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Effects of Nd:Yag Laser on some virulence factor genes of *Pseudomonas aeruginosa* bacteria

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ABSTRACT

The aim of this study was to assess effects of the 532nm Nd-yag laser on the genes of Tox A, Exo S, and Opr L, of *Pseudomonas aeruginosa* (*P. aeruginosa*) bacteria isolated from clinical (wounds, burns, otitis media) and environmental (water, soil) samples. Clinical samples were collected from patients coming to Saladdin General Hospital from wound, burns and middle ear infections while environmental samples were extracted from water and soil for Saladdin General Hospital . Bacterial samples irradiated by Nd-Yag laser with wavelength of 532 nm using energies (300mj,500mj) with (15 and 25 sec) and genomic DNA were extracted from all samples after the diagnosis of *P. aeruginosa* bacteria depending on the macroscopic and biochemical examination, then the PCR technique was performed. The results have shown an impact on *P. aeruginosa* bacteria of Nd-Yag laser by comparing PCR results of treated samples with control (unexposed) as loss of normal bands. This indicates that the laser had a genetic effect on the *P. aeruginosa* bacteria. We conclude that the laser induces genetic changes in *P. aeruginosa's* DNA so that lasers can be used in treatment and sterilization for clinical and environmental . The PCR technique could be used as a biomarker study to determine the biological effects of radiation on bacteria.

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is an omnipresent bacteria that is responsible for nosocomial infections that can affect immunocompromised individuals. It has several virulence factors, such as biofilm formation, which protects the pathogen from the antibody of a host and antibiotics. It is an opportunistic pathogen causing specific infectio ns [1,2]. These bacteria plays a significant role in patients with wounds and burns as an etiological agent of serious infections[3]. It is possible to recover opportunistic disease caused by *P. aeruginosa* bacteria from clinical cases [4]. Several infections in the hospital can be because of *P. aeruginosa* bacteria such as wound, burns, urinary tract, and outer ear infections [5].

One of the virulence factors is Exoenzyme S that is a ADP-ribosyltransferase is such which is secreted directly into the cytosol of epithelial cells by a type-III secretion mechanism [6]. Exotoxin A encoded by the gene A, which is capable of inhibiting protein biosynthesis such as toxin diphtheria [7]. The first step in an infection is the adhesion of a pathogen to the epithelial surface. In several studies, *P. aeruginosa* exoproducts, especially proteinases and neuraminidase, are noted that such receptors are accessible by cleaving terminal sialic acid residues from glycosides of the cell surface [8]. Opr L and Opr I lipoprotein are two *P.aeruginosa* outer membrane proteins responsible for *P.aeruginosa's* natural antibiotic and antiseptic resistance. Because of these proteins are originate merely in *P.aeruginosa*, it's may be reliable factors in clinical samples for the rapid identification of *P.aeruginosa* [9,10].

Laser treatment is particularly important in ophthalmology, dermatology and otolaryngology [11]. Just CO2 (carbon dioxide), the diode and the Er: YAG laser can be used for decontaminating implant surfaces from all lasers used in the field of dentistry, this is because of their bactericidal activity and

because titanium is poorly absorbed in their specific wavelength [12]. Nd-yag is a type of laser that emits 1064nm in the infrared region and it used in sterilization and treatment because of its ability to kill microorganisms such as bacteria by laser photons because the water molecules inside the living cell absorb this laser significantly leads to inhibition the bacterial cell is caused by a genetic change in the DNA of the living cell, even if the dose is low [13]. The laser can impact gram negative and gram positive bacteria, the antimicrobial and bactericidal effects of laser are confirmed by several studies [14]. Previous research demonstrated the antibacterial effects of Nd-YAG laser in patients treated for oral diseases, where pulsed Nd-YAG laser with power settings of more than 2 W was used to cause a photothermal effect [15,16]. The effect of laser irradiation on other bacteria such as *Escherichia coli*, *Staphylococcus*, *Actinomyces naeslundii*, *Enterococcus faecalis* and *Streptococcus anginosa* has been demonstrated [15,17]. *P.aeruginosa* bacteria have a high history of antibiotic resistance to antibiotic use. The use of laser that is independent of the bacteria's antibiotic resistance pattern could therefore be useful in the treatment of burning and wound infection [18]. There are some studies that provide general information about the fungicidal and bactericidal influence of laser therapy, using various types of lasers of different wavelengths, energy and doses of irradiation [19].

Many PCR-based, DNA test methods have been developed to detect various pathogens from clinical, water and food samples. Nevertheless, there are a few reports on the application of PCR to control the environment of pathogenic Pseudomonas strains [20]. Quick identification of causative agent isolates is indeed necessary for patients to choose subsequent treatment [21]. Polymerase chain reaction (PCR) was an important technique using specific sequences of the particular microbial to identifying microbial species rapidly [22].

The present study aimed to using the Nd-YAG laser to effect on some virulence factor genes of *P.aeruginosa* bacteria using PCR technique.

Materials and Methods

Isolation and identification of bacteria: During the period from December 2018 to March 2019, 40 number of clinical samples were collected from patients with middle ear infection, burns and wounds attending salahuddin General Hospital using cotton swab in addition to environmental samples from the soil of the and from the drainage water of salahuddin General Hospital, then was cultured on nutritious cultures stramide agar for 24 h at 37 C◦ for the growth of bacterial colonies. The bacteria was identified at the level depending on Macroscopic and biochemical test.

Irradiation of Bacteria: For the purpose of irradiation of the bacterial samples, a disk was taken by 7mm diameter diaphragm from the growing colonies on the petri dish of *P.aeruginosa* and placed in a tube containing Nutrient broth and incubated for 24h at 37C° then exposure to laser Nd-Yag continuous pulse and 1064nm wavelength with energy (300,500)mj and a time of (15,25)sec where the sample was placed 15cm away from the laser nozzle.

Isolation of Genomic DNA: Before and after exposing bacteria *P.aeruginosa* to the laser radiation genomic DNA was isolated according to the method [23]. integrity and purity of DNA was determined by agarose gel electrophoresis stained with red safe.

PCR analysis: The PCR technique was optimized using three specific primers as shown in Table (1).

Table 1: Gene names sequences of primers used in PCR:

No.	Primer	Sequence	Size bp
1	Tox A	GGT AAC CAG CTC AGC CAC AT	352 bp
		TGA TGT CCA GGT CAT GCT TC	
2	Opr L	ATG GAA ATG CTG AAA TTC GGC	500 bp
		CTT CTT CAG CTC GAC GCG ACG	
3	Exo S	CTT GAA GGG ACT CGA CAA GG	504 bp
		TCC AGG TCC GCG TAG TGA AT	

The Accupower PCR Master Mix of the Korean Bioneer company was used for conducting PCR reactions, adding a total volume of 20 µL comprising 50 ng of DNA template and 10 Pico moles of forward and reverse primers in each tube. Before PCR amplifications, each tube was a vortex then a thermal cycler from Applied Biosystems using with the following program for PCR amplifications, for the first denaturation, one cycle at 94 seconds followed by 35 cycles, each with 30 seconds at 94 ◦C for the denaturation, 30 seconds at 55 ◦C for the annealing and 45 seconds at 72 ◦C for the extension, and 7 minute at 72 ◦C for the final extension according to [24]. PCR products are analyzed by (1.5 or 2%) agarose gel electrophoresis stained with red safe in 1X SB buffer at (5 volt / cm) and visualized using gel documents. The 100 bp DNA ladder (BioLabs) was used as a molecular marker. Reproducible and clear bands have been scored. The marked changes observed in PCR profiles are assessed as band disappearance compared to untreated or control.

Results

In this study, the effect of the Nd-Yag laser with different Energys and times are studied on *P. aeruginosa* samples as a gram-negative bacteria. The Nd-Yag laser irradiation caused a significant

difference in the growth of the bacterial isolates. Thus, the importance of our study confirms the suggestion that when irradiated with the specified parameters, Nd-Yag laser effectively inhibits the growth of bacteria.

This study was carried out to determine the effect of the Nd-Yag laser on three genes (Tox A, Exo S and Opr L) of p.aeruginosa. The results of PCR showed the Nd-Yag laser affected all genes were using in this study in varying proportions from gene to gene and from one sample to another as in Figure 1 and Table 2.

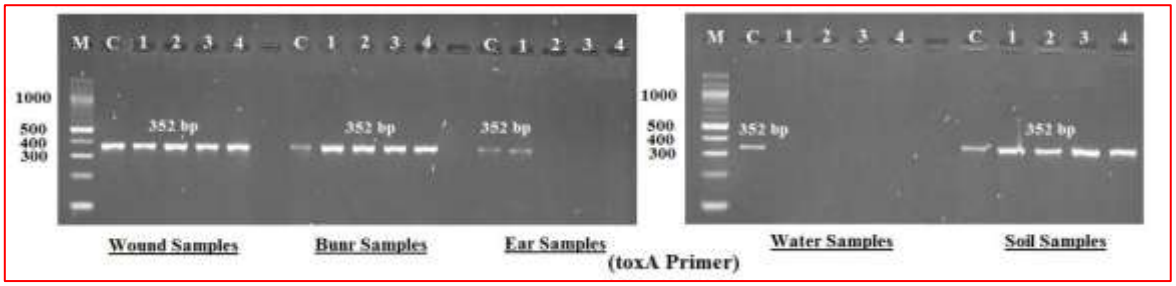

Fig. 1: Shows PCR product of toxA gene (352 bp) of Pseudomonas aeruginosa on 2 % agarose gel electrophoresis. Lane C (control or unexposed); Lane 1 (exposed to 15 sec with Energy 300mj); Lane 2 (exposed to 25sec with Energy 300mj); Lane 3 (exposed to 15sec with Energy 500mj); Lane 4 (exposed to 25 sec with 500mj).

Table 2: Show the percentage of the effect of *P.aergenosa* gene (Tox A) on Nd-Yag laser cards and different time

Genes	Samples	Control	Energy:300mj		Energy:500mj		%	∑%
			15 sec	25 sec	15 sec	25 sec		
Tox A	Wound	+	+	+	+	+	0	35 %
	Burn	+	+	+	+	+	0	
	Ear	+	+	-	-	-	75	
	Water	+	-	-	-	-	100	
	Soil	+	+	+	+	+	0	
No. of lost band			1	2	2	2		
Total No. of lost band			3		4			
Polymorphism			30%		40%			
∑ Polymorphism			35%					

(+) presence of new bands, (-) disappearance of normal bands

The results in figure and table 2 showed effect of Nd-Yag laser on the bacterial isolates through the disappearance of the band of the gene such as isolated bacteria from otitis media where the laser was able to affect the genetic material using energy of 300mj and a time of 25sec and 500mj and time (15,25sec), while the energy 300mj and time 15sec did not affect the bacteria. The environmental bacterial isolations that isolated from water, we find that the laser has effect by using all energies and times, so this is evidence of a malfunction in the arrangement of nitrogen bases, while other isolations (wounds, burns, soil) was not affected in any way by the laser so the ratio of the effect of the Nd-Yag laser on this gene is 35% as shown in table 2.

The effect of the Nd-Yag laser on the Exo S gene for three bacterial isolates are wounds, the middle ear and water in varying proportions. The Nd-Yag laser affected the location of the gene at 500mj and 25sec wounds isolations, while the sites of this gene disappeared in ear and water isolates at 500mj and time energy (15, 25sec), while burn and soil isolates were not affected by the laser beam. The laser effect on this gene was 25%, as shown in Table 3.

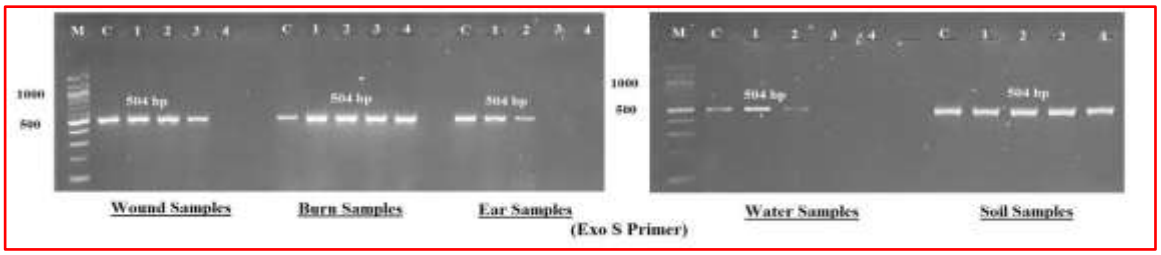

Fig. 2: Shows PCR product of ExoS gene (352 bp) of Pseudomonas aeruginosa on 2 % agarose gel electrophoresis. Lane C (control or unexposed); Lane 1 (exposed to 15 sec with Energy 300mj); Lane 2 (exposed to 25sec with Energy 300mj); Lane 3 (exposed to 15sec with Energy 500mj); Lane 4 (exposed to 25 sec with 500mj).

Table 3: Show the percentage of the effect of *P.aergenosa* gen (Exo S) on Nd-Yag laser cards and different time

Genes	Samples	Control	Energy:300mj		Energy:500mj		%	∑%
			15 sec	25 sec	15 sec	25 sec		
Exo S	Wound	+	+	+	+	-	25	25 %
	Burn	+	+	+	+	+	0	
	Ear	+	+	+	-	-	50	
	Water	+	+	+	-	-	50	
	Soil	+	+	+	+	+	0	
No. of lost band			0	0	2	3		
Total No. of lost band			0		5			
Polymorphism			0%		50%			
∑ Polymorphism			25%					

(+) presence of new bands, (-) disappearance of normal bands

The least impact (5%) of the Nd-Yag laser was on the Opr L gene for isolated bacteria isolate from water at 300mj and 25sec, while the rest of the isolations was not affected at all by the laser because of its ability to preserve its genetic material from damage due to its resistance to the laser, because the isolations were able to resist the laser as in Table 4.

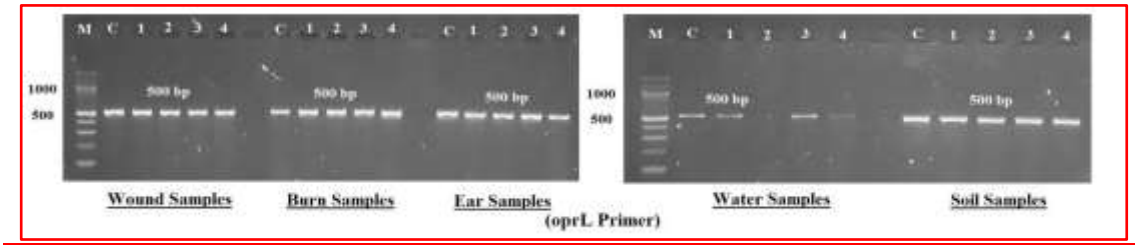

Fig. 3: Shows PCR product of oprL gene (352 bp) of Pseudomonas aeruginosa on 2 % agarose gel electrophoresis. Lane C (control or unexposed); Lane 1 (exposed to 15 sec with Energy 300mj); Lane 2 (exposed to 25sec with Energy 300mj); Lane 3 (exposed to 15sec with Energy 500mj); Lane 4 (exposed to 25 sec with 500mj).

Table 4: Show the percentage of the effect of *P.aergenosa* gen (Opr L) on Nd-Yag laser cards and different time

Genes	Samples	Control	Energy:300mj		Energy:500mj		%	∑%
			15 sec	25 sec	15 sec	25 sec		
Opr L	Wound	+	+	+	+	+	0	5 %
	Burn	+	+	+	+	+	0	
	Ear	+	+	+	+	+	0	
	Water	+	+	-	+	+	25	
	Soil	+	+	+	+	+	0	
No. of lost band			0	1	0	0		
Total No. of lost band			1		0			
Polymorphism			10%		0%			
∑ Polymorphism			5%					

(+) presence of new bands, (-) disappearance of normal bands

From Table 5, we found that the highest effect of the Nd-Yag laser was on the Tox A gene by 35% where the number of bands lost as a result of laser treatment of all energies and times for all samples 7 locations, while we found that the Exo S gene was affected by 25% where the number of packages lost was 5 bands The lowest effect of the laser was the Opr L gene and by 5% where only one band was lost as a result of laser treatment.

Table 5: Shows the number and percentage of lost bands of all genes and samples treated with Nd-Yag laser

Sample	Tox A		Exo S		Opr L	
	No.	%	No.	%	No.	%
Wound	0	0	1	25	0	0
Brain	0	0	0	0	0	0
Ear	3	75	2	50	0	0
Water	4	100	2	50	1	25
Soil	0	0	0	0	0	0
Total	7	35%	5	25%	1	5%

Discussion

P. aeruginosa is a leading cause of nosocomial infections, often involving multiple infections and life-threatening and difficult to treat as the organism is highly immune to many drug cases (MDRs) and is capable of developing resistance to all powerful antimicrobials. Over the years, P.aeruginosa contributes significantly to morbidity and mortality associated with surgical infections worldwide [25].

Such bacteria have many factors of virulence, including cilia, whips and exoenzyme S [26]. As well as having toxins that cause fever and the septicemia and exotoxin A-related shock, endotoxin [27]. Production of these genes encoding proteins of the outer shell such as Opr L, Opr [9]. The laser is the new hope in bacterial infection control, even those that are immune to the current drug [28].

The effect on a living cell of laser radiation is linked to several factors including energy, time, and wavelength. Several wavelengths are similar to the cell's DNA, which causes damage to the DNA of the bacterial cell [29]. The bacteria were influenced by the laser beam due to the protein's presence in the cell's DNA in the absence of a pulse [30,31]. Or because of radiation, the explanation for the disappearance may be the result of DNA damage. Such damage can occur when a single or double DNA strand is disrupted [32]. Lasers are a physical insuring agent that disengages water molecules in the bacterial cell and produces the roots of hydroxel that cause oxidative damage. Free radicals interact with bio-matter molecules, among which DNA is removed from them, destroying the structural structure of DNA. During PCR reaction, when enzyme Taq polymerase meets destroyed DNA, this will lead to the closure of the binding sites, the enzyme's inability to bind, meaning the loss of sites that existed prior to radiation exposure [33].

In this research, PCR technique was used to determine the effect of laser on Tox A, Exo S, and Opr L, of *P.aeruginosa* bacteria. When the polymerase enzyme experiences broken DNA during the PCR, this will result in the binding sites being closed, which is the enzyme's inability to bind. This means the loss of sites before radiation exposure [34,35].

We note that the laser beam influenced the positions of all the genes identified by their absence, and we find that the lowest laser beam effect was the Opr L gene because it is part of the antibiotic and toxin flow regimens that influence bacterial cells [9].

We note that the laser beam affected the locations of all the genes represented by their disappearance, and we find that the lowest effect of laser beam was the Opr L gene because it is part of the flow regimens of antibiotic and toxins that affect bacterial cell [9]. Laser therapy is an advanced medical technique because a particular cellular feature may be prevented by laser beam exposure. This procedure is referred to as laser processing [36].

In conclusion, the Nd-Yag that laser had genetic effects on the genes of *P. aeruginosa*, so lasers can be used in treatment and sterilization. The PCR method could be used to evaluate the biological effects of radiation on bacteria as a biomarker assay.

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تأثير ليزر الندميوم–ياك على بعض جينات عوامل الضراوة لبكتريا الزوائف الزنجارية

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الملخص

هدفت هذه الدراسة لتقييم التأثيرات الوراثية لليزر الندميوم–ياك 1064nm على جينات Tox A, Exo S, Opr L لبكتريا الزوائف الزنجارية والمعزولة من العينات السريرية (الجروح، الحروق، التهاب الأذن الوسطى) والبيئية (الماء والتربة). تم جمع العينات السريرية من المرضى الراقدين في مستشفى صلاح الدين العام اما العزلات البيئية فقد تم جمعها من مياه وتربة المشفى ذاته، بعد ذلك تم تعريض العينات كافة الى شعاع ليزر الندميوم–ياك ذي الطول الموجي 532nm وبطاقات مختلفة (500mj,300mj) وبزمن (25sec,15sec)، وقد تم استخلاص الحمض النووي قبل التشعيع وبعده، وتم تشخيص البكتريا بالاعتماد على الصفات المجهرية والكيموحيوية. واستخدمت تقنية الـPCR لمعرفة مدى تأثير شعاع الليزر على هذه الجينات، وبينت النتائج تأثير واضح لليزر النديوم–ياك على هذه الجينات من خلال فقدانها لمواقع ارتباطها وذلك بالمقارنة مع عينات السيطرة (الغير معرضه لليزر)، نستنتج ان الليزر يحدث التغيرات الجينية في الحمض النووي للبكتريا الزنجارية بحيث يمكن استخدام اشعة الليزر في العلاج والتعقيم. ويمكن استخدام تقنية PCR لتحديد الآثار البيولوجية للإشعاع على البكتريا.