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EFFECT OF ND:YAG (1064NM) ON *STAPHYLOCOCCUS AUREUS* ISOLATED FROM WOUNDS

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Abstract

Between October and December 2018, 27wounds and burn swab specimens were collected by laboratories at Al-Yarmook hospital, and cultured on Mannitol salt agar. the isolate was subjected to Nd: YAG laser in different power (400mJ, 500mJ, 800mJ and 900mJ). In general the laser showed effect on bacterial growth that reach to complete killing, the statistical analysis showed that there is weak correlation between laser at 400mJ with killed percentage. While in 500mJ its exhibit complete correlation with killing percentage, this correlation was decreased with increasing in power to 800mJ and 900Mj.
Keywords: *Staphylococcus aureus*, Nd:YAG laser

Introduction

Staphylococcus aureus is a G^{+ve} cocci , facultative anaerobic bacteria. Staph have another name is golden staph. It is non-spore former and nonmotile (Hon *et al.,* 2012). *Staph aureus* or *Staph a.* commonly used name that referred to this bacterium in medical literatures. Grape-like clusters is detectable shape of *S. aureus* appearance under microscope, on agar plate, it appear golden-yellow colonies, with hemolysis on blood agar plates (Nakatsuji *et al.,* 2016). *S. aureus* is not belonging to upper respiratory tract normal microbiota of humans, rather it is a colonization (Zago *et al.,* 2015) and on skin and in the gut mucosa (Nandakumar *et al.,* 2013).

Pathobionts it another term that used for *S. aureus*, that act symbiotically and can colonize, they cause disease if they overcome the tissues defenses and they have colonized or invade other tissues (Zago *et al.,* 2015).

Role in disease

Pus shed *S. aureus* from infected wound that represent as a source of infections with the bacterium and can spread to other persons through contact or objects used by an infected person such as clothing, sheets, or athletic equipments.

Extra precautions must be taken by diabetics and injection drug users, because they are represent cases with the highest risk, so they should avoid infection with *S. aureus*.

Some of infection pathway of *S. aureus* including chronic biofilm infections on medical implants, and the repressor of toxins (Chung and Toh, 2014).

Skin infections

Most common skin infections form of *S. aureus* including small benign boils, impetigo, folliculitis, and cellulitis, while invasive soft-tissue infections represent as more severe infections (Morikawa *et al*., 2012; Ryan and Ray, 2004).

Eczema, is commonly name of atopic dermatitis as prevalent in patients with *S. aureus*. It is mostly found in active places, fertile, including the hair, armpits, and scalp. Large pimples may lead to Staphylococcal scalded skin syndrome (SSSS), that appear in those areas may exacerbate the infection if lacerated. a severe form of which can be seen in newborns (Boost *et al* 2008).

Food poisoning

Toxins generated by *Staph aureus* is capable to produce food poisoning in the human body (Medical Laboratory Manual For Tropical Countries vol two).

Bone and joint infections

Major bone and joint infections may be as a result *S. aureus* that may be appear as one of three forms: septic arthritis, osteomyelitis, and infection from a joint replacement surgery (Lina *et al* 1999).

Bacteremia

In industrialized world, *S. aureus* is one of an important causative agents of bloodstream infections that commonly associated with mucosal membranes or skin breaks due to injury, surgery, or use of intravascular devices, or injected drugs. [10] various organs infected when bacteria entering in the blood stream., causing infective septic arthritis, endocarditis, and osteomyelitis (Lina *et al.,* 1999).

Medical implant infections

Implanting of medical devices in the human tissue or body may be lead to implanting some microorganisms such as *S. aureus* biofilms and commonly found with another one like, *Candida albicans*, that leading to form multispecies biofilms. helping in penetration of human tissue by *S. aureus* (Schenck *et al.,* 2016).

Nd: YAG lasers

Nd: YAG lasers using a laser diodes or flashtube for optically pumped. These are many different applications used and represent the most common laser types. Nd:YAG lasers emit light with a wavelength of 1064 nanometer, in the infrared (Van Tiggelen and Lagendijk, 1998). There are also transitions near 946, 1120, 1320 and 1440 nm. Nd:YAG lasers operate in both pulsed and continuous mode. Pulsed Nd:YAG lasers are operated in a manner called Q-switching mode.

The amount of the neodymium dopant in the material is varied according to its use. The doping for pulsed lasers is significantly higher than for continuous wave output.

Using of Nd: YAG lasers

Diabetic patients are used frequency-doubled Nd:YAG lasers with retinopathy for pan-retinal photocoagulation. Nd: YAG lasers are also used to treat eye floaters (Strutt 2009).

1064 nm represent as the most widely Nd:YAG lasers used emitting light for laser-induced thermotherapy, in which benign or malignant lesions in different organs are ablated by the beam.

The lasers can be used in oncology for removing of skin cancers (Hersules, 1960). They are used also for reducing benign thyroid nodules,(Martin and Lindavist1965) and for destroying primary and secondary malignant lesions in liver. (Shapiro 2006;Vero and Sfiligoj-Smole 2009). Nd:YAG lasers are also used extensively for laser hair removing and for minor vascular defects treating such as spider veins on the face and legs. Venous Lake lip lesions also can be treated with Nd:YAG lasers. (Barltropand and Coyle1975). Recently used for a rare skin disease such as Dissecting cellulitis of the scalp (Eisinger and Navon 2003).

Nd:YAG laser has been used for uterine septa removing (Thalhammer and Penzkofer, 1983). The Nd:YAG laser is also being used for onychomycosis treating (Pellitier, 2017).The merits of laser treatment of these infections are not yet clear, and research is being done to establish effectiveness. (Gibbons and Murray 1978; Gupta 2000).

Materials and Methods

Isolates Collection

Between October and December 2018, 27 wounds and burn swab specimens were collected by laboratories at Al-Yarmook hospital, and cultured on Mannitol salt agar. Isolates were obtained from these laboratories by sub culturing on Mannitol salt agar, and kept at 4ºC during transportation, then incubated at 37ºC for 18-24hours.

Laser System

The laser system used in the project includes, on the Nd: YAG laser device, as the system described contains the sample in which the bacteria used for the laser beam used towards it so that the distance between the laser device and dish (prepared sample) in which the bacteria are located is 20 cm (the best distance obtained after experimenting with different distances less or more than the above distance to control and inhibit the bacteria used) finally the glasses used to protect from laser radiation. **Nd: YAG**.

Different energies were used with a different repetition rate also, after experimental study to obtain the appropriate energy control and inhibition of that type of bacteria.

Nd: YAG Laser Source

Nd: YAG laser (Huafei Tongda Technology-DIAMOND - 288 pattern EPLS) which is used to fully control the bacteria in the plates (prepared samples). The whole system is consist of light route system, power supply system, computer controlling system, cooling system, and the light route system is installed into the hand piece, but power supply, controlling and cooling system are installed into the machine box of power supply.

Main Technical Parameters

Laser model: Q-switched Nd: YAG Laser. Laser wavelength: (1064 /532) nm. Pulse energy: (100-1000) mJ. Pulse width: 10 ns.

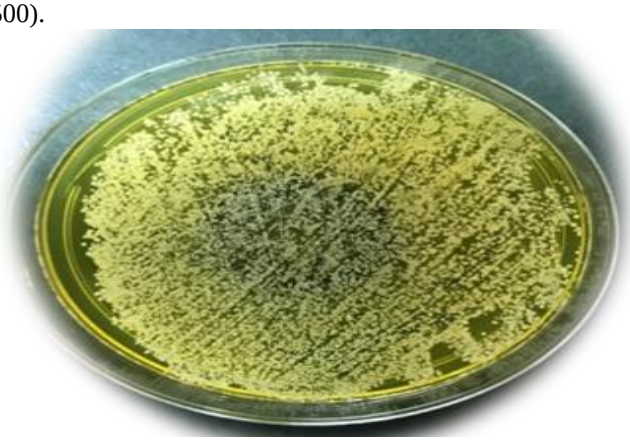
Repetition frequency: 6 Hz. Focal spot size is ~ 4 mm. Cooling method: inner circulation water cooling. Power supply: 220V.

Results and Discussion

Identification of Bacteria

Cultural Characteristics

Golden colony on mannitol salt agar. laser Nd:YAG was used with wave length 1064 nm, frequency 6Hz, power 400, 500, 800 and 900 mJ with different shoots number (100-500).



The experiment done by putting the inoculums in the center of agar media (Mannitol salt agar) then hit it with a pulse action of laser, after treatment complete, the inoculums spread on all the media surface by sterile swab and incubate at 37ºC for 24 hr.

The results (shown in tables) were expressed as viable count percentage comparing with control plate (the same medium with the same inoculums size but without any laser treatment).

Table 1 :400 mJ

Laser	1064 nm, 6Hz, 400 mJ			No of colony in control = 238 CFU		
No. of shoot	CFU (1)	CFU (2)	CFU (3)	Mean	Survival %	Killed %
100	218	215	213	215	96.6	3.4
200	138	140	143	141	59	41
300	109	105	101	105	44	56
400	74	75	77	76	32	68
500	21	20	19	20	8	92

Table 2 :500 mJ

Laser	1064 nm, 6Hz, 500 mJ			No of colony in control = 238 CFU		
No. of shoot	CFU (1)	CFU (2)	CFU (3)	Mean	Survival %	Killed %
100	189	190	190	189	79	21
200	154	156	161	157	65.9	34.1
300	93	98	102	97	40.7	59.3
400	24	25	29	26	11	89
500	0	0	0	0	0	100

From the statistical analysis of results, its concluded that the correlation of laser with 400mJ (Table 1) had very weak relation with the killing percentage (0.369) but the correlation been very high with laser in 500mJ (0.989) (Table 2) and its correlation is higher than other laser power (800mJ and 900mJ) (Table 3 and 4) that gave strong relation with killing percentage at 800mJ (0.765) and its more than obtained with 900mJ that gave intermediate correlation with killing percentage (0.707). The results of other researchers concluded that the low level laser improved the third degree burns of rat that infected with *Staphylococcus aureus* (16).

Table 3 :800 mJ

Laser	1064 nm, 6Hz, 500 mJ			No of colony in control = 238 CFU		
No. of shoot	CFU (1)	CFU (2)	CFU (3)	Mean	Survival %	Killed %
100	96	93	92	94	39.4	60.6
200	0	1	22	11	4.6	95.4
300	0	0	0	0	0	100
400	0	0	0	0	0	100
500	0	0	0	0	0	100

Table 4:900 mJ

Laser	1064 nm, 6Hz, 500 mJ			No of colony in control = 238 CFU		
No. of shoot	CFU (1)	CFU (2)	CFU (3)	Mean	Survival %	Killed %
100	84	86	91	87	36.5	63.5
200	0	0	0	0	0	100
300	0	0	0	0	0	100
400	0	0	0	0	0	100
500	0	0	0	0	0	100

Diode laser with pulsed and continuous mode induced a significant reduction of viable count of bacteria. It indicate that laser irradiation bactericidal effect can also inhibit activation of macrophage that is LPS-induced leading to blunt the inflammatory response. (17)

The reduction on cell growth was observed in *S. aureus* in polymicrobial biofilms. That mean it represents an initial in vitro approach to study the influence of NIR laser treatment on bacterial biofilms in order to explain its potentially advantageous effects in the healing process of chronic infected wounds (18).

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