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Antimicrobial Photodynamic inactivation of using Rose Bengal and Low-Level Laser Light against *Staphylococcus aureus*

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Abstract: Background: *Staphylococcus aureus* is Gram-positive bacteria that lives as a normal flora in living organisms but can be pathogenic to humans. Although a relatively unspectacular, nonmotile coccoid bacterium, *S. aureus* is a dangerous human pathogen in both community-acquired and nosocomial infections. Due to the increasing emergence of new strains of this antibiotic-resistant bacteria, it has become essential to approach different methods to control this pathogen. One of these methods is the antimicrobial photodynamic inactivation process using a low-level laser, in this paper, the Photodynamic effects of Rose Bengal and LLLL on the virulence factors of *S.aureus* were evaluated. **The aim of the study** The present study aims to evaluate the Photodynamic effects on *S. aureus* using laser irradiation and Rose Bengal as an external photosensitizer**. Methods:** sixty samples from sputum were taken. Then ten isolated from these samples were chosen to be under the study, where RB was used at a concentatin of 100 µg/ml that is activated by diode laser (532 nm) with power density of 1 W/m^{2}and exposure time (1, 2& 3) minute. **Results**: show that there is no effect on the inhibition of virulence factors except at the last minute, that is, the virulence factors decrease at the third minute only in the absence of a photosensitizer agent, while there is a direct effect of activated Rose Bengal on *S. aureus* isolated from the sputum of Iraqi patients with pneumonia, where all times of exposure of (RB + 532 nm) were effect on the virulence factors by inhibiting it. **Conclusions:** show that the diode laser of 532 nm has no effect on the virulence factor of *S. aureus* isolated from sputum except at the third minute, while RB activated by diode laser (532 nm) have an effective action on all virulence factors of *S. aureus* isolated from sputum at all times of exposure, accordingly, it was concluded that when using a laser diode alone, the bacterial viability decreases at the third minute only, While when using Rose Bengal activated by a diode laser, the viability of bacteria is reduced at all times of exposure.

Keywords: photodynamic effect; *Staphylococcus aureus*; Rose Bengal dye; photosensitizer

1. Introduction

S. aureus is Gram-positive bacteria that lives as a normal flora in living organisms but can be pathogenic to humans (Bien, Sokolova, & Bozko, 2011). Although a relatively unspectacular, nonmotile coccoid bacterium, *Staphylococcus aureus* is a dangerous human pathogen in both community-acquired and nosocomial infections. A fundamental biological property of this bacterium is its ability to asymptomatically colonize healthy individuals. *S. aureus* carriers are at higher risk of infection, and they are presumed to be an important source of the *S. aureus* strains that spread among individuals (Chambers & DeLeo, 2009). The pathogen can cause a wide variety of infections, which can be divided into three types: (i) superficial lesions such as wound infection, (ii) toxinoses such as food poisoning, scalded skin syndrome and toxic shock syndrome, and (iii) systemic and life-threatening conditions such as endocarditis, osteomyelitis, pneumonia, brain abscesses, meningitis, and bacteremia (Sousa, 2004).

The majority of staphylococci can spread illness in both anaerobic and aerobic environments. There are currently more than 80 species and subspecies in the genus, many of which can be located on mammalian mucous membranes and skin (Ahmed & Al-Daraghi 2022).

2. Virulence factors of *Staphylococcus aureus*

They are particles present or secreted by bacteria that play important and multiple roles in infection and pathogenesis, such as the colonization of the host cells, cell adhesion, and invasion of the host immune system. As well as inhibition of the host's immune response, several virulence factors possessed by Staphylococcal bacteria play an essential role in their pathogenesis (Jekle,Yoon, Zuck, Najafi, Wang, Shiau & Debabov, 2013).

3. Exoproteins

Some Staphylococci secrete a group of proteins, including Exotoxins and enzymes such as nuclease, protease, lipase & coagulase. Notably, the important goal of these proteins is to transform the local tissue of the host into a food substance used by the bacterial cell for growth (Dinges, Orwin, & Schlievert, 2000).

Hemolysins; Hole-forming toxins represent the most crucial group of toxins secreted by *S. aureus*. As these toxins break down red blood cells and release hemoglobin, the formation of pores or holes on the membranes of the host cell subject to the toxin leads to a loss of the integrity of the membrane by the formation of holes (Ratner, Hippe, Aguilar, Bender, Nelson & Weiser, 2006). This group is diverse and numerous, including alpha-hemolysin. It is the first bacteria exotoxin that has been identified among the hole-forming hemolytic toxins. It is a polypeptide with a molecular weight of (3302) KDa. This toxin disrupts the smooth muscle in blood vessels **(**Czajkowsky, Sheng, & Shao, 1998).

Proteases: *S. aureus* produces different types of protease, including metal protease, serine protease & cysteine protease. Many studies have suggested that these enzymes are essential virulence factors and that about 21% of the strains of these bacteria give areas of proteolysis on the casein agar medium. In contrast, the rest gives a negative result (Bose, Daly, Hall, Kenneth & Baylesa , 2014).

Dnase: A small globular protein composed of (149) amino acids with a molecular weight of (16.8) Kda and an optimal PH range between (8.6-10.3). It has recently been known that this enzyme is involved in the formation of the biofilm, as well as its contribution to the distribution and diffusion of that membrane and transmission or Subsequent promotion of bacterial propagation (Mann, Rice, Boles, Endres, Ranjit, Chandramohan, & Bayles, 2009).

Lipases: It is an enzyme known as FAME, which is produced by Staphylococci and harms the immune functions of the host, as all strains of *S. aureus* and more than 30% of other types of Staphylococci produce this enzyme, which breaks down lipids as a primary function to ensure the survival of bacteria in the Sebaceous area of the body.

The activity of this enzyme in Staphylococci was noticed in 1901, as there are strains of *S. aureus* that secrete

two types of this enzyme, one is Geh and the other is lipase, both of which are required for the analysis of fats and the breakdown of nutrients in favor of bacteria (Rollof, Braconier, Söderström, & Nilsson-Ehle, 1988). The laser was invented more than 60 years ago and developed very quickly, and its applications expanded to include biology, medical, chemical, technology, and other fields (Chopra & Chawla, 1992). laser light has unique properties like monochromaticity, high intensity, and low beam divergence (Wahhab, Mahdi, Faris & Altiafy, 2017). Numerous studies have proven that the bactericidal effect of a diode laser (810 nm) is based on thermal properties; furthermore, bacteria cannot develop resistance to laser exposure (Asnaashari, Ebad & Shojaeian, 2016). low-level laser therapy work by a photochemical mechanism in which photons from the laser source interact with cells that result in stimulation them or biochemical changes (Mohammed & Al-ameri, 2021). Anti-microbial PDT has been recognized for approximately one hundred years. Numerous significant variables affect the sensitivity of diverse microorganisms to photodynamics. Staphylococcus aureus is Gram-positive bacteria that live in a normal flora in living organisms but can be pathogenic to humans. Because changing the place will produce new strains of this antibiotic-resistant bacteria, it has become essential to approach different methods to control this pathogen. This method uses an antimicrobial photodynamic use process, which depends on the presence factor of photosensitizer and oxygen, which cause irreversible damage to bacteria cells. Antibiotic-resistant microorganisms are increasing at an alarming rate. Consequently, innovative antimicrobial strategies are required without delay. Photodynamic therapy (PDT) consolidates oxygen with light-activated photosensitizers (PS) to create cytotoxic species and tissue death (Dolmans, Fukumura & Jain 2003). Also PDT shows promise as a treatment for several cancerous and non-malignant disorders, using a photosensitizer that is targeted specifically in the targeted site and exposing the lesions to visible light to cause photodamage and consequent cell death (Abbas, Al-Taee & Mahmood, 2012).

4. Material and methods

Table (1) Ready Culture Media

Seq.	The name of the medium	manufacture	Purpose
1	Mannitol salt agar	Himedia	Selective medium for *S. aureus*
2	Nutrient agar	Himedia	for the growth of bacteria
3	Blood agar	Himedia	Hemolysis test medium
4	DNase test agar	MERCK	DNA degradation test medium
5	Nutrient broth	Himedia	For the growth of bacteria
6	Sugar fermentation medium	Himedia	Carbohydrate fermentation test medium
7	Lipid agar	Himedia	Lipolysis test medium
8	Protease agar	Himedia	Proteolytic test medium

4.1 Rose Bengal (photosensitizer)

In 100ml of distilled water, 0.01g of Rose Bengal powder was dissolved to produce stock with a 100g/ml concentration. Filtration was used to sterilize the solution through a 0.20µm syringe filter. The stock was kept in the dark till use. This dye was used to inhibit bacterial isolates (Sabbahi, Ben Ayed & Jemli, 2018).

4.2 Physiological Saline Solution

It was produced by solubilizing 0.85g of Na Cl in 100 ml of distilled water and sterilizing it in an autoclave.

4.3 Catalase reagent

The bacteria' susceptibility to the catalase enzyme production was examined using a 3 % concentration of hydrogen peroxide. It was stored in an opaque glass bottle protected from light (MacFaddin, 2000).

4.4 Cytochrome Oxidase Indicator
This reagent was made by dissolving (1) g of a substance (N, N, N, N Tetra methyl -p- phenylene di amine di hydrochloride) in (100) ml of distilled water and storing it in an opaque glass vial (Tang & Stratton 2006).

4.5 Gram Stain
The different between G-positive and G-negative bacteria.

4.6 The plasma used in the coagulase test
It was obtained from the blood bank in Baghdad either in the form of plasma or blood, where plasma is obtained by centrifuging at 3000 cycles per minute for 20 minutes, and a sterile syringe separates the plasma.

4.7 Samples collection
The collection of samples was conducted using tiny swabs. Sixty samples from sputum were taken. Ten isolated from these samples were chosen to be under the study.

4.8 Isolation of *S. aureus*
After patient samples were collected, they were cultivated in a selective media (mannitol salt) medium and incubated aerobically for 24hrs at 37℃. Then the plates of the medium were examined.

4.9 Identification of *S. aureus*
Microscopical and biochemical tests are used to identify bacteria.

4.10 Microscopically examination
In order to identify bacteria, microscopy and biochemical analysis are utilized.

A- Colonial morphologies
Colonial characteristics of *S. aureus* on the mannitol salt agar medium were examined by dissecting a microscope at a magnification of (X8). Where include colony shape, size, height, edges, color, and effect on the medium, such as hemolysis and fermentation of mannitol.

B- Cell morphologies
Cells arrangement, chain formation, and Gram staining for *S. aureus* were examined microscopically by the Gram stain method (Matar, 2004).

4.11 Catalase test
By removing a tiny sample of colonies from the agar with a sterile loop and combining them with a few drops of 3 percent H_2O_2 on a slide, the catalase activity of S. aureus was determined. The lack of bubble formation indicates the absence of enzyme catalase (Heinz & Mortimer 1981).

4.12 Cytochrome Oxidase Test
Colonies were evaluated by removing a small number of colonies from the plate with a sterile loop and mixing it with a few drops of Cytochrome Oxidase Indicator on a glass slide. The appearance of the violet color within (10-20) seconds is a positive result (Atlase, Brown, & Parks, 1995).

4.13 Coagulase test
The plasma coagulation enzyme was investigated using the tube method, where 0.8 ml of blood plasma was added to 0.2 Brain heart infusion broth medium inoculated with growing bacterial isolates at the age of (18-24) hours in small tubes. Furthermore, incubated for 4 hours at 37 degrees Celsius, during which coagulation occurred, indicating the test was positive. The tubes that did not show clotting were left at room temperature until the next day (Holt, Krieg, Sneath, Staley & William, 1994).

4.14 Carbohydrate Fermentation Test
The fermentation medium of sugars containing sugars such as the mannitol to be tested was inoculated with a bacterial culture at age (18-24) hours and incubated at 37°C for seven days with daily follow-up of the color. Changing the medium's color from red to yellow signifies a positive test (Mahon, Lehman & Manuselis, 2018).

5. Virulence factors

5.1 Blood hemolysis test

On a blood agar medium, the examined bacterial isolates were cultured for 24 hours at 37 ° C. Observation of plate decomposition is evidence of bacterial secretion of the Hemolysin enzyme (Mathai, Sindhu , Sulochana & Sathyabhama, 2003).

5.2 Lipase test

The bacterial colonies were inoculated on a lipid agar medium) by the planning method, the plates were left for 24 hours at 37°C. Observation of decomposition in dishes is evidence of bacterial secretion of lipase enzyme (Slifkin, 2000).

5.3 DNase test

The bacterial colonies were inoculated on a Toluidine blue DNA agar by the planning method, and the dishes were incubated at 37°C for 24 hours; the blue color of the medium turned pink, indicating that the bacteria secreted a nuclease enzyme that breaks down DNA into nucleotides that combine with the dye toluidine blue to form a pink color, meaning that the test is positive.

5.4 Protease test

The bacterial colonies were inoculated on a lipid agar medium by planning procedure. The plates were left for 24 hours at 37 degrees Celsius; observing decomposition in dishes is evidence of the bacterial secretion of protease Enzyme (Hynes & Tagg, 1985).

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6. Laser Irradiation

6.1 Diode laser irradiation without Rose Bengal

One ml of A diluted bacterial suspension at $1*10^5$ CFU/ml concentration is put in each Eppendorf tube and exposed to a diode laser (532 nm) at different exposure times (1, 2, and 3) minutes (except one of these Eppendorf tubes did not expose to laser light in order to keep it as a control) with power density of 1 W/cm^2. The bacterial suspension in Eppendorf tubes was grown on nutrient agar and incubated aerobically for 24 hrs. The virulence factors were tested. The irradiation of bacteria by diode laser without Rose Bengal is shown in fig. (1)

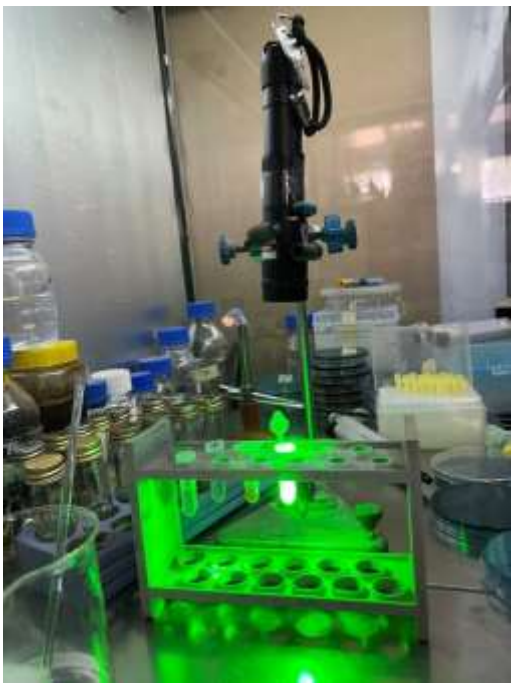

Figure (1): Irradiation of *S. aureus* by diode laser without Rose Bengal

6.2 Diode laser irradiation with Rose Bengal

Rose Bengal is considered a photosensitizer and has a maximum absorption at 550 nm, close to the wavelength of diode laser light, so it is chosen. Rose Bengal of 0.5 ml, with a 100 µg/ml concentration. It was added to these tubes containing bacterial suspension at a concentration of $1*10^5$ to get a final concentration equal to 50µg / ml. All these were exposed to laser light at different exposure times (1, 2, and 3) minutes, except one of the Eppendorf tubes did not expose to laser light (containing bacterial suspension and photosensitizer (Rose Bengal)) to act as a control. All bacterial suspensions in all tubes (exposed and non-exposed) were grown on nutrient agar for 24hrs at 37°C in an aerobic jar; then, the virulence factors were tested. The irradiation of bacteria by a diode laser with Rose Bengal is shown in fig. (2)

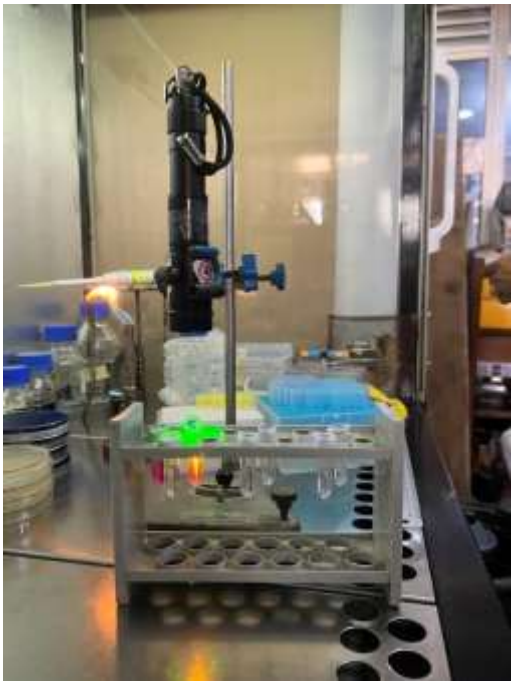

Figure (2): Irradiation of *S. aureus* by a diode laser with Rose Bengal

7. Statistical analysis

The statistical computer program IBM SPSS version 27.0 (IBM Corp. Released 2020) was used to calcultae the frequency and percentage frequency.

8. Results and Discussions:

8.1 Bacterial Identification

Two methods are used in the bacterial identification of three isolates of *S. aureus;* microscopic examination and biochemical tests.

8.2 Microscopic Examination

This method includes colonial morphologies and cell morphologies of *S. aureus*.

8.3 Colonial morphologies

In this study, under dissecting microscope, *S. aureus* that had grown on mannitol salt medium, where this medium is selective and differential because it contains a high percentage of salts (7.5-10) %, which can be tolerated by the genus *Staphylococcus,* as well as the medium contains mannitol sugar and mythel red reagent, as the bacteria of *S. aureus*. Yellow colonies with a diameter of (2-3) mm can ferment mannitol sugar and produce acidic products; consequently, the color of the medium changes from red to yellow (Leboffe & Pierce, 2019).

8. 4 Cell morphologies of *S aureus*

The S. aureus bacteria appeared through the microscopic examination of the slides stained with gram stain using the ordinary light microscope using an oil lens. Where the shape of the cells, their regularity, and the way they collect and interact with gram stain were observed. They are gram-positive cells with a spherical shape with a diameter of approximately 1µm, clustered in clusters, and they are in single spherical shapes, pairs, or quadrilaterals, and this corresponds to what was mentioned by (Matar, 2004) as shown in fig. (3).

8.5 Biochemical & culture factors tests

The results of diagnostic biochemical tests appeared similar for all ten isolates of *S. aureus* isolated from sputum, as shown in table (2)

Table (2) the results of diagnostic biochemical test

Type of test	Result
Suger Fermentation Test	+
Catalase test	+
Oxidase test	-
Coagulase test	+

8.6 Bacterial irradiation with Diode laser

One type of laser irradiated bacterial suspensions of ten isolates of *S. aureus*: diode laser.

This laser is a CW mode laser and emits light at 532 nm. Its output power was 80 mW, and its spot size was 1cm. The bacterial suspension was exposed for different exposure times (1, 2 & 3) minutes.

8.7 Virulence factors (without Rose Bengal)

8.7.1 Before and after irradiation

The virulence factors of *S. aureus* isolates as shown in the table (3):

Table (3) *S. aureus* virulence factors in sputum isolates without RB

Tests		Exposure time in minutes							
		0		1		2		3	
		N.	%	N.	%	N.	%	N.	%
Hemolysin	+Ve	10	100.0	10	100.0	10	100.0	6	60.0
	-Ve	0	0	0	0	0	0	4	40.0
Lipase	+Ve	10	100.0	10	100.0	10	100.0	4	40.0
	-Ve	0	0	0	0	0	0	6	60.0
Protease	+Ve	10	100.0	10	100.0	10	100.0	4	40.0
	-Ve	0	0	0	0	0	0	6	60.0
DNAse	+Ve	9	90.0	9	90.0	9	90.0	5	50.0
	-Ve	1	10.0	1	10.0	1	10.0	5	50.0

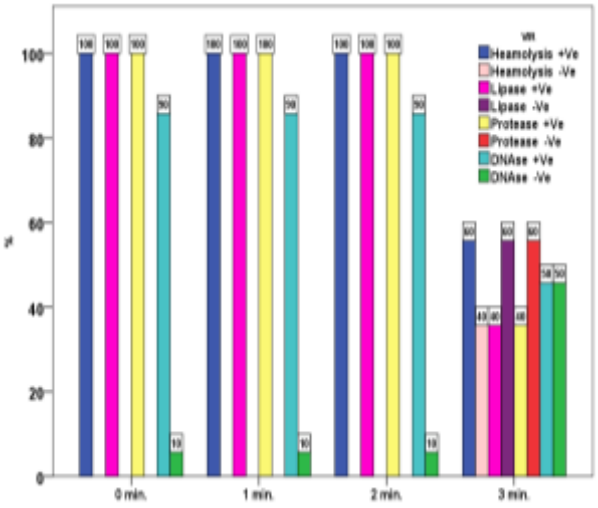

Figure (3): percentage frequency of *S. aureus* virulence factors in sputum isolates without RB

Statistical analysis of the results of bacterial irradiation by diode laser radiation without photosensitizer at different exposure time (1, 2& 3) minute, indicated that there is no effect on the inhibition of virulence factors except at the last minute, that is, the virulence factors decrease at the third minute only in the absence of a photosensitizer agent, the results agree with Fila and Grinholc's (Fila, Kawiak, & Grinholc, 2017).

The mechanism of action for the diode laser on virulence factors of *S. aureus* include the following:

When using a laser in range visible and near IR laser radiation occur one or more of the following physical and / or chemical changes can occur as a result of photoexcitation of their electronic state:

1-Alteration of redox properties and an acceleration of electron transfer, which can initiate of folding of protein and in this way influence the activity of the enzyme, as it was demonstrated for reduced cytochrome c. (important component of respiratory chain and can be considered as a primary photoacceptor) leads to inhibition in the manufacture of enzymes responsible for virulence factors.

2-during light excitation of electronic state, a noticeable fraction of the excitation energy is inevitably converted to heat, which causes a local transient increase in the temperature of the absorbing chromophres, this may cause structural (e.g. conformational) changes and trigger biochemical activity such as activation or inhibition of virulence factors enzymes,and the results of this study agree with many studies that used low-level laser, for instance, (DeSimone, Christiansenand Dore, 1999) the study showed the mechanisms of inhibiting virulence factors in *S. aureus* when using a low-level laser light.

8.7.2 Virulence factors (with Rose Bengal)

Before and after irradiation, The virulence factors of *S. aureus* isolates as shown in the table (4):

Table (4) *S. aureus* virulence factors in sputum isolates with RB

Test		Exposure time in minutes							
		0		1		2		3	
		N.	%	N.	%	N.	%	N.	%
Hemol-ysin	+	10	100.0	8	80.0	6	60.0	0	0
	-	0	0	2	20.0	4	40.0	10	100.0
Lipase	+	10	100.0	7	70.0	2	20.0	0	0
	-	0	0	3	30.0	8	80.0	10	100.0
Protease	+	9	90.0	6	60.0	3	30.0	1	10.0
	-	1	10.0	4	40.0	7	70.0	9	90.0
DNAse	+	9	90.0	5	50.0	3	30.0	0	0
	-	1	10.0	5	50.0	7	70.0	10	100.0

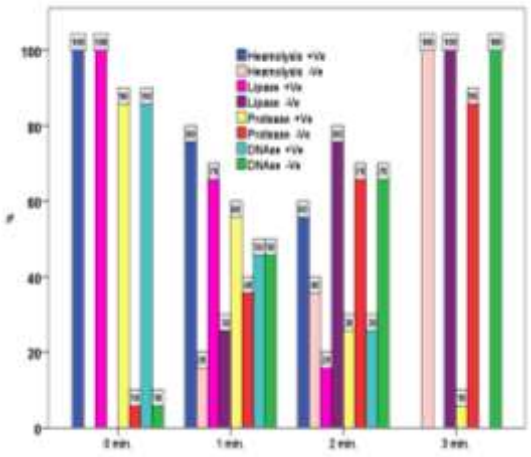

Fig. (4) percentage frequency of *S. aureus* virulence factors in sputum isolates with RB

Statistical analysis of the results that there are effects in the inhibition of virulence factors at all times of exposure in the presence of the photosensitizer agent (Tubby, Wilson & Nair, 2009).

The mechanism of action for the RB on virulence factors of *S. aureus* include the following:

1-The precise mechanism of inhibition of virulence factors may be due to the reactive oxygen species formed during photosensitization that can oxidize the proteins that make up the enzymes and thus disrupt their work.

2-The enzymes produced by the *S. aureus* bacteria, which are virulence factors that cause human diseases, have genes responsible for their secretion, such as *hlp, sspA*, *cidA,* and *gehA*. These genes encode beta-hemolysin, protease, Dnase, and lipase, respectively. Once Photodynamic action occurs through ROS generation, such as singlet oxygen (formed during the irradiation process), oxidative damage can occur to a variety of cellular components that play a significant role in the maintenance of bacterial stability, such as the structures of genes encoding enzymes and molecular components (proteins and lipids) of external structures. This alteration in the structures of genes encoding enzymes inhibits the enzymes, ,and the results of this study agree with many studies that used low-level laser with appropriate quantity of the photosensitizer, for instance, (Bartolomeu, Rocha, Cunha, Neves, Faustino, & Almeida, 2016) the objective of this work was to evaluate the effect of antimicrobial photodynamic inactivation (PDI) on virulence factors of *S. aureus* and to assess the potential development of resistance of this bacterium as well as the recovery of the expression of the virulence factors after successive PDI cycles, and the PDI process is based in the combined use of light, oxygen, and an intermediary agent (a photosensitizer)

9. Conclusions

1-The diode laser of 532 nm has no effect on the virulence factor of *S. aureus* isolated from sputum except at the third minute.

2-Rose Bengal activated by diode laser (532 nm) have an effective action on all virulence factors of *S. aureus* isolated from sputum at all times of exposure.

3- Accordingly, it was concluded that when using a laser diode alone, the bacterial viability decreases at the third minute only, but when using a diode laser in the presence of RB, the bacterial viability decreases at all times of exposure.

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التأثير الديناميكي الضوئي لروزالبنغال المنشط بواسطة ضوء ليزر منخفض المستوى على *S. aureus*

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الخلاصة: المكورات العنقودية الذهبية هي بكتيريا موجبة الجرام تعيش كفلورا طبيعية في الكائنات الحية ولكنها يمكن أن تكون مسببة للأمراض للإنسان. على الرغم من كونها بكتيريا كروية غير متحركة نسبيًا ، إلا أن المكورات العنقودية الذهبية هي أحد مسببات الأمراض الخطيرة البشرية في كل من العدوى المكتسبة من المجتمع واصابات المستشفيات. نظرًا لتزايد ظهور سلالات جديدة من هذه البكتيريا المقاومة للمضادات الحيوية ، فقد أصبح من الضروري اتباع طرق مختلفة للسيطرة على هذا العامل الممرض. إحدى هذه الطرق هي عملية التعطيل الضوئي الديناميكي المضاد للميكروبات باستخدام ليزر منخفض المستوى ، في هذا البحث ، تم تقييم التأثيرات الديناميكية الضوئية لروز البنغال وضوء الليزر منخفض المستوى على عوامل ضراوة *S. aureus* تم أخذ ستين عينة من البلغم. ثم تم اختيار عشر عينات معزولة من هذه العينات لتكون قيد الدراسة ، حيث تم استخدام روز البنغال بتركيز 100 ميكروغرام / مل يتم تنشيطه بواسطة ليزر الصمام الثنائي (532 نانومتر) بجرعات مختلفة (6،12 و 18) جول / سم 2. أظهرت النتائج أنه لا يوجد تأثير على تثبيط عوامل الضراوة إلا عند كثافة الطاقة الأخيرة (18 جول / سم 2) ، أي أن عوامل الضراوة تنخفض عند كثافات الطاقة العالية فقط في حالة عدم وجود عامل متحسس ضوئي. بينما كان هناك تأثير مباشر لعقار روز البنغال المنشط على بكتيريا *S. aureus* المعزولة من بلغم المرضى العراقيين المصابين بالالتهاب الرئوي ، حيث أثرت جميع الجرعات (532 نانومتر+ روز البنغال) على عوامل الضراوة عن طريق تثبيطها. أظهرت النتائج أن ليزر الصمام الثنائي البالغ 532 نانومتر ليس له أي تأثير على عوامل ضراوة *S. aureus* المعزولة من البلغم إلا بجرعات عالية ، بينما روز البنغال المنشط بواسطة ليزر الصمام الثنائي (532 نانومتر) له تأثير فعال على جميع عوامل ضراوة *S. aureus*المعزولة من البلغم في جميع الجرعات (6،12 و 18 جول / سم 2) ، وبناءً عليه ، استنتج أنه عند استخدام الصمام الثنائي ليزر لوحده ، تقل قابلية بقاء البتكريا بالجرعات العالية فقط ، ولكن عند استخدام روز البنغال المنشط بواسطة ليزر ثنائي الصمام تنخفض قابلية بقاء البكتيريا في جميع الجرعات