---|---|
| *Staphylococcus aureus* (clinical isolates) | 0.058 ± 0.013 | 0.271 ± 0.021 | 0.284 ± 0.018 | 0.279 ± 0.022 | 0.258 ± 0.012 | 0.254 ± 0.016 |
| *S. aureus* ATCC 25923 | 0.071 ± 0.010 | 0.289 ± 0.020 | 0.309 ± 0.015 | 0.309 ± 0.025 | 0.267 ± 0.015 | 0.269 ± 0.020 |
| *Candida albicans* (clinical isolates) | 0.063 ± 0.011 | 0.380 ± 0.016 | 0.401 ± 0.021 | 0.401 ± 0.019 | 0.367 ± 0.022 | 0.361 ± 0.017 |
| *C. albicans* ATCC 10231 | 0.059 ± 0.010 | 0.391 ± 0.020 | 0.413 ± 0.018 | 0.405 ± 0.024 | 0.374 ± 0.017 | 0.371 ± 0.016 |

*Note*: data analysis (ANOVA) showed that the difference between control and experimental groups is not considered to be statistically significant (P > 0.05).

Figure 2 shows the growth on Petri dishes containing mannitol salt agar all five groups of the *S. aureus* strain after inoculation with 10 µL of various dilutions of biofilm scrapings. No pronounced differences in growth intensity were observed among microorganisms in groups 1–3, whereas a somewhat lower number of colonies was noted in group 4, and almost complete absence of growth was observed in group 5.

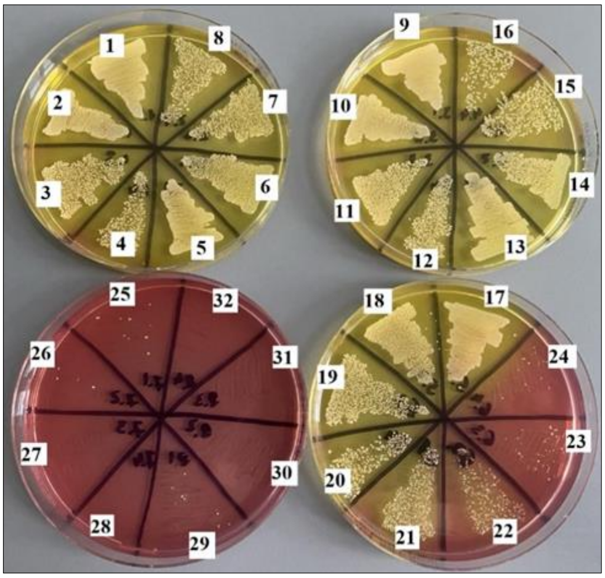

**Fig. 2.** Growth of *Staphylococcus aureus* clinical isolate on Petri dishes with mannitol-salt agar after subculture of different dilutions: 1–4 – control; 5–8 – irradiation of microorganisms by laser radiation; 9–12 – irradiation of microorganisms by PILER; 13–16 – irradiation of biofilm by laser radiation; 17–20 – irradiation of biofilm by PILER; 21–24 – adding methylene blue; 25–28 – adding methylene blue with subsequent irradiation by laser radiation; 29–32 – adding methylene blue with subsequent irradiation by PILER

Similar patterns were observed for *C. albicans* (Fig. 3). As in the case of the studied *S. aureus* strains, complete absence of growth was noted in some series at high dilutions ($1/10^3$ and $1/10^4$) of biofilm scrapings from microorganisms of group 5.

Table 2 presents statistically processed data on the number of colonies of the studied microorganisms on Petri dishes after inoculation with 10 µL of the highest dilutions ($1/10^4$) of biofilm scrapings.

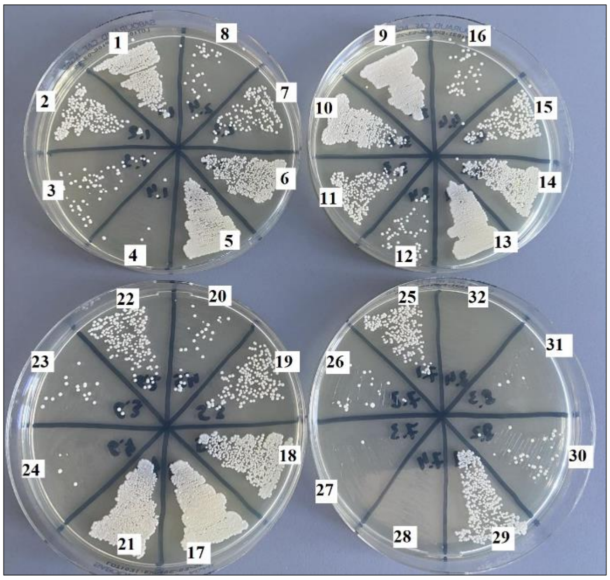

**Fig. 3.** Growth of *Candida albicans* clinical isolate on Petri dishes with Sabouraud dextrose agar after subculture of different dilutions: 1–4 – control; 5–8 – irradiation of microorganisms by laser radiation; 9–12 – irradiation of microorganisms by PILER; 13–16 – irradiation of biofilm by laser radiation; 17–20 – irradiation of biofilm by PILER; 21–24 – adding methylene blue; 25–28 – adding methylene blue with subsequent irradiation by laser radiation; 29–32 – adding methylene blue with subsequent irradiation by PILER

**Table 2**
Number of colonies of investigated microorganisms on solid nutrient media (x ± SD; n = 5)

| Species of bacteria | Group 1 (control) | Group 2 | | Group 3 | | Group 4 (methylene blue) | Group 5 | |
|---|---|---|---|---|---|---|---|---|
| | | Group 2 A (irradiation of inoculum with laser) | Group 2 B (irradiation of inoculum with PILER) | Group 3 A (irradiation of biofilm with laser) | Group 3 B (irradiation of biofilm with PILER) | | Group 5 A (methylene blue + irradiation with laser) | Group 5 B (methylene blue + irradiation with PILER) |
| *S.aureus* (clinical isolates) | 44.2 ± 9.4 | 54.4 ± 8.4 | 55.4 ± 10.0 | 40.2 ± 8.7 | 42.0 ± 6.7 | 24.0 ± 5.2** | 4.8 ± 3.7** | 3.8 ± 3.3** |
| *S. aureus* ATCC 25923 | 53.4 ± 7.0 | 64.2 ± 8.3 | 66.6 ± 9.0* | 48.2 ± 5.4 | 49.2 ± 7.7 | 32.8 ± 8.1** | 4.4 ± 3.0** | 2.8 ± 2.6** |
| *C. albicans* (clinical isolates) | 42.6 ± 7.8 | 49.2 ± 7.5 | 54.6 ± 10.1 | 39.6 ± 7.9 | 40.8 ± 10.1 | 23.2 ± 6.1** | 4.2 ± 2.6** | 5.0 ± 1.6** |
| *C. albicans* ATCC 10231 | 44.6 ± 8.9 | 55.8 ± 9.9 | 58.2 ± 8.8* | 40.2 ± 9.0 | 41.2 ± 7.3 | 26.0 ± 5.9** | 2.8 ± 2.4** | 2.4 ± 2.1** |

*Note*: the difference between control and experimental groups is significant: * – P < 0.05; ** – P < 0.01.

The results obtained with clinical strains of *S. aureus* indicate that the addition of methylene blue to the biofilms of group 4 led to a reduction in the number of colonies after subculturing biofilm scrapings onto Petri dishes by an average of 45.7% ($P = 0.003$). The combined effect of methylene blue and low-intensity laser radiation resulted in a reduction in microbial growth intensity by 89.0% ($P < 0.0001$). With the combined application of the photosensitizer and PILER irradiation, an average reduction in the number of colonies by 91.4% was observed ($P < 0.0001$). At the same time, no statistically significant differences in growth intensity were observed between the control group and groups 1–3 ($P > 0.05$).

Similar patterns were observed for the collection test strain *S. aureus* ATCC 25923: the growth intensity of microorganisms in group 4 was on average 38.6% lower compared with the control ($P = 0.0025$). The number of colonies of microorganisms in groups 5A and 5B after subculturing biofilm scrapings was on average 91.7% and 94.7% lower, respectively, compared with the control group ($P < 0.0001$). Irradiation of the microbial inoculum with PILER radiation resulted in a certain increase in the biofilm density of *S. aureus* ATCC 25923, which was manifested by an average increase in the growth intensity of biofilm scrapings by 24.7% ($P = 0.03$). As in the case of clinical isolates of *S. aureus*, no statistically significant differences were observed between the control group and groups 2A, 3A, and 3B ($P > 0.05$).

In the case of clinical isolates of *C. albicans*, the growth intensity of microorganisms in group 4 decreased on average by 45.5% compared with the control. The combined effect of the photosensitizer and low-intensity laser radiation led to a reduction in the number of colonies of microorganisms in group 5A by 90.1% compared with the control group. A similar effect of methylene blue combined with PILER irradiation reduced the biofilm density of *C. albicans* in group 5B by an average of 88.2%.

Irradiation of the microbial inoculum of the collection test strain *C. albicans* ATCC 10231 with red-spectrum PILER radiation resulted in an average increase in the growth intensity of biofilm scrapings by 30.5% compared with the control ($P = 0.04$). The growth intensity of *C. albicans* ATCC 10231 in group 4 was 41.7% lower compared with the control ($P = 0.005$). The number of colonies from biofilm scrapings in groups 5A and 5B was on average 93.7% and 94.6% lower, respectively, compared with group 1 ($P < 0.0001$). No statistically significant differences were detected between the control group and groups 2A, 3A, and 3B.

## Discussion

The obtained results indicate a significant antibiofilm effect of the combined application of a photosensitizer – a 0.1% aqueous solution of methylene blue – with both coherent and non-coherent radiation, and are comparable with the results of our previous studies in which LED radiation served as the source of low-intensity red-spectrum radiation (Pantyo et al., 2020, Pantyo et al., 2025). Data from a number of authors (Souza et al., 2010; Soria-Lozano et al., 2015; Mahmoudi et al., 2018; Songca & Adjei, 2022) indicate a high level of antimicrobial activity of photodynamic therapy when using methylene blue as well as other photosensitizers against a wide range of pathogenic and opportunistic microorganisms.

Although antimicrobial photodynamic therapy relies on light to activate a photosensitizer and induce the generation of reactive oxygen species (ROS), the role of light – a key factor in ROS production – has often been overlooked (Piksa et al., 2023). When comparing the effects of coherent (low-intensity laser) and non-coherent (PILER) radiation on biofilm formation (groups 2A and 2B), on established 5-day-old biofilms (groups 3A and 3B), and when used in combination with methylene blue (groups 5A and 5B), their similarity and the absence of statistically significant differences between them were noted ($P > 0.05$). The above-mentioned findings allow us to suggest that the principal parameters of low-intensity radiation when used for antimicrobial photodynamic therapy are wavelength and power density. This assumption is based, in particular, on the fact that the investigated types of low-intensity radiation had identical power densities (40 mW/cm²) and similar wavelengths. At the same time, parameters such as coherence and polarization do not appear to have a determining influence on the biological effects of low-intensity radiation. This is supported by similar results in antibiofilm activity observed with the combined use of methylene blue and red-spectrum LED radiation (Pantyo et al., 2025).

The absence of a statistically significant effect of 20-minute exposure to low-intensity laser radiation and PILER on the biofilm density of the studied strains may be explained by the markedly greater resistance of biofilms to physical and chemical factors compared with planktonic forms. In most cases, 10-minute irradiation of the microbial inoculum did not affect the biofilm density of microorganisms in groups 2A and 2B compared with the control groups, as confirmed both by measurements of biofilm optical density and by the growth intensity of biofilm scrapings on solid nutrient media. Exceptions were observed for the reference test strains exposed to PILER radiation, which resulted in an average increase in biofilm density of 24.7-30.5% compared with the control.

When comparing the effects of radiation on biofilm formation (groups 2A and 2B) and on established biofilms (groups 3A and 3B), a statistically significant difference in biofilm density between these groups was observed for the majority of the studied strains. This finding indicates a stimulatory effect of short-term exposures to low-intensity radiation on planktonic forms of microorganisms, as well as a certain degree of reduction in biofilm density with longer radiation exposures, which in turn confirms a dose-dependent effect of the biological action of radiation. An exception was observed for clinical isolates of *C. albicans*, for which the differences in the growth intensity of biofilm scrapings between groups 2A and 3A ($P = 0.08$), 2A and 3B ($P = 0.19$), and 2B and 3B ($P = 0.07$) were not statistically significant.

Due to a number of advantages, including a broad spectrum of activity against bacteria, viruses, fungi, and protozoa, low invasiveness, and the absence of a risk of resistance development in microorganisms (Cieplik et al., 2018; Mahmoudi et al., 2018), antimicrobial photodynamic therapy is considered as an adjunct to, and in some cases even an alternative to, conventional antibiotic therapy. This method is used in clinical practice for the treatment of pathological conditions associated with the development of dental biofilms, inflammatory processes of the oral mucosa, wound infections, ventilator associated pneumonia, rhinosinusitis, and related conditions (Hu et al., 2018; Mahmoudi et al., 2018; Youf et al., 2021). A better understanding of the significance of low-intensity radiation parameters for photosensitizer activation and the subsequent induction of photochemical reactions will make antimicrobial photodynamic therapy more versatile and, as a result, promote its wider implementation in clinical practice.

## Conclusions

The obtained results indicate the absence of a statistically significant effect of irradiation of the microbial inoculum with either PILER or red-spectrum low-intensity laser radiation on biofilm formation by the studied microbial strains. Likewise, 20-minute irradiation of already formed biofilms with both coherent and incoherent low-intensity radiation did not produce a pronounced effect on their density. A 20-minute exposure to a 0.1% aqueous solution of the photosensitizer methylene blue resulted in a reduction in the growth intensity of subcultured biofilms by 38.6–45.7% compared to the control groups. The most pronounced antibiofilm effect was observed when methylene blue was used in combination with subsequent irradiation of the biofilm, which resulted in an average reduction in the growth intensity of biofilm scrapings by 88.2–94.7%. When comparing the antibiofilm efficacy of antimicrobial photodynamic therapy using low-intensity laser radiation and red-spectrum PILER light, their effects were found to be comparable, with no statistically significant differences between them.

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## References

Azeredo, J., Azevedo, N. F., Briandet, R., Cerca, N., Coenye, T., Costa, A. R., Desvaux, M., Di Bonaventura, G., Hébraud, M., Jaglic, Z., Kačániová, M., Knøchel, S., Lourenço, A., Mergulhão, F., Meyer, R. L., Nychas, G., Simões, M., Tresse O., & Sternberg, C. (2017). Critical review on biofilm methods. Critical Reviews in Microbiology, 43(3), 313–351.

Cieplik, F., Deng, D., Crielaard, W., Buchalla, W., Hellwig, E., Al-Ahmad, A., & Maisch, T. (2018). Antimicrobial photodynamic therapy – what we know and what we don't. Critical Reviews in Microbiology, 44(5), 571–589.

Costerton, J. W., Stewart, P. S., & Greenberg, E. P. (1999). Bacterial biofilms: A common cause of persistent infections. Science, 284(5418), 1318–1322.

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews, 74(3), 417–433.

Djordjevic, D., Wiedmann, M., & McLandsborough, L. A. (2002). Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. Applied and Environmental Microbiology, 68(6), 2950–2958.

Foster, T. J. (2017). Antibiotic resistance in *Staphylococcus aureus*. FEMS Microbiology Reviews, 41(3), 430–449.

Franco-Duarte, R., Černáková, L., Kadam, S., Kaushik, K. S., Salehi, B., Bevilacqua, A., Corbo, M. R., Antolak, H., Dybka-Stępień, K., Leszczewicz, M., Relison Tintino, S., Alexandrino de Souza, V. C., Sharifi-Rad, J., Melo Coutinho, H. D., Martins, N., & Rodrigues, C. F. (2019). Advances in chemical and biological methods to identify microorganisms – from past to present. Microorganisms, 7(5), 130.

Hall-Stoodley, L., Costerton, J. W., & Stoodley, P. (2004). Bacterial biofilms: From the natural environment to infectious diseases. Nature Reviews Microbiology, 2(2), 95–108.

Høiby, N. (2017). A short history of microbial biofilms and biofilm infections. Apmis, 125(4), 272–275.

Hu, X., Huang, Y.-Y., Wang, Y., Wang, X., & Hamblin, M. R. (2018) Antimicrobial photodynamic therapy to control clinically relevant biofilm infections. Frontiers in Microbiology, 9, 1299.

Koo, H., Allan, R. N., Howlin, R. P., Stoodley, P., & Hall-Stoodley, L. (2017). Targeting microbial biofilms: Current and prospective therapeutic strategies. Nature Reviews Microbiology, 15(12), 740–755.

Kwiatkowski, S., Knap, B., Przystupski, D., Saczko, J., Kędzierska, E., Knap-Czop, K., & Kulbacka, J. (2018). Photodynamic therapy – mechanisms, photosensitizers and combinations. Biomedicine and Pharmacotherapy, 106, 1098–1107.

Mahmoudi, H., Bahador, A., Pourhajibagher, M., & Alikhani, M. Y. (2018). Antimicrobial photodynamic therapy: An effective alternative approach to control bacterial infections. Journal of Lasers in Medical Sciences, 9(3), 154–160.

Mansouri, V., Arjmand, B., Rezaei-Tavirani, M., Razzaghi, M., Rostami-Nejad, M., & Hamdieh, M. (2020). Evaluation of efficacy of low-level laser therapy. Journal of Lasers in Medical Sciences, 11(4), 369–380.

Murugaiyan, J., Kumar, P. A., Rao, G. S., Iskandar, K., Hawser, S., Hays, J. P., Mohsen, Y., Adukkadukkam, S., Awuah, W. A., Jose, R. A. M., Sylvia, N., Nansubuga, E. P., Tilocca, B., Roncada, P., Roson-Calero, N., Moreno-Morales, J., Amin, R., Kumar, B. K., Kumar, A., Toufic. A.-R., Zaw, T. N., Akinwotu, O. O., Satyaseela, M. P., & van Dongen, M. B. M. (2022). Progress in alternative strategies to combat antimicrobial resistance: Focus on antibiotics. Antibiotics, 11(2), 200.

Mussttaf, R. A., Jenkins, D. F. L., Jha, A. N., & McQuaid, S. (2019). Assessing the impact of low level laser therapy (LLLT) on biological systems: A review. International Journal of Radiation Biology, 95(2), 120–143.

Otto, M. (2014). *Staphylococcus aureus* toxins. Current Opinion in Microbiology, 17, 32–37.

Pantyo, V. V., Koval, G. M., Danko, E. M., & Pantyo, V. I. (2020). Complex impact of polarized and non-polarized low intense light and methylene blue on growth rate of some opportunistic microorganisms. Regulatory Mechanisms in Biosystems, 11(4), 520–523.

Pantyo, V. V., Pallah, O. V., Boiko, N. V., Danko, E. M., Koval, G. M., Pantyo, V. I., & Chobey, A. S. (2025). Impact of methylene blue and LED radiation on microbial biofilms. Regulatory Mechanisms in Biosystems, 16(1), e25014.

Piksa, M., Lian, C., Samuel, I. C., Pawlik, K. J., Samuel, I. D., & Matczyszyn, K. (2023). The role of the light source in antimicrobial photodynamic therapy. Chemical Society Reviews, 52(5), 1697–1722.

Rolim, J. P., de-Melo, M. A. S., Guedes, S. F. F., Albuquerque-Filho, F. B., de Souza, J. R., Nogueira, N. A. P., & Rodrigues, L. K. (2012). The antimicrobial activity of photodynamic therapy against *Streptococcus mutans* using methylene blue. Journal of Photochemistry and Photobiology B, 106, 40–46.

Shamim, A., Ali, A., Iqbal, Z., Mirza, M. A., Aqil, M., Kawish, S. M., Siddiqui, A., Kumar, V., Naseef, P. P., Alshadidi, A. A. F., & Saheer Kuruniyan, M. (2023). Natural medicine a promising candidate in combating microbial biofilm. Antibiotics, 12(2), 299.

Songca, S. P., & Adjei, Y. (2022). Applications of antimicrobial photodynamic therapy against bacterial biofilms. International Journal of Molecular Sciences, 23(6), 3209.

Soria-Lozano, P., Gilaberte, Y., Paz-Cristobal, M. P., Pérez-Artiaga, L., Lampaya-Pérez, V., Aporta, J., Pérez-Laguna, V., García-Luque, I., Revillo, M. J., & Rezusta, A. (2015). *In vitro* effect photodynamic therapy with differents photosensitizers on cariogenic microorganisms. BMC Microbiology, 15, 187.

Souza, R. C., Junqueira, J. C., Rossoni, R. D., Pereira, C. A., Munin, E., & Jorge, A. O. (2010). Comparison of the photodynamic fungicidal efficacy of methylene blue, toluidine blue, malachite green and low-power laser irradiation alone against *Candida albicans*. Lasers in Medical Science, 25, 385–389.

Watkins, R. R., Bonomo, R. A., & Rello, J. (2022). Managing sepsis in the era of precision medicine: Challenges and opportunities. Expert Review of Anti-Infective Therapy, 20(6), 871–880.

Youf, R., Müller, M., Balasini, A., Thétiot, F., Müller, M., Hascoët, A., Jonas, U., Schönherr, H., Lemercier, G., Montier, T., & Le Gall, T. (2021). Antimicrobial photodynamic therapy: Latest developments with a focus on combinatory strategies. Pharmaceutics, 13(12), 1995.