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Changes of the susceptibility of *Staphylococcus aureus* bacteria to the local therapeutic agent by using Nitrogen laser

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Abstract

This study involves the investigation of the effect of nitrogen laser with 337.1 nm wavelength on the sensitivity of *Staphylococcus aureus* bacteria by using local therapeutic due to burns.

Thirty six isolate of *Staphylococcus aureus* bacteria were isolated from 25 patients suffering from sever burns, each isolate of bacteria was irradiated with nitrogen laser at (5, 10, 15 and 30) pulses/second repetition rates for 1, 5, 10, 20 and 30 minutes for each repetition rate.

The effects of nitrogen laser on the local therapeutics sensitivity of bacteria were obtained using Kirby Baur method.

Changes in the sensitivity of bacteria to local therapeutics (Tetracyclin, Chloramphenicol, Flumizin and Fucidin) occur at high repetition rate(30 pulses/second) and for long exposure times (10, 20 and 30 minutes) with (2×10^{-3} J/cm^2) fluence.

Keywords: ***Staphylococcus aureus*, Local therapeutic agent, Nitrogen laser, Susceptibility.**

Introduction

Following a burn injury, the wound is a site with serious bacterial contamination and infection. Moreover, toxins released from bacteria concurrently will cause lesions to local wound as well as the whole body [1].

The most probable bacteria that infects burn wounds; are *Pseudomonas aeroginosa, Staphylococcus aureus, Escherichia coli* *Bacillus spp* and *Bacteroids.*

The multi resistant strains of S. aureus play important role in contamination of burn wounds in the most burn care units [2].

The antibiotic resistance of S. aureus has been depated in recent years; for example, increase the resistance to methicillin was indicated in various hospitals in the world [3].

Resistance of S. aureus to streptomycin, gentamycin and tetracycline has been increased after a few years of using them [4].

Laser reactions offer a new field of research. Laser is an expanding technological discipline in biology and medicine. It will ultimately contribute to a broad rapid expansion of both diagnostic and treatment procedures in microbiology, entomology, botany, immunobiology, cytofluorography, photobiology, and genetics [5,6].

Understanding the mechanisms of laser tissue interaction is the basis for further development of diagnostic and therapeutic applications of lasers [5].

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In sever burns; contamination with multi resistant Staphylococcus aureus bacteria is common and lead to inflammatory and septic effects.

Many studies have demonstrated the effects of laser radiation on pathogenic or opportunistic bacteria, since the introduction of laser in this field [6]. Bacteria in supragingival plaque samples, *Streptococcus* can be killed by (He-Ne) laser light with 632.8 nm wavelength in the presence of photosensitizer (TBO) [7].

Staphylococcus aureus bacteria can be killed after exposure to 11 mW Gallium aluminum arsenide laser for 300 seconds [8]. He-Ne laser with 35 mW and with prescence (Toluidine blue O)(TBO) makes *Staphylococcus aureus* bacteria susceptible to killing by the laser light within 30 seconds[9]. Inhibition of the phagocytic activity of polymorphnuclear leukocytes and the viability of bacteria were noticed after irradiation with nitrogen laser at high repetition rates pulsed (15 and 30 pulses/second) and for long exposure times (10, 20 and 30 minutes) [10].

In 2007 Al-Taie, shows that a noticeable changes in some biochemical characteristic of the *Pseudomonas aeruginosa* bacteria after irradiation with N_2 laser at (15, 20 and 30) minutes, the changes were more when the acridine orange as a photosensitizer was used [11].

The present study aims to investigate the effects of short wavelength N2 laser on sensitivity of *Staphylococcus aureus* to local therapeutics.

Materials & Methods

Samples Preparation:

The samples of bacteria were obtained from (25) patients suffering from sever burns, (10) of them were males and (15) were females, the age of patients ranges from 9- 56 years. These patients attending the burn and surgical units of two hospitals in Baghdad; Al-Yarmouk hospital and Al- Kindy hospital during the period from October 2001 until April 2001.

Sterile cotton swabs were used for taking the samples from the burning skin. The samples were transferred in cooled boxes to the laboratory, were then cultured on blood agar and nutrient agar.

Isolation and Identification

The isolates of bacteria were identified according to the microscopic examination & the characteristics of the culture on the plates. The characteristics include the shape, color, hight, & edges of the colonies in addition to the hemolysis zones on blood agar. Also, identification was attempted according to Biochemical tests [12].

Depending on Bergys Manual of Determinative Bacteriology [12], final identification of Thirty-six isolates of *Staphylococcus aureus* were obtained, using the following tests:

1) Microscopic examination

A smear of each isolated bacteria was stained using gram stain. *Staphylococcus aureus* bacteria appear after staining as gram positive, & the cells were arranged as clusters [13].

2) Growth on mannitol Salts agar:

The isolates of bacteria were cultured on a mannitol salt agar (MSA). This media permits the selection of *Staphylococci* due to the high salt concentration of the medium. Since *Staphylococcus aureus* ferments manitol it can be distinguished due to the change in colour of the phenol red

indicator in the medium from red to yellow [14].

3) Biochemical tests includes:-

A-Coagulase tests:
B- Catalase test:
C- Fermentation of sugars and acid production

The vials of ointment were obtained from Al-Razi Company. Types of locally therapeutics are listed in table (1)

Table (1) Types of local therapeutics.

Local therapeutic	Basic material	Source
Samacyclin (Ointment)	Tetracycline 3%	SCD
Smsphenecol (Ointment)	Chloramphenicol 1%	SCD
Flumizin (Cream)	Silver Sulphadiazine 1%	SCD
Fucin (Ointment)	Fucidic acid 2%	SCD

SCD: Samara Company for Drugs

Pulsed N_2 – laser was used in this study. It is of Molectron UV 24-model. The laser emits radiation in the ultra violet region of the electromagnetic spectrum, 337.4 nm.

It can be operated with repetition rate from 1 to 50 pulses per second. The pulse energy is 1 mJ with pulse duration of 10 nano second.

Experimental Setups

1) Bacterial samples preparation:
2) Irradiation procedures:

The samples of bacterial dilutions were subdivided into subgroups in epndrof tubes containing 0.5 ml of bacterial suspension with three replicates for each sample, with exception the control group.

3) Effect of Nitrogen laser on the sensitivity of *Staphylococcus aureus* to local therapeutics:

For each of 36 isolate of bacteria, the sensitivity testes were done before and after irradiation with Nitrogen laser, as follows:

1- A pure colony of bacteria from a fresh culture were inoculated in 5ml of Muller-Hinton broth, that was incubated for 5-6 hr at a temperature of 37 ^{o}C to develop the turbidity.

2- Suspension of the bacteria was adjusted to a 0.5 McFarland standard, which gives Absorbance of 0.10 at 600nm wavelength, using spectrophotometer (Optima 2000).

3- The suspension of each isolate was irradiated with Nitrogen laser with a repetition rates of 5, 10, 15 and 30 pulses/second and for exposure times of (1, 5, 10, 20 and 30) minutes for each repetition rate with ($2x10^{-3}$ J/cm^2) fluence, the spot diameter of laser beam was 8 mm.

4- After irradiation, the Muller-Hinton agar plates were inoculated by wetting a swab with bacterial suspension, the entire surface of the plate was swabbed in a number of different directions.

5- After 5 minutes of inoculation, a holes of 5mm diameter and 3 mm of depth were done in the agar to put the local therapeutics.

6- The plates were incubated at 37^{o}C for 18 to 24 hours.

7- After the incubation interval the zones of inhibition in plates of irradiated bacteria and control plates were measured (in mm) with a ruler.

8- According to standard tables, the bacterial susceptibility were determined as resistant or sensitive or intermediate.

Results & Discussion

The results show that four isolates of *S. aureus* bacteria exhibit noticeable change in their sensitivity to local therapeutics after exposure to nitrogen laser beam 30 P/second repition rate for different exposure time, as it is illustrated in table (2).

Table (2) Changing of *Staphylococcus aureus* Sensitivity to local therapeutics after irradiation with nitrogen laser

Isolate	Antibiotic or local therapeutic	Diameter of Inhibition zone (mm) before irradiation	Diameter of Inhibition zone (mm) after irradiation	Laser parameters (repetition rate & exposure time)
S_4	Tetracycline	7	20	30 P/second 10 minutes
S_{31}	Chloramphenicol	8	25	30 P/second 30 minutes
S_9	Flumizin Fucidin	15 20	35 25	30 P/second 20 minutes
S_{11}	Tetracycline	8	26	30 P/second 20 minutes

P: Pulses

1- Isolate (S_4): this isolate is resistant to the local therapeutic tetracycline, figure (1) and exhibit sensitivity to this local therapeutic after irradiation with (30 pulses/second) repetitions rate and for 10 minutes exposure time, figure (2).

2- Isolate (S_{31}): this isolate is resistant to the local therapeutic chloramphenicol before exposing to laser beam, figure (3). The sensitivity to this local therapeutic is noticed after exposure to laser beam with (30 pulses/second) repetitions rate and for 30 minutes exposure time, figure (4).

3- Isolate (S_9): this isolate is resistant to the local therapeutics flumizin and fucidin before exposing to laser beam, figure (5). The sensitivity to these local therapeutics is noticed after exposure to laser beam with (30 pulses/second) repetitions rate and for 20 minutes exposure time, figure (6)

4- Isolate (S_{11}): this isolate is resistant to the local therapeutic Tetracyclin before exposing to laser beam, figure (7). The sensitivity to this local therapeutic is noticed after exposure to laser beam with (30 pulses/second) repetitions rate and for 20 minutes exposure time, figure (8).

Figure (1). Photograph of sensitivity test for isolate (S4), resistance to the local therapeutic tetracycline before irradiation with nitrogen laser

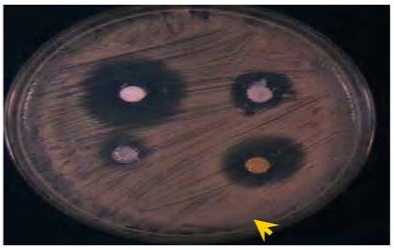

Figure (2). Photograph of sensitivity test for isolate (S4), sensetive to the local therapeutic tetracycline after irradiation with nitrogen laser (30 p/sec) repition rate for 10 minutes exposure time

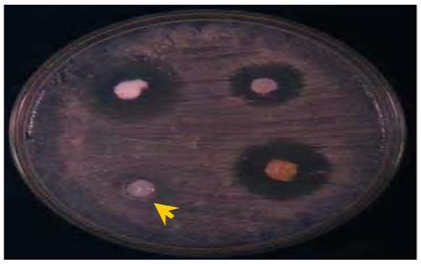

Figure (3). Photograph of sensitivity test for isolate (S31), resistance to the local therapeutic chloramphinecol before irradiation with nitrogen laser

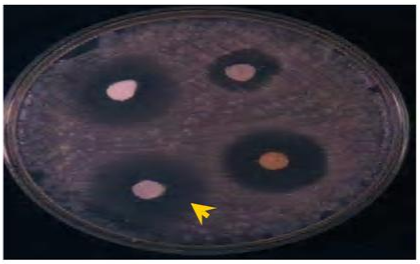

Figure (4). Photograph of sensitivity test for isolate (S31), sensetive to the local therapeutic chloramphinecol after irradiation with nitrogen laser (30 p/sec) repition rate for 30 minutes exposure time

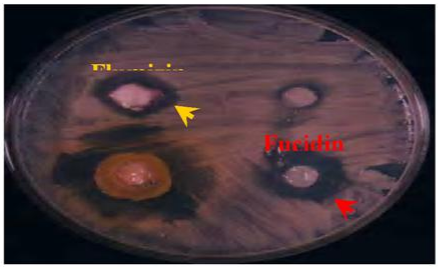

Figure (5). Photograph of sensitivity test for isolate (S9), resistance to the local therapeutic flumizin & fucidin before irradiation with nitrogen laser

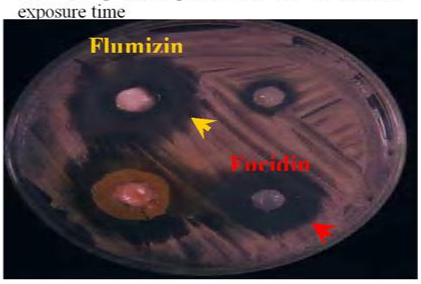

Figure (6). Photograph of sensitivity test for isolate (S9), sensetive to the local therapeutic flumizin & fucidin after irradiation with nitrogen laser (30 p/sec) repition rate for 20 minutes exposure time

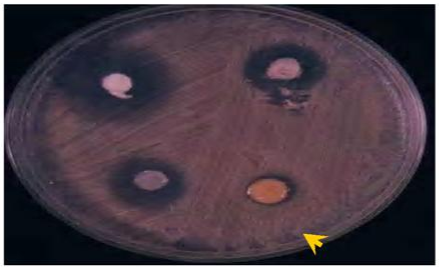

Figure (7). Photograph of sensitivity test for isolate (S11), resistance to the local therapeutic tetracyclin before irradiation with nitrogen laser

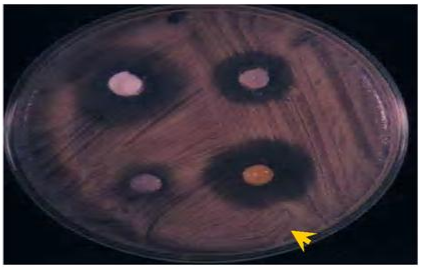

Figure (8). Photograph of sensitivity test for isolate (S11), sensetive to the local therapeutic tetracyclin after irradiation with nitrogen laser (30 p/sec) repition rate for 20 minutes exposure time

Discussion

The effects of laser with high repetition rates and for long exposure times lead to changing the susceptibility of bacteria to local therapeutics from resistance to sensitivity. As it is clear in the results, the changes in sensitivity to local therapeutics was observed at high repetition rates (30 pulses /second) and for long exposure times (10, 20 and 30 minutes). The changes in the sensitivity occur to (Tetracycline, chloramphenicol, flumizin and fucidin) local therapeutics.

Changing the sensitivity of bacteria to local therapeutics after irradiation with nitrogen laser is may be due to:

1- Failure of bacterial cells to use the alternative mechanisms of bacterial resistance such as; alteration in the protein target, over production of the target, production of specific enzymes that cleave or chemically modify specific ointments

2- Uncontrolled entering of ointment molecules inside the bacterial cell as a result of changing in proton motive force in the plasma membrane.

3- Accumulative effect of both laser light and local therapeutic making the bacteria more sensitive to local therapeutic.

4- Direct effect of laser radiation at high doses on the bacterial cell wall and on the permeability of plasmic membrane which may lead to increase the sensitivity of bacteria to local therapeutics.

Al- khafajy (2002) found that irradiation of *Pseudomonas aerogenusa* with He – Ne laser and with presence of Toluidin blue as photosensitizer, cause changing in the sensitivity of bacteria to (Amikacin , Tobramycin, Ceftazidime,Piperacillin ,Tazocin ,Norfloxacin ,Ciprofloxacin , Chloroamphenicol) antibiotics due to probable effect of laser on the resistance mechanisms in bacteria [15].

The effect of nitrogen laser on changes of some metabolic characteristics of bacteria without photosensitizer may be explained in term of photochemical interaction due to absorption of 337 nm laser light by certain chromophores; like Nicotinamide dinucleotide dehyrogen that has wavelength maximum at UV region. Laser light at certain energy density and exposure time may lead to induce changes in the redox activity of NADH dehydrogenase, this event can in turn cause other redox changes and modulations of biochemical reactions [16, 17].

The effect of nitrogen laser on changes of some metabolic characteristics of bacteria without photosensitizer may be due to the modification of amino acid residues.

Direct effect of UV light results in photomodification of amino acid residues, usually yielding decreased enzyme activity. Lysozyme photoinactivation could be taken as an example [18].

Ghazi in (2003) proposed the effect of N_2 laser at high doses (high repition rates and long exposure times) on both of bacteria and phagocytic cells were noticed in the manner of decreasing the viability and changing the antibiotics susceptibility of bacteria and inhibition of phagocytic activity of polymorphoneuclear leukocytes. The most probable mechanism of interaction of the N_2 laser light with the living cells is photochemical interaction [10].

Conclusions

Nitrogen laser with high repetition rates and for long exposure times lead to change the sensitivity of bacteria to antibiotics and local therapeutics from resistance to

sensitivity, especially which that effect on protein synthesis in bacterial cells.

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التغير في استجابة بكتريا المكورات العنقودية الذهبية *Staphylococcus aureus* للعلاجات الموضعية بأستخدام ليزر النتروجين

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الخلاصة

يتضمن البحث دراسة تأثير اشعة ليزر النتروجين بالطول الموجي 337.1 نانوميتر على استجابة بكتريا المكورات العنقودية الذهبية *Staphylococcus aureus* المعزولة من الحروق للعلاجات الموضعية.

تم عزل 36 عزلة من البكتريا من 25 مريض يعانون من تلوثات الحروق شعت كل عزلة بليزر النتروجين بالنبضات 5, 10, 15 و30 نبضة/ثانية وللفترات الزمنية 1, 5, 20 و 30 دقيقة لكل تكرارية نبضة.

تم تقدير تأثير الليزر على استجابة البكتريا للعلاج الموضعي بأستخدام طريقة قياس اقطار منطقة التثبيط Kirby-Baur Method.

أدت نبضات الليزر العالية واوقات التشعيع الطويلة الى تغير استجابة البكتريا للعلاجات الموضعية(تيتراسايكلين, كلورامفينيكول, فلامايزين فيوزدين) من المقاومة الى الحساسية عند تكرارية نبضات (30 نبضة/ثانية) واوقات تشعيع (10, 20 و 30) دقيقة وكثافة طاقة (2×10^{-3}) جول/سم^2.