Effect of high-power Nd:YAG laser on the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*: an experimental study

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**Abstract.** [Purpose] The aim of this study was to evaluate the effect of high-power Nd:YAG laser on *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacterial growth. [Materials and Methods] Seven samples of *S. aureus* and seven samples of *P. aeruginosa* were prepared in the microbiology lab, one used as a control sample and the remaining six samples used as experimental samples, which were irradiated by a high-power laser (LASERSIX ME, 15W) with a total dose of 500 and 700 J. The primary measure was the semi-qualitative assessment of turbidity and bacterial count; the turbidity was assessed 24 h after laser application. [Results] There was a significant decrease in turbidity in all experimental samples of *S. aureus* and *P. aeruginosa* after 24 h of high-power laser application for 500 and 700 J and a significant decrease in the colony-forming unit (CFU) value in both types, and there were no significant differences in turbidity and CFU when comparing 500 and 700 J. [Conclusion] A high power Nd:YAG laser was found to be an effective modality for inhibition of *S. aureus* and *P. aeruginosa* growth.

**Key words:** High-power-laser, *Staphylococcus aureus*, *Pseudomonas aeruginosa*

INTRODUCTION

Laser is a unique physical therapy modality used for the treatment of acute and chronic pathological conditions, such as chronic osteoarthritis, carpal tunnel syndrome, wound healing, shoulder pain, inhibition of bacterial growth, and post-operative incisional wounds1–3).

High-power neodymium-doped yttrium aluminum garnet (Nd:YAG) laser therapy is among the most common types of laser therapy. Nd:YAG laser therapy is a non-invasive method used to treat many pathological conditions, improving functional abilities and quality of life. It is a modern technology used in medicine and physical therapy. In general, Nd:YAG lasers emit light at a wavelength of 1,064 nm in the infrared region, which allows it to spread and penetrate tissue3–5).

The efficacy of laser irradiation has been confirmed against *Escherichia coli*, *Staphylococcus aureus*, *Actinomyces naeslundii*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Streptococcus anginosus*7). Also, there are studies that provide general information about the fungicidal and bactericidal influence of laser therapy using various types of lasers of different energy, wavelengths, and doses of irradiation8).

Laser therapy can kill pathogens (e.g., bacteria) by inducing alteration in DNA. Also, water molecules within pathogens absorb laser photons, which leads to inhibition or death9). Lasers can influence both Gram-negative and Gram-positive
bacteria; the anti-microbial and bactericidal effects of laser have been confirmed by several studies.  

*S. aureus* is a major bacterial human pathogen repeatedly found on the skin and in the upper respiratory tract infection. *S. aureus* is one of the main bacterial agents accountable for both hospital-acquired and community infections. The acquisition of this microorganism in hospital and society settings is a serious public health problem.

*Pseudomonas aeruginosa* can be involved in respiratory and nosocomial infections, often involving multiple infections. *P. aeruginosa* infections can be life-threatening and difficult to treat because it is extremely immune to many drugs and is capable of building resistance to all powerful antibiotics. It has numerous virulence factors, such as biofilm creation, which shields the pathogen from the antibody of a host and antibiotics. Over the years, *P. aeruginosa* has contributed significantly to morbidity and mortality connected with surgical infections throughout the world. These bacteria play a substantial role in patients with burns and wounds as an etiological agent of serious infections.

There is a growing need for an innovative, convenient, and easy-to-use process that can clear and/or control pathogens in vivo. The use of a laser that is autonomous of the bacteria’s antibiotic resistance could thereby be useful in the management of wound infection and burns. There was a lack of information and relevant studies about the optimal dose, application time, frequency of application, wavelength, and duration of treatment in killing or inhibition of bacteria. Therefore, the purpose of the present study was to evaluate the effect of high-power Nd:YAG laser on the growth of *S. aureus* and *P. aeruginosa*.

**MATERIALS AND METHODS**

The examined organisms in this study were *S. aureus* and *P. aeruginosa*, both of which are aerobes. The bacterial strains were obtained from the Department of Medical Microbiology lab at the College of Medicine, Umm Al-Qura University. The media used in this study was tryptophan broth medium (TB), which is a liquid medium poured into tubes, sterilized by autoclaving for approximately 2 h, then stored at 4 °C until the day of bacteria inoculation and testing.

*S. aureus* and *P. aeruginosa* were used in this study; the two species of bacteria used in our research are a pure culture of each species obtained from the microbiology lab in Maternity and Children hospital in Holy Capital, Makkah. A fresh subculture of each bacteria was made for the experiment (24 h culture).

For laser exposure in SET A, a large tube containing 15 mL of tryptophan broth (TB) was inoculated with 3 mL of fresh *P. aeruginosa* broth (master stock tube). Next, 2 mL was transferred from the master mix to each of seven tubes [six tests (P1, P2, P3, P4, P5, and P6) and one control (PC)]. In SET B, a large tube containing 15 mL of TB was inoculated with 3 mL of fresh *S. aureus* broth (master mix tube). Next, 2 mL was transferred from the master mix tube to each of seven tubes [six tests (S1, S2, S3, S4, S5, and S6) and one control (SC)].

The present study used a neodymium-doped yttrium aluminum garnet Nd:YAG laser (LASERSIX ME (15W), Mauro Marrucci, Sixtus Italia SRL) at 1,064 nm wavelength, with an adjustable handpiece. The apparatus provided continuous 1,064-nm wavelength light (maximum output, 15 W; fluency, 1.806 J/cm²; impulse, 59,000 μs; frequency, 25 Hz; probe diameter, 0.5 cm; spot area, 4.9,000 cm²). The handpiece was positioned perpendicular to all samples during the application, with the same manner of high-power laser application and the same position of tubes. Slow manual scanning was performed to cover all areas of the colony in the tube. A total energy doses of 500 and 700 J were administered, and the application time for all doses was measured approximately. The LASERSIX ME 15W device calculated the energy applied during each dose and the total energy delivered to the colony during the application session (i.e., measured by Nd:YAG laser software).

The laser application was conducted in three steps with the same procedure for both *S. aureus* (S1, S2, S3, S4, S5, and S6) and *P. aeruginosa* (P1, P2, P3, P4, P5, and P6) experimental tubes. The first step includes irradiation of the S1 and P1 sample with 500 J (duration of irradiation, 4 min and 38 s) and S4 and P4 with 700 J (duration of irradiation 6 min and 29 s) for only one-time irradiation. The second step includes irradiation of S2 and P2 with 500 J (duration of irradiation, 9 min and 16 s) and S5 and P5 with 700 J (duration of irradiation 12 min and 58 s) twice, with a 10-min time interval between each application. The third step includes irradiation of S3 and P3 with 500 J (duration of irradiation 13 min and 25 s) and S6 and P6 with 700 J (duration of irradiation 19 min and 27 s), three-time irradiation with a 10-min time interval between each application. After laser irradiation, the control and experimental tubes were incubated at 35 °C overnight. The next day the bacterial inhibition was evaluated visually by comparing the control tube (based on turbidity proportional to bacterial density) and counting of the bacterial colony (colony-forming unit, CFU). A colony-counting procedure was used for determining viable cell counts by counting the number of colonies that develop on a solid medium that has been inoculated with the sample or bacterial suspension.

The outcome measure is turbidity proportional to bacterial density (reduction of transparency of a liquid caused by the presence of undissolved and/or colloidal matter and small organisms). This method indicated that increased turbidity of the solution corresponds to an increasing number of microorganisms, and that a clear solution means no growth of bacteria. Results were recorded as (Clear 0, +, ++, ++++, and +++++). Also, the colony count was assessed before and after laser application.

Statistical analyses were done using SPSS for Windows (IBM, Inc.) version 22. The differences between the control and experimental samples were analyzed using an unpaired t-test. One-way analysis of variance (ANOVA) was used to analyze the data to detect the overall differences between the means (Bonferroni multiple comparison tests). Means and standard deviations were reported, and the alpha level of significance was 0.05.
RESULTS

No significant differences in colony count or turbidity were observed between all samples of *S. aureus* and *P. aeruginosa* at baseline ($p>0.001$). The turbidity and colony count data were similar when comparing the 500 and 700 J conditions. The colony count and turbidity were significantly decreased in all experimental samples after laser application.

Higher power and longer irradiation produced significant reductions in turbidity in all *P. aeruginosa* samples; this effect was most pronounced in the P3 sample (clear 0) relative to the control sample (PC) (Table 2 and Fig. 1). Also, there was a significant reduction of turbidity in all *S. aureus* experimental samples, with the greatest effect observed in the S4 and S5 samples (clear 0) relative to the control sample (SC) (Table 3 and Fig. 2).

Higher power and longer irradiation time resulted in a significant reduction ($p<0.0001$) in colony-forming units (CFU/mL) of *P. aeruginosa* and *S. aureus* after the application (Tables 1, 4 and Figs. 3, 4).

DISCUSSION

Many studies on low-intensity laser therapy (LILT) with wavelength below 1,000 nm over the past years have provided a positive effect and results in *in vitro* studies and clinical practice to improve wound healing, treat inflammation, treat chronic and infected wounds, inhibit bacterial and fungal growth, and reduce acute and chronic pain.

| Table 1. | The mean values of colony count for control and experimental samples for *S. aureus* and *P. aeruginosa* |
|------------------|----------------------------------------------------------------------------------------------------------------|
| **P. aeruginosa** | **Control (CFU/mL)** | **Experimental (CFU/mL)** | **p value** | **Control (CFU/mL)** | **Experimental (CFU/mL)** | **p value** |
| 500 J | 172.40 ± 0.56 | 0 | p<0.0001* | 1,276.60 ± 0.90 | 0 | p<0.0001* |
| 700 J | 173.46 ± 0.90 | 0 | p<0.0001* | 1,277.80 ± 0.70 | 0 | p<0.0001* |

CFC: colony forming unit; ml: milliliters, *: significant.

| Table 2. | Turbidity of *P. aeruginosa* after laser application of experimental and control samples. |
|------------------|----------------------------------------------------------------------------------------------------------------|
| **P. aeruginosa** | **P6** | **P5** | **P4** | **PC** | **P1** | **P2** | **P3** |
| Turbidity | + | + | + | ++++ | ++ | ++ | 0 |
| Irradiation time | 3-time | 2-time | 1-time | 1-time | 2-time | 3-time |
| Total time | 19 min and 27 s | 12 min and 58 s | 6 min and 29 s | 4 min and 38 s | 9 min and 16 s | 13 min and 54 s |
| Bacterial suspension | 2 mL | 2 mL | 2 mL | 2 mL | 2 mL | 2 mL |

mL: milliliters; min: minute; s: second.

**Fig. 1.** Turbidity of *P. aeruginosa* after laser application of experimental and control samples.

**Fig. 2.** Turbidity of *S. aureus* after laser application of experimental and control samples.
However, the development of high-power Nd:YAG laser with wavelengths greater than 1,000 nm and new optical systems has resulted in its widespread use in many fields of medicine and physical therapy, including in surgery, as an anti-microbial, to reduce or eliminate pathogenic organisms, to treat dental and musculoskeletal problems, and many varieties of bacteria-infected wounds.

The present study aimed to evaluate the effect of high-power Nd:YAG irradiation on the in vitro growth of *S. aureus* and *P. aeruginosa*, and the major finding of the study is that high power Nd:YAG laser resulted in the reduction of experimental *S. aureus* and *P. aeruginosa* growth, compared with the control tubes. Irradiation using high power Nd:YAG laser resulted in a decrease in both tested bacteria. These findings indicate that a 1064-nm wavelength Nd:YAG can reduce the total number of irradiated microorganisms, as measured by turbidity methods of microorganisms counting.

By a semi-qualitative turbidity method, we found that Nd:YAG laser irradiation significantly reduced the number of both *S. aureus* and *P. aeruginosa* of bacteria. The use of a long-pulsed 1,064 nm Nd:YAG laser for the treatment of onychomycosis

### Table 3. Turbidity of *S. aureus* after laser application of experimental and control samples

| S. aureus | Control |
|-----------|---------|
| S3        | S2      | S1 | SC | S4 | S5 | S6 |
| Turbidity | +       | +  | +  | +++ | 0  | 0  | + |
| Repetitions | 3-time Irradiation | 2-time Irradiation | 1-time Irradiation | 1-time Irradiation | 2-time Irradiation | 3-time Irradiation |
| Total time | 13 min and 54 s | 9 min and 16 s | 4 min and 38 s | 6 min and 29 s | 12 min and 58 s | 19 min and 27 s |
| Bacterial suspension | 2 mL | 2 mL | 2 mL | 2 mL | 2 mL | 2 mL |

### Table 4. Colony-forming units of *P. aeruginosa* of experimental and control samples

| P. aeruginosa | Control |
|---------------|---------|
| P6 | P5 | P4 | PC | P1 | P2 | P3 |
| CFU/mL | 0 | 0 | 0 | 192 | 0 | 0 | 0 |
| Repetitions | 3-time Irradiation | 2-time Irradiation | 1-time Irradiation | 1-time Irradiation | 2-time Irradiation | 3-time Irradiation |
| Total time | 19 min and 27 s | 12 min and 58 s | 6 min and 29 s | 4 min and 38 s | 9 min and 16 s | 13 min and 54 s |
| Bacterial suspension | 10 uL | 10 uL | 10 uL | 10 uL | 10 uL | 10 uL |

### Table 5. Colony-forming units of *S. aureus* of experimental and control samples

| S. aureus | Control |
|-----------|---------|
| S3        | S2      | S1 | SC | S4 | S5 | S6 |
| CFU/mL | 0 | 0 | 0 | 1,276 | 0 | 0 | 0 |
| Repetitions | 3-time Irradiation | 2-time Irradiation | 1-time Irradiation | 1-time Irradiation | 2-time Irradiation | 3-time Irradiation |
| Total time | 13 min and 54 s | 9 min and 16 s | 4 min and 38 s | 6 min and 29 s | 12 min and 58 s | 19 min and 27 s |
| Bacterial suspension | 10 uL | 10 uL | 10 uL | 10 uL | 10 uL | 10 uL |

CFU: colony forming unit; UL: Unit liter; mL: milliliters.
has revealed positive results due to the photothermal effects of the laser\textsuperscript{22}. It has been reported that the laser light generated by infrared lasers with wavelengths in the range of 600–1,100 nm affects a wider cell-light response\textsuperscript{23}.

Studies using LILT and high-power Nd:YAG laser light at 1,064 nm have suggested that the laser can cause photoexcitation of endogenous microbial porphyrin molecules contained in microorganisms, thereby evoking oxidative damage through reactive oxygen species (ROS), which have a high killing potential for bacteria, fungus, and viruses\textsuperscript{7}.

Our literature review identified many studies on the effect of light on the inhibition of bacterial without the administration of photosensitizers\textsuperscript{10}.

Also, laser irradiation can inhibit the physiological function of bacterial cells through the suppression of DNA metabolism and cell division, degenerative changes, cytomorphology, and cell pyknosis. The degree of destruction differs according to the dose used, laser parameters and types of laser, ranging from decreased cell growth to inhibition, loss of metabolic activity, and physical structural damage\textsuperscript{24}. Increasing the pulse energy, pulse rate, or time of irradiation creates an extended diameter of the pyknotic cell zone\textsuperscript{25}.

The dose-dependent effects of lasers can be described by Arndt Schultz’s curve\textsuperscript{23, 26}. This suggests that different stimuli evoke different cellular reactions (e.g., increased stimulus inhibits activity)\textsuperscript{26}.

Application of laser with specific parameters leads to contracting or shrinking of the bacterial cell and deoxyribonucleic acid (DNA), which alters the gene expression of microorganisms and, ultimately, inhibits bacterial growth and activity. Also, laser light affects cell integrity directly after the application, including inhibited cell division and increased number of metabolically inactive cells\textsuperscript{7, 27}.

The effect of laser on bacterial/fungal destruction was also described by Sommer, where the expansion and contraction of the intracellular water volume and fluidity generate bidirectional flow\textsuperscript{28}. This phenomenon likely accounts for our findings, where the irradiation with the two different doses (500 J and 700 J at 15 W/25 Hz) resulted in a reduction in \textit{S. aureus} and \textit{P. aeruginosa} amount. Further research is still needed to translate our data into the clinical setting (e.g., the treatment of infected wounds and ulcers) and to clarify the most effective dose and time. Also, additional work will be needed to determine whether Nd:YAG 1,064 nm laser therapy is effective in the treatment of bacterial infections.

There were some limitations to our study. The study was conducted on a small sample size, with a single type of laser, so a more isolated suitable environment for application should be considered, and another alteration using higher doses than lower doses in both types of bacteria.

Despite these limitations, we believe that this study served as a good report for the effect of high-power Nd:YAG laser on bacterial inhibition and growth. Finally, our results demonstrate that a higher dose of Nd:YAG laser and repeated exposure are more effective than one-time exposure, which is important to know in the context of treating an infected wound. The high-power Nd:YAG laser (15 W) was found to be a rapid and effective method for inhibiting the growth of \textit{S. aureus} and \textit{P. aeruginosa} after single and repeated applications, and we suggest that laser-based anti-microbial treatment can significantly reduce the quantity of \textit{S. aureus} and \textit{P. aeruginosa}. Next, this approach can be applied to rats to determine clinical suitability.
for humans.

High power Nd:YAG laser light at specific wavelengths, adjustable pulse, high emission frequency, and continuous mode might have some positive effects on the reduction of *S. aureus* and *P. aeruginosa* growth; however, clinical studies should be conducted. There are many advantages of laser irradiation; it offers a lower treatment cost, a short period of treatment duration, no or minimal side effects, and is an alternative to the systemic administration of antibiotics. A high-power Nd:YAG laser can significantly reduce *S. aureus* and *P. aeruginosa* growth in infected wounds.

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**Conflicts of interest**

All authors declare no conflicts of interest.

**REFERENCES**

1) Dundar U, Turkmnen U, Toktas H, et al.: Effect of high-intensity laser therapy in the management of myofascial pain syndrome of the trapezius: a double-blind, placebo-controlled study. Lasers Med Sci, 2015, 30: 325–332. [Medline] [CrossRef]

2) Ebid AA, Alhammad RM, Alhendi RT, et al.: Immediate effect of pulsed high-intensity neodymium-doped yttrium aluminum garnet (Nd: YAG) laser on staphylococcus aureus and pseudomonas aeruginosa growth: an experimental study. J Phys Ther Sci, 2019, 31: 925–930. [Medline] [CrossRef]

3) Parra PF, Ghinassi S, Ciuti F: The neodymium-YAG laser in defocused for its evolution increasingly effective treatment of the injured athlete. Laser Technol, 1992, 2: 13–16.

4) Parra PF: New laser method for rapid recovery of the injured athlete: the neodymium-YAG high-power defocused. Laser News, 1990, 2: 27–30.

5) Pins L: Use of class IV, high intensity laser therapy as an adjunct in treating a patient with an acute shoulder injury: a case report. Iowa Res Online 2017, 27: 1–10.

6) Ebid AA, Ibrahim AR, Omar MT, et al.: Long-term effects of pulsed high-intensity laser therapy in the treatment of post-burn pruritus: a double-blind, placebo-controlled, randomized study. Lasers Med Sci, 2017, 32: 693–701. [Medline] [CrossRef]

7) Grzech-Leśniak K, Nowicka J, Pajączkowska M, et al.: Effects of Nd:YAG laser irradiation on the growth of Candida albicans and Streptococcus mutans: in vitro study. Lasers Med Sci, 2019, 34: 129–137. [Medline] [CrossRef]

8) Carvalho A, Rodrigo A, Luciana PM, et al.: Analysis of low-level laser therapy in vitro cultures of bacteria and fungi. MTP & Rehab Journal, 2015, 1–6.

9) Machado RS, Viana S, Sbruzzi G: Low-level laser therapy in the treatment of high power laser phototherapy in the treatment of periodontal disease. A review. Lasers Med Sci, 2010, 25: 781–792. [Medline] [CrossRef]

10) Vescovi P, Conti S, Merigo E, et al.: In vitro bactericidal effect of Nd:YAG laser on *Staphylococcus aureus* strains to broadband visible light. Photochem Photobiol, 2009, 85: 255–260. [Medline] [CrossRef]

11) Lipovsky A, Nitzan Y, Friedmann H, et al.: Sensitivity of *Staphylococcus aureus* strains to broadband visible light. Photochem Photobiol, 2009, 85: 255–260. [Medline] [CrossRef]

12) Lowy FD: Staphylococcus aureus infections. N Engl J Med, 1998, 339: 520–532. [Medline] [CrossRef]

13) Williams RE: Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. Bacteriol Rev, 1963, 27: 56–71. [Medline] [CrossRef]

14) Mundhada S, Sharma A, KishorIngole S, et al.: Prevalence of pseudomonas aeruginosa in surgical site infection in a tertiary care center. Int J Curr Microbiol Appl Sci, 2017, 6: 1202–1206. [CrossRef]

15) Church D, Elsayad S, Reid O, et al.: Burn wound infections. Clin Microbiol Rev, 2006, 19: 403–434. [Medline] [CrossRef]

16) Lipovsky A, Nitzan Y, Friedmann H, et al.: Sensitivity of *Staphylococcus aureus* strains to broadband visible light. Photochem Photobiol, 2009, 85: 255–260. [Medline] [CrossRef]

17) Naji EN, Ali AA, Hamzah BF: The bactericidal effect of COz Laser on *Pseudomonas aeruginosa* isolated from wound and burn infections, in vitro. Baghdad Sci J, 2015, 12: 485–495. [CrossRef]

18) de Paula Eduardo C, de Freitas PM, Esteves-Oliveira M, et al.: Laser phototherapy in the treatment of periodontal disease. A review. Lasers Med Sci, 2010, 25: 781–792. [Medline] [CrossRef]

19) Ren C, McGrath C, Yang Y: The effectiveness of low-level diode laser therapy on orthodontic pain management: a systematic review and meta-analysis. Lasers Med Sci, 2015, 30: 1881–1893. [Medline] [CrossRef]

20) Vescovi P, Conti S, Merigo E, et al.: In vitro bactericidal effect of Nd:YAG laser on Actinomyces israelii. Lasers Med Sci, 2013, 28: 1131–1135. [Medline] [CrossRef]

21) Maver-Biscanin M, Mravak-Stipetic M, Jerolimov V: Effect of low-level laser therapy on Candida albicans growth in patients with denture stomatitis. Photomed Laser Surg, 2005, 23: 328–332. [Medline] [CrossRef]

22) Piccolo D, Kostaki D, Del Duca E, et al.: Long-pulsed 1064-nm Nd: YAG laser for the treatment of onychomycosis. Photomed Laser Surg, 2017, 35: 213–216. [Medline] [CrossRef]

23) Huang YY, Sharma SK, Carroll J, et al.: Biphasic dose response in low level light therapy - an update. Dose Response, 2011, 9: 602–618. [Medline] [CrossRef]

24) Yuan X, Song Y, Song Y, et al.: Effect of laser irradiation on cell function and its implications in Raman spectroscopy. Appl Environ Microbiol, 2018, 84: 02508–02517. [Medline] [CrossRef]

25) Gutknecht N, Kanellis C, Moritz A, et al.: Effects of Nd:YAG-laser irradiation on monolayer cell cultures. Lasers Surg Med, 1998, 22: 30–36. [Medline] [CrossRef]
27) Cabrera JE, Cagliero C, Quan S, et al.: Active transcription of rRNA operons condenses the nucleoid in Escherichia coli: examining the effect of transcription on nucleoid structure in the absence of transversion. J Bacteriol, 2009, 191: 4180–4185. [Medline] [CrossRef]

28) Sommer AP: Antiinfectives and low-level light: a new chapter in photomedicine. Photomed Laser Surg, 2007, 25: 150–158. [Medline] [CrossRef]