



APPLICATION OF LASER INHIBITS THE BACTERIAL GROWTH CONTRASTING TO THE ANTIBIOTICS

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ABSTRACT Respectably studies provided a unique examples of most frequent types of germs such as *E. coli* and *S. aureus* as results to their presence in large quantities around the human being as normal flora to human intestinal canal and their foods specially salty dairy, causes various disease such as diabetic foot. Laser uses in medical procedures mostly are therapeutic, diagnostic, and imaging application. Like important and highly sensitive procedures of ophthalmometry surgery in corrections and lizak, dentistry, dermatology, and close the bleeding of the tiny vessels within the operations. Samples collected from forty patients suffered different injury inflammations, using sterile cotton, the samples diagnosed depending on a number of morphological examinations, biochemical tests and culturing on selective media. The bacterial isolates obtained including the followings: *Staphylococcus aureus* (24.68 %), *Escherichia coli* (21.31 %). The sensitivity of the bacterial isolates before irradiation to a number of antibiotics were examined, they all gave high resistance to the antibiotics, except two types (Streptomycin & Ciprofloxacin), which the bacterial isolates were sensitive to. The bacterial isolates irradiated with laser using wavelengths (770, 880, and 990 nm) to increase their sensitivity reaching to a step of attenuating or killing the bacteria with increasing exposure times. Laser effect on the bacterial isolates showed a significant decrease in the viability of the bacteria of all species when the dose was increased, where occur killing the bacteria after 20 min and more of laser dose. It is likely to be increasing the motility of antibiotics molecules by the radiation heat rather than direct effects. Increasing the motility of water and media molecules by the radiation heat rather than direct effects is well known drama of irradiation through free radical formation process as hydroxyl and superoxide. The results encourage for more work to seek the interpretation of free radical formation and irradiation to give clear direct evident in using laser as disinfectant.

KEYWORDS :

Introduction

The skin is the first line of defense to the external environment. The skin's ability to resist infection is likely a key factor in a germs transition from commensal to pathogenic dermatology, with highly intention for Diabetic Foot. *Staphylococcus aureus* is a gram-positive bacterium that colonizes the skin and is present in the anterior nares in about 25–30% of healthy people. The ability of *S. aureus* to attach to surfaces and form a biofilm is a critical determinant of the course of chronic disease¹. *Escherichia coli* is a gram-negative bacteria, can be definitely pathogenic especially when it is in pure growth or as part of a polymicrobial deep infection. It is isolate from skin and soft tissue infections and a deep surgical wound infection has been characterized, in patients of all age groups².

Laser is a device that converts electrical or chemical energy into light energy. In contrast to ordinary light that is emitted spontaneously by excited atoms or molecules, the radiation emitted by lasers including both visible and invisible light was more generally termed as electromagnetic radiation. All these forms of electromagnetic radiation are fundamentally similar, in that they travel at the speed of light (186,000 miles/s). The difference between them is their wavelength and energy; the shorter the wavelength, the higher the energy. For example, Radio waves have the longest wavelength (tens to hundreds of meters) and the lowest energy³. The mechanism of amplification is perhaps the most fundamental difference between electrical and optical oscillators. The radiation emitted by lasers including both visible and invisible light is more generally termed as electromagnetic radiation⁵.

Semiconductor light emitting devices are grouped into two categories:

- Light emitting diodes (LEDs), which are basically general-purpose replacements for vacuum tube-based light emitters.
- Laser diodes that emit sharply focused monochromatic light beams,^{6,7}

Laser surgery is a highly sterile process since contact does not occur between the surgical tools and the tissues being cut. Further advantage is that the laser does not only cuts but also "welds" blood vessels being cut. Operations are done very fast and patients do not feel pain. The first big success of lasers in medicine was in the treatment of eye. Argon laser has been in use for several years to treat the detachment of the retina. Laser photocoagulations of retinopathies are a common treatment⁸. Ordinary, when a new method of treatment have broken the scientific market, its accompanied by suspicious and confirmative researches, furthermore, the laser applications have approved its capability in surgery, the subject that stimulates the authors to use it at

tiny tissue sample of single cells count of microbiology by Laser BacterioScan. The research is practical attempt to measure the effects of laser at recorded wavelength on the bacterial growth, and to seek explanations of its mode of actions.

Materials & Methods

Collection Of Samples: Forty samples were collected from patients admission in Al-Hussain Teaching Hospital, Al-Muthanna Province, suffering from wound infections, using sterilized cotton swabs. The specimens transferred to the laboratory under cool conditions. Required culture media were prepared according to the information of the manufacturing company such as, Blood agar, MacConkey agar, EMB, and Mullar-Hinton agar¹⁰.

Isolation and Identification of Bacteria: After collection the samples from wound infections, samples were identified according to Bergeys manual using different morphological and biochemical tests¹⁰. First the samples were grown on Brain heart infusion broth, incubated at 37 °C for 24h. The growing bacteria on the medium were cultivated on MacConkey agar and Blood agar and incubated at 37 °C for 24h. To assist in diagnosing the microbial infections, microorganisms were examined microscopically for their motility, morphology, and staining reactions. Under the compound light microscope after staining with gram stain, smears of *Staphylococcus* showed as gram – positive cocci arranged in clusters¹¹, While *E. coli*, smears of these bacteria after gram staining were gram–negative, short rod shaped, arranged as single bacilli or diplobacilli. *Staphylococcus* colonies on blood agar are opaque, and yellow or white color and appear a wide zone of clear (beta-type) hemolysis¹².

Antibacterial Susceptibility Testing: Susceptibility testing was performed by Kirby-Bauer technique. The test organism was cultured over the Mueller-Hinton agar surface and exposed to a concentration gradient of antibiotic diffusing from antibiotic - impregnated paper disk into the agar medium. The isolate was then incubated at 37 °C for 24 hours. Organisms sensitive to the antibiotic were inhibited from growing in a circular zone around the antibiotic impregnated paper disk. A comparison of the inhibition zone diameter that was produced by a control strain was used to interpret the antimicrobial sensitivity^{13,14}.

Laser Parameters: The laser system used in this study was the Diode laser (Omega). It had wavelengths between (600 – 1000) nm, with a series of probes, some of probes with hold clusters of diode emitting at the same time⁷. The Diode laser which was considered as CW laser and had the following parameters: The wavelength was (770, 880 & 990)

nm, Frequencies (1 kHz, 5 kHz, 10 kHz), The exposure times varied (10, 15, 20, 30, 40 & 50) minutes and the Power density (PD): 300, 600 & 1000 J/cm².min .

Results

Forty clinical specimens were collected from 40 patients suffering from injury inflammation infections. During the sampling of the specimens 60 bacterial isolation were obtained from the original 40 samples, 21 samples of them two types of bacteria, while the remaining 19 samples showed only one type of bacteria, bacterial species included the following (*Staphylococcus aureus*, *Escherichia coli*).

The bacterial isolates obtained including the followings:

Staphylococcus aureus (24.68 %), *Escherichia coli* (21.31 %), The sensitivity of the bacterial isolates before irradiation to a number of antibiotics were examined, they all gave high resistance to the antibiotics by Kirby-Bauer technique, except two types (*Streptomycin* & *Ciprofloxacin*), which the bacterial isolates were sensitive to as shown in Figure¹².

After irradiation of the bacterial suspensions with the diode laser for different exposure times and different frequencies, and the wavelengths used were (770, 880 & 990) nm, the results showed a noticeable change in the sensitivity of all the bacterial isolates in contrast to most of the used antibiotics. In order to increase their sensitivity reaching to a step of attenuating or killing the bacteria with increasing exposure times. Laser effect on the bacterial isolates showed a significant decrease in the viability of the bacteria of all species when the dose was increased, where occur killing the bacteria after 20 min and more of laser dose as shown in Figure¹².

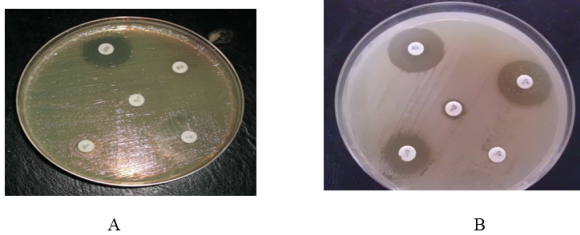


Fig. (1): the inhibition zone diameters of *E. coli* for 10 min, and 5 kHz. A: before irradiation, B: after irradiation with 990 nm.

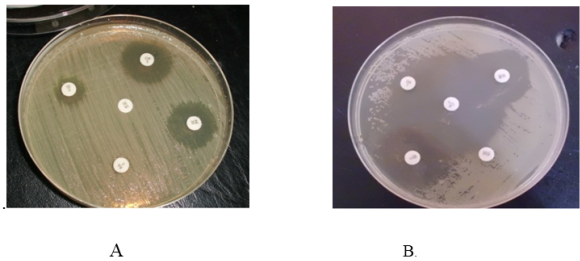


Fig. (2): the inhibition zone diameters of *Staphylococcus aureus* A: before irradiation B: after irradiation with 770 nm for 10 min. and 10 kHz.

Discussion

The skin become the most wealthy properties ever that human beings have, throughout this cover, he could prevent a lot of kinds of harmful agents, whatever these kinds of living and non-living, the skin could hold all the organs a way of stress. Agents like a widely spread microbes around the wounds as example to gram-positive we examined the *Staphylococcus aureus*, and of gram-negative we examined the *Escherichia coli*.

The various type of lasers that have been developed so far, display wide range of physical and operating parameters. The lasers are characterized by the wavelength of the emitted radiation; refers to infrared lasers, visible lasers, UV, and X-ray lasers, while, according to the physical state of active material, it has been classified as solid, liquid and gaseous laser. The use of lasers in medical procedure can be grouped into two distinct categories: therapeutics and diagnostics or imaging applications⁵. Forty clinical specimens were collected from patients suffering from injury infections. It have been diagnosed a 16 samples of *staphylococcus aureus* and 14 samples as a *E. coli* by using

a differential medias. The therapeutic agent activities against the bacterial isolates were variable depending on the bacterial species and the mode of action of each agent¹⁵.

After irradiation of the bacterial suspensions with the diode laser for different exposure times and different frequencies, and the wavelengths used were (770, 880 & 990) nm which are the well recommended doses, other work show effective doses ranging from 3 to 6 J/cm² appear to be more effective doses 10 J/cm² are associated with deleterious effect. The wavelength ranging from 632.8 to 1000 nm remain as those that provide more satisfactory results in the wound healing process¹⁶. At 10 minutes exposure time, and frequency 10 KHz, with wavelength 770 nm, the isolates were very sensitive with higher MIC zone.

When using 880 nm, 10 kHz, for 10 minutes, it gave approximately the same results, but seems different when using 990 nm, 10 kHz, at 20 minutes. Wavelength (770) nm was more effective in killing the bacteria. Laser effects on the Bacterial Isolates, where the results of irradiation showed a significant decrease in the viability of the bacteria of all species by increasing the doses. Not so far from the other results that showed wound healing by low level laser throughout enhancing the fibroplasia making changes to myofibroblastes¹⁷.

It is clear to see that the survival rates decreased as the laser exposure time increased. After irradiation of *Staphylococcus aureus* with diode laser using wavelengths 770, 880 & 990 nm, frequency 5 & 10 kHz, and exposure time 10 & 15 min., the bacteria still survive, but at 20 min. and more over, the growth decreased reaching to killed of bacteria at 20, 30 & 40 min. Sensitivity of the bacterial isolates to the laser increased with increasing of exposure time for irradiation of laser, but still unknown how the laser rays could penetrate the citins and cuticle layers of the enveloped kinds. In other way, seems that microbe's effects are gradually depending on the wavelength and frequency, so, that is not a character of physical agents. It is likely to be increasing the motility of water and media molecules by the radiation heat rather than direct effects or through free radical formation process as superoxide. The results encourage for more work to seek the interpretation of free radical formation and irradiation to give clear direct evident in using laser as disinfectant.

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