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College of Medicine

# Evaluation of the effectiveness of different doses from alexandrite laser on *Staphylococcus aureus* bacteria growth *in vitro*

A Thesis
Submitted to the Council of the College of Medicine University of Baghdad, as a Partial Fulfillment of the Requirements for the Degree of Master in Medical Physics


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صدق الله العظيم

سورة يوسف الآية [٧٦]

## Supervisor's Certification

We certify that this thesis entitled " **Evaluation of the effectiveness of different doses from Alexandrite laser on *Staphylococcus aureus* bacteria growth *in vitro*** " was prepared under my supervision at the University of Baghdad /College of Medicine/ Department of Medical Physics in partial fulfillment of the requirements for the degree of Master of Science in Medical Physics .

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# DEDICATION

To my father and mother ,

To my husband and my sisters

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# Summary

**Background:** Laser is a novel physical therapy technique used to treat a variety of conditions, including wound healing, inhibition of bacterial growth, and postoperative wounds. High-Power pulsed alexandrite laser therapy is one of the most prevalent forms of laser therapy, which is a noninvasive method for treating a variety of pathological conditions, thereby enhancing functional capacities and quality of life. It is a modern medical and physiotherapeutic technology. Generally, the Alexandrite laser emits infrared light with a wavelength of 755 nm, allowing it to propagate and penetrate tissues. **Objective:** The study focused on the application of a high-power pulsed alexandrite laser in vitro to evaluation the effect of a pulsed alexandrite laser on antibiotic-resistant bacteria utilizing varying exposure times, pulse durations, and laser fluencies to determine which dose is more effective on *Staphylococcus aureus* bacteria. **Method**: The laser system was fixed vertically on mechanical jack supported with height tuner screw on plane bench; so the laser beam can fall vertically on the test sample and the laser aperture was stick to the test sample. The alexandrite laser that was used in the study which was considered as pulsed laser and had the following **parameters**: The wavelength was 755 nm, the beam diameter was (14 mm), the exposure times varied (30, 60, 90) seconds, the laser fluency (5, 10, 15 and 20 $J.cm^{-2}$) and pulsed duration (5 , 10 , 20 ms ). The study was carried out after the bacteria were diagnosed as being resistant to antibiotics, they were exposed to different doses of Alexandrite laser. Three isolates of bacteria were exposed to laser beams for 30 seconds with a 5ms of pulse duration and with a laser fluency of 5J/cm2 and the process were repeated with laser fluencies of 10, 15, and 20. The procedure was repeated using exposure times of 60sec and 90sec. As well as, the process was repeated by expose with 30 sec, 60 sec and 90 sec exposure times, 10ms pulse duration and with laser fluencies 5, 10, 15 and $20J/cm^2$, separately. Also, the previous process was repeated by expose the bacteria with different exposure times (30 sec, 60 sec and 90 sec), 20ms pulse duration and with different laser fluencies (5, 10, 15 and 20$J/cm^2$), separately. **Results**: At 30, 60 and 90 sec exposure times, there are significant reduction ($p = <0.0001$) in mean of the bacteria colonies was observed with the increase of laser fluency doses at the same pulse duration. As well as, a significant reduction ($p = <0.0001$) in mean of the bacteria colonies was observed with in comparison between two laser fluencies at the same pulse duration. However, there are no significant differences in mean values of colony count between control and 5 $J.cm^{-2}$ at 20ms pulse duration. At 5ms and 10ms pulse durations, there are highly significant reduction ($p < 0.0001$) in mean of the colonies was observed with the increase of laser fluency doses at the same pulse duration. As well as, a highly significant reduction ($P < 0.0001$) in mean of the bacteria colonies was observed with in comparison between two laser fluencies at the same exposure time. However, at 20ms, there is no significant differences ($P > 0.05$) were noticed in mean of the bacteria colonies between the exposure times at 30 sec and 60 sec with all of the laser fluencies were used in current study. As well as, there are no significant differences ($P > 0.05$) in 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 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 mean of the bacteria colonies between exposure times (30 sec and 90 sec) at all of the laser fluencies were used in our study except at 15 $J.cm^{-2}$ laser fluency. **In conclusion** the exposure times, pulse durations and laser fluencies of pulsed alexandrite laser shown effect on the mean of bacterial count of *S. aureus* bacteria and determine effective dose.

# List of Contents

## *List of Symbols and Abbreviations*

| Symbols | Meaning | Unit |
|---|---|---|
| **(BA)** | **Blood agar base** | **----** |
| **(BHIA)** | **Brain heart infusion agar** | **----** |
| **(BHIB)** | **Brain heart infusion broth** | **----** |
| **(D.W)** | **distilled water** | |
| **($H^2O^2$)** | **hydrogen peroxide** | |
| **(ID-GPB)** | **identification of gram -positive bacteria.** | **----** |
| **(LILT)** | **Low-intensity laser therapy** | **----** |
| **(MHA)** | **Muller Hinton agar** | **----** |
| **(MRSA)** | **Methicillin- resistant *S. aureus*** | **----** |
| **(MSA)** | **Mannitol salt agar** | **----** |
| **(N.A)** | **Nutrient agar** | **----** |
| **(N.B)** | **Nutrient broth** | **----** |
| **(ROS)** | **Reactive oxygen species** | **----** |
| **(SSSS)** | ***Staphylococcal* Scalded Skin Syndrome** | **----** |
| **(SSTIs)** | **Skin and soft tissue infections** | **----** |
| **(TSA)** | **Trypton soy agar** | **----** |
| **(TSB)** | **Trypton soy broth** | **----** |
| **(VRE)** | **Vancomycin -resistant enterococci** | **----** |
| **(VRSA)** | **Vancomycin resistance *Staphylococcus aureus*** | **----** |
| **($\lambda$)** | **The wavelength** | **nm** |
| **ANOVA** | **Analysis of Variation** | **----** |
| **CFU** | **Colony forming unit** | **----** |
| **CW** | **Continuous wave** | **nm** |

| | | |
|---|---|---|
| **Er:YAG** | **Erbium-doped Yttrium Aluminium Garnet laser** | **nm** |
| **GaAlAs** | **GalliumAluminum-Arsenide** | **nm** |
| **Ho:YAG** | **Holmium-doped Yttrium Aluminum Garnet** | **nm** |
| **InGaALP** | **Indium-Gallium-AluminumPhosphide** | **nm** |
| **LSD** | **Least Significant Difference** | |
| **MASER** | **Microwave amplification by stimulated emission of radiation** | **nm** |
| **Nd:YAG** | **Neodymium-doped Yttrium Aluminum Garnet** | **nm** |
| **PBP** | **(penicillin binding protein)** | **----** |
| **Rpm** | **Round per minute** | **----** |
| **rRNA** | **Ribosomal RNA** | **----** |
| **SAS** | **The Statistical Analysis System** | **----** |
| **SE** | **Standard error mean** | **----** |
| **VITEK-2** | **Automated microbiology system utilizing growth- based technology** | **----** |

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# Chapter one

## INTRODUCTION AND LITERATURE REVIEW

## 1.1 Introduction

In this chapter, some definitions and concepts of electromagnetic radiation will be discussed, such as the laser, the principles of the laser, spontaneous and stimulated emission, population inversion, and the fundamental elements of lasers. It is possible to categorize both the active medium of the laser and the characteristics of the laser beam with respect to the state of the active medium. As well as, the effects of laser light on tissue, Bacterial Infection, like *Staphylococcus* will be presented.

## 1.2 Electromagnetic Radiation (EMR)

The electromagnetic radiation spectrum encompasses a wide range of wave lengths, from the extremely short wavelengths of X-rays and gamma rays to the extremely long wavelengths of radiowaves and microwaves. The majority of lasers have wavelengths that are within or very close to the visible range, which spans the range from 400 to 700 nanometers and is more commonly known as light. This is the range, but it is convenient and intuitively appealing when discussing other parts of the electromagnetic spectrum also to refer to them as light, even though they are invisible( Jelnková *et al* ., 2013).

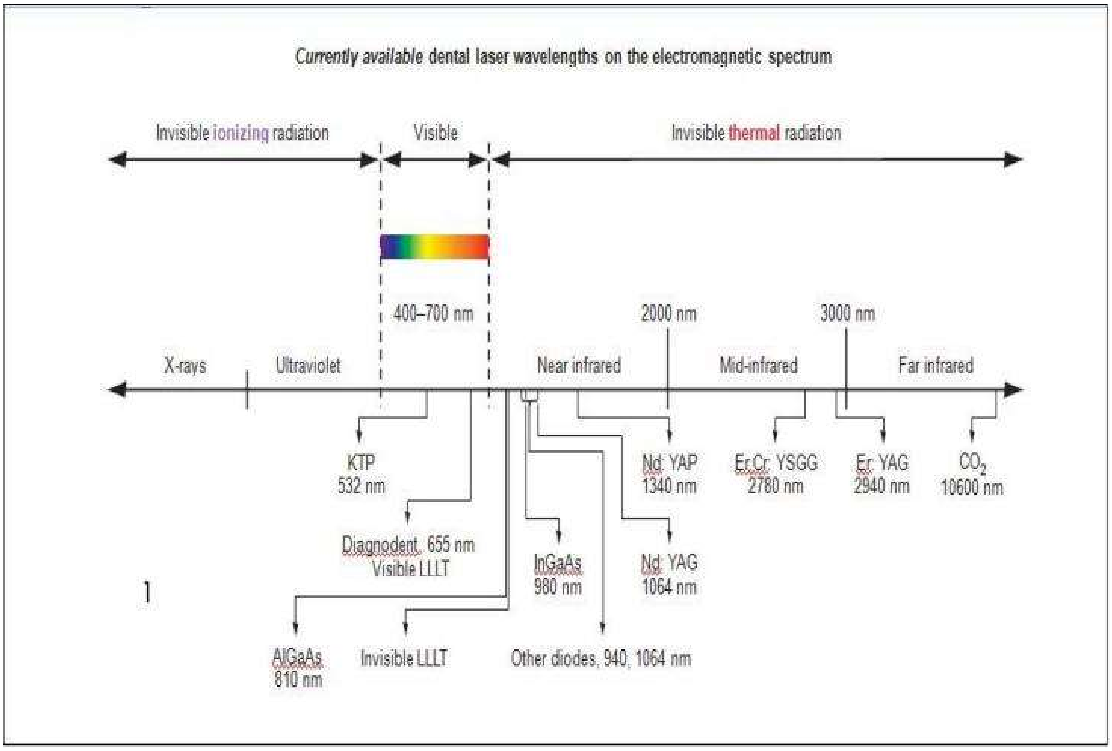



Figure (1.1): Parts of electromagnetic spectrum (Convissar *et al.,* 2011).

## 1.3 Laser Historical Overview

Albert Einstein's publication in 1917 of the theoretic concepts and principles of stimulated emission of radiation as a part of his quantum theory played a significant part in the development of lasers and other light-emitting devices in the decades to come (Donald *et al* ., 1977 ; Richard *et al* ., 1980 ; and William,. *et al* 1999)

CH. Townes and A. L. Shawlow (1958) published a proposal suggesting that the principles utilized in microwave amplification by stimulated emission, to produce the MASER, could be extended to the amplification of light. ') In the year 1960, T.H. Maiman was able to create the very first laser that actually worked. It was made up of a ruby rod that had mirrored ends and was encircled by a helical flash lamp all the way around. Soon after, a wide variety of additional materials that had the potential to act as active lasing media were discovered (Donald *et al* ., 1977 ; Richard *et al* ., 1980 ; and William,. *et al* 1999)

## 1.4 Laser

Is a device that works by stimulating atoms or molecules to emit light at specific wavelengths and then amplifying that light, which results in the production of an extremely focused beam of radiation. In most cases, the emission is only detectable in a narrow band of wavelengths across the visible, infrared, or ultraviolet of spectrum. There are a great number of distinct kinds of lasers that have been developed, each with their own set of properties. "Light amplification by the stimulated emission of radiation" is the full meaning of the acronym "laser" (Allmen *et al* ., 2012).

### 1.4.1 Principles of Laser

The word "laser" is an acronym that stands for "light amplification through stimulated emission of radiation" (Allmen *et al.,* 2012). The most fundamental component of the universe is matter, which are atoms and molecules are the basic units of construction for all different kinds of matter. Each and every kind of atom and molecule has its own one-of-a-kind configuration of electrons in their orbits around the nucleus. This specific configuration is what's meant when people talk about the "state of the atom." It is a synthesis of the states that each electron possesses in its entirety. Each state of an electron is associated with a particular level of energy, and these energy levels are distinct from one another. The quantum state of an atom or molecule is referred to as its energy level. This energy can be measured in a particular way (Wright & Fisher *et al* ., 1993). This state can begin at a low level, also referred to as the ground level, and progress all the way up to a high level from there.

Atoms and molecules are typically found in the ground state, also known as the resting state, which corresponds to the lowest level of energy. This state is reached by driving them to an excited state. Their energies can be increased by a variety of processes, such as absorbing photons with the appropriate amount of energy or colliding with ions or electrons .(Bartella *et al* ., 2019)

### 1.4.2 Basic Elements of Laser

All the devices that produce laser regardless of the style, size, or application, must have the following components

Active Medium, Resonant Optical Cavity, and Source of Excitation are three components that are essential to any device that generates a laser, regardless of its form, size, or function. These elements are required for any laser generator.

1. Active Medium: The active medium could be made up of solids, liquids, or gases (Ryer *et al* ., 1998). The electrons of the atoms that make up the active medium have the potential to be excited to a metastable energy level by an external energy source. This transition between certain energy levels is what causes the generation of laser radiation. Because of this transition, the frequency and wavelength of the laser radiation that is emitted can be calculated (Wright & Fisher *et al* .,1993).
2. Resonant Optical Cavity: The active medium is encased in a cavity and surrounded on both sides by two mirrors; together, these components make up the resonant optical cavity (Bartella *et al* .,2019).

a) High reflectance mirror: this particular mirror is capable of reflecting nearly one hundred percent of the laser light.

b) Partial reflectance mirror: this reflects less than one hundred percent of the laser light, which is approximately eighty percent, and transmits the remaining laser light (Shahrokh *et al.,* 2019).

3. The Source of Excitation is a population inversion state of the higher and lower levels of laser transition cannot be produced in a laser system without a source of excitation. It is important to keep things in this abnormal state for the duration of the laser system's operation. Therefore, in order to achieve higher overall system efficiency, the input energy must be pumped continuously into the laser medium in a manner that has been thoroughly researched and considered (Bartella *et al.,* 2019). The fundamental components of lasers are depicted in figure (1-2)

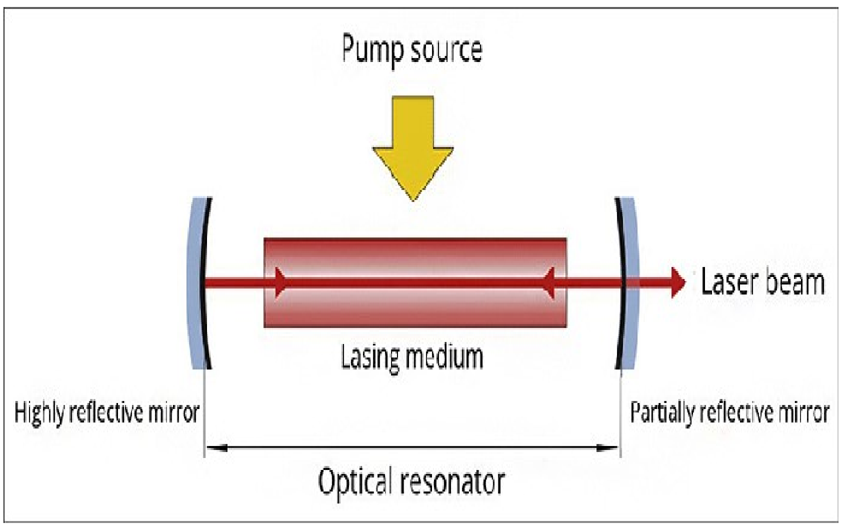



Figure (1.2 ) Basic components of laser (Black & Jobling *et al*., 2014).

### 1.4.3 Types of laser:

Crystals, glasses, semiconductors, gases and liquids represent the active medium of laser and they can generate laser beams.

• According to the state of the active medium, a laser can be categorized in a number of different ways (Harris and Pick *et al* ., 1995; Convissar *et al*., 2011; Jelnková *et al*., 2013).

1. Solid state lasers: for example, Ruby laser, Ho:YAG, Nd: YAG, Er: YAG and alexandrite.
2. Gas lasers: for example, $CO^2$ , Helium-Neon, Argon, and Excimer.
3. Liquid laser: for example organic dye laser.
4. Semiconductor lasers: for example, GalliumAluminum-Arsenide (GaAlAs) diode laser. Indium-Gallium-AluminumPhosphide (InGaALP).

o In accordance with the mode of emission. According to (Convissar *et al*., 2011 and Jelnková *et al*., 2013), lasers can be classified into the following three groups:

1. Continuous wave (CW): When operating in this mode, lasers produce an unvarying amount of power and operate without pausing.
2. Pulsed mode: periodic alterations of the laser's energy in which the laser is emitted in short bursts of high power at a pulse repetition rate that can be adjusted by the user. There is no emission of laser energy in the intervals between pulses. The average powers of a CW laser are significantly lower than its peak powers.

Lasers have completely altered the experimental landscape of spectroscopy. This is because lasers are light sources with very particular characteristics. However, the ability to operate lasers in a pulsed mode is far more significant. The laser's oscillation at the fundamental frequency of the employed atomic transition lasts for only a very brief period of time (a few femtoseconds, for example).

3. Free running pulsed mode (Q-Switching): large peak energies of laser light are emitted for a period of time that is typically measured in microseconds, and then the laser is turned off for a period of time that is relatively lengthy.

#### 1.4.3.1 Long-Pulse Alexandrite (755 nm)

The long-pulse alexandrite laser was initially designed for the treatment of hair removal ; however, it became apparent very quickly that the wavelength, fluence, and pulse duration could also be used for telangiectasias (Mitchel *et al* ., 2017). In clinical and histologic studies, it has been shown that a wavelength of 755 nm can penetrate two to three millimeters below the epidermis and is effective in thermocoagulating blood vessels. These findings are summarized below.

In the field of medicine, alexandrite lasers have been utilized specifically for dermatological procedures such as tattoo removal (both professionally and through trauma), removal of nevus of (Ota) , treatment of leg veins, and hair removal (20-23). Because of its suitable wavelength, which is absorbed in the middle of the melanin-absorbing spectrum and specifically targets the melanin, its clinical use has been well established for the purpose of hair removal.

Cryogen spray skin cooling and a 755 nm Alexandrite laser used near the clinical response threshold fluence result in few severe adverse events. Erythema, edema, purpura, pain, and permanent hair loss are the most common after effects, and they should be discussed with the patient or parents before treatment begins. With the help of perioperative narcotic analgesics and sedatives, treatment can be carried out safely under general anesthesia in cases of extensive lesions, in very young patients, or in cases of extreme anxiety or pain sensitivity. When working with Alexandrite lasers, an intraocular shield made of stainless steel should be used for maximum eye safety (Hammes *et al*., 2007). Although there is a theoretical risk of heating a metal eyeshield during treatment with a 755 nm wavelength laser (Sherisse *et al.,* 2019), no such complications have been reported to date. 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.

## 1.5 Energy levels

### 1.5.1 Spontaneous Emission

An electron moves from a state with a higher energy level to a state with a lower energy level will lead to the emission of a photon. The emission process can either be spontaneous or it can be stimulated. In the process known as spontaneous emission, the photon is released into the environment in a direction that is chosen at random and does not require any outside stimulation.

### 1.5.2 Stimulated Emission

In the process known as stimulated emission, an incoming photon either induces or stimulates the electron to change its energy level (Cutnell,. & Johnson *et al*.,2001). However, in order to produce stimulated emission, the incoming photon needs to have an energy that is exactly comparable to the gap in energy that exists between the two levels. In the process of stimulated emission, one photon is taken in and two photons are given off. This photon shares the same wavelength, phase, and level of spatial coherence as the previous one. Both photons possess the capability of inducing the emission of additional photons (Allmen *et al* .,2012 & Lili *et al* .,2018), (Figure 1 - 3).

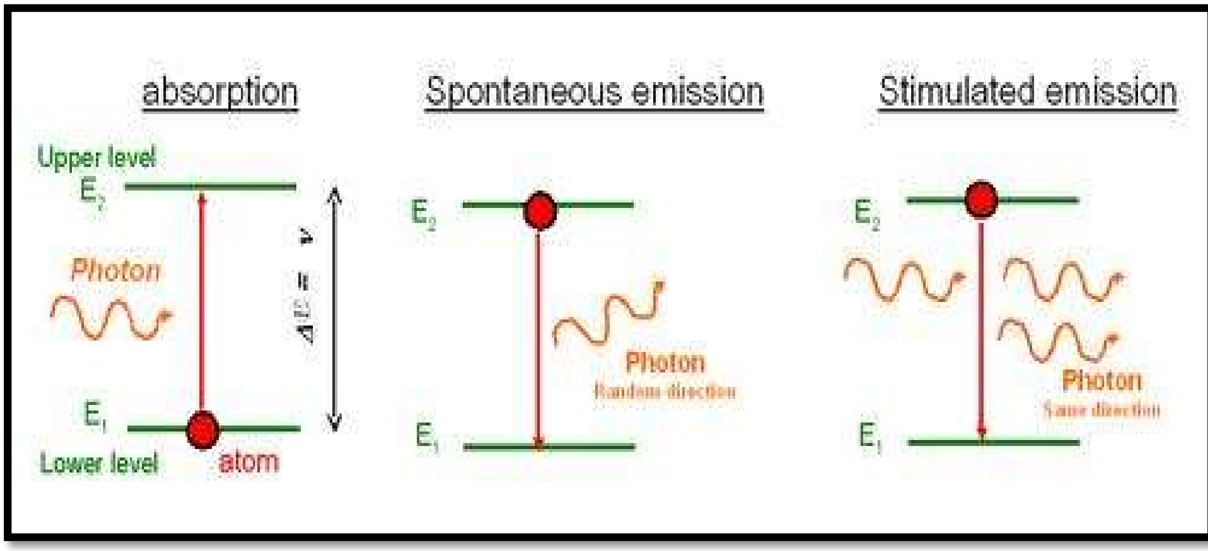



Figure (1.3) Absorption spontaneous emission and stimulated emission (Hitz, . *et al* .,1990)

### 1.5.3 Population Inversion

Taking into account two-level energy systems, each of which represents an atom in either an excited or a ground state, and assuming that the energy of a photon is equal to the difference in energy between the two levels (Beesly *et al*.,1978). Einstein demonstrated that both processes are equally probable under normal conditions. This means that the probability of an induced transition from the upper level to the lower level is the same as that from the lower level to the upper level. Additionally, Einstein demonstrated that the probability of an induced transition from the lower level to the upper level is the same (Abster *et al* .,1991). The conclusion that can be drawn from this is that the dominant process in a system with a very high total number of atoms (or molecules) will be determined by the proportion of atoms that are in the higher state compared to those that are in the lower state (Beesly *et al.,* 1978). The distribution of atoms or molecules across energy levels is given by the Boltzmann equation under conditions of thermal equilibrium. This equation demonstrates that the population of atoms (or molecules) at any given level of energy is always much lower than at levels below this given level (Wright & Fisher *et al.,* 1993). This is referred to as a "normal population distribution," and the most important process at play here is absorption. In order for stimulated emission to be more dominant than absorption, there must be a greater number of atoms in the higher state than there are in the lower state. This peculiar occurrence is referred to as population inversion, and it is possible to accomplish it through the application of external excitation to the atomic (Heinemann *et al*., 2022). To bring about population inversion, we must either ensure that the higher energy level is more densely populated than the lower level or devise a strategy that will allow the lower level to lose its inhabitants at a rate that is significantly higher than that of the higher level. The processes of pumping are the names given to the physical mechanisms that are responsible for populating and depopulating energy levels (Thyagarajan,. & Ghatak *et al* .,1981).

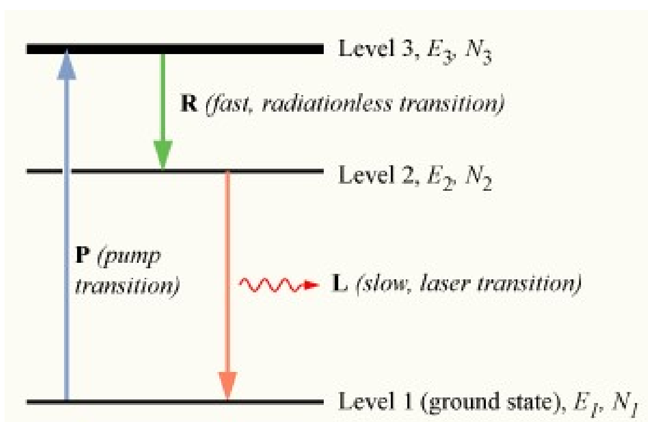



Figure (1.4) Three-level laser

A burst of energy excites electrons in more than half of the atoms from their ground state to a higher state, creating a population inversion. The electrons then drop into a long-lived state with slightly less energy, where they can be stimulated to quickly shed excess energy as a laser burst, returning the electrons to a stable ground state. Population inversion 3-level diagram, by (Bob Mellish *et al.,* 2012 )

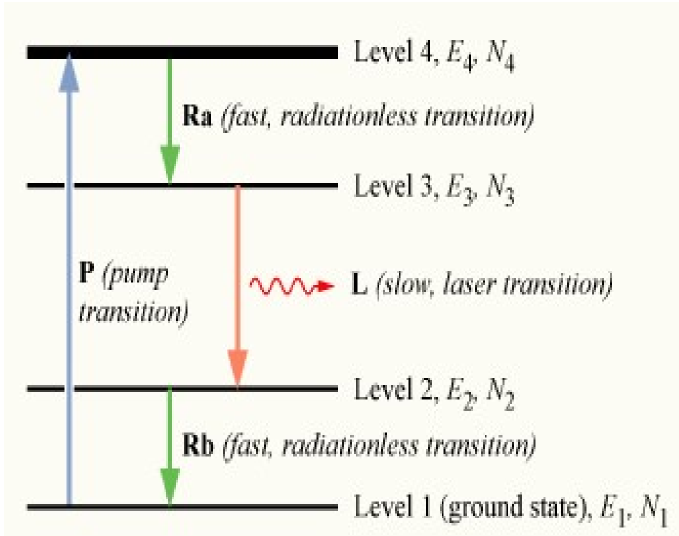



Figure (1.5 ) Four-level laser Population inversion 4-level diagram. By (Bob Mellish *et al* .,2012 )

A sustained laser beam can be achieved by using atoms that have two relatively stable levels between their ground state and a higher-energy excited state. As in a three-level laser, the atoms first drop to a long-lived metastable state where they can be stimulated to emit excess energy. However, instead of dropping to the ground state, they stop at another state above the ground state from which they can more easily be excited back up to the higher metastable state, thereby maintaining the population inversion needed for continuous laser operation.

Utilizing atoms that have two energy levels that are relatively stable between their ground state and a higher-energy excited state is one method for maintaining a laser beam's intensity over time. The atoms first enter a long-lived metastable state, where they are susceptible to being stimulated to emit an excess amount of energy, just as they do in a three-level laser. However, rather than falling to the ground state, they stop at another state above the ground state, from which they can be excited back up to the higher metastable state more easily. This allows the population inversion that is necessary for continuous laser operation to be maintained.

## 1.6 Laser elements:

### 1.6.1 The production of laser radiation:

The lasing medium is contained within the laser tube, which has one fully reflective mirror at one end and another partially reflective mirror at the other end to let light into the laser beam. A high-energy light source or electricity is used to pump and excite the lasing medium, causing an atomic population to undergo a transition into a high-energy state. If a population inversion is to be achieved, then stimulated emission will occur as excited atoms collide and emit photons that stimulate even more excited atoms to emit photons in the same direction as the original stimulating photons. This process is called stimulated emission. These photons are emitted in a direction perpendicular to the laser tube's axis. The photons are reflected back into the lasing medium by mirrors, where they collide with other excited atoms and emit more photons along the tube's axis. As the laser light's energy rapidly accumulates in the tube and is then released as a beam through the partially reflective mirror, a cascading effect is produced (Carruth *et al* .,1997).

The active medium of a laser may take the form of a solid, liquid, gas, or semiconductor. Electrical discharge, a flashlamp, radio frequency emission, or a different laser could all serve as pump sources. Laser radiation can be emitted as either a continuous wave (CW) or a series of pulses, depending on the circumstances. The power output of a laser is determined by the amount of active medium present in the resonant cavity and the efficiency with which the pump source output is matched to the medium, leading to a significant amount of energy from the pump source being used to excite the active medium. (Carruth. *et al.,* 1997).

### 1.6.2 Characteristics of laser beam:

- Monochromaticity: all laser rays have same wave length and frequency when they are emitted from the same source.
- Coherence: laser light has wave length that spatially and temporally in phase.
- Collimation: laser light is nearly parallel and non divergent.
- Brightness: The resulted laser beam can be much brighter or more powerful than conventional light source as the coherence of a laser beam allows it to be focused to a very high intensity (Herd *et al*., 1997; Convissar, 2011; Jelínková, 2013).

### 1.6.3 Parameters:

The most important radiometric terms in the medical laser application:

1.Wavelength

2. Power and Energy : Energy density (fluence): the energy delivered per unit area, expressed in joules per square centimeter ($J/cm^2$). It is gained by multiplying the output power of the laser in milliwatts by exposure time in seconds equals the energy has been produce.

3. Spot size

4.Pulse Duration ; In pulse mode laser: Fluence = laser output (W) × number of pulses × exposure time per pulse  Area of the treatment site ($cm^2$) .

5. Exposure time: time characteristic is a significant parameter of the generated output radiation, because it determines duration of tissue exposition or therapeutic dose, as well as the power of the radiation.

6. Dose : the most important parameter in low level laser therapy is always the dose. (Herd *et al.,* 1997; Convissar *et al.,* 2011; Jelínková *et al.,* 2013).

The therapeutic dose is influenced by many factors: the depth of target tissue; type of tissue either mucosa, bone or muscle; another complicating factor is the amount of chromophore in the target tissue, such as melanin.

In addition to that hemoglobin in blood in which highly vascular tissue would absorb these certain wavelengths well, and less vascular tissue would absorb these wavelengths poorly (Nussbaum *et al*., 2002; Convissar *et al* .,2011). That laser light dosimetry is an important part of the cell photostimulation (Frigo *et al*., 2010)

## 1.7 Action of Laser Light with tissue

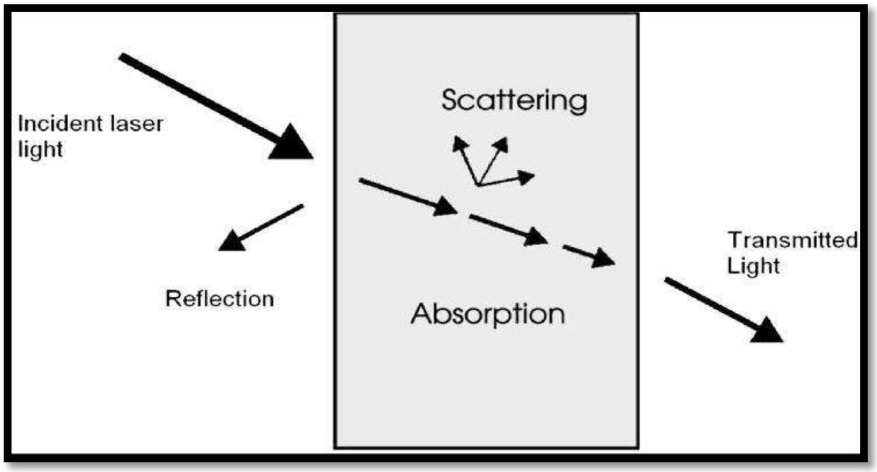
There are two questions that need to be answered in order to gain an understanding of the nature of the interaction between the laser and the tissue: what happens to the laser light when it strikes a tissue? In addition, what ends up happening to the tissue? When the laser light is absorbed, what happens then?

When a laser beam strikes a tissue, there is the potential for four fundamental physical phenomena to take place (figure 1.6).

1. The phenomenon of reflection and refraction
2. The act of absorbing.
3. The act of scattering.
4. Transmission (Markolf *et al*., 1996, Sharma *et al* .,2019, and Ginsburg,. & Geshwind *et al.,* 1992)

Both the relative and absolute magnitudes of these phenomena are determined by the wavelength of the laser light as well as the characteristics of the tissue itself (Keye *et al.,* 1990).

Figure (1.6): The reflection, absorption, scattering and transmission process (Heithoff *et al*., 2001)




## 1.7.1 Reflection and Refraction

The phenomenon known as reflection refers to the sending back of electromagnetic radiation to its source by a surface upon which it has been incident (Markolf *et al* .,1996, Xuelan *et al* .,2022). The ratio of the intensities of light that are reflected and those that are incident is referred to as a medium's reflectance. The angle of incidence, the polarization of the radiation, and the indices of refraction of the materials that form the boundary surface all have an effect on the reflectance of the light (Markolf *et al.,* 1996). When a reflection surface separates two media with different indices of refraction, a phenomenon known as refraction takes place. It is caused by a fluctuation in the speed at which light waves travel (Markolf *et al* ., 1996).

## 1.7.2 Absorption

The passage (During absorption) of an incident light through a medium causes a partial conversion of the light's energy into heat, motion, or certain vibrations of molecules within the absorbing material. This results in an attenuation of the light's intensity, which is referred to as absorption. The ability of a medium to absorb

electromagnetic radiation is contingent upon a number of factors, the most important of which are the electronic constitution of the medium's atoms and molecules, the wavelength of the radiation, the thickness of the absorbing layer, and internal parameters such as the temperature or concentration of the medium (Markolf *et al* .,1996).When it comes to bio-substances, absorption is most commonly brought about by molecules of water or by macromolecules like proteins and pigments. Molecules of water are thought to be responsible for the absorption of infrared light, whereas proteins and pigments are thought to be responsible for the absorption of ultraviolet and visible light (Hamdy O *et al* .,2022).

## 1.7.3 Scattering

When laser light travels through living tissue or a biosubstance, it is subjected to a number of different scattering processes, which causes it to transform from a collimated narrow beam into a diffused broad beam (Slarkin*et al*., 2002, Khalkhal *et al*., 2019). It is possible to differentiate between elastic scattering and inelastic scattering based on the proportion of the incident photon's energy that is converted during the process of scattering (Markolf *et al*., 1996). When the incident photons and the photons that are scattered have the same energy, we have achieved elastic scattering. When the frequency of the incident light is different from the frequency of the scattered light, a phenomenon known as inelastic scattering takes place. The phenomenon known as Rayleigh scattering is a subtype of elastic scattering. This type of scattering occurs when the particles that are dispersed are smaller than the wavelength of the incident radiation (Markolf *et al* .,1996).

### 1.7.4 Transmission

The process of transmission takes place when the light energy from the laser passes through the bio-substance. As a result, it either does not have any effect on the bio-substance at all or only a very slight effect (Xuelan *et al* ., 2022).

## 1.8 The Effect of Laser on Bio-substance

It is possible for the absorption of laser light by a biosubstance (such as a tissue, a bacterial suspension, etc.) to have a variety of effects, the specific nature of which is determined by the wavelength of the laser radiation, the power density of the laser radiation, the pulse duration, and the type of bio-substance (Convissar *et al., 2011;* Jelinková *et al .,2013)*

### 1.8.1 Wavelength Dependent Interaction Mechanisms

#### 1.8.1.1 Photochemical interaction

Irradiation of cells at a particular wavelength at low laser intensities can cause some of the native components in bio-substance to undergo bolivalization. Alterations can be made not only to particular biochemical reactions but also to the metabolism of the entire cell in this way. It is believed that this reaction is at the heart of the low-power laser effect, also known as biostimulation (Fadhali *et al.,* 2011). In recent years, there has been a rise in interest in the biostimulating effects of low-intensity laser light. On the other hand, the mechanism of biostimulation appears to be highly incredible and even mysterious, and there is a requirement for additional explanation at the molecular level of cells (Markolf*et al*., 1996). Radiation from lasers with low intensities has been applied successfully in a number of medical fields. The quantitative studies conducted with cells of varying degrees of complexity to demonstrate or refute the action of stimulation caused by low-intensity laser light are presented here. Irradiating cells with a specific laser at a certain parameter setting can make some of the native components active, and this can result in changes to both particular biochemical reactions and the overall metabolic state of the cell (M. Fadhali *et al*.,2011).

### 1.8.1.2 Photo thermal interaction

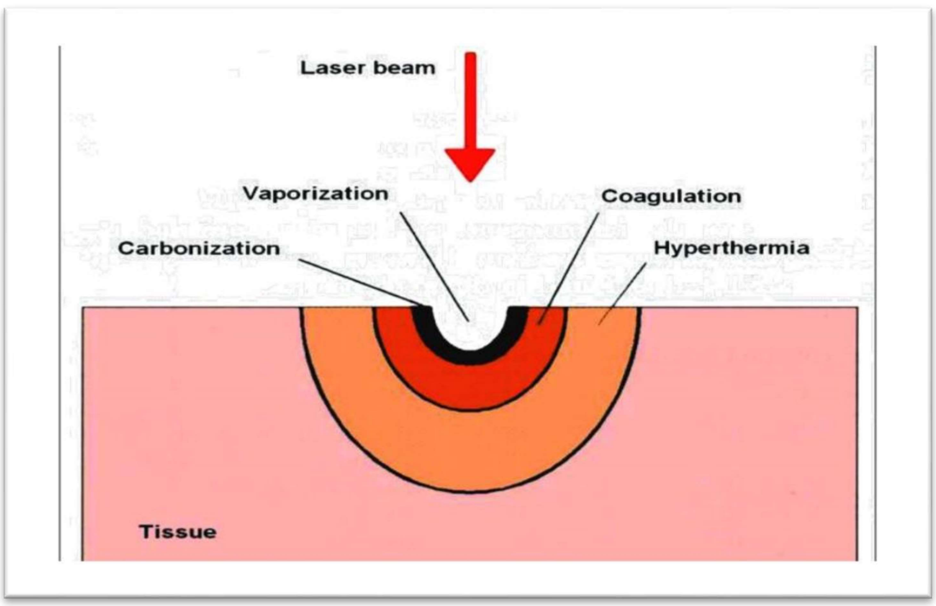
Photothermal interactions: this interaction is caused by the change of photon energy (absorbed by tissue fluids) into heat energy that arises as a result of molecular vibration and collisions between molecules. This can lead to photothermal effects on the tissue, such as coagulation, vaporization (thermal ablation) and carbonization or melting.(Youssef *et al*., 2022)

The term "thermal interaction" refers to a broad category of interaction types, each of which is characterized by an increase in one significant parameter as a result of the interaction. In laser cases, both continuous wave (CW) and pulsed laser radiation have the potential to produce thermal effects. Different effects, such as coagulation, vaporization, carbonization, and melting, can be differentiated depending on the duration of the tissue temperature increase as well as the peak value of the temperature attained. The denaturation of proteins and collagen that occurs at a temperature of 60º is what leads to the coagulation of tissue and the necrosis of cells. (Youssef *et al* ., 2022)

At a temperature of 100º, the water molecules that are present in the majority of tissues begin to vaporize. Because the vapor that is produced by the evaporation of water carries away excess heat and helps to prevent any increase in the temperature of adjacent tissue, the high vaporization heat that water possesses is beneficial. (Youssef *et al* .,2022)

At temperatures greater than 150º, a process called carbonization takes place. This can be seen as a darkening of the surrounding tissue as well as the release of smoke. In order to prevent the tissue from becoming carbonized, it is typically cooled with either water or gas.

At temperatures above 300 degrees Celsius, melting may occur, depending on the material being heated (Markolf *et al.,* 1996). (Figure 1 - 7).




Figure( 1.7): Location of thermal effects inside biological tissue (Markolf., *et al* 1996) .

## 1.9 Medical applications

The physical treatment modality known as laser has several positive attributes, including its ability to treat a wide range of pathological diseases without causing any discomfort to the patient. (Alayat *et al.,* 2014; Dundar *et al .,* 2015). Carpal tunnel syndrome, persistent osteoarthritis, shoulder pain, and post-operative pain are only a few examples of the acute and chronic conditions that benefit greatly from its potent analgesic action (Alayat *et al.,* 2014 , Dundar *et al* ., 2015 ; Ebid *et al* ., 2015).

The use of low-intensity laser therapy (LILT) has been shown to be effective in the treatment of musculoskeletal disorders, soft tissue injuries, and wound healing, all of which may be colonized by bacterial species (Chung *et al.,* 2014; de Sousa *et al .,*2016 ; Barbora *et al*., 2021,), and there is evidence from the pre-clinical literature that LILT has an inhibitory effect on (Peplow *et al .,* 2010 , Nussbaum *et al* ., 2009, Santamato *et al* ., 2009).

By changing the characteristics of the light (such as its wavelength and coherence) lasers are able to affect cellular and tissue function. Which permits efficient coupling to chromophore peak absorption, allowing for maximum photoactivation and stimulation of biological processes (Solmaz *et al* ., 2017).

## 1.10 Laser safety:

According to safety precautions, lasers are divided into four categories (Smally *et al* .,2013):

Class 1: safe under conceivable condition of use in which is viewing without optical aids, but potentially hazardous when using magnification aids (microscopes, loupes, binoculars).

Class 2: Visible wavelengths (400–700 nm). It is safe if viewed for less than 0.25 seconds. Subclass in which visible wavelengths not safe even with optical viewing aids.

Class 3R: Unsafe for viewing of intrabeam of beams with diameters >7mm.

Class 3B: Unsafe for viewing of intrabeam, causing eye and skin injury from direct, but not diffuse, energy.

Class 4: High power lead to injury of skin and eye from direct and reflected radiation.

## Literature review

### 1.11 General description of *Staphylococcus spp.*

*Staphylococcus* spp , belongs to the family Staphylococcaceae. The name` *Staphylococcus* ` comes from the Greek words 'staphyle' (grape bunch) and 'kokkos' (berry) (Gnanamani *et al* .,2017).

The phylum Firmicutes includes the genue *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 simulans* , coagulase-negative and novobiocin-resistant species groups which includes : *Staphylococcus* saprophyticus and *Staphylococcus sciuri* and coagulase-positive and novobiocin-susceptible species groups includes *Staphylococcus intermedius* and *Staphylococcus* aureus ( Schleifer and Bell *et al.,* 2009). *Staphylococcus* have 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* (Heo *et al*., 2020).

*Staphylococcus* have a Spherical shape and are arranged in grape-like clusters that resemble a bunch of grapes. They are non forming spres , non-motile, oxidase coagulase negative , coagulase positve , and fermented mannitol (Egerton- Warburon *et al*., 2014) .

*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. (Hamzah *et al.,* 2015).

### 1.11.1 Classification of *Staphylococeus aureus*

According to ( Schleifer and Bell*et al* .,2009) *S. aureus* was classified as follows:

Domain: bacteria

Kingdom: Eubacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Staphylococcaceae

Genus: *Staphylococcus*

Species: *aureus*

### 1.11.2 *Staphylococcus aureus*

*Staphylococcus 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 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 ( Taylor and Unakal *et al* .,2017 ).

### 1.11.3 Pathogenicity of *Staphylococcus aureus*

*Staphylococcus aureus* uses a variety of surface- bound proteins to bind to the host tissue and invade host cells in order to start an infection following internalization and adherence, they can grow and activate their toxin production, which is largely controlled by global regulators like quorum sensing systems (Löffler and Tuchscherr *et al.,* 2021).

The virulence factor is reported as useful in the pathogenesis of many diseases caused by *S.aureus*, including meningitis , pneumonia , toxic shock syndrome, endocarditis, and sepsis. (Monteiro *et al.,* 2019).

*Staphylococcus aureus* infections can be divided into four categories depending on the location and mechanism of infection . (1) local infections ,associated with skin and soft tissue infections (SSTIs), (2) systemic infections include sepsis , pneumonia , bacteremia etc., (3) Invasive implant infection linked to dialysis patients and intravascular catheters etc., and (4) toxin associated diseases include toxic shock syndrome and Staphylococcal Scalded Skin Syndrome (SSSS) Figure (1-9 ) (Häggström, Mikael. "Medical gallery of Mikel Häggstriöm 2014" )

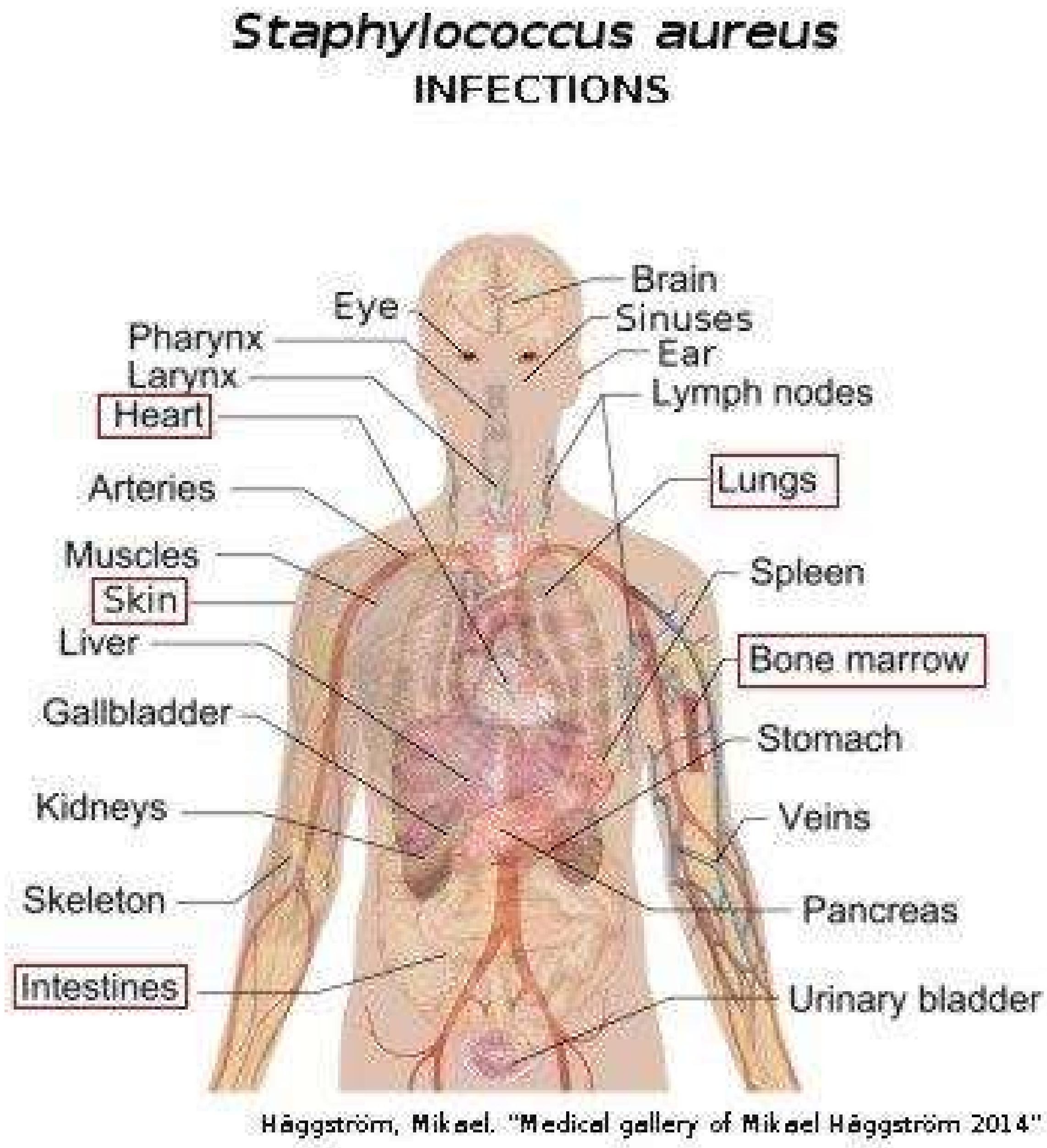



Figure (1.8 ) part of the body and disease caused by *Staphylococcus* aureus

(Häggström, Mikael. "Medical gallery of Mikel Häggstriöm 2014" )

## 1.12 Antibiotic resistance

*Staphylococcus aureus* strains which are resistant to penicillin emerged immediately after the antibiotic's discovery in the early 1940s . They produced a β-lactamase enzyme that hydrolyzed the crucial β-lactam bond , proving the antibiotic ineffective against bacteria. Penicillin's native aminoadipoy| chain was replaced with bulkier moieties, resulting in semisynthetic versions that were not -lactamase substrates and methicillin was developed. Methicillin resistance was discovered shortly after it was released, and methicillin- resistant *S. aureus* (MRSA) was born (Foster *et al* ., 2017).

Resistance mechanism to Methicillin and Oxacillin is throughout the acquisition of a gene that encodes a homologue of PBP2 known as PBP2a (penicillin binding protein 2a) which resists drug action (King *et al*., 2017)

Methicillin- resistant *S. aureus* is a multidrug- resistant bacteria that is resistant to penicillins, cephalosporins , tetracyclines ,chloramphenicol, lincomycin ,quinophthalones, aminoglycosides, macrolides, sulfonamides, and rifampicin ( Bateman *et al*., 2016 ).

Furthermore , MRSA infection has been identified as one of the world's major infectious illnesses, due to its elevated rates of morbidity and mortality, which have higher risk to human health (Hassoun *et al*., 2017).

MRSA resistance is mostly caused by plasmids, or drug- resistant gene transmission mediated by plasmids, which may extend the genome and transfer resistance genes between *S. aureus* and other bacteria (Vestergaard *et al* ., 2019).

In the late 1980s, vancomycin was approved as a therapy for severe Infections caused by MRSA. The first vancomycin resistance *Staphylococcus aureus* (VRSA) strain was discovered in Michigan, USA, in 2002.

The *vanA* operon expressed on transposon Tn1546 , which was originally part of avancomycin -resistant enterococci (VRE) conjugative plasmid, provides complete vancomycin resistance in *S. aureus*. During discrete conjugation events, *S. aureus* can acquire enterococcal plasmids. *S. aureus* maintains vancomycin resistance by keeping an enterococcal plasmid or transposing Tnl546 from the VRE plasmid into a *Staphylococcal* resident plasmid (Guinness *et al.,* 2017).

## 1.13 The Aim of the study

The aim of the current work is to study

1- The effect of the different laser radiation doses on bacterial growth *in vitro.*

2- The select of the best dose of laser that effect on growth of bacteria.

# Chapter Two

## Materials and Methods

## 2.1 The biological part: Materials and Methods

### 2.1.1 Apparatus and Instruments

Table (2.1): Apparatuses and equipment used

In the study's experiments, the following equipment and instruments were utilized

| Apparatus or instrument | Manufacturer and origin |
|---|---|
| Autoclave | Diako (Germany) |
| Balance ( electrical) | Sartourus (Germany) |
| Centrifuge | Iraqi airways (Iraq) |
| Colony counter | Memmert (Germany) |
| Compound Light Microscope | Olympus (Japan) |
| Electrical Oven | Memmert (Germany) |
| Hot Plate With Magnetic Stirrer | L.IP. (England) |
| Incubator | GallenKamp (England) |
| Micropipette | Gilson (France) |
| pH – meter | Philip Harris (England) |
| Refrigerator | Concord(Germany) |
| Sensitive Balance | Sartourus (Germany) |
| Vortex | Griffin (Germany) |
| Viteck-2 compact system | Biomerieux (France) |
| Water distellator | G.FL. (Germany) |

### 2.1.2 Chemicals and biological materials

Table (2.2): The chemicals and biological materials used in this study with their companies

| Chemicals | Company and origin |
|---|---|
| Absolute ethanol | ROMIL pure chemistry , UK |
| Catalase reagent | Analar – England |
| Coagulase rabbit plasma | Coagulase rabbit plasma |
| Glycerol | Fluka – Switzerland |
| Grams stain kit including crystal violet ,alcohol , Iodine and safranine | Syrbio – Syria |
| Human blood | Central blood bank |
| Hydrogen peroxide | Fluka (Switzerland) |
| Oxidase reagent | Himedia/(India) |
| Standard McFarland's solution (0.5) | BioMérieux/France |

### 2.1.3 Bacterial culture media

Bacteriological media used in the study are listed in table (2-3).

Table (2.3) Ready-made bacterial media

| Media | Company and origin |
|---|---|
| Blood agar base (BA) | Oxoid – England |
| Brain heart infusion agar (BHIA) | Oxoid – England |
| Brain heart infusion broth (BHIB) | Oxoid – England |
| Chromogenic agar | CHROMagar (Spain) |
| Nutrient agar (N.A) | Himedia – India |
| Nutrient broth (N.B) | Himedia – India |
| Trypton soy agar (TSA) | Oxoid – England |
| Trypton soy broth (TSB) | Oxoid – England |
| Mannitol salt agar (MSA) | Himedia – India |
| Muller Hinton agar (MHA) | Oxoid – England |

## 2.2 Methods

### 2.2.1 Media Preparation

A: Culture media

All media were sterilized in an autoclave for 15 minutes at 121 °C and 15 lb/in2 of pressure, with the pH adjusted to 7.0 ± 0.3. These media were prepared in accordance with the manufacturer's instructions. As previously stated, the media were brought to a boil on a magnetic hot plate stirrer at 100 °C until the constituents were completely dissolved and then autoclaved. The media were then dispensed as needed into sterile petri dishes or tubes, incubated for 24 hours at 37°C to ensure sterility, and stored at 4°C until use. Except chromogenic agar that was not sterilized by autoclave. All the Petri dishes put in an incubator after preparing to ensure that Petri dishes did not contain any contamination.

### 2.2.2 Laboratory prepared culture media

#### 2.2.2.1 Blood agar medium

Blood agar was made by adding 5–10% sterilized fresh human blood to 40gm/L of agar that had been autoclaved and cooled to 45–50°C, as according to the manufacturer's instructions. The ability to hemolysis blood and the specific type of hemolysis were tested for numerous harmful bacteria in these media like *S. aureus* (Harley and Prescott *et al*., 2007).

#### 2.2.2.2 Mannitol-Salt agar medium

It was prepared according to the instructions provided of the provider. This medium has a high salt concentration of 7% NaCl and an indicator of Phenol Red. It was placed in sterilized Petri dishes after cooling to 45-55 °C. Agar is a *S. aureus* differential medium (Berger-Bachi *et al*., 2002).

### 2.2.2.3 Chromogenic agar medium

This medium can be prepared by dissolving 110 grams of the medium in 1 liter of distilled water (D.W), mixing thoroughly, and then dissolving for heating with frequent agitation. The mixture is then brought to a boil for one minute, or until total dissolution.

## 2.2.3 Reagents, stains and solutions

### 2.2.3.1 Catalase reagent

This reagent was made by combining 1 ml of 30 percentage concentrated H202 with 9 ml of D.W. Final concentration was 3 %. The reagent was used to examine the ability of bacteria to produce catalase enzyme (Forbes *et al.,* 2007).

### 2.2.3.2 Oxidase reagent

Freshly dissolving 0.1 g of N, N, N-tetra-methyl-P-phenylene diamine dihydrochloride in 10 ml of D.W to be stored in a dark container produced the reagent. It was immediately used to detect the ability of bacteria to produce oxidase enzyme (Vandepitte *et al*., 2003).

### 2.2.3.3 Coagulase test

In blood plasma, the coagulase enzyme catalyzes the conversion of fibrinogen to fibrin. Adding 0.5 ml of the tested bacterial isolate and 0.5 ml of citrated plasma solution to a test tube and incubating it at 37°C initiates the reaction. The tubes are examined for coagulation after 0.5, 1, 2, and 4 hours. During four hours, the formation of a clot indicates a positive result. After twenty-four hours, waek or delayed coagulase production must be detected by bringing the tubes to room temperature (Kader *et al*., 2011).

### 2.2.3.4 Gram stain

A ready-to-use kit includes four containers: a) Crystal violet solution, b) Lugol's iodine, c) Decolorization solvent, and d) 0.5% standard safranin as a counterstain. This stain distinguishes between two types of gram-positive bacteria (Atlas *et al.,* 1995).

### 2.2.3.5 Normal saline solution

The solution was prepared by dissolving 8.5 grams of NaCl in 900 ml of distilled water, then filling to 1000 ml with distilled water, autoclaving, and storing at 4º until use (Benson *et al.,* 2001).

### 2.2.3.6 Standard McFarland solution (tube No. 0.5)

The following is how Baron and Finegold (1994) recommend making standard McFarland solution No. 0.5:

- 1.175 g of barium chloride was dissolved in 90 ml of D.W., and the final volume was brought up to 100 ml to make solution (1).
- To make solution (2), we mixed 1 ml of concentrated sulfuric acid into 90 ml of D.W. and brought the volume up to 100 ml.

Added 0.5 ml of solution and stirred to combine (1). Ninety-nine and a half milliliters of the solution were added (2). This solution was used to measure the turbidity of bacterial suspensions at a cell density of $1.5 \times 10^8$ CFU/ml.

## 2.2.4 Collection of isolates

Ten isolates of *Staphylococcus aureus* isolated from patients suffering from skin infection from two teaching hospital in Baghdad . A loop full of each isolates were culture in test tube containing brain _heart infusin agar and them examined at the clinical communicate disease research unit laboratories and other additional tests.

## 2.2.5 Identification of clinical *Staphylococcus aureus* isolates

### 2.2.5.1 Morphological Examination

Morphological characteristics of bacterial colonies grown on blood agar, MacConkey agar, mannitol salt agar, and chromogenic agar were investigated as potential primary diagnostic tests (Harley and Prescott *et al*., 2002).

### 2.2.5.2 Microscopic examination

Isolates were Gram stained and studied under an oil immersion lens microscope to determine their sizes, morphologies, stain reactions, and cell configurations (Gillet *et al.,* 2002).

#### 2.2.5.2.1 Growth on mannitol salt agar

Bacteria can be isolated and grown in controlled conditions using this medium. Streaks of bacteria were examined after being incubated at 37 °C for 24 hours on a plate containing mannitol salts in order to determine whether or not they were able to ferment the mannitol sugar "(Gillet *et al.,* 2002)".

### 2.2.5.2.2 Growth on Chromogenic agar medium

To diagnose *S. aureus*, use this medium. It is put to work in the process of isolating and growing desired bacteria in controlled conditions. Positive results were indicated by the development of a mauve color after 24 hours of incubation at 37 °C on a Chromogenic agar plate after streaking (Flayhart *et al.,* 2005).

### 2.2.5.3 Biochemical Tests

### 2.2.5.3.1 Catalase test

To conduct the test, a sterile inoculating wood stick was used to collect a colony from a pure, fresh culture that had been growing for 24 hours, and this colony was then combined with a drop of 3 percentage of the hydrogen peroxide ($H_2O_2$) reagent on a clean glass slide. (Brown *et al*., 2005).

### 2.2.5.3.2 Oxidase test

One isolated colony was placed on filter paper, and then two or three drops of oxidase reagent were added. A positive test would show a rapid color change to deep purple, happening within 20-30 seconds. Color change is not something that would be caused by oxidase-negative bacteria. This was found by a group of researchers led by Harley and Prescott in 2007 (Harley and Prescott *et al.,* 2007).

### 2.2.5.3.3 Free coagulase test (Test tube)

To routinely identify *S. aureus*, the tube coagulase test is used. A loop was used to move several bacterial colonies from a petri dish into a tube containing 5 ml of brain heart infusion broth. Overnight, the tube was placed in an incubator at 37 degrees Celsius with the lid on to prevent evaporation. An internal centrifuge was used to mix the contents of the tube. After that, half a milliliter of the supernatant was combined with the same volume of rabbit plasma and placed in a water bath at 37 degrees Celsius for several hours. If the organism caused the plasma to clot, it has coagulase activity. Whether it took 30 minutes or several hours, coagulation did occur. Even if it took 24 hours to form, a positive result was considered to be any degree of coagulation, from a clot floating in the plasma to a solid clot that couldn't be moved (Brown *et al.,* 2005).

#### 2.2.5.3.4 Hemolytic activity

Striking the pure isolates on blood agar plates uncovered their hemolytic activity. The presence of a hemolytic zone around the bacterial colony indicates the presence of hemolytic activity after 24 hours of incubation at 37°C (Benson *et al.,* 2001).

### 2.2.6 Identification and Antibiotic susceptibility test of *Staphylococcaceae* using VITEK® 2 System

VITEK-2 is an automated microbiology system utilizing growth-based technology, which represents advanced colorimetric technology for bacterial identification and antibiotic susceptibility.

**The kit contains the following**

1. Gram positive card (ID-GPB) used for identification of gram -positive bacteria.
2. Gram positive susceptibility card: it contains 20 microwells , contained Benzylpenicillin, Oxacillin, Gentamicin, Tobramycin, Clindamycin, Erythromycin, Linezolid, Levofloxacin, Moxifloxacin,

Nitrofurantoin, Tigecycline, Rifampicin, Teicoplanin, Tetracycline, Fusidic Acid.

Trimethoprim-Sulfamethoxazole and Vancomycin Procedure

The following steps were done depending on the manufacture's instruction:

- In a plan tube, 3ml of normal saline were inoculated with a loopful of isolated colony.
- Test tube was standardization of colony with McFarland solution to obtain cell density 1.5 x $10^8$ (CFU/ml).

The standard inoculum was put within the cassette, and the sample identification number was entered via barcode into the software program. A reader scanned the barcode on the VITEK-2 card for identification and antibiotic susceptibility testing.

## 2.2.7 Preservation technique of bacterial isolates

The following bacterial isolates were kept by Vandepitte *et al*. (2003): as

### 2.2.7.1 Preservation for short-term

The procedure of (Vandepitte *et al*., 2003) was carried out to store the isolate for 1 to 3 months on plates or slants, respectively. A single pure colony of bacterial isolate is streaked on the nutrient agar culture plate and on the nutrient agar slants, incubated for 24 hours at 37°C, and then stored at 4°C in the refrigerator.

### 2.2.7.2 Preservation for Long-term

To maintain bacterial isolates for an extended period of time (at minimum three months); the bacteria were cultured at a low temperature on a medium containing 20 percent of the glycerol. The medium was prepared by adding 2ml of glycerol to 8ml of brain heart infusion broth, then dispensing the mixture into a small bottle with a screw-on cap and autoclaving it. After cooling, the tubes were inoculated with a single pure, isolated colony and incubated for one day (24 hours) at 37°C. The tubes were kept at -20°C in deep freezing (Vandepitte *et al.,* 2003).

## 2.2.8 Experimental Setups

### 2.2.8.1 System Setup

The laser system was fixed vertically on mechanical jack supported with height tuner screw on plane bench; so the laser beam can fall vertically on the test sample and the laser aperture was stick to the test sample. Figure (2.2) shows the irradiation setup.

### 2.2.8.2 Laser Parameters

The laser that was used in this study was the alexandrite laser which was considered as pulsed laser and had the following parameters:

- The wavelength (λ) was 755 nm.
- The beam diameter was (14 mm).
- 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:** E= is the power of the laser multiplied of pulse width (watt x second).

A = is the exposed area to laser beam ($cm^2$)


Figure (2.1) Light Evo laser device

### 2.2.8.3 Bacterial Samples Preparation

From the nutrient agar, agar slants, a loopful of the resistant isolate culture was transferred to a tube containing 10ml of brain/heart infusion broth, and then incubated at 37°C for 18–24 hrs. Serial dilutions were made in tubes containing physiological saline to obtain appropriate CFU. The bacterial broth was compared with McFarland tubes to determine the number of bacteria equal 1.5 x $10^8$ mI/CFU.

### 2.2.8.4 Irradiation Procedures

Irradiation for was as follows:

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

The study was carried out using different exposure times (30, 60 and 90 sec) with different fluencies (5,10,15 and 20 $J.cm^{-2}$), as well as was using different pulse durations (5,10 and 20ms).

An alexandrite laser was used to expose the *S. aureus* bacteria (resistance to antibiotic) to different exposure times and different pulse durations with different fluencies. The first exposure time was 30 seconds at 5 ms pulse duration to expose three samples of *S. aureus* using four laser fluencies at 5, 10, 15 and 20 J/cm2. This process was repeated in 10ms and 20ms pulse durations. Then all the previous process was repeated with the second exposure time of 60 seconds and also repeated with the third exposure time of 90 seconds, as shown in figure (2-2)

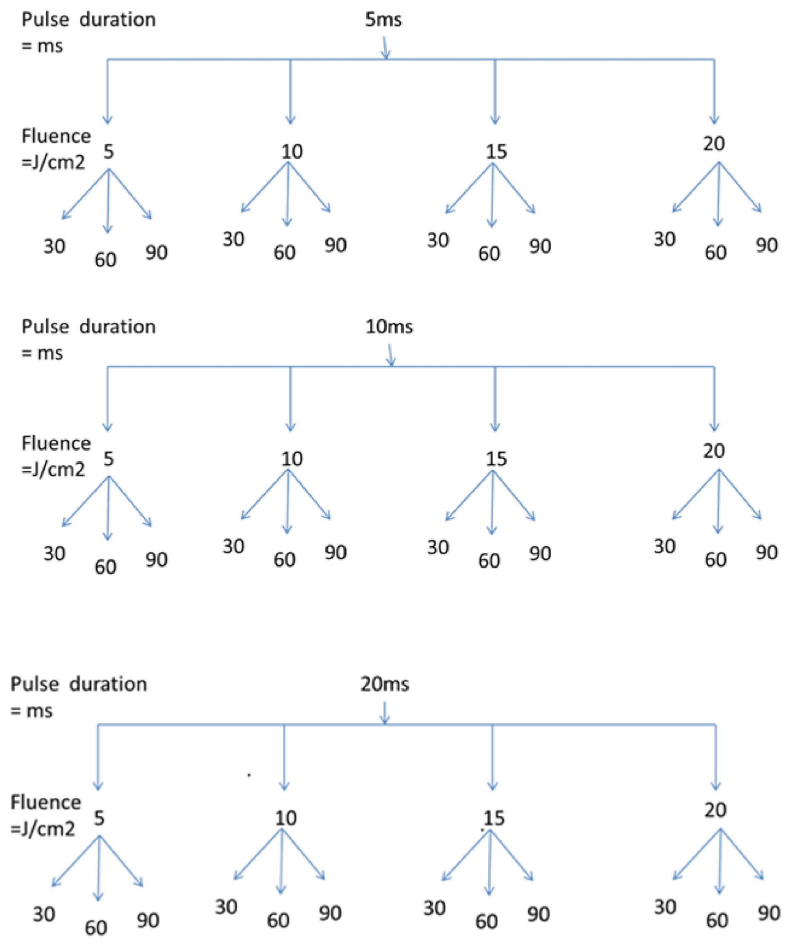



Figure (2-2): Diagram Irradiation Procedures of pulse durations, flounce, and time exposure

## 2.2.8.5 Inoculation of irradiated isolates

0.1ml of each irradiated group of bacteria was transferred to the surface of media (three plates for each group). After that inoculum was speared missing glass spreader. Left at room temperature for 10 minutes, and these plates incubated aerobically for 24 hour at 37 °C. The number of colonies counted by using colony counter.

## 2.2.9 Statistical analysis:

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference (LSD) test (Analysis of Variation- ANOVA) was used to make a comparison between means. A chi-square test was used to compare percentages (0.05 and 0.01 probability) in this study.

# Chapter Three

## RESULTS

## 3.1 Isolation and Identifction of Bacteria

### 3.1.1Cultural cheraterzations

All isolates obtained by culture on Nutrient agar were then subcultured on manital salt agar, and blood agar. The results indicated that *Staphylococuus aureus* isolates were able to grow on mannition salt agar medium yielding white or yellow colonies, large, smooth, round colonies with entier margin.

### 3.1.2 Microscopic characterization

Microscopic examination showed that *S. aureus* reacted with Gram stain, cocci, arranged in pairs or in cluster and non- spore forming.

### 3.1.3 Biochemical characterization

The biochemical characteristic of all *S. aureus* ioslates were catalase and coagulase positive, but oxidase negative ( table 3.1)

**Table (3.1) Gram stain and Biochemical tests for *S. aureus***

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

### 3.1.4 Identification of *Staphylococcus aureus* by VITEK© compact system

Identification of bacterial isolates done by VITEK-2 System using gram positive card which gave 96 % probability of *S. aureus.*

### 3.1.5 Antibacterial susceptibility of *Staphylococcus aureus*

Ten isolates of *S. aureus* revealed a various resistance level toward 16 antimicrobial agent by VITEK as following :

benzyl Penicillin 10/10 (100%) , Oxacillin 9/10 (90%) , Gentamicin 4/10 (40%) Tobarmycin 5/10 (50%) , 6/10 (60%) for Rifampicin and Erythromycin , Clindamycin 7/10 (70%) ,linezolid 2/10 (20%) , Tecoplanin 2/10 (20%) ,Vacomycin 3/10 (30%) , Trimethoprim sulfamethoxazole 2/10 (20%) while all isolates were sensitive to Tigecycline .

The results of the effect of an Alexandrite pulsed laser on *Staphylococcus aureus* bacteria growth (by the mean values of colony count) will presented in this chapter. The study was carried out using different exposuer 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 was using different pulse durations (5,10 and 20 ms) .

## 3.2 Effect of Puls Alexandrite Laser according to expouser time.

### 3.2.1Expouser time 30 sec

Table 3.2: The mean values of colony count for experimental isolates and control of *Staphylococcus arueus* bacteria after treated with different fluencies of an Alexandrite Laser pulse (5,10,15 and 20 $J.cm^{-2}$) and different pulse durations 5, 10, 20ms) at exposure time 30 sec .

Different small letters (a, b, c, d and e) in row are significant at $p \leq 0.05$ and same letters are Non-Significant, SE: Standard error mean. Different capital letters (A, B and C) in in column are significant at $p \leq 0.05$ and same letters are Non-Significant, SE: Standard error mean.

The mean values of colony count of *S. aureus* bacteria for the control were 216±7.35, 246±4.18 and 241±7.12 for puls duration 5ms, 10ms and 20ms, respectively ( table 3.2). For laser fluency 5$J.cm^{-2}$, the mean values of colony count of *S. aureus* were 126.67±2.9, 214.67±4.4and 224.67±8.2 for puls duration 5ms, 10ms and 20ms, respectively. Whereas, for laser fluency 10$J.cm^{-2}$, the mean values of colony count of *S. aureus* were 87.67±4, 174.67±8.17 and 185.33±6.85 for puls duration 5ms, 10ms and 20ms, respectively (see table 3.2). The mean values of colony count of *S. aureus* treated with the laser fluncy 15J.cm-2 were 74.67±3.6, 142±6 and 160.67±7.25for puls duration 5ms, 10ms and 20ms, respectively. As well as, for laser fluency 20J.cm-2 , the mean values of colony count of bactira were 41.67±2.3, 80.33±6.17 and 85.67±6.88for puls duration 5ms, 10ms and 20ms, respectively (Table 3.2).

Statistical analysis of the results, there is a reduction in the mean values of colony count after treated with different fluencies ($J.cm^{-2}$) of an

Alexandrite Laser pulse when in comparison with untreated control at the same pulse durations (ms). For the 5ms pulse duration, 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 pulse in comparison with the control were by 41%, 59% , 65% and 81%, respectively. Also, for the 10ms pulse duration, the reduction in the mean values of colony count treated with 5J.cm$^{-2}$, 10J.cm$^{-2}$, 15J.cm$^{-2}$ and 20J.cm$^{-2}$ fluencies of an Alexandrite Laser pulse in comparison with the control were by 13%, 29% , 42% and 67%, respectively. As well as, for the 20ms pulse duration, the reduction in the mean values of colony count of *S. aureus* bacteria treated with 5J.cm$^{-2}$, 10J.cm$^{-2}$, 15J.cm$^{-2}$ and 20J.cm$^{-2}$ fluencies of an Alexandrite Laser pulse in comparison with the control were by 6%, 23% , 33% and 64%, respectively (Table 3.2)

According to the resulted presented in Table (3.2), the mean values of colony count for control was in comparison with experiment samples based on different laser fluency at one pulse duration and 30 sec exposure time, respect to the rows. A significant reduction ($p$ = <0.0001) in the mean values of colony observed with the increase of laser fluency doses in comparison with control at the same pulse duration. As well as, a significant reduction ($p$ = <0.0001) in the mean count of the colonies were observed with in comparison between two laser fluenceis at the same pulse duration. However, there are no significant differences in mean values of colony count between control and 5 J.cm$^{-2}$ at 20ms pulse duration, as shown in figure 3.1.

With respect to different pulse durations (5, 10 and 20 ms) at same fluency effect on bacteria colonies and at 30 sec exposure time, there is noticed an increased in mean values of colony count with increased pulse duration to 10ms and 20ms comparing with 5ms pulse duration.

Significant difference was ($p \leq 0.05$) noticed in mean values of colony count between pulse durations (5ms and 10ms, 5ms and 20ms), however there is no significant differences the mean values of colony count between 10ms and 20ms pulse duration at 5, 10 and 20 $J.cm^{-2}$ laser pulse fluencies as shown in figure 3.1.

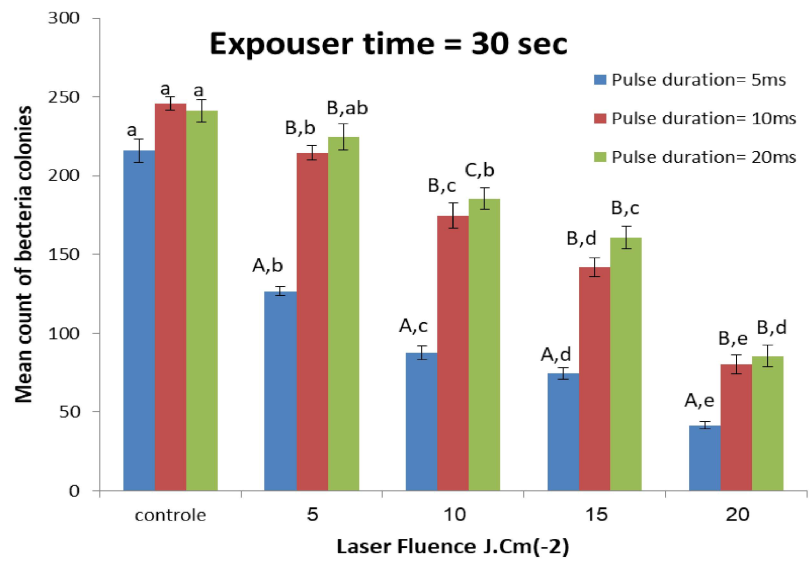



Figure 3.1: Relationship between mean of bacteria colonies and four fluencies of an Alexandrite pulsed Laser (5, 10, 15 and 20 $J.cm^{-2}$) with three pulse durations (5, 10 and 20ms) at 30 sec exposure time

## 3.2.2 Expouser time 60 sec

Table 3.3: The mean values of colony count for control and experimental samples of *Staphylococcus arueus* bacteria after treated with different fluencies of an Alexandrite Laser pulse (5,10,15 and 20 $J.cm^{-2}$) and different pulse durations 5, 10, 20ms) at exposure time 60 sec.

Different small letters (a, b, c, d,e) in row are significant at $p \leq 0.05$ and same letters are Non-Significant, SE: Standard error mean. Different capital letters (A ,B) in in column are significant at $p \leq 0.05$ and same letters are Non-Significant, SE: Standard error mean.

The mean values of colony count of *S. aureus* bacteria for the control were 216± 7.35, 246 ± 4.18 and 241±7.12 for puls duration 5ms, 10ms and 20ms, respectively (see table 3.3). For laser fluency 5J.cm$^{-2}$ , the mean values of colony count were 116.67±2.96, 196.67±3.38 and 204.67±7.8 for puls duration 5ms, 10ms and 20ms, respectively. Whereas, for laser fluency 10J.cm$^{-2}$, the mean values of colony count were 80.67±2.4, 165.67±6.47 and 180±3.8 for puls duration 5ms, 10ms and 20ms, respectively. The mean values of colony count treated with the laser fluncy 15J.cm$^{-2}$ were 70±2.55, 120.67±8.44 and 156±7.52 for puls duration 5ms, 10ms and 20ms, respectively. As well as, for laser fluency 20J.cm$^{-2}$ , the mean values of colony count were 29.67±3.88, 58.33±5 and 78.67±3.9 for puls duration 5ms, 10ms and 20ms, respectively (Table 3.3).

From the analysis of the results, it can be seen that there is a reduction in the mean values of colony count after treated with different fluencies (J.cm$^{-2}$) of an Alexandrite Laser pulse in comparison with control at the same pulse durations (ms) involved in the current study and at exposure time 60 sec. For the 5ms pulse duration, the reduction in the mean values of colony count treated with 5J.cm$^{-2}$, 10J.cm$^{-2}$, 15J.cm$^{-2}$ and 20J.cm$^{-2}$ fluencies of an Alexandrite Laser pulse in comparison with the control were by 46%, 63% , 68% and 86%, respectively. Also, for the 10ms pulse duration, the reduction in the mean values of colony count treated with 5J.cm$^{-2}$, 10J.cm$^{-2}$, 15J.cm$^{-2}$ and 20J.cm$^{-2}$ fluencies of an Alexandrite Laser pulse in comparison with the control of *S. aureus* were by 20%, 33% , 51% and 76%, respectively As well as, for the 20ms pulse duration, the reduction in the mean values of colony count of *S. aureus* treated with $5J.cm^{-2}$, $10J.cm^{-2}$, $15J.cm^{-2}$ and $20J.cm^{-2}$ fluencies of an Alexandrite Laser pulse in comparison with the control were by 15%, 25% , 35% and 67%, respectively (Table 3.3).

According to the resulted presented in Table (3.3), the mean values of colony count of control was in comparison with experiment samples based on different laser fluency doses at one pulse duration and 60 sec exposure time, respect to the rows. A significant reduction ($p = <0.0001$) in mean of the bacteria colonies was observed with the increase of laser fluency doses at the same pulse duration. As well as, a significant reduction ($p = <0.0001$) in mean of the bacteria colonies was observed with in comparison between two laser energies at the same pulse duration (see figure 3.2).

With respect to different pulse duration (5, 10 and 20 ms) at same laser fluency effect on bacteria colonies and at 60 sec exposure time (respect to the column), there is noticed an increased in mean of the colonies with increased pulse duration to 10ms and 20ms comparing with 5ms pulse duration. Significant difference was ($p \leq 0.05$) noticed in mean of the colonies between pulse durations 5ms and 10ms at different fluencies except at $10 J.cm^{-2}$ the $p$ value was less than 0.05. Significant differences in mean values of colony count between 10ms and 20ms pulse duration were ($p \leq 0.05$) with all laser fluencies except at $5 J.cm^{-2}$ and $10 J.cm^{-2}$ were no significant, as shown in figure 3.2.

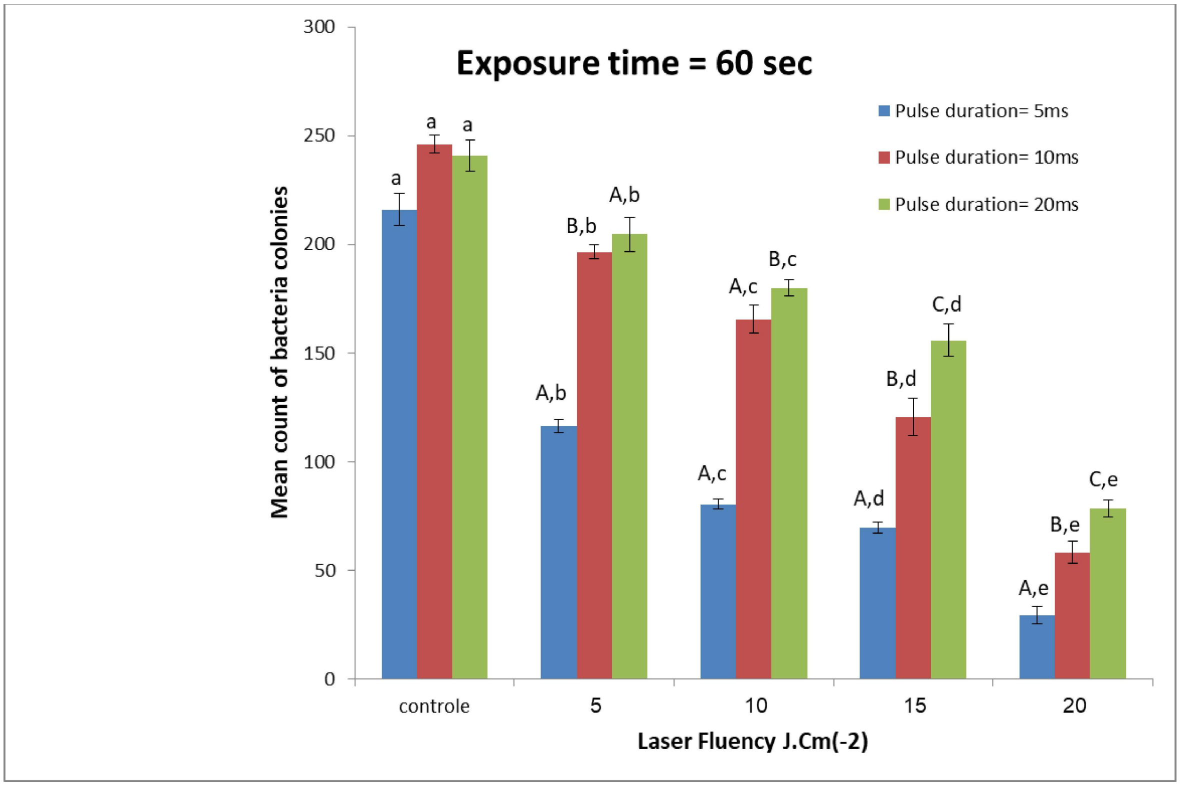



Figure 3.2: Relationship between mean of bacteria colonies and four fluencies of an Alexandrite Laser pulsed (5, 10, 15 and 20 J.cm$^{-2}$) with three pulse durations (5, 10 and 20ms) at 60 sec exposure time.

## 3.2.3 Expouser time 90 sec

Table 3.4: The mean values of colony count for control and experimental samples of *Staphylococcus arueus* bacteria after treated with different fluencies of an Alexandrite Laser pulse (5,10,15 and 20 J.cm$^{-2}$) and different pulse durations 5, 10, 20ms) at exposure time 90 sec

Different small letters (a, b, c, d,e) in row are significant at $p \leq 0.05$ and same letters are Non-Significant, SE: Standard error mean. Different capital letters (A ,B) in in column are significant at $p \leq 0.05$ and same letters are Non-Significant, SE: Standard error mean.

The mean values of colony count of the control were 216± 7.35, 246 ± 4.18 and 241±7.12 for puls duration 5ms, 10ms and 20ms, respectively. For laser fluency 5J.cm$^{-2}$ , the mean values of colony count were 99.67±4.17, 162.67±5.18 and 181.33±3.9 for puls duration 5ms, 10ms and 20ms, respectively. Whereas, for laser fluency 10J.cm$^{-2}$, the mean values of colony count were 74.67±3.5, 140.33±5.86 and 166.33±3.5 for puls duration 5ms, 10ms and 20ms, respectively. The mean values of colony count treated with the laser fluncy 15J.cm$^{-2}$ were 54±5.77, 90.67±4.76 and 142±2.34 for puls duration 5ms, 10ms and 20ms, respectively. As well as, for laser fluency 20J.cm$^{-2}$ , the mean values of colony were 15.67±2.6, 38.33±3.45 and 55.33±4.48 for puls duration 5ms, 10ms and 20ms, respectively (Table 3.4).

From the analysis of the results of (table 3.4), there is a reduction in the mean values of colony count after treated with different fluencies (J.cm$^{-2}$) of an Alexandrite Laser pulse when in comparison with control of colonies at the same pulse durations (ms) involved in the current study and at exposure time 30 sec. For the 5ms pulse duration, 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 pulse in comparison with the control were by 54%, 65%, 75% and 93%, respectively. Also, for the 10ms pulse duration, 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 pulse in comparison with the control of *S. aureus* bacteria (untreated) were by 34%, 43% , 56% and 84%, respectively As well as, for the 20ms pulse duration, 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 pulse in comparison with the control of *S. aureus* bacteria (untreated) were by 25%, 31% , 41% and 77%, respectively.

According to the resulted presented in (Table 3.4), the mean values of colony count for control was compared with experiment samples based on different laser fluency doses at one pulse duration and 90 sec exposure time, respect to the rows. A very highly significantly reduction ($p$ = <0.0001) in mean values of colony count was observed with the increase of laser fluency doses at the same pulse duration. As well as, a significant reduction ($p$ = <0.0001) in mean colonies was observed with in comparison between two laser fluencies at the same pulse duration, as shown in figure 3.3.

With respect to different pulse duration (5, 10 and 20ms) at same laser fluency effect on bacteria colonies and at 90 sec exposure time (respect to the column), there is noticed an decreased in mean of the colonies with increased pulse duration to 10ms and 20ms comparing with 5ms pulse duration. Significant difference was ($p \leq 0.05$) noticed in mean of the bacteria colonies between pulse durations (5ms and 10ms, 5ms and 20ms), see figure 3.3.

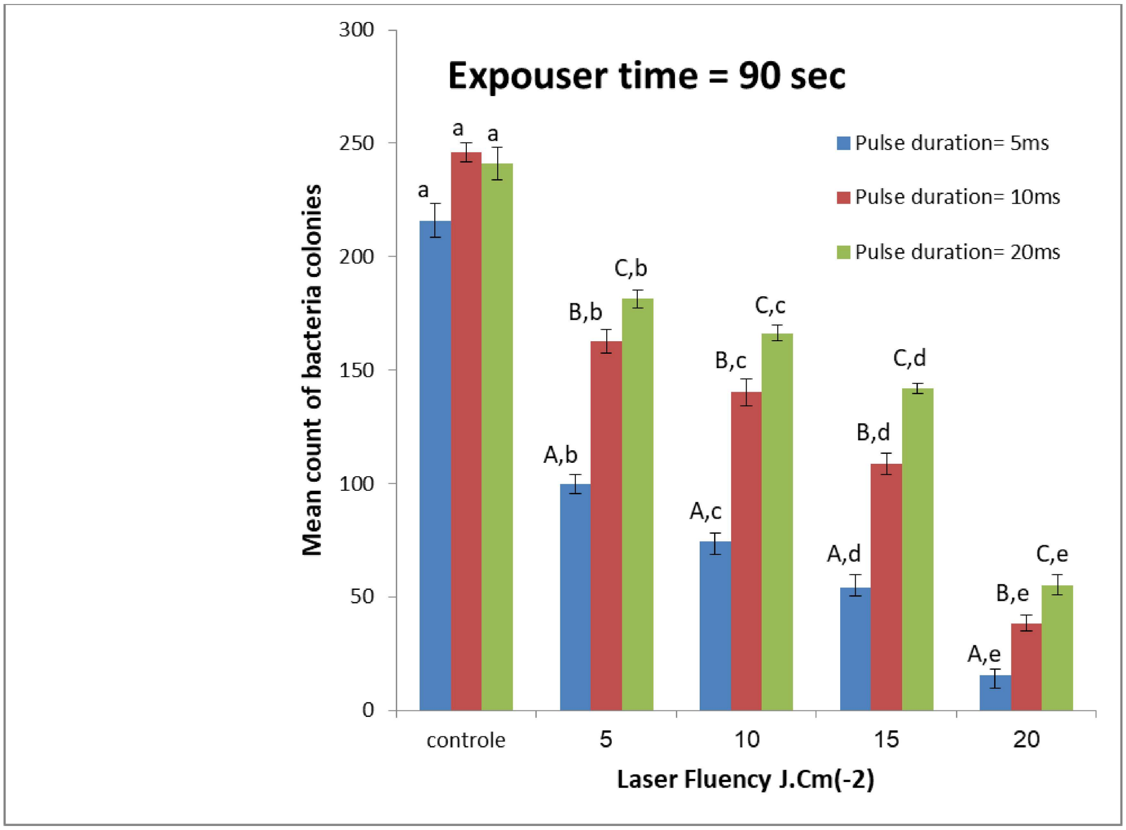



Figure 3.3: Relationship between mean of bacteria colonies and four fluencies of an Alexandrite Laser pulsed (5, 10, 15 and 20 $J.cm^{-2}$) with three pulse durations (5, 10 and 20ms) at 90 sec exposure time

## 3.3 Effect of Puls Alexandrite Laser according on Pulse duration

### 3.3.1Pulse duration 5ms

Table 3.5: The mean values of colony count for control and experimental samples of *Staphylococcus* bacteria after treated with different fluencies of an Alexandrite Laser pulsed (5,10,15 and 20 $J.cm^{-2}$) and different exposuer times (30,60 and 90 sec) at pulse duration 5ms

Different small letters (a, b, c, d,e) in row are significant at $p \leq 0.05$ and same letters are Non-Significant, SE: Standard error mean. Different capital letters (A ,B) in in column are significant at $p \leq 0.05$ and same letters are Non-Significant, SE: Standard error mean.

The mean colonies of the control was 216±7.35 for time expouse 30 sec ,60 sec and 90 sec. For laser fluency 5J.cm$^{-2}$ , the mean bacteria colonies were 126.67±2.96, 116.67±2.96 and 99.67±4.17 for time expouse 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 expouse 30 sec ,60 sec and 90 sec, respectively. The mean colonies trated with the 15J.cm$^{-2}$ laser fluency were 74.67±3.6, 70±2.55 and 54±5.77 for time expouse 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* bactira trated with the laser fluency 20J.cm$^{-2}$ were 41.66±2.33, 29.66±3.88 and15.66±2.6 for time expouse 30 sec ,60 sec and 90 sec, respectively (Table 3.5).

The results in (table 3.5) indicated that there is a reduction in mean values of colony count after 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 in comparison with the control were 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 were 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 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%, 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 3.5).

According to the resulted presented in (Table 3.5), the mean value of control was in comparison with experiment samples based on different energy doses at one exposure time and 5ms pulse duration, respect to the rows. A significant reduction ($p$ = <0.0001) in mean of the bacteria colonies was observed with the increase of laser energy doses at the same pulse duration. As well as, a highly significant reduction ($p$ = <0.0001) in mean of the bacteria colonies was observed with in comparison between two laser energies at the same exposure time (see figure 3.4).

With respect to different exposure times (30, 60 and 90 sec) at same energy effect on bacteria colonies and at 5ms pulse duration, there is noticed a decreased in 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 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 is significant differences in the mean of the colonies between exposure times at 30 sec and 60 sec when laser energies was at 20 J.cm$^{-2}$. A significant difference was ($p \leq 0.05$) noticed in mean of the colonies between exposure times (90 sec and 30 sec) at all of laser energies used in 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 3.4).

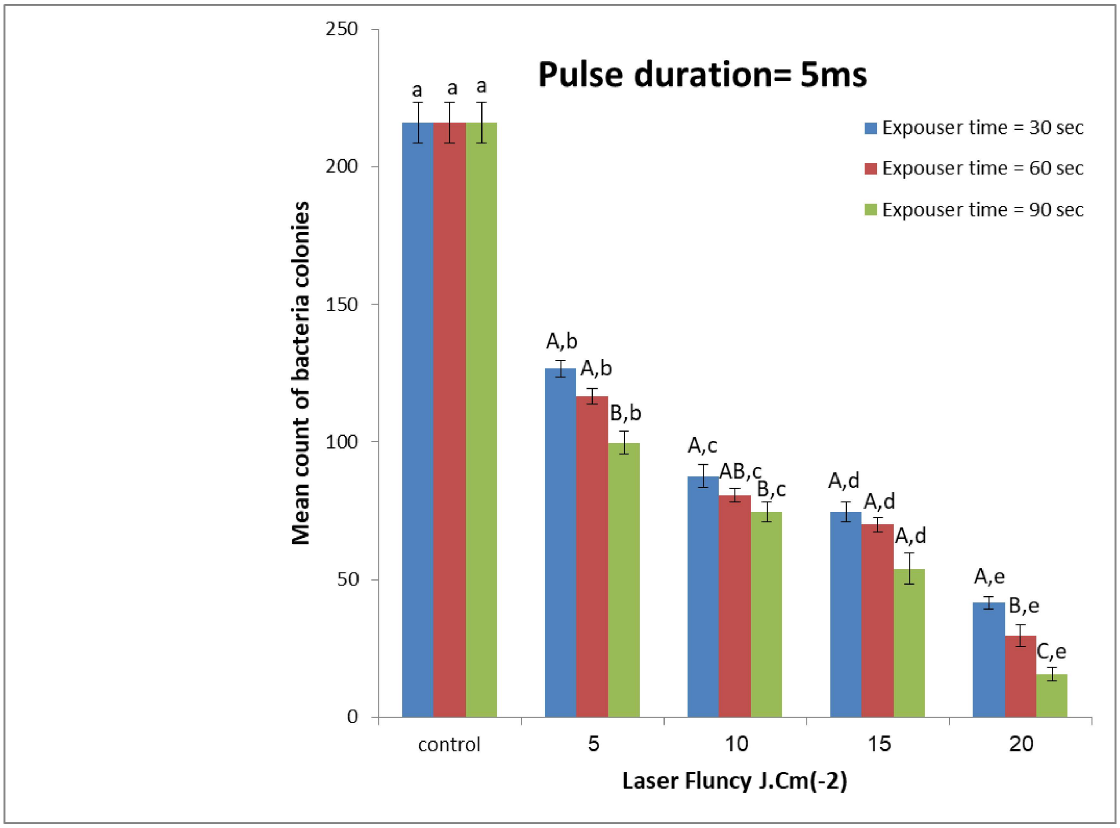



Figure 3.4: Relationship between mean of bacteria colonies and four fluencies of an Alexandrite Laser pulsed (5, 10, 15 and 20 J.cm$^{-2}$) with three exposure times (30, 60 and 90 sec) at 5ms pulse duration.

## 3.3.2Pulse duration 10ms

Table 3.6: The mean values of colony count for control and experimental samples of *Staphylococcus* bacteria after treated with different fluencies of an Alexandrite Laser pulsed (5,10,15 and 20 $J.Cm^{-2}$) and different exposuer times (30,60 and 90 sec) at pulse duration 10ms

Different small letters (a, b, c, d,e) in row are significant at $p \leq 0.05$ and same letters are Non-Significant, SE: Standard error mean. Different capital letters (A ,B) in in column are significant at $p \leq 0.05$ and same letters are Non-Significant, SE: Standard error mean.

The mean colonies of the control was 246±4.185 for time expouse 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 expouse 30 sec ,60 sec and 90 sec, respectively, whereas for laser fluency $10J.cm^{-2}$ , the mean values of colony were 174.67±8.17, 165.67±6.47 and 140.33±5.86 for time expouse 30 sec ,60 sec and 90 sec, respectively. The mean colonies trated with the $15J.cm^{-2}$ laser fluency were 142±6, 120.67±8.44 and 90.67±4.76 for time expouse 30 sec ,60 sec and 90 sec, respectively. As well as, for laser fluency $20J.cm^{-2}$ , the mean values of colony trated with the laser fluency $20J.cm^{-2}$ were 80.33±6.17, 58.33±5 and 38.33±3.45 for time expouse 30 sec ,60 sec and 90 sec, respectively (Table 3.6).

The statistical analysis of the results (table 3.6), the result indicated that there is a reduction in mean values of colony count of bacteria after 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 were 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 were 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 resulted presented in Table (3.6), the mean values of colony count for control was compared with experiment samples based on different laser fluency doses at one exposure time and 10ms pulse duration, respect to the rows. A highly significant reduction ($p < 0.0001$) in mean of the colonies was observed with the increase of laser fluency doses at the same pulse duration. As well as, a highly significant reduction ($P < 0.0001$) in mean of the bacteria colonies was observed with in comparison between two laser energies at the same exposure time (Figure 3.5).

With respect to different exposure times (30, 60 and 90 sec) at same laser fluency effect on bacteria colonies and at 5ms pulse duration (respect to the column), there is noticed a decreased in 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 colonies between exposure times at 30 sec and 60 sec when laser fluencies were at 5 $J.cm^{-2}$ , 10 $J.cm^{-2}$ and 15$J.cm^{-2}$, however there is significant differences in 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 were used in our study as illustrate in (Figure 3.5).

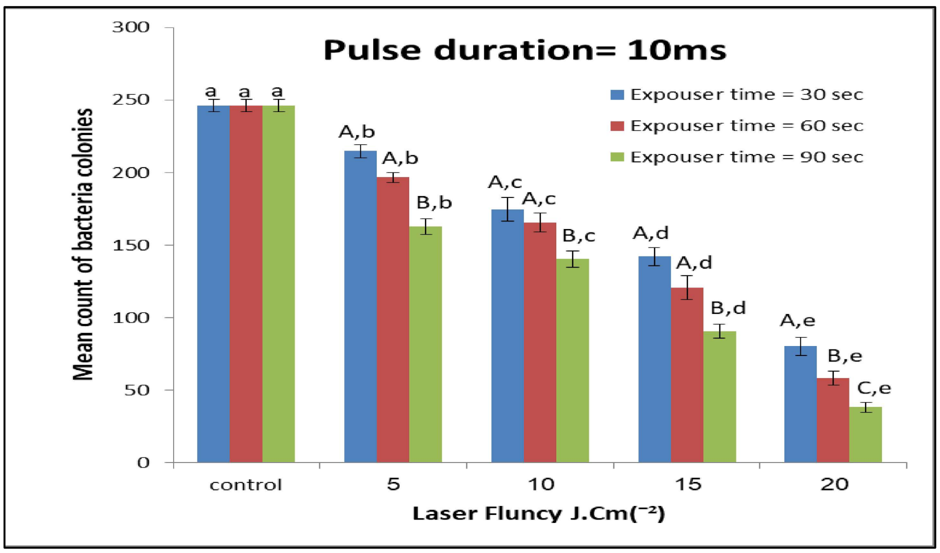



Figure 3.5: Relationship between mean of bacteria colonies and four fluencies of an Alexandrite Laser pulsed (5, 10, 15 and 20 $J.cm^{-2}$) with three exposure times (30, 60 and 90 sec) at 10ms pulse duration.

### 3.3.3 Pulse duration 20ms

Table 3.7: The mean values of colony count for control and experimental samples of *Staphylococcus aureus* bacteria after treated with different

fluencies of an Alexandrite Laser pulsed (5,10,15 and 20 $J.Cm^{-2}$) and different exposuer times (30,60 and 90 sec) at pulse duration 20ms

Different small letters (a, b, c, d,e) in row are significant at $p \leq 0.05$ and same letters are Non-Significant, SE: Standard error mean. Different capital letters (A ,B) in in column are significant at $p \leq 0.05$ and same letters are Non-Significant, SE: Standard error mean.

The mean colonies of the control was 241±7.12 for time expouse 30 sec ,60 sec and 90 sec For laser fluency $5J.cm^{-2}$ , the mean colonies were 224.67±8.2, 204.67±7.8 and 181.33±3..9 for time expouse 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 expouse 30 sec ,60 sec and 90 sec, respectively. The mean colonies of bactira trated with the $15J.cm^{-2}$ laser fluency were 160.67±7.25, 156±7.52 and 142±2.34 for time expouse 30 sec ,60 sec and 90 sec, respectively. As well as, for laser fluency $20J.cm^{-2}$ , the mean values of colony trated with the laser fluency $20J.cm^{-2}$ were 85.67±6.88, 78.66±3.9 and 55.33±4.48 for time expouse 30 sec ,60 sec and 90 sec, respectively (Table 3.7).

Statisicaly analysis of the results (Table 3.7) indicated that there is a reduction in mean values of colony count of *S. aureus* bacteria after 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 $5J.cm^{-2}$, $10J.cm^{-2}$, $15J.cm^{-2}$ and $20J.cm^{-2}$ fluencies of an Alexandrite Laser pulsed in comparison with the control were by 7%, 23%, 33% and 64%, respectively. Also, for the 60 sec exposure time, the reduction in the mean value of colonies treated with $5J.cm^{-2}$, $10J.cm^{-2}$, $15J.cm^{-2}$ and $20J.cm^{-2}$ laser fluencies of an Alexandrite Laser pulsed in comparison with the control of bacteria were 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 $5J.cm^{-2}$, $10J.cm^{-2}$, $15J.cm^{-2}$ and $20J.cm^{-2}$ by 19%, 10%, 12% and 35%, respectively (Table 3.7).

According to the resulted presented in (Table 3.7), the mean values of colony count for control in comparison with experiment samples based on different laser fluency doses at one exposure time and 10ms pulse duration, respect to the rows. A significant reduction ($p < 0.0001$) in mean of the bacteria colonies was observed with the increase of laser fluency doses at the same pulse duration. As well as, a significant reduction ($p < 0.0001$) in mean of the colonies was observed with in 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 exposure time 30 sec, as shown in figure 3.6.

With respect to different exposure times (30, 60 and 90 sec) at same fluency effect on bacteria colonies and at 5ms pulse duration (respect to the column), there is noticed a decreased 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 mean of the bacteria colonies between exposure times at 30 sec and 60 sec with all of the laser fluencies were used in current study. As well as, there are no significant differences ($P > 0.05$) in 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 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 mean of the bacteria colonies between exposure times (30 sec and 90) at all of the laser fluencies were used in our study except at 15 $J.cm^{-2}$ laser fluency as illustrate in (Figure 3.6).

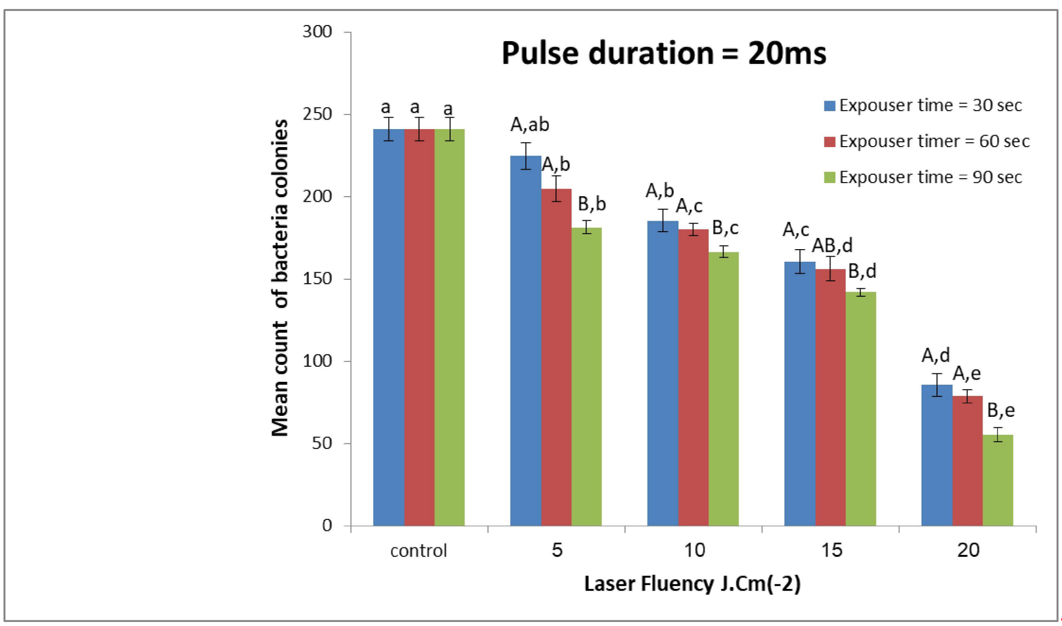



Figure3.6: Relationship between mean of bacteria colonies and four fluencies of an Alexandrite Laser pulsed (5, 10, 15 and 20 $J.cm^{-2}$) with three exposure times (30, 60 and 90 sec) at 20ms pulse duration

# Chapter Four

## Discussion

## 4.1 Discussion

### *Staphylococcus aureus*

*Staphylococci* are able to ferment carbohydrates and produce pigments ranging in color from white to a golden yellow when cultured on a variety of laboratory media. Although some of these 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 even septicemia (Karen *et al* ., 2013).

Mannitol salt agar is used to isolate *S. areuas* from theris , including clinical and enviromental samples . Mannitol salt agar contain about 7.5% sodium chloride most other bacterial speaies will be inhibited except *staphylococcus* and phenol red indicator indicated that *S. areuas* ferment the mannitol to form yellow zone in mannitol salt agar due to fermentation and producion of acid that decrease that PH of the medium ,converting the color of phenol red to yellow . The test allow to distinguished from S. epidermis which produce coloring of red zone (Vande pitt *et al* .,2003 )

*Staphylococcus aureus* solates revealed various resistance levels to antimicrobial agent (Cennet *et al*., 2016 ) found 32% of S. areuas resistant to gentamicin which agree with the present study . Antibiotics have a crucial role in treating and preventing common diseases, but antibiotic resistance is rising due to natural selection, overuse, and abuse (zhen *et al* ., 2019).

### The effect of laser on *Staphylococcus aureus*

The use of LILT for the treatment of musculo-skeletal disorders, soft tissue injuries, and wound healing, all of which can be colonized by bacteria, has been found to be effective. (Gamaleya *et al* ,. 1977 ; Chung *et* *al*.., 2014 ;de Sousa *et al*.,. 2016) 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 inactivation of proliferation of human and animal cells in vitro (Nussbaum *et al*., 2009, Peplow *et al*., 2010, Santamato *et al.,*2009).

Light's characteristics (such as wavelength and coherence) are used by lasers to affect cellular and tissue function. One of the most fundamental mechanisms of lasers is mono-chromaticity (Santamato *et al*., 2009). To maximize photo-activation and stimulation of biological processes, this efficient coupling must be timed with the peak absorption of chromophores (Conlan *et al*., 1996).

Wound healing, bacterial growth inhibition, and postoperative wounds are just a few of the disorders that can be treated with laser, and unique physical therapy technology (Walid *et al*., 2020). High-Power pulsed alexandrite laser therapy is one of the most used forms of laser therapy, which is a noninvasive way for treating a number of pathological illnesses and improving functional skills and quality of life. It is a cutting-edge medical and physiotherapeutic device. In general, the Alexandrite laser emits infrared light with a wavelength of 755 nm, which enables it to propagate and enter tissues (Pancar *et al*., 2020). When bacteria are exposed to the Alexandrite laser, their temperature rises. We observed that the temperature was greater the greater the energy density and the shorter the pulse duration.

## Interaction of laser with bacteria

Generally speaking, the laser's effect is thermal in nature. By creating a heat effect and closing the irradiated dentinal tubules, high-power diode lasers are utilized to kill germs. (Kaiwar *et al*., 2013;Ashofteh *et al*., 2014). Large amounts of energy are dissipated as heat when the laser is activated (Gutknecht *et al*., 1998;Alfredo *et al*., 2008).

The present investigation set out to determine whether or not a high-power alexandrite laser would have an effect on 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 755nm wavelength.

By inhibiting DNA metabolism and cell division, altering cyto-morphology de-generative, and even causing pyknosis in some cases, laser irradiation can disrupt the normal functioning of bacterial cells, inhibition cell growth and metabolic function, and damage of the physical structural occur at varying rates and intensities depending on the dose ( Yuan *et al*.., 2018). The pyknotic cell diameter zone was increased with either an increase in the pulse energy, the pulse rate, or the duration of the irradiation (Gutknecht *et al*., 1998). Bacterial cells and their accompanying strands of deoxyribonucleic acid (DNA) shrink in response to laser treatment, causing a change in gene expression that ultimately stunts the bacteria's development and activity (Cabrera *et al*., 2009).

In laser therapy, the irradiation photosensitiser induces reactive oxygen species (ROS) that have a high lethal potential for bacteria (Seyedmousavi *et al.,* 2014) by accelerating electron transport in certain regions of the respiratory chain. 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 (DeSimone *et al*., 1999 ).

By measuring the photo-thermal activity on bacteria, we can see that the bacteria absorb the laser light, which then causes the cells to heat up and die (Schoop *et al*., 2004 ). The chromophores within bacteria are extremely light sensitive (Esteban *et al*., 2005), leaving the bacteria defenseless against the intense photodynamic of light, the dramatic increase in local tissue temperature, and electromagnetic poisoning (Cabrera *et al.,* 2009), all of which lead to thermal resonance and, in turn, cause protein denaturation, tissue shrinkage, tissue disintegration, vaporization, cutting, ablation, etc (Cortes *et al*., 2003 ). In addition, the selective bactericidal effect of pulsed high-intesity 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 (Hellingwerf *et al*,. 1996; Esteban *et al*., 2005 , Gokhale *et al* ., 2010).

The bacteria are killed when the laser light is strongly absorbed by the substrate they are adhered to (as described by Schoop *et al*., 2004). This local increase in temperature is lethal for the bacteria.

It has been shown in other research that the wavelength, power, and dose of the laser all have a role in its inhibitory impact. A more damaging effect could be achieved by using bigger doses ( Brugger *et al*., 2012 , Wietzikoski *et al*., 2018 ). On the other hand, Schoop *et al*. (2004) indicated that the laser light is substantially absorbed by the material to which the bacteria adhere, increasing the temperature to a degree where the bacteria can be killed.

Recent research using lasers of varying wavelengths and energies for therapy has shown promising results in the lab and in clinical practice for improving wound healing, treating inflammation and infection, and preventing the spread of bacteria and fungi (Grzech-Leniak *et al*.,2019; de Paula *et al*., 2010). 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 bacterial-infected wounds (Ebid *et al.,* 2019 ; Ren *et al*., 2021).

By adjusting the laser's wave length, exposure time, pulse duration, and laser fluence, bacterial cells and DNA can be shrunken and their gene expression altered to prevent further growth and activity. It has been shown that laser light has an immediate effect on cell integrity, halting cell division and increasing the amount of metabolically dormant cells (Grzech-Leniak. *et al*.,2019).

One of the primary mechanisms of lasers is monochromic, which, depending on the nature of the light, can alter cellular and tissue function. (for example, wavelength, coherence) (Santamato *et al*., 2009), this allows for efficient coupling to chromophores' maximal absorption, leading to enhanced photoactivation and biological activity (Conlan *et al*., 1996).

The current study aims to evaluate the effects of a high-power alexandrite laser on *S. aureus* growth in vitro, and the primary finding is that the experimental *S. aureus* grew more slowly when exposed to the laser than the control *S. aureus* did. High-power alexandrite laser irradiation was found to be effective in killing off the tested germs. This finding suggests that the overall number of bacteria detected by the colony counting method can be reduced when the wavelength is set 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 in comparison 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. 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) (Santamato *et al*., 2009). In contrast, when working with bacteria, the laser light is strongly absorbed by the substance to which the bacteria adhere, raising the temperature to a point where the organisms are killed by their own heat (Schoop *et al*., 2004).

Radiant energy dose is proportional to laser exposure time; the longer the exposure, the higher the dose. The dose of laser with a 30 second exposure time, 5 millisecond pulse duration, and a laser fluency of 20 J/cm2 was more effective against *S. aureus* than doses of 5, 10, and 15 J/cm2. In addition, the laser dose with a 60-second exposure time, 5-millisecond pulse duration, and a laser fluency of 20 J/cm2 was more successful in reducing the *S. aureus* count than doses with laser fluencies of 5, 10, and 15 J/cm2. This method utilizes a 90-second exposure time. Therefore, exposure time and laser dose (pulse duration and laser fluency) are two elements that determine the effective dose of a pulsed alexandrite laser when calculating colonies, and more exposure time in conjunction with a higher dose may be necessary for optimal outcomes (DeSimone NA. *et al*.,1999). Additional exposure may augment the laser's photo-thermal effects on microorganisms.

The laser's photo-thermal effects on bacteria may be amplified with longer exposure and a single application of pulsed high-intensity alexandrite laser was proven to be an effective method for preventing the growth of *S. aureus*. In accordance with earlier research (Maver *et al*., 2005, Dadras *et al*., 2006; Peplow *et al*., 2010, Risovi *et al*., 2014) the optimal effect of the laser was detected at greater doses than at lower doses.

# Chapter Five

## CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusions

Exposure times, pulse duration, and laser fluency of the pulsed alexandrite laser showed an effect on the mean number of *Staphylococcus aureus* colonies and the determination of the effective dose depending on:

1. Increased exposure time leads to increased bacterial killing when laser fluency and pulse time are stabilized.
2. Increased laser fluency leads to increased bacterial killing when exposure time and pulse time are determined.
3. Reducing the duration of the pulse leads to an increase in the killing of bacteria when the exposure time and the fluency of the laser are constant.

## 5.2 Recommendations

1. Using other types of laser to study their effect on bacteria and compare them with our results .

2. Studying the effect of the another doses of Alexandrite laser (energies, pulse durations and fluencies) on *S. aureus* bacteria and comparing them with our results .

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# Appendix

# (table 3.2 ) Expouser time 30 sec

| | | Laser Fluency J.cm$^{-2}$ | | | |
|---|---|---|---|---|---|
| **Pulse duration** | **Control Mean ± SE** | **5 Mean ± SE** | **10 Mean ± SE** | **15 Mean ± SE** | **20 Mean ± SE** |
| **P.d= 5ms** | 216±7.35 A | 126.67±2.9 A,b | 87.67±4 A,c | 74.67±3.6 A,d | 41.67±2.3 A,e |
| **P.d=10ms** | 246±4.18 A | 214.67±4.4 B,b | 174.67±8.17 B,c | 142±6 B,d | 80.33±6.17 B,e |
| **P.d=20ms** | 241±7.12 A | 224.67±8.2 B,a | 185.33±6.85 C,b | 160.67±7.25 B,c | 85.67±6.88 B,d |

# (table 3.3 ) Expouser time 60 sec

| | | Laser Fluency J.cm$^{-2}$ | | | |
|---|---|---|---|---|---|
| **Pulse duration** | **Control Mean ± SE** | **5 Mean ± SE** | **10 Mean ± SE** | **15 Mean ± SE** | **20 Mean ± SE** |
| **P.d= 5ms** | 216±7.35 A | 116.67±2.96 A,b | 80.67±2.4 A,c | 70±2.55 A,d | 29.67±3.88 A,e |
| **P.d=10ms** | 246±4.18 A | 196.67±3.38 B,b | 165.67±6.47 B,c | 120.67±8.44 B,d | 58.33±5 B,e |
| **P.d=20ms** | 241±7.12 A | 204.67±7.8 B,b | 180±3.8 B,c | 156±7.52 C,d | 78.67±3.9 C,e |

# (table 3.4 ) Expouser time 90 sec

| | | Laser Fluency J.cm$^{-2}$ | | | |
|---|---|---|---|---|---|
| **Pulse duration** | **Control Mean ± SE** | **5 Mean ± SE** | **10 Mean ± SE** | **15 Mean ± SE** | **20 Mean ± SE** |
| **P.d= 5ms** | 216±7.35 A | 99.67±4.17 A,b | 74.67±3.5 A,c | 54±5.77 A,d | 15.67±2.6 A,e |
| **P.d= 10ms** | 246±4.18 A | 162.67±5.18 B,b | 140.33±5.86 B,c | 90.67±4.76 B,d | 38.33±3.45 B,e |
| **P.d= 20ms** | 241±7.12 A | 181.33±3.9 C,b | 166.33±3.5 C,c | 142±2.34 C,d | 55.33±4.48 C,e |

# (table 3.5 ) Pulse duration 5 ms

| | | Laser Fluency J.cm$^{-2}$ | | | |
|---|---|---|---|---|---|
| **Exposure time** | **Control Mean ± SE** | **5 Mean ± SE** | **10 Mean ± SE** | **15 Mean ± SE** | **20 Mean ± SE** |
| **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 |

# (table 3.6 ) Pulse duration 10 ms

| | | Laser Fluency $J.cm^{-2}$ | | | |
|---|---|---|---|---|---|
| **Exposure time** | **Control Mean ± SE** | **5 Mean ± SE** | **10 Mean ± SE** | **15 Mean ± SE** | **20 Mean ± SE** |
| **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,b | 140.33±5.86 B,c | 90.67±4.76 B,d | 38.33±3.45 C,e |

# (table 3.7 ) Pulse duration 20 ms

| | | Laser fluency $J.Cm^{-2}$ | | | |
|---|---|---|---|---|---|
| **Exposure time** | **Control Mean ± SE** | **5 Mean ± SE** | **10 Mean ± SE** | **15 Mean ± SE** | **20 Mean ± SE** |
| **T.E = 30 sec** | 241±7.12 a | 224.67±8.2 A,ab | 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 AB,d | 78.66±3.9 A,e |
| **T.E = 90 sec** | 241±7.12 a | 181.33±3.9 B,b | 166.33±3.5 B,c | 142±2.34 B,d | 55.33±4.48 B,e |

## الخلاصة :

الخلفية: الليزر هو أسلوب علاج طبيعي يستخدم لعلاج مجموعة متنوعة من الحالات ، بما في ذلك التئام الجروح ، وتثبيط نمو البكتيريا ، وجروح ما بعد الجراحة. يعد العلاج بليزر الألكسندريت النبضي عالي الطاقة أحد أكثر أشكال العلاج بالليزر انتشارًا ، وهو طريقة غير جراحية لعلاج مجموعة متنوعة من الحالات المرضية ، وبالتالي تعزيز القدرات الوظيفية ونوعية الحياة. إنها تقنية طبية وعلاج طبيعي حديثة. بشكل عام ، يصدر ليزر الكسندريت ضوء الأشعة تحت الحمراء بطول موجي 755 نانومتر ، مما يسمح له بالانتشار واختراق الأنسجة.

الهدف: ركزت الدراسة على تطبيق ليزر ألكسندريت النبضي عالي الطاقة في المختبر لتقييم تأثير ليزر ألكسندريت النبضي على البكتيريا المقاومة للمضادات الحيوية باستخدام أوقات تعرض ومدد النبض وكثافات طاقة الليزر محتلفة لتحديد الجرعة الأكثر فعالية. على بكتيرياS. aureus

.طريقة العمل: تم تثبيت نظام الليزر عموديًا على جاك ميكانيكي مدعوم ببرغي موالف ارتفاع على مقعد سطح مستو ؛ لذلك يمكن أن يسقط شعاع الليزر عموديًا على عينة الاختبار وفتحة الليزر كانت ملتصقة بعينة الاختبار. تم استخدام ليزر الكسندريت المستخدم في الدراسة وأعتمادا على المعلمات التالية: الطول الموجي 755 نانومتر ، وقطر الحزمة (14 ملم) ، أوقات التعرض (30 ، 60 ، 90) ثانية ، كثافة الطاقة الليزر (5 ، 10 ، 15 ، 20 ) جول/ سم2. أجريت الدراسة بعد تشخيص البكتيريا على أنها مقاومة للمضادات الحيوية وتعرضت لجرعات مختلفة من ليزر الكسندريت. تم تعريض ثلاث عينات من البكتيريا لأشعة الليزر لمدة 30 ثانية مع 5 مللي ثانية من مدة النبضة وكثافة طاقة ليزر 5 جول / سم 2 وأعيدت العملية بكثافة طاقة ليزر 10 و 15 و20 جول/سم2 أعيد الإجراء باستخدام أوقات التعرض 60 ثانية و 90 ثانية. بالإضافة إلى ذلك ، تم تكرار العملية بالتعرض مع أوقات تعرض 30 ثانية و 60 ثانية و 90 ثانية ومدة نبضة 10 مللي ثانية وبطلاقة ليزر 5 و 10 و 15 و 20 جول / سم2 ، بشكل منفصل. أيضًا ، تم تكرار العملية السابقة عن طريق تعريض البكتيريا بأوقات تعرض مختلفة (30 ثانية ، 60 ثانية ، 90 ثانية) ، مدة نبضة 20 مللي ثانية وبطلاقة ليزر مختلفة (5 ، 10 ، 15 و 20 جول / سم2) ، بشكل منفصل.

ألنتائج: في أوقات التعرض 30 و 60 و 90 ثانية ، لوحظ انخفاض معنوي ($p = <0.0001$) في متوسط مستعمرات البكتيريا مع زيادة جرعات طلاقة الليزر في نفس مدة النبضة. بالإضافة إلى ذلك ، لوحظ انخفاض معنوي ($p = <0.0001$) في متوسط مستعمرات البكتيريا بالمقارنة بين طلاقة ليزر في نفس مدة النبضة. ومع ذلك ، لا توجد فروق ذات دلالة إحصائية في القيم المتوسطة لعدد الطوائف بين

التحكم و 5 جول/سم2 في مدة نبضة 20ملي ثانية. في فترات النبض 5ملي ثانية و 10ملي ثانية ، لوحظ انخفاض كبير للغاية (p <0.0001) في متوسط المستعمرات مع زيادة جرعات طلاقة الليزر في نفس مدة النبضة. بالإضافة إلى ذلك ، لوحظ انخفاض معنوي (P <0.0001) في متوسط مستعمرات البكتيريا مقارنة بين طلاقة ليزر في نفس وقت التعرض. ومع ذلك ، عند 20 مللي ثانية ، لا توجد فروق ذات دلالة إحصائية (P> 0.05) لوحظت في متوسط مستعمرات البكتيريا بين أوقات التعرض عند 30 ثانية و 60 ثانية مع جميع كثافات طاقة الليزر المستخدمة في الدراسة الحالية. بالإضافة إلى ذلك ، لا توجد فروق ذات دلالة إحصائية (P> 0.05) في متوسط المستعمرات بين أوقات التعرض عند 60 ثانية و 90 ثانية عندما كانت طلاقة الليزر عند 15 جول/ سم2، بينما هناك فرق معنوي ( p = <0.05) عندما كانت طلاقة الليزر عند 5 و 10 و 20 J سم2. لوحظ وجود فرق معنوي (p <0.05) في متوسط مستعمرات البكتيريا بين أوقات التعرض (30 ثانية و 90 ثانية) في جميع حالات طلاقة الليزر التي تم استخدامها في دراستنا باستثناء طلاقة الليزر 15 جول/سم2. .

ألأستنتاجات ، أظهرت فترات التعرض ومدة النبض وكثافات الطاقة لليزر الإسكندرايت النبضي تأثيرًا على متوسط عدد مستعمرات بكتيريا Saureus وتحديد الجرعة الفعالة.

جمهورية العراق
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جامعة بغداد كلية الطب

# تقييم فعالية الجرعات المختلفة من ليزر الالكسندرايت على نمو بكتريا المكورات العنقودية الذهبية

رسالة

مقدم الى مجلس كلية الطب بجامعة بغداد في كجزء من متطلبات الحصول على درجة الماجستير في الفيزياء الطبية

من قبل الطالبة

ايلاف احمد مصطفى

بكلوريوس علوم فيزياء طبية / 2018

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2022 م 1444 ه