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Original Research Article

In Vitro Assessment of the Effects of Varying Dosages of Alexandrite Laser on the Development of Staphylococcus Aureus

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Abstract

Background*:** This study aimed to apply a high-power pulsed alexandrite laser in vitro, the researchers tested different exposure periods, pulse lengths, and laser fluencies to see which dosage was most successful against S. aureus bacteria, which had developed resistance to many antibiotics. ***Method: Three bacteria samples were exposed to laser beams for 30 seconds with a 5ms pulse duration and a laser fluency of 5J/cm^2. The process was repeated with laser fluencies of 10, 15, and 20. ***Results***: The study was carried out by using different doses of Alexandrite laser. ***Results***: There are significant differences ($p = 0.05$) in the mean number of bacteria colonies exposed for 30 and 60 seconds at any laser fluencies utilized in the present investigation. Except for the 15 J.cm-2 laser fluency; there was a statistically significant difference ($p < 0.001$) between 60 sec and 90-sec exposure durations. ***Conclusion*:** In conclusion, the exposure periods, pulse durations, and laser fluencies of pulsed alexandrite laser were found to have an influence on the mean colony count of S aureus bacteria and to establish the effective dose.

Keywords: Varying Dosages, Alexandrite Laser, Staphylococcus aureus.

INTRODUCTION

Staphylococcus bacteria belong to the family Staphylococcaceae. The name` *Staphylococcus* ` comes from the Greek words 'staphyle' (grape bunch) and 'kokkos' (berry) [1]. The phylum Firmicutes includes the genus *Staphylococcus*, using the comparative 16S rRNA sequence analysis. On the other hand, Staphylococcal species can be classified based on coagulase and novobiocin susceptibility into three groups; coagulase-negative and novobiocin-susceptible species groups, which include: *Staphylococcus* epidermidis and Staphylococcus simulants, coagulase-negative and novobiocin- resistant species groups which include: *Staphylococcus* saprophyticus and *Staphylococcus* sciuri and coagulase-positive and novobiocin-susceptible species groups include *Staphylococcus* intermedius and *Staphylococcus* aureus [2]. *Staphylococcus* has 53 species and 27 subspecies, the majority of which are only present in lower mammals, the most frequently *Staphylococcus* associated with human diseases are *S. aureus*, S. epidermidis, S. haemolyticus, and S. saprophyticus [3].

Staphylococcus has a Spherical shape and is arranged in grape-like clusters that resemble a bunch of grapes. They are non-forming spores, non-motile, oxidase coagulase-negative, coagulase-positive, and fermented mannitol [4].

Staphylococcus spp. can grow on a wide range of media and produce pigments that range from yellow to deep yellow to white. The optimum temperature is between 30 and 37 °C. On solid media, the growing colonies are round, smooth, and raised [5].

S. aureus is Gram-positive with a spherical shape when examined under a light microscope after Gram staining. It is often found in clusters that resemble grape bunches. Facultative anaerobic grows well in a medium containing 10 - 15 % sodium chloride, hemolysis, coagulase, and catalase are all positive, oxidase is negative, non-spore-forming, non-motile, and encapsulated on rare occasions, *S.aureus* can be present in the environment as well as in normal microbiota in humans, where it can be found on the skin and mucous membranes most often in the nasal cavity of healthy people [6].

High-Power pulsed alexandrite laser treatment is one of the most used kinds of laser therapy, which is a non- invasive way of treating several pathological illnesses and improving functional skills and quality of life. It is a cutting-edge medical and physiotherapeutic device. In general, the Alexandrite laser generates infrared light with a wavelength of 755 nm, which enables it to propagate and enter tissue [7].

Laser irradiation is efficient against Staphylococcus aureus, Streptococcus anginosus, and other bacterial species [8]. In addition, several research studies give extensive data on the antifungal and antibacterial effects of laser treatment using varied energy, wavelengths, and doses [9].

Laser therapy can eliminate bacteria by altering DNA. In addition, infections' water molecules absorb laser photons, resulting in Inhibition or death [10]. Multiple studies have proven that laser therapy has antibacterial effects against Gram-negative and Gram-positive bacteria [11].

Staphylococcus generally does not transmit illness unless it enters the body via broken skin or mucous membranes or if the immune system is impaired. If certain circumstances are met, these bacteria will become transmissible and cause moderate or severe disease in the community, in addition to hospital-acquired sickness [12]. Bacteria can colonize various environments, but animals and humans provide the most favorable circumstances for bacterial development. The rapid growth rate of S. The fact that S. aureus develops resistance to several antibiotic classes, complicating treatment, is a major clinical problem associated with this bacterium. Traditionally, S. aureus resistance evolved within two years after the introduction of penicillin [13].

The widespread use of pulsed Alexandrite laser in various fields of physical therapy is attributable to its efficient application and excellent outcomes [14]. Bacteria can colonize various environments, but animals and humans provide the most favorable circumstances for bacterial development. The remarkable pace at which S. The fact that S. aureus gains resistance to several types of antibiotics, which complicates treatment, is a major clinical problem associated with this bacterium. In the past, S. aureus developed resistance to penicillin within two years of its introduction [13]. Due to its efficacious use and excellent outcomes, pulsed Alexandrite laser is frequently utilized in several fields of physical therapy.

The long-pulse alexandrite laser was first created for hair removal; however, it rapidly became evident that the wavelength, fluence, and pulse duration could also be utilized to cure telangiectasias. According to clinical and histologic research, a wavelength of 755 nanometers may penetrate two to three millimeters below the epidermis and is efficient in the thermocoagulation of blood vessels [15]. Although there is a theoretical risk of heating a metal eye shield during treatment with a 755 nm wavelength laser, no such complications have been reported. Although there is a dearth of research on drug-laser interactions, it is recommended that patients refrain from taking aspirin and other platelet-function-reducing medications for at least a week before and several weeks after laser treatment [16]. This study aims to reduce the pulse duration and increase the killing of bacteria when the exposure time and the fluency of the laser are constant.

MATERIALS AND METHODS

The laser that was used in this study was the alexandrite laser which was considered a pulsed laser and had the following parameters such as the wavelength was 755 nm, the beam diameter was (14 mm), and the exposure times varied (30, 60, 90 seconds, the laser fluency (5, 10, 15 and 20 $J.cm^{-2}$).

Fluency = Energy (J) /Area (cm^2) = $J.cm^{-2}$

Where:
Energy = is the laser's power multiplied by pulse width (watt × second).
Area = is the exposed area to the laser beam (cm^2)

Bacterial samples prepared from agar slants and a loopful of resistant isolates culture were transferred to a test tube containing 10ml of brain-heart infusion broth, then incubated at 37°C for 18 – 24 hours. Serial dilutions were made by tubes containing physiological saline to obtain appropriate CFU.

The bacteria sample was centrifuged at a speed of (3500 rpm) for 6 minutes, and the precipitant was kept; the normal saline was added and centrifuged again. A series of dilutions was made till the solution had Turbidity. (1ml) from the bacterial suspension was taken by a micropipette and placed in a sterile Eppendorf tube. The bacteria sample was exposed to the alexandrite laser with an exposure time (of 30, 60, and 90) seconds. The number of bacteria was the same for each dose which $=1.5 \times 10^8$ CFU / ml.

Three bacteria samples were exposed to laser beams for 30 seconds with a 5ms pulse duration and a laser fluence of 5 J/cm^2. This was repeated using exposure times of 60sec and 90sec. Also, the previous process was repeated by exposing the bacteria with different exposure times (30 sec, 60 sec, and 90 sec).

Statistical Analysis

A one-way analysis of variance (ANOVA), Least Significant Difference (LSD), and correlation were done to determine whether the variation between groups was significant. Data were presented as mean, standard deviation (SD), and statistical significance was determined using version 26 of the SPSS software.

RESULTS

Cultural characterizations perform the identification of bacteria. All isolates obtained by culture on Nutrient agar were then subcultured on matinal salt agar and blood agar. The results indicated that all Gram-positive bacteria, including Staphylococcus aureus isolates, could grow on mannitol salt agar medium, yielding white or yellow colonies, large, smooth, round colonies with an entire margin.

The microscopic examination showed that *S. aureus* reacted with Gram stain, cocci, arranged in pairs or the cluster, and was non-spore-forming. The biochemical characteristic of all *S. aureus* isolates was catalase and coagulase positive, but oxidase negative (table 1)

Table 1: Gram stain and Biochemical tests for *S. aureus*

Biochemical test	S. aureus
Mannitol salt agar	Yellow colonies
Gram stain	Gram-positive cocci
Coagulase test	Positive
Catalase test	Positive
Oxidase test	Negative

Identification of *Staphylococcus Aureus* by VITEK© Compact System

Identification of Bacterial Isolates Done by VITEK-2 System Using Gram-Positive Card Gave 96 % Probability of *S. Aureus.*

Ten isolates of *S. aureus* revealed a various resistance level toward 16 antimicrobial agent by VITEK as following: Benzylpenicillin 10/10 (100%), oxacillin 9/10 (90%), gentamicin 4/10 (40%) tobramycin 5/10 (50%), 6/10 (60%) for rifampicin and Erythromycin, clindamycin 7/10 (70%), linezolid 2/10 (20%), teicoplanin 2/10 (20%), vancomycin 3/10 (30%), Trimethoprim-sulfamethoxazole 2/10 (20%) while all isolates were sensitive to tigecycline.

The results of an Alexandrite Laser pulsed effect on staphylococcus aureus bacteria growth (by the mean values of colony count) will be presented in this chapter. The study was carried out using different exposure times (30, 60, and 90 sec) with different fluencies of an Alexandrite Laser pulse (5,10,15 and 20 J.cm^{-2}), as well as using different pulse durations (5,10 and 20 ms) with different fluencies of an Alexandrite Laser pulse (5,10,15 and 20 J.Cm^{-2}).

The effect of Pulse Alexandrite Laser according to Pulse duration with 5ms is shown in table (2). The mean values of colony count for control and experimental samples of staphylococcus bacteria after being treated with different fluencies of an Alexandrite Laser pulsed (5, 10, 15 and 20 J.cm^{-2}) and different exposure times (30, 60 and 90 sec) at pulse duration 5ms.

Table 2: Alexandrite Laser pulsed (5, 10, 15 and 20 J.cm^{-2}) and different exposure times (30, 60 and 90 sec) at pulse duration 5 ms

Laser Fluency J.cm^{-2}					
Exposure time	**Control**	**5**	**10**	**15**	**20**
E.T = 30 sec	216±7.35 a	126.67±2.96 ^{a,b}	87.67±4 ^{a,c}	74.67±3.6 ^{a,d}	41.66±2.33^{a,e}
E.T = 60 sec	216±7.35^{a}	116.67±2.96 ^{a,b}	80.67±2.4$^{ab,\ c}$	70±2.55 ^{a,d}	29.66±3.88 ^{b,e}
E.T = 90 sec	216±7.35 ^{a}	99.67±4.17 ^{b,b}	74.67±3.5 ^{b,c}	54±5.77 ^{a,d}	15.66±2.6 ^{c,e}
Small letters (a, b, c, d,e) in row are significant at p ≤ 0.05, and the same are Non-Significant, SE: Standard error mean. Different capital letters (A, B) in the column are significant at p ≤ 0.05 and the same are Non-Significant, SE: Standard error mean.					

The mean colonies of the control were 216±7.35 for time espouse 30 sec,60 sec, and 90 sec. For laser fluency 5 J.cm$^{-2,}$ the mean bacteria colonies were 126.67±2.96, 116.67±2.96, and 99.67±4.17 for time espouse 30 sec,60 sec, and 90 sec, respectively, whereas for laser fluency 10J.cm^{-2}, the mean values of colony count were 87.67±4, 80.67±2.4 and 74.67±3.5 for time exposure 30 sec,60 sec and 90 sec, respectively. The mean colonies were treated with the 15J.Cm^{-2} laser fluency was 74.67±3.6, 70±2.55, and 54±5.77 for time exposure of 30 sec,60 sec, and 90 sec, respectively, as well as for laser fluency 20J. cm^{-2}, the mean values of colony count of *S. aureus* bacteria treated with the laser fluency 20J.cm^{-2} were 41.66 ± 2.33, 29.66±3.88, and15.66±2.6 for time exposure 30 sec,60 sec, and 90 sec, respectively (Table 3).

The results in (table 3) indicated that there is a reduction in mean values of colony count after being treated with different fluencies (J.cm^{-2}) of an Alexandrite Laser pulsed when in comparison with control at the same exposure (sec) involved in the current study and at 5ms pulse duration. For the 30 sec exposure time, the reduction in the mean values of colony treated with

5J.cm^{-2}, 10J.cm^{-2}, 15J.cm$^{-2,}$ and 20J.cm^{-2} fluencies of an Alexandrite Laser pulsed with the control was by 41%, 59%, 65%, and 81%, respectively. Also, for the 60 sec exposure time, the reduction in the mean value of colonies treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm$^{-2,}$ and 20J.cm^{-2} laser fluencies of an Alexandrite Laser pulsed in comparison with the control was by 46%, 63%, 68%, and 86%, respectively as well as, for the 90 sec exposure time, the reduction in the mean value colonies treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm^{-2} and 20J.cm^{-2} fluencies of an Alexandrite Laser pulsed in comparison with the control were by 54%, 65%, 75%, and 93%, respectively.

At the same time, the result indicated that there is a reduction in the mean value colonies treated with 60 sec exposure time compared with 30 sec exposure time at the same fluencies of an Alexandrite Laser pulsed 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm$^{-2,}$ and 20J.cm^{-2} by 8%, 9%, 6%, and 28%, respectively. In addition, there is a reduction in the mean value colonies treated with 90 sec exposure time in comparison with 30 sec exposure time at the same fluencies of an Alexandrite Laser pulsed 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm^{-2}, and 20J.cm^{-2} by 21%, 14%, 27%, and 62%, respectively (Table 5).

According to the results presented in (Table 5), the mean value of control was compared with experiment samples based on different energy doses at one exposure time and 5ms pulse duration concerning the rows. A significant reduction (p = <0.0001) in the mean of the bacteria colonies was observed with increased laser energy doses at the same pulse duration. As well as a highly significant reduction (p = <0.0001) in the mean of the bacteria colonies was observed in the comparison between two laser energies at the same exposure time (see figure 1).

Concerning different exposure times (30, 60, and 90 sec) at the same energy effect on bacteria colonies and 5ms pulse duration, a decrease in mean of the colonies with increased exposure time to 60 sec and 90 sec compared with 30 sec exposure time. No significant difference was noticed in the mean of the colonies between exposure times at 30 sec and 60 sec when laser energies were at 5 J.cm^{-2}, 10 J.cm$^{-2,}$ and 15 J.cm^{-2}. However, there are significant differences in the mean of the colonies between exposure times at 30 sec and 60 sec when laser energies were at 20 J.cm^{-2}. A significant difference was ($p \leq 0.05$) noticed in the mean of the colonies between exposure times (90 sec and 30 sec) at all of the laser energies used in the current study except at 15 J.cm^{-2} laser energy. A significant difference was ($p \leq 0.05$) noticed in the mean of the colonies between exposure times (90 sec and 60 sec) at 5 J.cm^{-2} and 20 J.cm^{-2} laser energies, while at 10 J.cm^{-2} and 15 J.cm^{-2} laser fluencies were no significant between them (Figure 1).

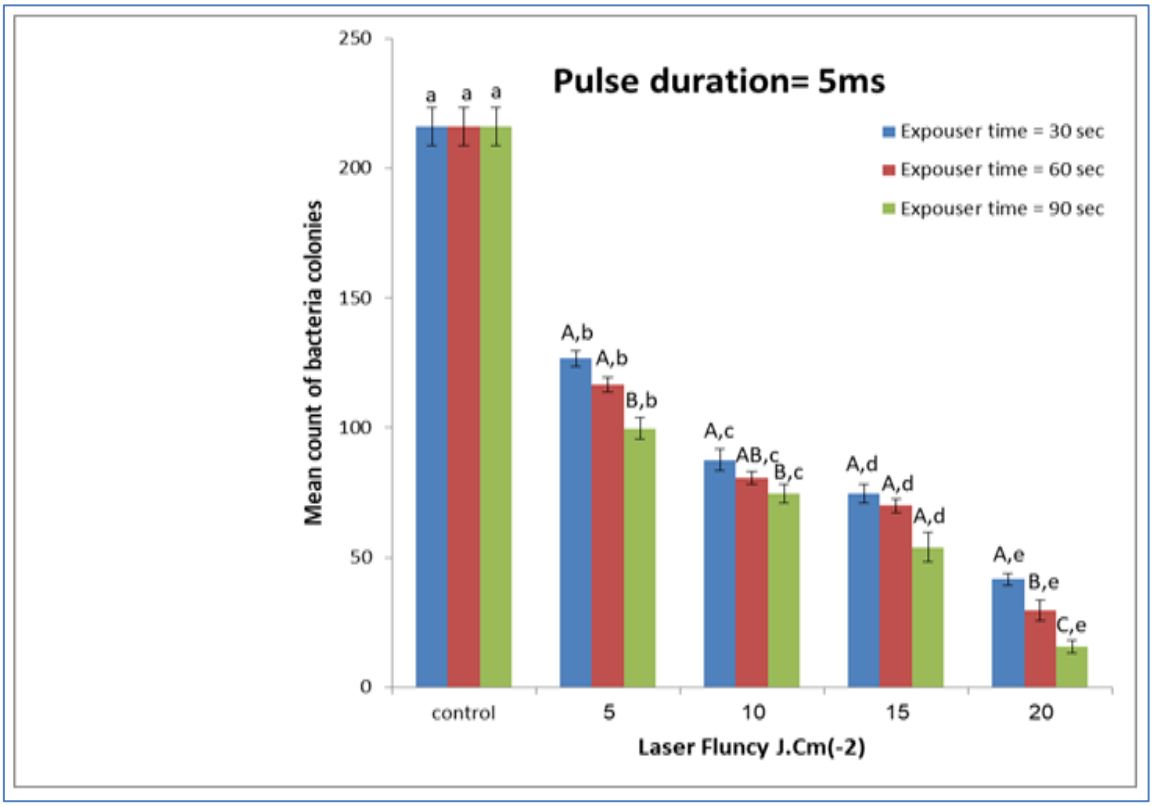

Figure 1: Relationship between mean of bacteria colonies and four fluencies of an Alexandrite Laser pulsed (5, 10, 15, and 20 J.cm $^{-2}$) with three exposure times (30, 60, and 90 sec) at 5ms pulse duration

The Pulse duration at 10ms is shown in table (3). The mean values of colony count for control and experimental samples of staphylococcus bacteria after being treated with different fluencies of an Alexandrite Laser pulsed (5, 10, 15 and 20 J.cm^{-2}) and different exposure times (30, 60, and 90 sec) at pulse duration 10ms.

Table 3: Alexandrite Laser pulsed (5, 10, 15 and 20 J.cm^{-2}) and different exposure times (30, 60 and 90 sec) at pulse duration 10ms

Laser Fluency J.Cm^{-2}					
Exposure time	**Control**	**5**	**10**	**15**	**20**
E.T = 30 sec	246±4.18 ^{a}	214.67±4.4 ^{a,b}	174.67±8.17^{a,c}	142±6 ^{a,d}	80.33±6.17^{a,e}
E.T = 60 sec	246±4.18 ^{a}	196.67±3.38^{a,b}	165.67±6.47^{a,c}	120.67±8.44^{a,d}	58.33±5 ^{b,e}
E.T = 90 sec	246±4.18 ^{a}	162.67±5.18^{b}	140.33±5.86^{b,c}	90.67 ± 4.76 ^{b,d}	38.33±3.45^{c,e}
Small letters (a, b, c, d,e) in row are significant at p ≤ 0.05, and the same are Non-Significant, SE: Standard error mean. Different capital letters (A, B) in the column are significant at p ≤ 0.05 and the same are Non-Significant, SE: Standard error mean.					

The mean colonies of the control were 246±4.185 for time exposure of 30 sec,60 sec, and 90 sec. For laser fluency 5J.cm$^{-2,}$ the mean values of the colonies were 214.67±4.4, 196.67±3.38, and 162.67±5.18 for time exposure 30 sec,60 sec, and 90 sec, respectively, whereas for laser fluency 10 J.cm^{-2}, the mean values of the colony were 174.67±8.17, 165.67±6.47 and 140.33±5.86 for time exposure 30 sec,60 sec and 90 sec, respectively. The mean colonies treated with the 15J.cm^{-2} laser fluency were 142±6, 120.67±8.44, and 90.67±4.76 for time exposure of 30 sec, 60 sec, and 90 sec, respectively. For laser fluency, 20 J.cm$^{-2,}$ the mean values of colony treated with the laser fluency 20J.cm^{-2} were 80.33±6.17, 58.33±5, and 38.33±3.45 for time exposure 30 sec,60 sec, and 90 sec, respectively (Table 4).

The statistical analysis of the results (table 4), the result indicated that there is a reduction in mean values of colony count of bacteria after being treated with different fluencies (J.cm^{-2}) of an Alexandrite Laser pulsed when in comparison with control of bacteria (untreated) at the same exposure (sec) involved in the current study and at 5ms pulse duration. For the 30 sec exposure time, the reduction in the mean values of bacteria colony count treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm$^{-2,}$ and 20J.cm^{-2} fluencies of an Alexandrite Laser pulsed in comparison with the control of bacteria was by 13%, 29%, 42%, and 67%, respectively. Also, for the 60 sec exposure time, the reduction in the mean value of colonies treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm$^{-2,}$ and 20J.cm^{-2} laser fluencies of an Alexandrite Laser pulsed in comparison with the control of bacteria was by 20%, 33%, 51%, and 76%, respectively. As well as, for the 90 sec exposure time, the reduction in the mean value of bacteria colonies treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm$^{-2,}$ and 20J.cm^{-2} fluencies of an Alexandrite Laser pulsed in comparison with the control of bacteria were by 34%, 43%, 63%, and 84%, respectively.

At the same time, the result indicated that there is a reduction in the mean value of bacteria colonies treated with 60 sec exposure time in comparison with 30 sec exposure time at the same fluencies of an Alexandrite Laser pulsed 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm^{-2} and 20J.cm^{-2} by 8%, 5%, 15%, and 27%, respectively. In addition, there is a reduction in the mean value of bacteria colonies treated with 90 sec exposure time in comparison with 30 sec exposure time at the same fluencies of an Alexandrite Laser pulsed 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm$^{-2,}$ and 20J.cm^{-2} by 24%, 19%, 36%, and 52%, respectively.

According to the results presented in Table (3.6), the mean values of colony count for control were compared with experiment samples based on different laser fluency doses at one exposure time and 10ms pulse duration concerning the rows. A highly significant reduction (p< 0.0001) in the mean of the colonies was observed with the increase of laser fluence doses at the same pulse duration as well as a highly significant reduction (P< 0.0001) in the mean of the bacteria colonies was observed with in comparison between two laser energies at the same exposure time (Figure 3.5). Concerning different exposure times (30, 60, and 90 sec) at the same laser fluency effect on bacteria colonies with 5ms pulse duration (respect to the column), there is noticed a decrease in the mean of the colonies with increased exposure time to 60 sec and 90 sec comparing with 30 sec exposure time. No significant difference was noticed in colonies between exposure times at 30 sec and 60 sec when laser fluencies were at 5 J.cm^{-2}, 10 J.cm$^{-2,}$ and 15J.cm^{-2}; however, there are significant differences in the mean value of bacteria colonies between exposure times at 30 sec and 60 sec when laser energy was at 20 J.cm^{-2}. A significant difference was ($p \leq 0.05$) noticed in the count of colonies between exposure times (30 sec and 60 sec, 30 sec and 90) at all of the laser fluencies that were used in our study, as illustrated in (Figure 2).

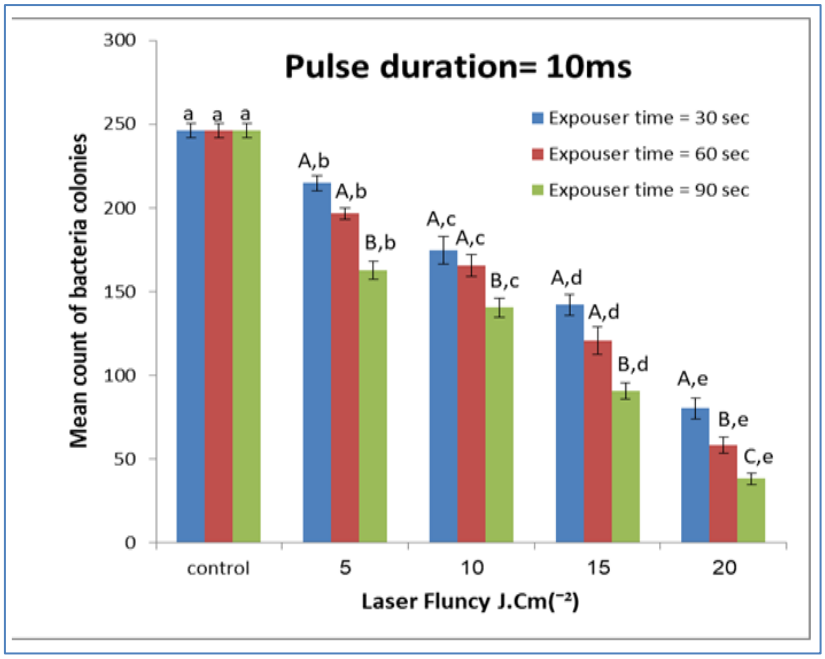

Figure 2: Relationship between mean of bacteria colonies and four fluencies of an Alexandrite Laser pulsed (5, 10, 15, and 20 J.cm^{-2}) with three exposure times (30, 60, and 90 sec) at 10ms pulse duration.

In table (4), The mean values of colony count for control and experimental samples of *staphylococcus aureus* bacteria after being treated with different fluencies of an Alexandrite Laser pulsed (5,10,15 and 20 J.cm^{-2}) and different exposure times (30,60 and 90 sec) at pulse duration 20ms.

Table 4: Alexandrite Laser pulsed (5, 10, 15 and 20 J.cm^{-2}) and different exposure times (30, 60 and 90 sec) at pulse duration 20ms.

Laser fluency J.Cm $^{-2}$					
Exposure time	**Control**	**5**	**10**	**15**	**20**
T.E = 30 sec	241±7.12 ^{a}	224.67±8.2 ^{a,b}	185.33±6.85^{a,b}	160.67±7.25 ^{a,c}	85.67±6.88^{a,d}
T.E = 60 sec	241±7.12 a	204.67±7.8 ^{a,b}	180±3.78 ^{a,c}	156±7.52 ^{a,b,d}	78.66±3.9 ^{a,e}
T.E = 90 sec	241±7.12 ^{a}	181.33±3.9^{b}	166.33±3.5 ^{b,c}	142±2.34 ^{b,d}	55.33±4.48^{b,e}
Small letters (a, b, c, d,e) in row are significant at p ≤ 0.05, and the same are Non-Significant, SE: Standard error mean. Different capital letters (A, B) in the column are significant at p ≤ 0.05, and the same are Non-Significant, SE: Standard error mean.					

The mean colonies of the control were 241±7.12 for time exposure of 30 sec,60 sec, and 90 sec. For laser fluency 5 J.cm^{-2}, the mean colonies were 224.67±8.2, 204.67±7.8, and 181.33±3.9 for time exposure of 30 sec,60 sec, and 90 sec, respectively, whereas for laser fluency 10J.cm^{-2}, the mean values of colony count were 185.33±6.85, 180±3.78, and 166.33±35 for time exposure 30 sec,60 sec and 90 sec, respectively. The mean colonies of bacteria treated with 15 J.Cm^{-2} laser fluency were 160.67±7.25, 156±7.52, and 142±2.34 for time exposure of 30 sec, 60 sec, and 90 sec, respectively. For laser fluency, 20 J.cm^{-2}, the mean values of colony treated with the 20J.cm^{-2} were 85.67±6.88, 78.66±3.9, and 55.33±4.48 for time exposure of 30 sec, 60 sec, and 90 sec, respectively (Table 5).

Statistically, analysis of the results (Table 5) indicated that there is a reduction in mean values of colony count of *S. aureus* bacteria after being treated with different fluencies (J.cm^{-2}) of an Alexandrite Laser pulsed in comparison with control at the same exposure (sec) involved in the current study and at 5ms pulse duration. For the 30 sec exposure time, the reduction in the mean values of colony count of the bacteria treated with 5J.cm^{-2}, 10J.cm^{-2}, 15 J.cm$^{-2,}$ and 20J.cm^{-2} fluencies of an Alexandrite Laser pulsed in comparison with the control was by 7%, 23%, 33%, and 64%, respectively. Also, for the 60 sec exposure time, the reduction in the mean value of colonies treated with 5J.cm^{-2}, 10 J.cm^{-2}, 15 J.cm$^{-2,}$ and 20J.cm^{-2} laser fluencies of an Alexandrite Laser pulsed in comparison with the control of bacteria was by 15%, 25%, 35%, and 67%, respectively. As well as, for the 90 sec exposure time, the reduction in the mean value of colonies treated with 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm$^{-2,}$ and 20J.cm^{-2} fluencies of an Alexandrite Laser pulsed in comparison with the control of bacteria were by 25%, 31%, 41%, and 77%, respectively.

At the same time, the result indicated that there is a reduction in the mean value of bacteria colonies treated with 60 sec exposure time in comparison with 30 sec exposure time at the same fluencies of an Alexandrite Laser pulsed 5J.cm^{-2}, 10J.cm^{-2}, 15J.cm^{-2} and 20J.cm^{-2} by 9%, 3%, 3%, and 8%, respectively. In addition, there is a reduction in the mean value of the colonies treated with 90 sec exposure time in comparison with 30 sec exposure time at the same fluencies of an Alexandrite Laser pulsed 5J.cm^{-2}, 10 J.cm^{-2}, 15J.cm$^{-2,}$ and 20J.cm^{-2} by 19%, 10%, 12%, and 35%, respectively.

According to the results presented in (Table 4), the mean values of colony count for control compared with experiment samples were based on different laser fluency doses at one exposure time and 10ms pulse duration for the rows. A significant reduction ($p<$ 0.0001) in the mean of the bacteria colonies was observed with increased laser fluency doses at the same pulse duration. As well as, a significant reduction ($p<$ 0.0001) in the mean of the colonies was observed in the comparison between two laser fluencies at the same exposure time. However, there are no significant differences ($P>$ 0.05) in the mean of the colonies between the control and the 5 J.cm^{-2} laser fluency, as well as between 5 J.cm and 10 J.cm at an exposure time of 30 sec, as shown in figure 3.

With respect to different exposure times (30, 60, and 90 sec) at the same fluency effect on bacteria colonies and at 5ms pulse duration (respect to the column), there is noticed a decrease in the count of colonies with increased exposure time to 60 sec and 90 sec comparing with 30 sec exposure time. No significant differences ($P>$ 0.05) were noticed in the mean of the bacteria colonies between 30 sec and 60 sec exposure times with all of the current study's laser fluencies. As well as there are no significant differences ($P>$ 0.05) in the mean of the colonies between exposure times at 60 sec and 90 sec when laser fluency was at 15 J.cm^{-2}, whereas there is a significant difference ($p = <0.05$) when laser fluencies were at 5, 10 and 20 J.cm^{-2}. A significant difference was ($p<$ 0.05) noticed in the mean of the bacteria colonies between exposure times (30 sec and 90) at all of the laser fluencies used in our study except at 15 J.cm^{-2} laser fluency, as illustrated in (Figure 3).

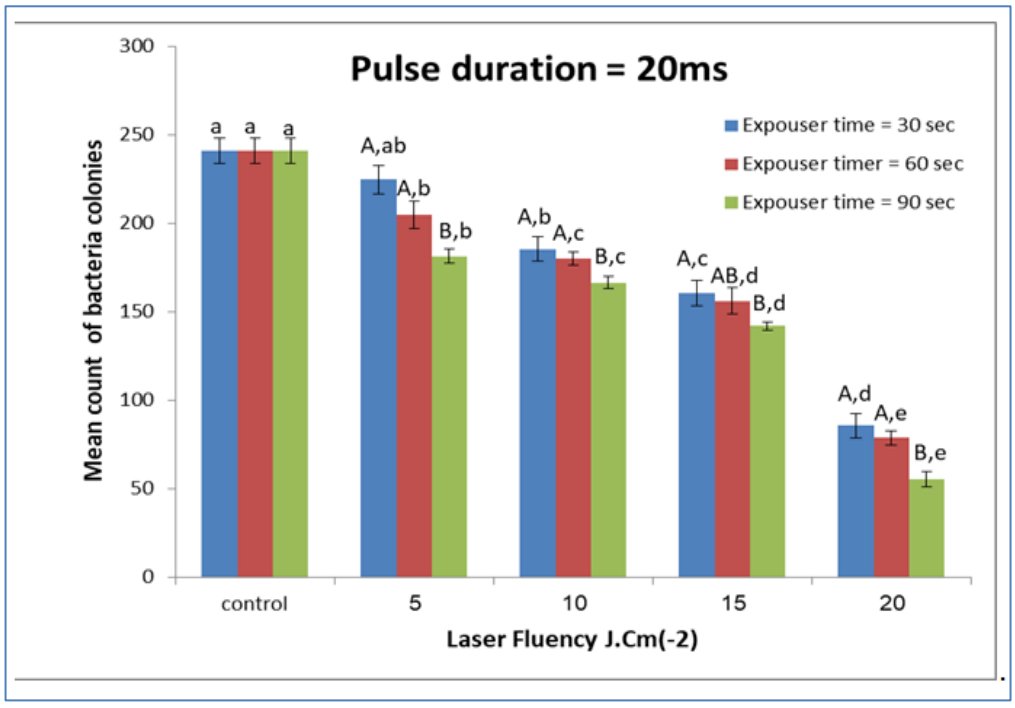

Figure 3: Relationship between mean of bacteria colonies and four fluencies of an Alexandrite Laser pulsed (5, 10, 15, and 20 J.cm^{-2}) with three exposure times (30, 60, and 90 sec) at 20ms pulse duration

DISCUSSION

Staphylococci can ferment carbohydrates and produce pigments ranging in color from white to golden yellow when cultured on various laboratory media. Although some bacteria are part of the healthy flora of human and animal skin and mucous membranes, others can lead to serious health problems such as abscess formation, suppuration, pyogenic infection, and septicemia [17].

Mannitol salt agar is used to isolate *S. aureus* from theirs, including clinical and environmental samples. Mannitol salt agar contains about 7.5% sodium chloride most other bacterial species will be inhibited except *Staphylococcus*, and the red phenol indicator indicated that *S. aureus* ferments the mannitol to form a yellow zone in mannitol salt agar due to fermentation and production of acid that decrease that PH of the medium, converting the color of phenol red to yellow. The test

allows it to distinguish from S. epidermis, which produces coloring of red zone [18].

As found in the current investigation, S. aureus isolates showed varying degrees of resistance to the antimicrobial agent, discovered that 32% of S. areuas are resistant to gentamicin [19]. Although antibiotics are vital in treating and preventing many illnesses, antibiotic resistance is on the rise owing to factors such as natural selection, misuse, and abuse [20, 21].

The use of low-intensity level laser therapy (LILT) for treating Musculoskeletal disorders, soft tissue injuries, and wound healing, all of which can be colonized by bacteria, is effective [22]. essential for recovery, and there is evidence in the pre-clinical literature that LILT has an inhibitory effect on bacterial growth via mono-chromaticity and a photo-biomodulator effect that assesses the inactivation of the proliferation of human and animal cells in vitro [23, 24].

Lasers employ the properties of light (such as wavelength and coherence) to impact cellular and tissue function. Monochromaticity is one of the most basic mechanics of lasers. This effective coupling must be synchronized with the peak absorption of chromophores to optimize photoactivation and biological process stimulation [25]. Wound healing, bacterial growth inhibition, and postoperative wounds are a few of the conditions that may be treated using laser and specialized physical therapy technologies [26].

High-Power pulsed alexandrite laser treatment is one of the most often utilized kinds of laser therapy, which is a non- invasive method for treating various pathological conditions and enhancing functional abilities and quality of life. It is medical and physiotherapeutic equipment of the highest caliber. In general, the Alexandrite laser generates infrared light with a wavelength of 755 nm, allowing it to penetrate tissues and propagate [7]. The temperature of bacteria that are exposed to the alexandrite laser increases, we discovered that the temperature was higher when the energy density and pulse length decreased.

The action of the laser is thermal. High-power diode lasers kill germs by causing a heating effect and sealing the irradiated dentinal tubules [27, 28]. When the laser is triggered, large quantities of energy are released as heat [29].

The present investigation set out to determine whether a high-power alexandrite laser would affect the in vitro growth of *S. aureus*, and its primary finding was that such a laser would have a suppressing effect on the growth of the experimental *S. aureus* compared to the growth of the control *S. aureus*. High-powered alexandrite laser irradiation decreased the number of test bacteria. This finding suggests that the overall number of bacteria detected by the colony counting approach can be decreased by switching to a 755 nm wavelength.

Laser irradiation may interfere with the normal functioning of bacterial cells by inhibiting DNA metabolism and cell division, influencing cytomorphology degeneration, and in certain cases producing pyknosis. Inhibition of cell growth, metabolic activity, and structural damage occur at varied rates and intensities depending on the dose [30]. An increase in the pulse energy, pulse rate, or irradiation period caused the pyknotic cell diameter zone to expand. In response to laser treatment, bacterial cells and their accompanying deoxyribonucleic acid (DNA) strands become shorter, causing a change in gene expression that ultimately restricts the bacteria's growth and activity [31].

In laser therapy, the irradiation photosensitizer induces reactive oxygen species (ROS) that have a high lethal potential for bacteria by accelerating electron transport in certain regions of the respiratory chain [32, 33]. At greater concentrations, the energy is transferred to oxygen to generate oxygen, which has a lethal impact at the level of the bacterial cell membrane, where their respiratory chain is located. However, the mitochondrial respiratory chain would still be disrupted, and the creation of free radicals and oxygen would result in the death of bacteria.

By measuring the photothermal activity of bacteria, we can see that the bacteria absorb the laser light, which then causes the cells to heat up and die [34]. The chromophores within bacteria are extremely light sensitive [35], leaving the bacteria defenseless against the intense photodynamic of light, the dramatic increase in local tissue temperature, and electromagnetic poisoning [31], all of which lead to thermal resonance and, in turn, cause protein denaturation, tissue shrinkage, tissue disintegration, vaporization, cutting, ablation, etc. In addition, the selective bactericidal effect of pulsed high-intensity alexandrite lasers is based on the absorption of the laser wavelength by the pigments inside the bacteria, which then causes the vaporization of water and cell lysis [36, 37].

Recent research using lasers of varying wavelengths and energies for therapy has shown promising results in the lab and clinical practice for improving wound healing, treating inflammation and infection, and preventing the spread of bacteria and fungi [8]. However, the introduction of high-energy pulsed alexandrite lasers with a wavelength of 755 nm and new optical systems has led to its widespread application in many areas of medicine and physical therapy, such as an antimicrobial to reduce or eliminate disease-causing organisms and numerous types of bacterially infected wounds.

By altering the laser's wavelength, exposure time, pulse duration, and laser fluence, bacterial cells and

DNA may be shortened, and the expression of their genes can be adjusted to limit future growth and activity. Laser light has been shown to impact cell integrity immediately, slowing cell division and increasing the number of metabolically quiescent cells [8].

Monochrome is one of the major processes of lasers, which, depending on the nature of the light, may affect cellular and tissue function. This permits effective coupling to chromophores' maximum absorption (e.g., wavelength, coherence) [24], resulting in improved photoactivation and biological activity.

This investigation aims to assess the impact of a high-powered alexandrite laser on S. aureus growth in vitro, and the key conclusion is that the experimental S. aureus strain is more resistant. Aureus developed slower when exposed to the laser than the control S. aureus. It was discovered that high-power alexandrite laser irradiation successfully eliminated the tested pathogens. This result implies that the colony counting technique's total number of bacteria discovered may be decreased when the wavelength is adjusted to 755nm.

When exposure time and pulse duration were held constant, the results showed that the laser fluencies resulted in statistically significant differences between the experimental and control groups, with the mean values of colony count for the experimental samples decreasing compared to the control group as the laser fluencies were increased. As the laser pulse length is shortened, the average colony count in the experimental samples drops in contrast to the control. The tissue stimulation phenomenon known as the "photobiology effect" results in the death of bacteria through the oxidative response of mitochondria and the creation of adenosine triphosphate (ATP), ribonucleic acid (RNA), or deoxyribonucleic acid (DNA) [25]. In contrast, when working with bacteria, the laser light is strongly absorbed by the substance the bacteria adhere to, raising the temperature to a point where the organisms are killed by their own heat [33].

The dosage of radiant energy is related to the duration of laser exposure; the longer the exposure, the greater the dose. S was more susceptible to the laser dosage with a 30-second exposure period, 5-millisecond pulse length, and a laser fluence of 20 J/cm2. aureus in comparison to dosages of 5, 10, and 15 J/cm2. The laser dosage with a 60- second exposure length, 5-millisecond pulse duration, and 20 J/cm2 laser fluence was more effective in reducing S. aureus than laser fluences of 5, 10, and 15 J/cm2. This approach employs an exposure period of 90 seconds. Therefore, exposure time and laser dosage (pulse length and laser fluency) are two factors that define the effective dose of a pulsed alexandrite laser when calculating colonies, and greater exposure time and a higher dose may be required for best results. Additional exposure may enhance the photothermal effects of the laser on microorganisms.

CONCLUSIONS

Exposure times, pulse duration, and laser fluency of the pulsed alexandrite laser influenced the mean number of Staphylococcus aureus colonies; increasing laser fluency leads to increased bacterial killing when exposure time and pulse time are determined, whereas decreasing pulse duration leads to increased bacterial killing when exposure time and laser fluency are constant.

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