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# *In vitro* antimicrobial activity of LED irradiation on *Pseudomonas aeruginosa*

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ABSTRACT

*Pseudomonas aeruginosa* is an opportunistic pathogen responsible of many deaths due to nosocomial pneumonia each year.
It is particularly resistant to many different classes of antibiotics and disinfectants. For all these reasons, there is the necessity to find novel approaches of treatment.
The aim of this study was to evaluate the effect of 880 nm light emitting diodes (LED) irradiation on *P. aeruginosa*, *in vitro*. Different LED irradiation parameters (time, energy output and the addition of methylene blue and chlorhexidine) have been tested in order to evaluate the effects on this bacterium. After treatment, the colony forming units per milliliter (CFU mL-1) were recorded and the data were submitted to ANOVA and Bonferroni *post hoc* tests at a level of significance of 5%.
A statistical significant reduction of bacterial count has been registered after 5 min of LED irradiation. The antibacterial effect was directly proportional to irradiation time and the output energy. The pre-treatment with methylene blue, seems to be not effective against *P. aeruginosa*, independently from irradiation parameters. On the contrary, the contemporary action of LED and chlorhexidine has shown a great reduction of bacterial count that was statistical significant respect chlorhexidine and LED alone. The effect of LED irradiation was visible also after 24 h, when a lower bacterial count characterized all irradiated samples respect controls.


## 1. Introduction

*Pseudomonas aeruginosa* is an opportunistic pathogen able to invade eukaryotic cells, whose size ranges from 0.5 to 1.0 μm wide and from 1.5 to 5 μm long [1–3]. Approximately 1400 deaths due to nosocomial pneumonia per year are caused by this bacterium in United States [4–5]. The oral cavity may be a major source of this respiratory pathogen, particularly in the presence of poor oral hygiene and periodontal infection [6]. The prevalence of *P. aeruginosa* in subjects with periodontal diseases may vary widely among different populations, and this pathogen has also been associated with treatment failure in patients with refractory periodontitis [6–8].

*P. aeruginosa* cell wall is characterized by a very low permeability, thanks also to the presence of efflux pumps that remove molecules and penetrate the intramembranous channels [9]; it is able to change the expression and function of the chromosomes and acquire resistant genes, thanks to mutations and mobile genetic elements such as plasmids, bacteriophages, and transposons [10]. All these factors make this bacterium particularly resistant to different classes of antibiotics and disinfectants. Moreover it also displays a certain degree of tolerance to photodynamic therapy (PDT) [11–12].

For all these reasons, there is the necessity to find novel approaches of treatment.

In a previous study we have shown that 880 nm light-emitting diodes (LEDs) irradiation is able to reduce the count of a very resistant strain of *Enterococcus faecalis* [13]. LED devices emit incoherent narrow spectrum light at wavelengths ranging from the ultraviolet (UV) to the visible to the near infrared (NIR). The bactericidal activity of LEDs is unclear, and their effects are dependent upon the wavelength, power density, quantity (or number) of bacteria, and microbial species [14]. The concurrent use of LED and (sodium hypochlorite) NaOCl 1% permitted to reach the total inactivation of *E. faecalis* in 5 min. NaOCl is a good endodontic irrigant, but it is not a viable option for periodontal use, due to its toxicity on periodontal ligament cells [13–15]. The use of light based therapies, are very promising because they contrast bacteria through mechanisms that are independently respect antibiotics and their connected problems [16]. Moreover, literature agrees that non-coherent light-emitting diodes (LEDs) are safe, non-thermal, nontoxic and noninvasive, respect high-power lasers and to date, no side effects have been reported from their use [17].

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The aim of this study is to verify if LED light irradiation is characterized by antibacterial activity on *P. aeruginosa*, to evaluate the influence of light parameters and such substances like methylene blue and chlorhexidine on bacterial count and to check if antibacterial effects are maintained after 24 h.

## 2. Materials and Methods

### 2.1. Bacteria and Culture Conditions

For preparation of a pure culture of *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™, isolated from blood culture) the test organism was first plated onto fresh Cetrimide agar (Oxoid LTD, Basingstoke, Hampshire, UK) and incubated for 24 h at 37 °C. Suspension was made from the culture by diluting a few colonies in nutrient broth (NB) (Oxoid) to a density of 0.5 McFarland Standard (1 × 108 Colony Forming Units per mL - CFU/mL), confirmed by spectrophotometer analysis (Agilent Technologies 8453 UV, Santa Clara, CA, USA).

*P. aeruginosa* solution was prepared for 25-well (dimension: 20 ∗ 20 mm) flat-bottom plates with lids separately for several experiments (tests).

Aliquots of 1 mL were dispensed in triplicate into micro-titer plates for each treatment group, and all of the tests included a positive control (C+) and a negative one (C−).

### 2.2. Light Source and Irradiation Parameters

A NIR-LED device characterized by an 880 nm-wavelength was used as light source (PhaseTech, Bergamo, Italy).

The hand-piece was constituted by 6 LEDs but to simplify the comprehension of the methods used, we will refer throughout the text to the energy output (mJ/s) emitted by a single led. In all of the experiments, the LED hand-piece was mounted perpendicularly to the wells with a particular polystyrene box to maintain a constant distance from light source. As shown in a previous study, the irradiation was performed under a laminar flow hood in the dark under aseptic conditions in all of the experiments [13].

### 2.3. Test 1

In this experiment, we had 6 different groups: MB was prepared by adding methylene blue (Carlo Erba Analyticals - Italy) in positive controls (C+) in order to reach a concentration of 0.0005% MB.

C+ and MB were not irradiated. IR 5 (t = 5 min), IR 10 (t = 10 min) and IR 20 (t = 20 min) were characterized by a different irradiation time (t).

IR 20 + MB contemporary action of methylene blue and 20 min of LED irradiation.

All irradiations were performed at a measured energy output of 2.37 mJ/s (PROG A), Fig. 1.

### 2.4. Test 2

Four different groups were distinguished, Fig. 2. CHX was prepared by adding to C+ samples 20% of Chlorhexidine Gluconate Solution (Bio Cide Grade, R.N. Laboratories PVT. LTD., India) and Dulbecco's phosphate buffered saline 0.00095 M (DPBS) (Lonza, Belgium) at pH 7.1, in order to obtain a final concentration of 0.1% CHX.

C+ and CHX were not irradiated.

The simultaneous incubation in a CHX solution 0.1% and 30 min of LED irradiation at different energy output (e) characterized both PROG A + CHX and PROG B + CHX (e = 2.37 and 8.15 mJ/s, respectively).

After 30 min CHX was inactivated by using a solution containing 3% Tween 80 (Sigma-Aldrich, USA) and 0.3% L-α-lecithin (Sigma-Aldrich, USA) in Dulbecco Phosphate Buffer Solution DPBS (Lonza, Belgium) [18].

### 2.5. Test 3

The following groups have been included in the experiment: C+, PROG B, CHX and PROG B + CHX.

C+ and CHX were not irradiated. CHX and PROG B + CHX were incubated in a 0.1% Chlorhexidine solution for 30 min and then inactivated by Tween + lecithin solution.

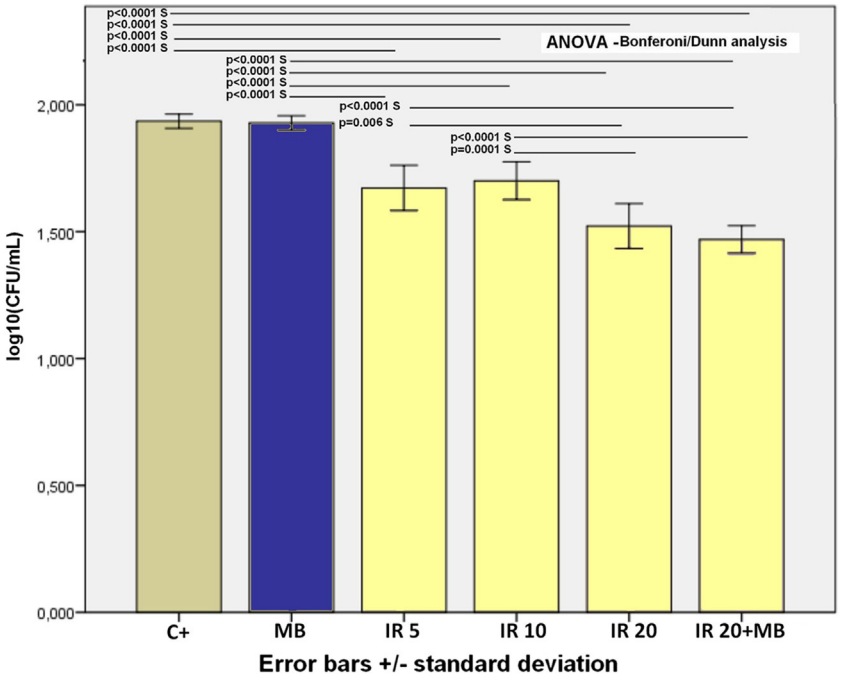


**Fig. 1.** The effect of led irradiation time with or without the presence of a photosensitizer methylene Blue (MB) on *Pseudomonas aeruginosa*. C+ = controls; MB = C+ Methylene Blue; IR 5 = samples irradiated 5 min; IR 10 = irradiated 10 min; IR 20 = irradiated 20 min; IR 20 + MB = irradiated 20 min + Methylene Blue. *Statistical significance at 0.05 in comparison to control group.