

# The effects of low level laser radiation on bacterial growth

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**Objective:** The low level lasers currently in the market vary in wavelength, dosage, and frequency. These devices are used with much different clinical pathology. Most notably, some studies claim that wounds heal faster with low level laser therapy due to the fact that bacteria commonly found in wounds are killed by laser light. Systemic and meta-analysis studies found the difficulty of comparison of numerous research studies because of differences in the intensities and frequencies of low level laser treatment (LLLT). The purpose of this study was to determine the effectiveness of LLLT on controlling bacterial growth.

**Design:** Cross-sectional study.

**Methods:** Variables included LLLT dosage and wavelength on 3 bacteria commonly seen in wounds, strains of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were used on commercially available 5.0-cm agar plates. Blue, green, and red, ultraviolet (UV) and infrared laser light sources were adjusted to either low or high intensity settings. Five Petri dishes at a time were placed directly beneath laser light sources with the exception of UV which was placed six inches below the suspended light and infrared which was placed directly on top of the Petri dish lid. Each group of five Petri dishes was irradiated for 15 minutes.

**Results:** The results showed no effect of any of 9 different LLLT intensities or colors on bacteria growth compared to sham light.

**Conclusions:** At least for claims of bacterial growth inhibition with LLLT, no support for this claim can be found here.

**Key Words:** Bacteria, Light, Low level laser, Wounds

## Introduction

Low level lasers have been used for decades for the treatment of a wide range of medical conditions including soft tissue injuries, musculoskeletal disorders, and wound healing [1]. Although low level laser treatment (LLLT) has been widely used, many research studies have shown little or no benefit of LLLT. The physiological mechanism of low level laser is poorly understood, and treatment parameters such as intensity, frequency, wavelength, and dosage are uncertain as well [2]. Even though the effectiveness of LLLT has not been proven, many practitioners continue to use LLLT to treat many conditions such as Carpal Tunnel Syndrome (CTS), musculoskeletal pain, inflammatory disease, venous

leg ulcers, and decubitus ulcers [3].

Naeser *et al.* [4] performed a randomized, double-blind, cross-over study of LLLT. The study population consisted of eleven patients with mild to moderate CTS. Patients were randomly assigned to receive nine to 12 sessions of active or sham LLLT and transcutaneous electrical nerve stimulation (TENS) treatment. The results showed that seven of the remaining eight subjects reported pain scores reduced by more than 50% post active LLLT and TENS treatment. All 11 subjects reported that they resumed their previous work activities with little to no pain.

The population of this study was small (n=11), and TENS was used with LLLT and therefore, the results of the study do not show an effect of LLLT alone [5].

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Irvine conducted a double blind randomized controlled trial of LLLT in August 2004. Fifteen CTS patients, 34 to 67 years of age, were randomly assigned to either the control group (n=8) or treatment group (n=7). Both groups were treated three times per week for five weeks. For this study 860 nm lasers were applied at a dosage of 6 J/cm<sup>2</sup> to the treatment group over the carpal tunnel, and those in the control group were treated with sham laser. After completion of treatment, there was no significant difference in any of the outcome measures between the two groups [6].

Gam *et al.* [2] conducted a meta-analysis of 23 trials on LLLT for musculoskeletal pain. The mean difference between treatment and placebo on a pain visual analogous scale was 0.3% indicating no effect of LLLT on pain in musculoskeletal syndromes. The differences were not weighted by the sample because trials varied in the musculoskeletal diseases treated, LLLT dose, laser type, and wavelength.

Mulcahy *et al.* [7] conducted a randomized double-blind placebo controlled trial to study the effect of LLLT on localized, painful, soft tissue conditions. Twenty-three patients were randomized to active or placebo groups. LLLT with an intensity of 35 mW was applied two times per week for four weeks to the treatment group. The results showed that 87% of the placebo felt their pain had improved compared to 42% of the active group.

Flemming and Cullum [8] reviewed four randomized controlled trials to determine the effect of LLLT on venous leg ulcers. Two studies compared LLLT with placebo treatment. One study compared LLLT with ultraviolet (UV) therapy. The fourth study compared the effect of three treatments including LLLT, LLLT plus infrared light therapy, and non-coherent unpolarized red light therapy. Flemming and Cullum [8] did not find any evidence of the benefit of LLLT on venous leg ulcer healing.

Lucas *et al.* [9] conducted a randomized observer-blind trial in three nursing homes to assess the effect of LLLT on decubitus ulcers. Eighty-four subjects with Stage III decubitus ulcers participated in the study. There were 47 subjects in the control group and 39 in LLLT group. The control group received standard wound treatments including patient instruction, wound cleansing, moist dressings, and frequent alteration of position. Standard wound treatments were applied daily for six weeks. The LLLT group received standard treatment and LLLT. The 904 nm lasers with an average beam power of 12×8 mW were applied five times a week for six weeks. This study did not find any significant differences between the groups.

Saltmarche [10] in 2012, found significant improvement in 61% of wounds with LLLT. But there were several types of wounds and modalities for treatment were mixed. They felt that LLT killed common bacteria and there allowed for faster healing of wounds.

Thus there is confusion in the literature, especially on the healing of wounds with lasers. To start more simply, here we used laser exposure of bacteria to different frequencies to see if the claim as to lasers reducing simple bacterial growth are valid. Further, the bacteria used the most common 3 found in human wounds making this relevant to wound treatment. By using different intensities of light and different frequencies, we designed the study to see if any of these 3 bacteria would show reduced growth when exposed directly to laser light.

## Methods

### Bacteria culture

Strains of *Staphylococcus aureus* (ATCC# 49444), *Escherichia coli* (ATCC# 25922), *Pseudomonas aeruginosa* (ATCC# 35032), were purchased from Microbiologics (St. Cloud, MN, USA). Commercially available 5.0-cm agar plates were used in this study for optimal distribution of laser light irradiation over the plate surface [11,12]. The agar plates were prepared and purchased from Hardy Diagnostics. Nutrient broth was purchased from the Carolina Biological Supply Company. To prepare the nutrient broth, 0.8 g of tryptic soy broth was added to saline to make 100 ml. One ml of *P. aeruginosa* culture was transferred to a sterile test tube with nine ml of sterile 0.9 % NaCl saline to create a 10<sup>-6</sup> dilution of each bacterial culture. The Petri dishes were gently agitated for uniform distribution over the agar. This method was repeated for *S. aureus* and *E. coli* bacterial cultures.

### Laser parameters

An adjustable stand was used to stabilize the lasers directly on top of the Petri dish for an optimal angle of irradiation. The lasers were adjusted one inch over the Petri dish for high level intensity and six inches over the Petri dish for low level intensity. Irradiation of Petri dishes took place in a dark room at room temperature (37°C). Six different wavelengths of light were used (Unitech Systems Inc./LC LED Inc., Brooklyn, NY, USA). A light meter was used to read the output for each laser light. The lasers utilized were: red (630 nm), infrared (904 nm), green (525 nm), blue (465

nm), and UV (350 nm).

Commercially purchased cold lasers commonly use watts as a measure of intensity. A watt is a poor measure to determine the brightness of a laser. It refers to how much electrical power goes into the laser. However, lux is a measure of how much light is reaching a particular location. Therefore, lux was used as a light measure in this study. Lasers were calibrated to the following intensities (Table 1).

### Storage and counting

All bacteria were stored in a Forma Scientific incubator at 37.4°C. *S. aureus* and *E. coli* colonies were counted using a bacteria counter (Hardy Diagnostics) *P. aeruginosa* was counted as a percentage of growth covering each Petri dish using a grid (Bel-Art Products, Wayne, NJ, USA). One cm<sup>2</sup> squares were printed onto transparencies. The 5-cm diameter lids were traced onto the grids and were then placed on top of each Petri dish to trace the bacterial growth with a marker. The number of squares covered with growth was counted and divided by the total area of the Petri dish to determine percentage growth. The average of four trials was taken and represented in a Table. Reliability of colony count data was performed through using a SD method.

### Procedures

Forty four Petri dishes were labeled with the bacterial species, dilution, date, and the intended laser light source to be

irradiated. Fifty  $\mu$ l of *P. aeruginosa* at 10<sup>-6</sup> dilution was distributed into each of these dishes using a 100- $\mu$ l pipette. Dishes were gently agitated immediately after distribution and covered with their respective lids.

Blue, green, and red laser light sources were adjusted to low intensity settings. Five Petri dishes at a time were placed directly beneath laser light sources with the exception of UV which was placed six inches below the suspended light and infrared which was placed directly on top of the Petri dish lid. Each group of five Petri dishes was irradiated for 15 minutes. After irradiation, the Petri dishes were immediately inverted and placed into an incubator for 24 hours. This procedure was repeated three times.

Blue, green, and red laser light sources were then adjusted to high intensity settings and the UV light was adjusted to one inch above Petri dish height. Groups of four Petri dishes at a time were then irradiated for 15 minute-intervals. Immediately following irradiation, these Petri dishes were also inverted and placed into an incubator for exactly 24 hours. Four shams were also placed in the incubator for exactly 24 hours. These preceding procedural steps were repeated for *S. aureus* and *E. coli*.

After incubation, results were recorded as a percentage of growth of *P. aeruginosa* over the Petri dishes. For *S. aureus* and *E. coli*, single bacterial colonies were counted. These results were entered into an Excel spreadsheet and analyzed for significant data and SD.

See the chart below for all Petri dishes irradiated with their corresponding laser light sources (Table 2).

### Data analysis

Data analysis was accomplished by calculating means and SD. ANOVA was conducted to compare mean percentage of growth among nine different colors of light and sham groups. LSD pairwise comparisons test for multiple comparisons was used to compare means of variables between any two different groups. The level of significance was  $p < 0.05$ .

**Table 1.** Laser types and intensities utilized

Laser	Intensity (lux)
Infrared	5,000
Low red	5,000
High red	240,000
Low blue	5,000
High blue	230,000
Low green	5,000
High green	16,000
Low ultraviolet	5,000
High ultraviolet	100,000

**Table 2.** Irradiation schedule for bacteria exposure to laser light

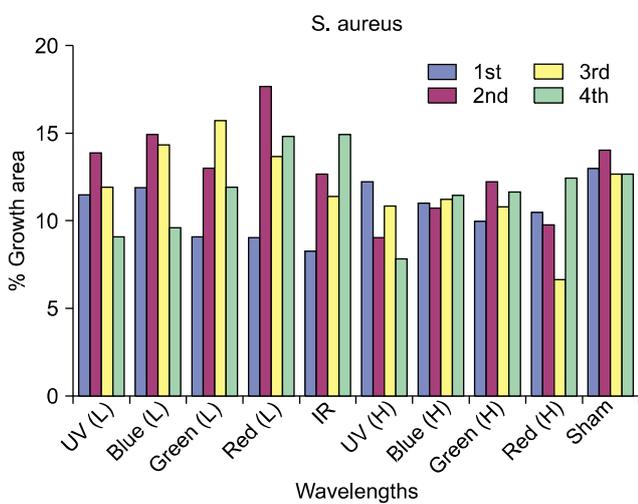
(unit: lux)

	IR	Low red	Low blue	Low green	Low UV	High red	High blue	High green	High UV
<i>Escherichia coli</i> ( $\times 3$ trials)	5,000	5,000	5,000	5,000	5,000	240,000	230,000	16,000	100,000
<i>Pseudomonas aeruginosa</i> ( $\times 3$ trials)	5,000	5,000	5,000	5,000	5,000	240,000	230,000	16,000	100,000
<i>Staphylococcus aureus</i> ( $\times 3$ trials)	5,000	5,000	5,000	5,000	5,000	240,000	230,000	16,000	100,000

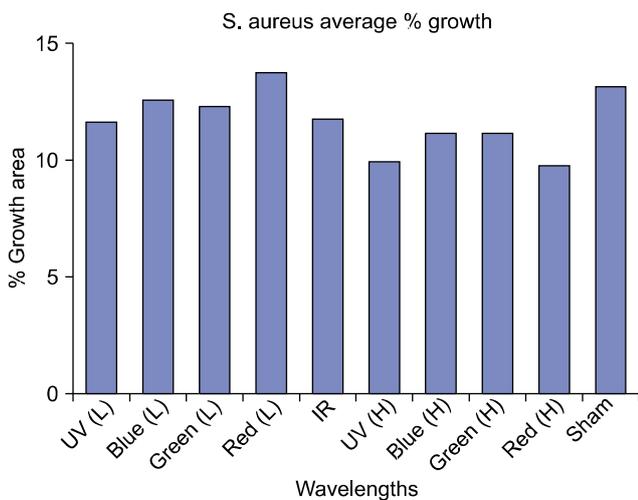
IR: infrared, UV: ultraviolet.

**Results**

*S. aureus* showed no significant difference in growth when irradiated with the nine different colors of light and two different laser intensities in comparison to the shams. Figure 1 shows four trials of *S. aureus*, and the percentage of growth areas after exposure to nine different light sources including the sham. Figure 2 is an average of the four trials for *S. aureus*. Statistical differences were not shown among the averages of exposed groups of bacteria when compared to the sham. Overall results are summarized in Table 3.



**Figure 1.** *Staphylococcus aureus* percentage of growth per four trials exposed to nine different sources of light. UV: ultraviolet, IR: infrared, (L): low, (H): high.



**Figure 2.** *Staphylococcus aureus* average percentage of growth among all four trials. UV: ultraviolet, IR: infrared, (L): low, (H): high.

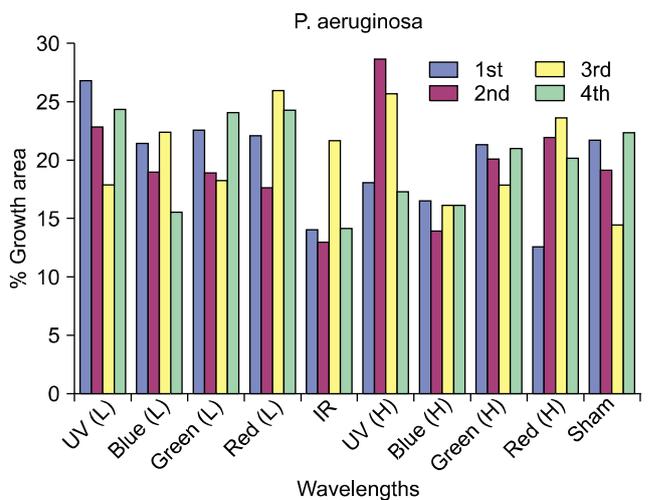
*P. aeruginosa* also showed no significant difference in growth when irradiated with the nine different light colors and two different laser intensities in comparison to the shams. Figure 3 shows four trials of *P. aeruginosa*, and the percentage of growth areas after exposure to nine different light sources including the sham. Figure 4 is an average of the four trials for *P. aeruginosa*. Although there was a trend for infrared (IR) and Blue (H) lasers to decrease bacterial growth, there were not statistical differences when compared to the average growth of the sham plates. Statistical differences were not shown among the averages of exposed groups of bacteria when compared to the shams. Overall results are summarized in Table 4.

Lastly, *E. coli* showed no significant difference in growth when irradiated with the nine different light colors and two

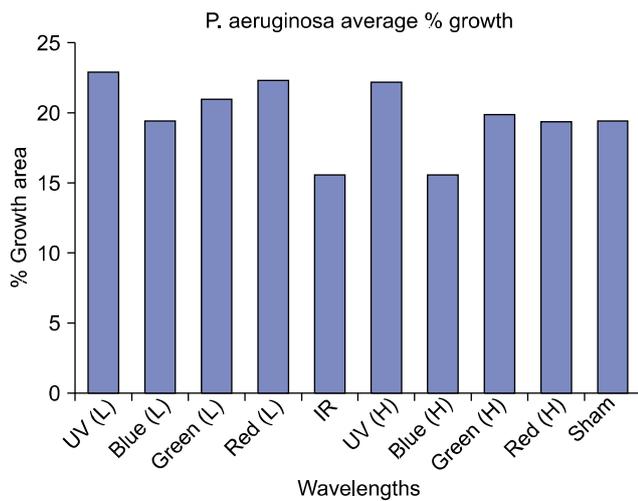
**Table 3.** *Staphylococcus aureus* summary of results

Group	Count	Sum	Average	Variance
Low UV	4	46.41	11.60	3.97
Low blue	4	50.87	12.72	6.38
Low green	4	49.53	12.38	7.08
Low red	4	55.2	13.80	13.29
IR	4	47.03	11.76	7.59
High UV	4	40.02	10.01	3.76
High blue	4	44.61	11.15	0.09
High green	4	44.63	11.16	0.97
High red	4	39.24	9.81	5.52
Sham	4	52.51	13.13	0.37

UV: ultraviolet, IR: infrared.



**Figure 3.** *Pseudomonas aeruginosa* percentage of growth per four trials exposed to nine different sources of light. UV: ultraviolet, IR: infrared, (L): low, (H): high.

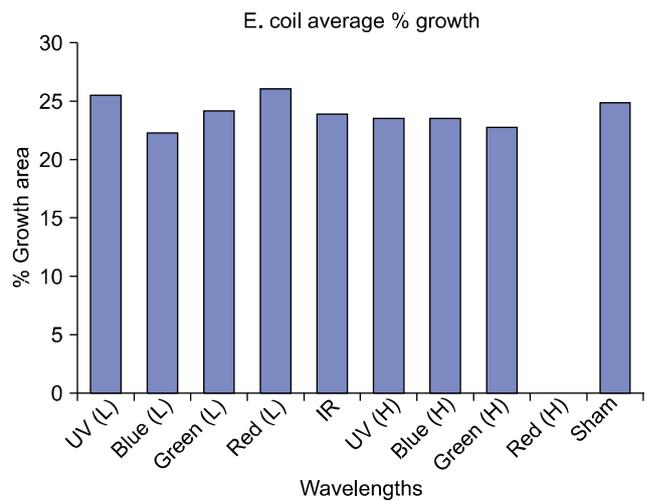


**Figure 4.** *Pseudomonas aeruginosa* average percentage of growth among all four trials. UV: ultraviolet, IR: infrared, (L): low, (H): high.

**Table 4.** *Pseudomonas aeruginosa* summary of results

Group	Count	Sum	Average	Variance
Low UV	4	92.5	23.13	13.35
Low blue	4	78.25	19.56	9.43
Low green	4	84.38	21.09	7.68
Low red	4	89.88	22.47	13.82
IR	4	62.63	15.66	15.43
High UV	4	89.38	22.349	30.54
High blue	4	62.75	15.69	1.31
High green	4	80.00	20.00	2.54
High red	4	78.00	19.50	23.83
Sham	4	77.75	19.44	12.56

UV: ultraviolet, IR: infrared.

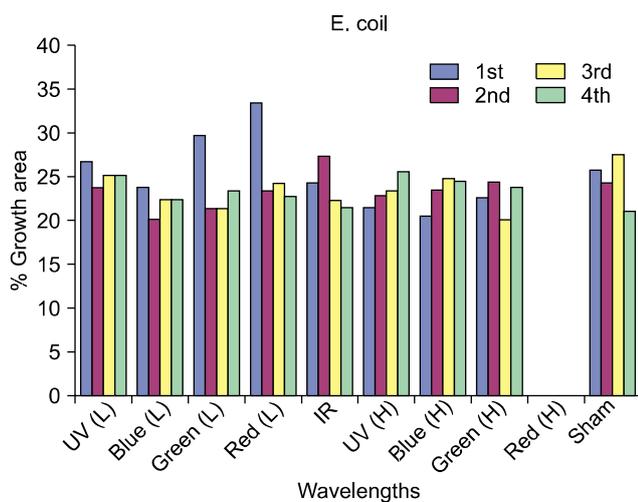


**Figure 6.** *Escherichia coli* average percentage of growth among all four trials. UV: ultraviolet, IR: infrared, (L): low, (H): high.

**Table 5.** *Escherichia coli* summary of results

Group	Count	Sum	Average	Variance
Low UV	4	100.83	25.21	1.96
Low blue	4	88.48	22.12	2.25
Low green	4	95.99	24.00	15.32
Low red	4	103.89	25.97	26.53
IR	4	94.92	23.73	6.53
High UV	4	93.12	23.28	2.65
High blue	4	93.14	23.29	3.85
High green	4	90.62	22.66	3.53
High red	4	0.00	0.00	0.00
Sham	4	98.15	24.54	7.64

UV: ultraviolet, IR: infrared.



**Figure 5.** *Escherichia coli* percentage of growth per four trials exposed to nine different sources of light. UV: ultraviolet, IR: infrared, (L): low, (H): high.

different laser intensities in comparison to the shams. The following Figure 5 shows four trials of *E. coli*, and the percentage of growth areas after exposure to nine different light sources including the sham. Figure 6 is an average of the four trials for *E. coli*. During the first trial of exposure to the Red (H) laser, the light source malfunctioned, resulting in a lack of data for those four trials. Statistical differences were not indicated among the averages of exposed groups of bacteria when compared to the shams. Overall results are summarized in Table 5.

### Discussion

Healthcare professionals have used LLLT for a wide range of conditions [3,8,13-16]. However, the clinical effectiveness of LLLT has not been proven. Mechanism and treatment parameters of LLLT have not been properly estab-

lished in previous studies. Many research studies show that LLLT has no effect on conditions claimed by manufacturers. Some show that LLLT is less effective than placebo treatments. Although some claim that LLLT is effective for certain conditions such as CTS, most of these research studies are poorly designed or have no control group [17].

Biostimulation theory is one hypothesis that has explained the mechanism of LLLT in recent studies. This theory states that LLLT will cause a biostimulation effect on target human tissues via non-thermal low intensity irradiation of laser light [18]. However, commercially certified LLLT products in United States are classified as infrared lamps, which are thermal-type devices [13,16,19]. Therefore, several current indications for LLLT usage contradict this biostimulation theory.

Many of LLLT devices emit light that is strong enough to cause thermal effects on human tissues. It was reported that twenty-nine patients had sustained thermal burns from use of the Anodyne Therapy System (890 nm infrared diodes). The manufacturer of this device received a warning from the Food and Drug Administration in 2005 for promoting laser devices for the treatment of non-certified conditions including soft tissue injuries, CTS, wounds, neuropathy and lymphedema [20].

Meta-analysis studies of LLLT have not been able to compare studies effectively, often lacking specific treatment parameters including intensity, time, frequency, and irradiated area. Most studies used Watts and Joules to measure how much light is emitted to the target area. The watt refers to how much electric power goes through the device, not how much light it produces. Joules measure how much work has been done per square cm on an area. In our study, lux was used as a light intensity measure because it is more accurate in establishing how many photons of light reach a particular location.

In our study, we irradiated three different bacteria species commonly found in human wounds (*S. aureus*, *E. coli*, or *P. aeruginosa*) with nine different wavelengths of laser lights. None of the laser lights caused significant difference in growth on any of the three bacteria species. Therefore, the significance of our study supports the possibility that low-level lasers are not effectively inhibiting or enhancing growth of bacteria when irradiated with the specified parameters.

Biostimulation effects of LLLT are possible when photons of laser light are absorbed into a target human chromophore. However, each chromophore absorbs only a

narrow-range specific wavelength of light. This specific wavelength of light is required to stimulate each chromophore in human tissue. For example, an absorption spectrum of lipid peaks at 930 nm and drops low significantly at 970 nm (Conway 1984). Therefore, in order to be effective, laser light must be emitted at a very specific wavelength of light.

An issue of tissue penetration is also a concern. Without the actual occurrence of tissue penetration, the effectiveness of laser light at the cellular level is meaningless. When photons in laser light enter tissues, they can be transmitted, scattered, reflected, or absorbed [21,22]. These behaviors of laser light are related to the penetration depth capacity of the human tissue [17].

Different human tissues have specific absorption characteristics depending on distinct components [22,23]. For example, infrared light is absorbed primarily by water, while visible and ultraviolet lights are absorbed mainly by hemoglobin and melanin, respectively. Because absorption coefficients are so specific to tissue components, there lies a tremendous possibility for error when utilizing LLLT to achieve desired cellular effects in the clinical setting.

In conclusion, LLLT in this study had no effect on bacteria growth. Therefore, if there is an effect on wound healing, it cannot be claimed to be due to killing bacteria. Further investigation is warranted to see what LLLT really can do. Certainly issues with tissue penetration probably limit the effect of LLLT to a placebo effect.

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