

# Analysis of decontamination of pressure injury through blue light - randomized trial clinical study

## Abstract

**Objective:** to analyze the effects of photobiomodulation using the Light-emitting Diode (LED), operating at wavelength 420 +/- 20 nm (Violet-Blue) in pressure decontamination (PI).

**Methods:** The sample consisted of 36 patients, 24 of whom met the inclusion criteria and were randomized into 3 treatment groups, where three different photobiomodulation We used a Cosmedical® LED plate Cicatrillux (figure 1); containing 36 LEDs, 420 +/- 20 nm, optical diameter 10 mm +/- 2, optical output 2-5 mW, device energy 106J, radiant exposure 3.8 J / cm<sup>2</sup>, irradiance 6.4 mW / cm<sup>2</sup> whit energy total Joules were applied in each, 53J (5 min) 106J (10 min) and 159J (15 min) respectively.

**Results:** The analysis of the results was based on the counting of Colony Forming Units (UFC), as well as characterization of the lesions using the National Pressure Ulcer Advisory Panel (NPUAP) and severity by the Pressure Ulcer Score Healing (PUSH) scale. The mean age of the study patients was 72 years, most of the pressure lesions were in the sacral region (70%), 62% female patients, 79% of the lesions were located in the sacral region and 54% were stage III.

**Conclusion:** The analysis of the results showed that the photobiomodulation of lesions at wavelengths of 420 nm to reduce contamination was not effective at the doses applied in the three study groups because, despite a slight reduction in CFU count in the time of 159J, was statistically significant.

**Keywords:** photo biomodulation, bacterial inactivation, pressure injury, bacterial infection, blue light

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## Introduction

The development of a pressure ulcer (also called pressure injury) has been considered an indicator for quality of care, as pressure ulcers are potentially preventable, a leading cause of morbidity for inpatients, and is a cause of substantial discomfort, prolonged hospitalizations, additional healthcare costs and, in some cases, death.<sup>1</sup> The National Pressure Ulcer Advisory Panel (NPUAP) defines a pressure ulcer as the impaired integrity of the skin caused by the compression of the soft skin tissue between the bony processes and an external surface.<sup>2</sup> This ulcer is caused by an impaired tissue perfusion and subsequent partial loss of the body cells progressively destroying the underlying layers. The pathogenesis of pressure ulcer is a complex problem, and three main factors are of great importance in this process: direct pressure, shear forces and friction. A patient who suffers from a combination of predisposing factors is more susceptible to this development.<sup>3</sup> The duration of compression that the skin tolerates until it breaks down varies from one patient to another; in many incapable patients, tissue damage occurs within less than two hours.<sup>2</sup> Complications arising from pressure ulcers are associated with significant morbidity and mortality. Bacterial infection is the most common complication associated with pressure ulcers. Infection of a pressure ulcer may result in soft tissue and bone infections: cellulitis, abscess formation, bursitis, and osteomyelitis of bone underlying the wound bed. Pressure ulcers are a common source of bacteremia.<sup>4</sup> Microbial imbalances and synergistic relationships between bacteria in medically important biofilms are poorly researched. Consequently, little is known about how synergy between bacteria may increase the net pathogenic effect of a biofilm in many diseases and infections, including chronic wounds. Microbial synergy in chronic wounds may increase virulence and pathogenicity, leading to enhanced

tissue degradation, malodour and in some cases, an impairment of the host immune response.<sup>5</sup> Microbial imbalances and synergistic relationships between bacteria in medically important biofilms are poorly researched. Consequently, little is known about how synergy between bacteria may increase the net pathogenic effect of a biofilm in many diseases and infections, including chronic wounds. Microbial synergy in chronic wounds may increase virulence and pathogenicity, leading to enhanced tissue degradation, malodour and in some cases, an impairment of the host immune response.<sup>6</sup> The application of antimicrobials in the management of wounds is a complex procedure requiring appropriate clinical decision making, judgment and a thorough understanding of antimicrobial therapies, together with their potential disadvantages. There is considerable direct and indirect evidence for the presence of bacterial biofilms in the chronic wound bed, and it has been demonstrated that bacteria within these biofilms may exhibit both specific and nonspecific antimicrobial tolerance.<sup>7</sup>

One such antimicrobial technology is 405 nm light. Violet-blue light in this region photo-excites intracellular porphyrins within microorganisms, producing a range of reactive oxygen species (ROS) which cause oxidative damage and cell death.<sup>2,4-5</sup> Although less germicidal than ultraviolet (UV) light, 405 nm light has broad-spectrum antimicrobial action against Gram positive and negative bacteria, bacterial biofilms, endospores, yeasts, fungi and in some circumstances viruses.<sup>8</sup>

Most of the publications on the antimicrobial effect of blue light have been confined to *in vitro* studies. There have been (rather surprisingly) no published preclinical or clinical reports to demonstrate blue light therapy for wound infections.<sup>9</sup>

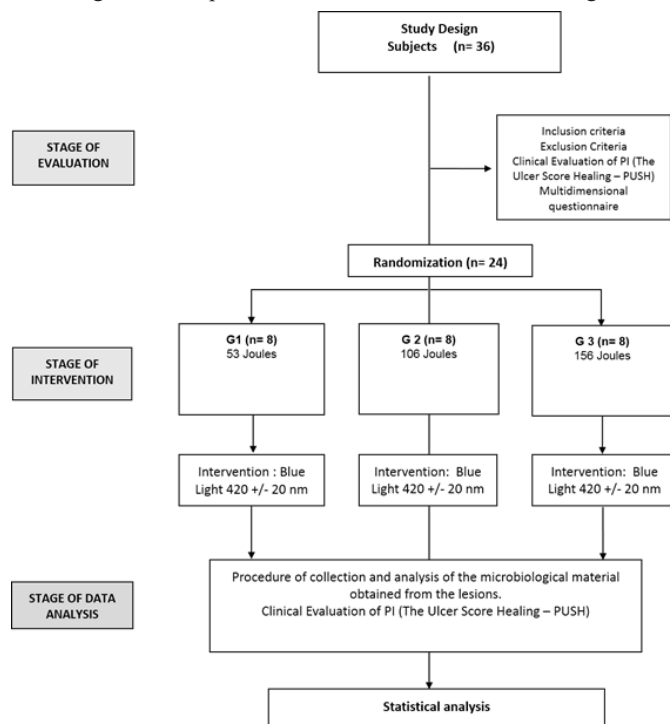
Violet-blue light, particularly 405 nm light, has significant antimicrobial properties against a wide range of bacterial and fungal pathogens and, although germicidal efficacy is lower than UV light, this limitation is offset by its facility for safe, continuous use in occupied environments. Promising results on disinfection efficacy have been obtained in hospital trials but the full impact of this technology on reduction of healthcare-associated infection has yet to be determined.<sup>10</sup>

Although 405 nm light has extensive antimicrobial action and safety advantages, little is known about the potential for the development of bacterial resistance or tolerance to violet-blue 405 nm light inactivation. It is hypothesized that tolerance is unlikely due to the mechanism of inactivation.<sup>8</sup> The results show that the use of blue light for treating infections is of great interest because it is a non-antibiotic approach that overcomes the antimicrobial resistance drawbacks. *P. aeruginosa* produces endogenous porphyrins that could play a significant role in the antimicrobial effect of blue light.<sup>10</sup> In view of the foregoing, we conducted this randomized clinical trial with the objective of verifying if the transposition of the in vitro and preclinical studies translates into an effective clinical application of blue light in the bacterial inactivation of cutaneous wounds (pressure lesion).

## Materials and methods

### Study design

It is a controlled clinical trial randomized. The patients were divided into three groups, both composed of 08 patients each, according to the sample calculation and described below: Figure 1.



**Figure 1** The consort flow chart of the eligible patients.

### Ethical aspects

The project was approved by the Ethics Committee of the Hospital Set of Mandaqui, São Paulo, under the number 53876416.2.0000.5551 and registered in the Brazilian Registry of Clinical Trials (ReBEC) under the number: REQ-4905 Patients who agreed to participate of the

study by signing the Term of Free and Informed Consent, according to Resolution 196/96. All participants were informed about the study's objectives and procedures and will be invited to sign an informed consent form agreeing to participate.

### Determination of Sample Size

For the calculation of the sample size, the t-test for two dependent samples was used and the mean of the differences and the standard deviation of the area of pressure ulcers for pre-intervention and post-intervention with low intensity laser were used according to Lucas et al.<sup>11</sup> The power of the test was 95% and the alpha level less than 5% ( $\alpha < 0.05$ ). The calculation was performed using the G \* Power program, which indicated 24 patients, with the expected loss of sample.

### Subjects

Twenty-four adult patients, mean age of both genders, with PI, stage III and / or IV, admitted to the Mandaqui Hospital Complex (MHC) were recruited. All patients who met the inclusion criteria were informed about the research objectives and after reading the Informed Consent Form (ICF) to the patient or to their legal guardian, they were invited to participate in the research and to voluntarily sign the consent form. ICF. The detection of patients with PI in MHC will be performed through active search, with weekly visits to the hospitalization units (medical and surgical clinics).

### Inclusion criteria

Patients older than 18 years of age, of both sexes with PI admitted to CHM; Patients with IP in stages III and IV; Patients without specific topical antibiotic coverage for treatment of infection in the pressure lesion; Patients who had expected hospitalization equal to or greater than 7 days; Patients who met the criteria for inclusion, aware of the research objectives and who agreed to sign the free and informed consent form from their own hand or their legal representative.

### Exclusion criteria

Patients with PI in stage I, II, ulcer not classifiable or with necrosis; Patients younger than 18 years; Patients with pathologies or health states that make it impossible to apply laser therapy, such as Carriers of thrombocytopenia and / or any coagulation disorder.

Epilepsy patients; Tumor malignancy localized or irradiated; Pregnant or lactating; History of highhypersensitivity. Patients with topical antibiotic therapy in PI.

### Clinical evaluation of PI

The evaluation of the pressure lesions included the anatomical location and the differentiation of the lesions in stages was performed in a qualitative way according to the classification proposed by the NPUAP [2]: Table 1.

### Randomization

Patients who were eligible to participate were randomized to the intervention group with 53 Joules of therapy or 106 Joules or longer for the third group irradiated for 159 Joules following a list of randomizations of 4 balanced blocks maintained in opaque sequentially sealed envelopes.

Randomization was generated with the online randomization.

The use of Blue Light in the intervention will be evident both for the participant and for the health professionals involved in the

treatment of their pressure ulcer. The primary outcome will, however, be confirmed by blind observer groups for randomization and all other outcomes will be analyzed by blinded groups in the randomized

(53, 106, and 159 joules) experimental group. Blinding occurred in relation to the microbiological count of colony forming units.

**Table 1** Classification pressure injury staging proposed by the NPUAP

<b>Stage 1</b>	Unbleached erythema of intact skin: Intact skin with a localized area of unbleached erythema, which may appear differently on dark pigmented skin.
<b>Stage 2</b>	Partial thickness loss of skin with exposed dermis: Loss of partial thickness of skin with exposed dermis. The wound bed is viable, pink or red, moist, and may present as a bubble filled with intact or ruptured serum.
<b>Stage 3</b>	Loss of Total Skin Thickness: Total loss of skin thickness, where fat is visible on the ulcer with granulation tissue and epiploic (edges of wound wraps) are often present.
<b>Stage 4</b>	Total loss of skin and tissue thickness: Total loss of skin thickness and tissues with exposed or directly palpable fascia, muscle, tendon, ligament, cartilage or bone in the ulcer. Eschar may be visible.
<b>Unclassifiable pressure injury</b>	Complete loss of skin and tissue thickness: Loss of skin and tissue at full thickness in which the extent of tissue damage within the ulcer cannot be confirmed because it is obscured by crushing or scarring.
<b>Deep tissue pressure injury</b>	Persistent and non-bleachable discoloration in dark red, brown or purple: Skin intact or not with localized area of non-bleach persistent discoloration in dark red, brown, purple or with epidermal separation, revealing a dark tissue or a bubble full of blood.

## Instruments for data collection

For the initial and final evaluations were used the questionnaires: Multidimensional: questionnaire prepared by the responsible researchers characterized by socio-demographic clinical questions. Sex, weight, height, age, marital status, length of hospital stay, schooling, associated conditions, medications in use, therapeutic regimen and dosage. Characteristics of the lesion: Location, Staging and characteristics of the tissue, Dimensions. Measurements will be made by direct measurement of the lesion. The Ulcer Score Healing - PUSH, which considers three parameters for evaluation of the wound healing process and intervention results: The wound area, related to the largest length versus the largest width; The amount of exudate present in the wound, evaluated after the removal of the cover and before the application of any topical agent; and the appearance of the wound bed, defined as the type of tissue prevalent in this region.

## Phototherapy equipment

We used a Cosmedical LED plate Cicatrilux® (Figure 2); containing 36 LEDs, 420 +/- 20 nm, optical diameter 10 mm +/- 2, optical output 2-5 mW, device energy 106J, radiant exposure 3.8 J / cm<sup>2</sup>, irradiance 6.4 mW / cm<sup>2</sup>.



**Figure 2** LED equipment used for irradiation of lesions.

## Intervention

After removal of the dressing, the lesions were cleaned by irrigation of sterile saline, following the standard PI cleaning procedure determined by the dressing team, which uses only a jet of physiological solution, without any contact of the lesion with any material. The PIs were irradiated for three consecutive days in the same period, that is, every 24 hours, with the predetermined dose of 420 nm (+/- 20 nm) LEDs and with energy density varying with time, as follows: Group 1 - 53 Joules - 1.9 J / cm<sup>2</sup>; Group 2 - 106 Joules - 3.8 J / cm<sup>2</sup>; Group 3 - 159 Joules - 5.7 J / cm<sup>2</sup>. The LED plate was wrapped in clear acetate film prior to application to the lesion. After the phototherapy procedure, the microbiological samples were collected, and the PI was covered with non-adherent membrane (Membracel® Monlink).

All patients in the study, after three days of the experiment, continued to treat the lesions using the conventional method used at the institution and determined by the Group of Dressings. For the decontamination of the lesions proposed in this study, three phototherapy sessions with 420 nm LEDs (+/-20 nm) were performed on three consecutive days. Before and after each application, a microbiological sample of the lesion was collected, and the plates were prepared for culture.

## Procedure of collection and analysis of the microbiological material obtained from the lesions

The collection of material for microbiological examination was performed before and after each application in the 3 consecutive sessions of blue LED. The collection was done after the lesion was cleaned with 0.9% saline solution, using sterile Swab and then pressing it and rotating it in 1 cm<sup>2</sup> of the injured area for 5 seconds to have tissue fluid expression.<sup>12,13</sup> The material collected at the tip of the Swab with +/- 5 cm of the stem (cut with sterile scissors) was placed in a glass tube (small test tube) containing 2 ml Brain Heart Infusion Broth (BHI) medium maintained between - 1 at 4° C where the collected material was inoculated in plates with nutrient agar and incubated in an oven for 24 hours at 35° C ± 2° C.

## Sample preparation procedure for culture

The samples collected before and after the phototherapy were placed at room temperature and homogenized in Vortex for 30 seconds. In the laminar flow, aliquots of 20  $\mu$ l of this solution were pipetted into a 96-well plate containing 180  $\mu$ l of Bovine Fetal Serum (PBS) - KASVI Lot 111216513 to compose the dilution criteria used in the statistical calculation. Diluted serially to set dilutions 10<sup>-1</sup> to 10<sup>-5</sup> times the original concentration. 10  $\mu$ l aliquots were drained in triplicate in demarcated Petri dishes containing Nutrient agar - KASVI Lot 012117504. The procedure was repeated for the sample collected after phototherapy. Subsequently they were incubated at 37°C for 24 hours, a period that preceded the count of CFU / ml. After 24h, the plates were read and the CFUs counted by diluting the stretch mark. The data obtained were tabulated in Microsoft Excel Software for later statistical analysis.

## Primary and secondary outcome variables

**Primary outcome:** variable was the difference between groups for the mean number of total CFUs in all plates (all locations).

**Secondary outcome:** were evaluated by the WSA and pressure ulcer scale for healing (PUSH) tool. The PUSH tool<sup>2</sup> was developed by NPUAP as a quick, reliable tool to monitor the change in pressure ulcer status. It categorized ulcers with respect to surface area, exudate, and type of wound tissue.

## Statistical analysis

The data was found to be normally distributed (using a Shapiro-Wilk test), and ANOVA two-way with Bonferroni post hoc test was used for comparisons between each group. All data are expressed as the mean  $\pm$  standard deviation (SD). The GraphPad Prism 5 software

program (GraphPad Software, San Diego, CA, USA) was used, and  $P < 0.05$  was considered to indicate a significant difference. The calculation of sample power using the G \* Power software and found 80% power for all outcomes analyzed.

## Results

Thirty-six volunteer subjects were evaluated to obtain the calculated sample size ( $n = 24$ ). All participants were evaluated according to the classification proposed by the NPUAP, (Figure 1). When this number of participants meeting the inclusion and exclusion criteria was reached, the enrollment process was terminated, and the subjects were randomized into one of three experimental groups. A total of 12 individuals were not eligible because they did not meet all the inclusion criteria. They reported no adverse effects of photobiomodulation at any stage of the experiment. Demographics and baseline characteristics of the population subjects are shown in Table 2.

Regarding the staging of the lesions, the pressure lesions in stage 3 and 4 were eligible for the study, and 54.2% were stage 3, to determine the severity of the lesions, were also evaluated using the PUSH - Pressure Ulcer Score Healing. Most of the lesions (41.7%) had scores  $> 16$ , which means extensive and deep lesions. The highest incidence of PI was in the sacral region, 79 % (Table 3).

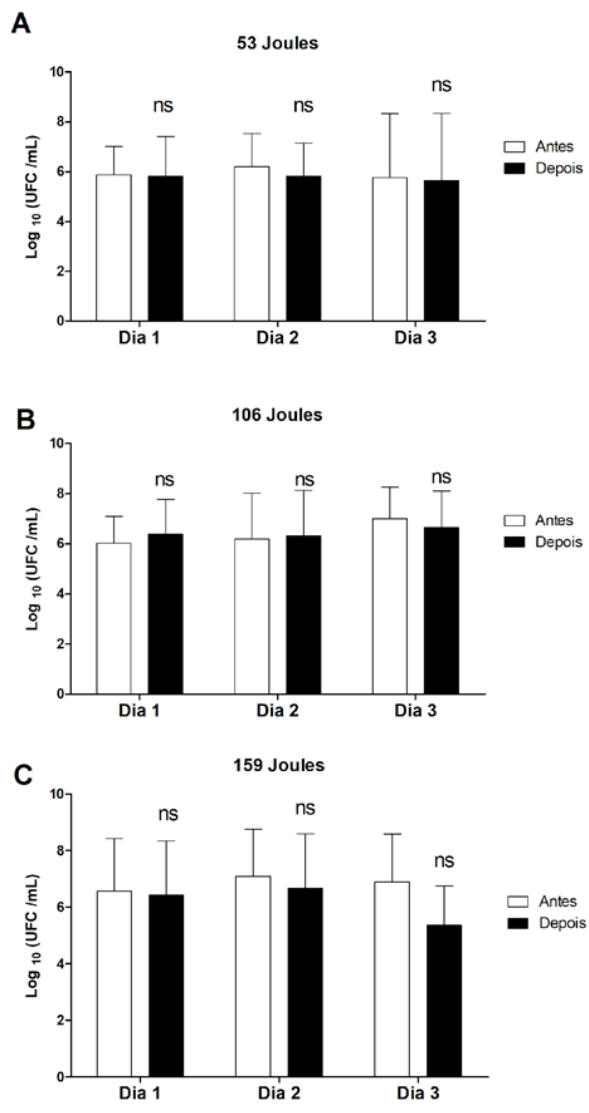
As for the photobiomodulation protocol, the counts of the Colony Forming Units (CFU) were analyzed in each of the three days of application, for the three groups. Considering that the individual is self-control with no interaction between groups of 53 Joules, 106 Joules min, and 159 Joules, therefore, two-way ANOVA with Bonferroni post hoc test was used, no intra-group statistical difference was observed ( $p < 0.05$ ) or intergroup (Figure 3 & 4).

**Table 2** Distribution of study participants according to gender, age, race and body mass index - BMI. ( $n=24$ )

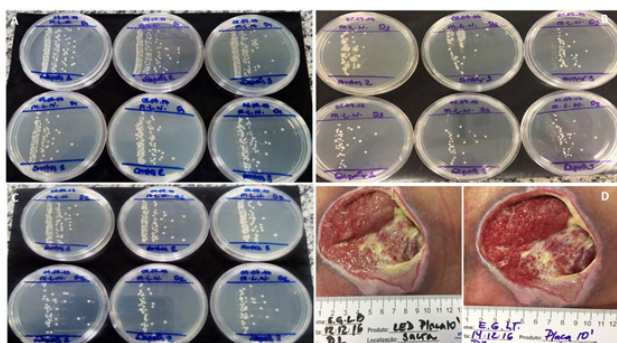
Variable	n (%)	Mean (SD)	Median range
Gender (Female / Male)	17 = (70,80%) -7=(29,20 %)	-	-
Age Year	-	72,4 $\pm$ 16,30	75,50
Ethnicity (White/Black)	17 = (70,80%) -7 = (29,20 %)	-	-
BMI Kg/m <sup>2</sup>	-	21,8 $\pm$ 5,71	21,99
Basic Pathology			
- Neurological	18 (75,00%)	-	-
- Cardiovascular	2 (8,30%)	-	-
- Hepatic	1 (4,20%)	-	-
- Orthopedic	1 (4,20%)	-	-
- Dermatological	1 (4,20%)	-	-
- Oncology	1 (4,20%)	-	-

**Table 3** Characterization of the lesion by pressure of the participants according to the stage of the injury and PUSH score ( $n = 24$ )

Variable	Score	N (%)
Stage pressure injury	3	13 (54,20%)
	4	11 (45,8%)
PUSH	16	10 (41,70%)
-	14	5 (20,80%)
-	15	4 (16,70%)
-	12	3 (12,50%)
-	11	1 (4,20%)
-	13	1 (4,20%)



**Figure 3** Mean and standard deviation of the CFU collected from the pressure lesions before and after the application of LEDs in the lesions: In (A) chart referring to 53 Joules by 5 minutes, In (B) chart referring to 106 Joules by 10 minutes and In (C) to 159 joules by 15 minutes (ns p> 0.05).



**Figure 4** Photographs of cultured plates, referring to one of the lesions submitted to irradiation by LED for 5 minutes. Panel A for day 1, day 2 and day 3, plates (A, B and C), respectively. In (D) Pressure injury before and after.

## Discussion

The objective of the present study was to analyze the antimicrobial effect of light on violet-blue wavelength (420 nm) in pressure lesions in stages III and IV. In the characterization of the sample, an average age of 72.4 years was found, predominantly female. The location of the lesions was mostly (79.2%) in the sacral region. The underlying disease of most patients (75%) was neurological and all of them were bedridden and diapered. In the analysis of the photobiomodulation in the three groups of the study there was no statistical difference in the dosimetry studied, 53J, 106J or 159J, respectively.

The increase in the incidence of pressure injuries in hospitalized patients, whether in a hospital environment or in institutions for the elderly, is directly related to the greater number of risk factors for this type of injury in this population, among them the reduction of mobility, age, presence of urinary and / or fecal incontinence, neurological disease, corroborate an incidence in Brazil of around 10% to 62% and worldwide, varying between 3.2% and 39%.<sup>14-16</sup>

In the present study, the PUSH score was quite high, being above 15 (score pressure ulcer score healing) in 79.2% of the lesions, demonstrating that the lesions were extensive and severe. This is because, the PUSH instrument considers three parameters of the lesion to analyze the healing process and the result of interventions in the wound bed, area (length x width), quantity of exudate and analysis of the wound bed by means of the type of classification of tissue predominant in the lesion.<sup>17</sup>

Managing bacterial burden is an important consideration in the care of PI; all contain a variety of bacteria. Bacterial contamination of PI should not be detrimental to patient health, but when a colony of bacteria reaches 10<sup>5</sup> or 10<sup>6</sup> organisms per gram in the lesion, it can be considered infected, and healing can be prevented when the lesion has high levels of bacteria.<sup>18</sup>

PI are often responsible for an increase in hospitalization time, especially for the infection associated with this type of injury, which leads to the use of antibiotic therapy. In a recently presented cohort study, with follow-up of patients in a teaching hospital for more than 30 years (January 1984 to December 2015) who presented with PI-associated bacteremia, 56 cases of this type were observed, with microorganisms with higher incidence in blood cultures and lesion cultures were: *Staphylococcus aureus* and *Proteus* spp, both appearing around 60% in blood cultures and more than 80% in PI cultures. In addition, a higher incidence of infected PI located in the sacral region was observed in 80% and 95% of the cases had PI in stages III and IV, demonstrating that the location and severity of the lesions are related to the occurrence of events and / or complications of local infection, Espejo E. et al<sup>19</sup> as in the present study.

PI can constitute an important reservoir of multiresistant microorganisms and are frequently colonized by several bacterial species. When they are grown on the surface, a polymicrobial culture is found, which includes Gram-positive and Gram-negative aerobic flora and species anaerobic. Several studies have shown that the presence of a chronic wound is an independent risk factor for persistent colonization of multiresistant microorganisms even after hospital discharge from Enterobacteriaceae, followed by *Staphylococcus aureus* and Gram-negative bacilli, mainly *Pseudomonas aeruginosa* and *Acinetobacter* spp, were found and in colonizations with multiresistant bacteria were found from *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter* among others.<sup>20-22</sup>

In the present study, the photobiomodulation was applied with the objective of reducing CFU by applying three consecutive days of

violet-blue light at 420 nm. Several studies have demonstrated the potential of light to inactivate pathogens.<sup>8,9,23-29</sup> However, clinical studies evaluating the effects of light on bacterial inactivation without the use of exogenous photosensitizers are scarce. It is widely reported that red or infrared light promotes tissue repair, while treatment with violet-blue light is known as antimicrobial. Therefore, infected lesions could benefit from combined red-violet-blue and red / infrared light therapy, however there may be a concern that violet-blue light may delay healing, studies in this regard demonstrate that blue-violet light produces an anti-inflammatory effect, through the reduction of IL-6 and increases the synthesis of proteins by fibroblasts.<sup>30</sup> In our study, although the anti-inflammatory effect of the applied photobiomodulation was not analyzed, it can be observed through the analysis of lesion images before and after treatment.

Some experimental studies point out that the use of violet-blue light, visible light length between 400-420 nm, peaking at 405 nm, for microbial inactivation would eliminate the need for exogenous photosensitizers. This is because the effectiveness of the photodynamic inactivation is not only dependent on the photosensitizer and its concentration, but also the dose, flow rate and light source combined with environmental and chemical conditions conducive to inactivation.<sup>31,32</sup>

The use of violet-blue light for bacterial inactivation has gained strength with studies on the treatment of Acne Vulgaris, several studies conducted to investigate the effects of violet-blue light on the treatment of this pathology demonstrate that the effect is based on observation that light at this wavelength exhibits a selective cytotoxic effect on the bacterium responsible for acne, through the excitation of bacterial porphyrins, which induces the production of singlet oxygen and reactive radicals leading to bacterial death. Despite having, limited penetration in the skin, violet-blue light is the one that would react better with porphyrins, which has now led to combined treatments between red light with its anti-inflammatory effect with violet-blue, bactericidal light, combining the treatment with the red-blue light.<sup>33-35</sup>

Regarding the type of light used, in a recent study comparing identical parameters of LED and Laser at 405 nm, there was no statistically significant difference when both were applied to evaluate the antimicrobial effect in cultures containing staphylococci resistant to metacycline, irrespective of the device used, LED or laser irradiation at each creep resulted in suppression of statistically significant bacterial growth compared to non-irradiated controls ( $p < 0.0001$ ). Regarding the exposure time, the cultures were irradiated for 15 and 30 minutes with fluence of 40, 54, 81 or 121 J / cm<sup>2</sup>.<sup>36</sup> In clinical practice, there is a limitation about very long exposure times.

Several in vitro and experimental studies have demonstrated the positive effects of violet-blue light on the inactivation of pathogens, presenting numerous advantages over conventional anti-infective agents, mainly because it is relatively safe in the application in several aspects and because there are no reports of development of resistance in anti-infective light-based microbial cells.<sup>8,22,23,33,34,37-40</sup> However, most studies do not report the exact dosimetry applied or present a time of exposure to very high violet-blue light, which makes it very difficult to translate the parameters of their experiments for the clinical application of photobiomodulation with violet-blue.

In the present study, three parameters of application of violet-blue light in the pressure lesions were used, by 53J, 106J and 159J. These parameters were chosen based on the model of device used, which uses as a standard dose 106 Joules in 10 minutes of exposure, being reduced to half the dose, 53 Joules in 5 minutes and 50%

of the standard dose plus 159 Joules in 15 minutes, thus forming the treatment groups studied. The scarcity of clinical studies investigating the effects of violet-blue light on the inactivation of pathogens in PI, therefore, led to the impossibility of comparing the dosimetry, and its effects with the results found here in this study.

The results found regarding the reduction of CFUs differ from the experimental studies reported in the literature and discussed here, as we observed a slight reduction in the CFU count, especially in the group with 159 J of treatment (Figure 3), but with a standard deviation rather accentuated. Therefore, in microbiological terms we cannot affirm that there was bacterial inactivation, nor an expected reduction of at least 75% in colon count.

Therefore, the use of light at wavelength 405 to 420 nm, blue spectrum, without the use of exogenous photosensitizers, although widely studied experimentally as an alternative therapy to conventional antimicrobial methods and having several experiments demonstrating that it actually reduces and even eliminates microorganisms, including those resistant to antibiotics, in the present study, it was observed that the violet-blue light, within the parameters used, was not shown to be effective for elimination of the colonies, when applied directly to the pressure lesions of the studied population.

## Limitations of the study

The doses studied between 53 and 159J; the localization of lesions, predominantly sacral; and the fact that the lesion patients are bedridden and present with urinary and / or fecal incontinence may have been determinant for the result. Therefore, we believe that there is a need for further investigations regarding the type of lesion studied; extension of the times, which are clinically feasible, of exposure of the lesion to light; amplification of the frequency of application of the photobiomodulation and use of different types of light. Therefore, other clinical studies are required for the results compared.

## Conclusion

The results obtained provide the first clinical evidence of the application of 420 nm wavelength for decontamination of pressure injury, without the use of exogenous photosensitizers. The patients' characterization showed a predominance of female patients with sacral lesions and with neurological pathologies, which determined the presence of urinary and fecal incontinence in many patients in the study. The severity of the lesions was demonstrated by the analysis of the PUSH score, which was quite high, being above 15 in most of the lesions. These factors were relevant to the result, where the CFU count was similar in the three doses studied (53J, 106J and 159J), which did not represent statistical relevance, which shows that, for the study population, there was no decontamination of the lesions by means of photobiomodulation with violet-blue light.

## Acknowledgments

None.

## Conflicts of interests

The authors declare no conflicts of interest.

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