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The Effect of Laser Light on Virulence Factors and Antibiotic Susceptibility of Locally Isolated *Pseudomonas Aeruginosa*

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Abstrac:t This study has been explored to cope determine whether continues diode laser with 805nm wavelength was able to affect the potency of bacterial production of virulence factors (alkaline protease ,hemolytic and pyocyanin),and susceptibility MIC of five antimicrobial agents (imipenem,ciprofloxacin,piperacillin,gentamicin and amikacin).The samples collected from 75 patients suffering from sever burn-wounds infections from burn units in Al-Kendy teaching hospital (Baghdad, Iraq) for a period of six month from (May 2009-November 2009). Samples were collected, stored and processed using standard laboratory procedures.Thirty five isolates of *Pseudomonas aeruginosa* bacteria were obtained depending on morphological and biochemical tests. Culture supernatant of three selected isolates of *Pseudomonas aeruginosa* were exposed to diod laser 805nm and 7.07W/cm^2 for (1,3and5) minutes .The activity of bacteria to produce virulence factors and its susceptibility to antimicrobial agents minimum inhibitory concentrations (MIC) were determined before and after irradiation. All *Pseudomonas aeruginosa* isolates, 100% were resistant to imipenem and ciprofloxacin, 91% resistant to piperacillin and 54% resistant to gentamicin and amikacin).The production of alkaline protease, blood heamolysis ,pyocyanine and MICs values of (amikacin and piperacillin)were reduced significantly by irradiation with red light with respect to both light energy dose and exposure times. Complete killing of cells was observed at 5 minutes exposure time. In conclusion, the ability of 2W diode laser with805nm wavelength and power density 7.07W/cm^2 to reduce production of virulence factors and increase sensitivity of *Pseudomonas aeruginosa* to antibiotics may be an additional benefit of using light in the treatment of infectious disease .

Key words: *Pseudomonas aeruginosa, diod* laser, nosocomial infection

INTRODUCTION

Since its invention, laser offers hope for new treatment of bacterial infections, even those that are resistant to current drug. The earliest operating laser was created by Theodore Maiman on May 16, 1960 at the Hughes Research Laboratory in California. The next revolution was semi-conductor lasers, first designed by Robert Hall and his associates at the General Electric laboratories in Schenectady, New York in 1962.[1] Diode lasers now involve many materials and forms, contributed in many fields. Antibiotic –resistant bacterial infections represent an important and increasing public health threat and a well-known problem facing modern medicine[2,3]. The gram negative bacterium *Pseudomonas aeruginosa* is an opportunistic nosocomial human pathogen of immunocompromised individuals [4,5] vIt cause a broad variety of infections ,ranging from minor infections of skin to post operative wound infections ,urinary tract and pulmonary tract infections[6] ,and one in ten hospital acquired infections are from *Pseudomonas aeruginosa*.[7] It possesses several virulence factors which aid in its pathogencity and resistance to antimicrobial agents[8,9]. One of the most worrisome characteristics of *Pseudomonas aeruginosa* is its low antibiotic susceptibility, and may demonstrate additional resistance after unsuccessful treatment[10]. Resistance following treatment with a single antimicrobial agent may be attributed to the action of multidrug efflux pumps with chromosomally –encoded antibiotic resistance genes, and low permeability of the bacterial cellular enzymes[11,]12].*P. aeruginosa* has been shown to be resistant to several antibiotics. In a study carried out in Tunis, Tunisia 60.9% of the *P.aeruginosa* isolates were resistant to piperacillin,53.4% to ceftazidime, 37.6% to imipenem, 70.6% to cefsulodime, 59.3% to tobramycin, 80% to gentamycin, 62.4% to amikacin, and 53.4% to ciprofloxacin[13]. Studies have demonstrated that laser –powered treatment will be useful in the treatment of many cases of infection that caused by bacteria such as *E.coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa.* A study on the effect of 35mW He-Ne laser on the proteolytic activity of *Pseudomonas*

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aeruginosa using toluidin blue O (TBO) as a photo sensitizer, the reduction in proteolytic activity was 94%,at high doses of both light and TBO concentration[14]. In another study, *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* when irradiated using a wavelength of 810 nm at irradiances of 0.015 W/cm^2 (0-50 J/cm^2) and 0.03 W/cm^2 (0-80 J/cm^2). Decrease in bacterial growth of *P. aeruginosa* after irradiation with 810nm laser, (0.03 W/cm^2) could potentially benefit wounds infection[15]. In invitro study, complete eradication of methicillin –resistant, *Staphylococcus aureus* was achieved following 15 minute exposure to a 632.8 nm HeNe laser in the presence of toluidin blue O photosensitizer[16]. The present study aimed to determine the effect of 805 nm diod laser light on some virulence factors and susceptibility of *Pseudomonas aeruginosa* to some antibiotics.

MATERIALS AND METHODS

Laser System: the laser used in his study was a Diode laser (K laser) with output power 2W.It emitted light in a collimated beam with diameter 6mm and a wavelength 805nm, which lies in the IR region of the electromagnetic spectrum. The power density was 7.07 W/cm^2.

Isolation and Identification of Bacteria: Seventy five bacterial samples were collected from patients suffering from burn-wounds infections using sterile swabs. The samples were inoculated on blood agar and MacConky agar plates and incubated at 37C for 24h. The colonies that give pale green were picked and cultivated on the cetrimid medium to observe the pigments production from the bacteria. Samples were identified as *Pseudomonas aeruginosa* according to Bergey's manual using different morphological and biochemical test[17]. Api 20 NE (Biomerieux,Marcy-I Etoile,France)was used to confirm *Pseudomonas aeruginosa* diagnoses. Inoculating King A medium with bacterial isolates to observe the pigments production from the bacteria and indicate pyocyanine production, for blood hemolysis test bacteria were inoculated on blood agar and incubated for 24h at 37C[18], the production of alkaline protease enzyme was determined after inoculation of bacterial samples on milk agar and incubated for 24h at 37C[19]. For stock preparation, *Pseudomonas aeruginosa* isolates were plated on nutrient agar. One isolated colony from each culture was grown to stationary phase in brain heart infusion broth and stored at -70C in sterial glycerol (final concentration 12%v/v) .These stocks were called original stocks and cultures obtained directly from them were designated original culture[16]. Minimum inhibitory concentration (MIC) of the antibiotics (piperacillin, gentamicin, amikacin, ciprofloxacin and imipenem), were determined by broth micro dilution in accordance with the methods of National Committee for clinical laboratory standards[20].

Irradiation procedure:

Bacterial Samples Preparation: A loopful of the culture was transferred from the nutrient agar slants to a test tube containing brain – heart infusion broth and incubated at 37C^0 for 18h .The suspension was centrifuged at (3500 r.p.m) for 10 minutes, supernatant was removed and the bacterial culture was resuspended using physiological saline . The suspension was mixed using vortex to get homogenous suspension, which compared with the McFarland solution to get suspension of 1.5x10^8 CFU/ml concentrations[21].

Two millilitre of the diluted bacterial suspension from each group was transferred to sterile Eppendroff tube and exposed to laser light at different exposure times, another Eppendroff tube also contains one millilitre of the diluted bacterial suspension did not exposed to laser light in order to keep it as a control, then one millilitre from the irradiated and non irradiated suspension in Eppendroff tubes was added to the serial dilutions of the antibiotics and incubated at 37C^0 for 16-18h, after incubation the minimum inhibitory concentration (MIC) for the antibiotics was determined .The other millilitre from each Eppendroff tubes were plated on nutrient agar plate and tested for biochemical characteristics.

RESULTS AND DISCUSSION

A total of thirty five *Pseudomonas aeruginosa* isolates out of seventy five specimens were identified according to their cultural characteristics microscopic and biochemical test. They were categorized as gram negative rod shaped flat, greenish with irregular edges .Pale on MacConkey agar and blue green on cetrimide agar. On blood agar isolates colonies were small brown, with clear zone around the colonies representing beta haemolysis.The production of blue green color on King A agar indicates pyocyanine production. While formation of transparent glory around the colonies on milk agar, indicates alkaline protease production. It was oxidase, catalase ,methyl red and citrate utilization positive ,and can grow at 42 C^0 .The results of Api 20 NE system came to ensure the biochemical identification of *Pseudomonas aeruginosa*.The thirty five isolates of *Pseudomonas aeroginosa* were tested for their sensitivity to antimicrobial agents by broth micro dilution method, and 100% of isolates were resistant to imipenem and ciprofloxacin ,91%resistant to pipercillin, 74% resistant to gentamicin and 54%rsistant to amikacin.. These findings compare

favorably with that in Sagamu, Nigeria in which a sensitivity of *P. aeruginosa* to aminoglycosides was in the range of 61.8%- 75%, fluoroquinolones, 82.8%-89.2% and co-trimoxazole, ampicillin, and tetracycline, 1.7%- 46.8%[22]. In a study in Ilorin *P. aeruginosa* sensitivity in the range 70%- 94% to ciprofloxacin, and 55%- 90% to gentamicin, ceftriaxone, azithromycin, and ampicillin [23]; Similar susceptibility patterns of *P. aeruginosa* were also reported in Saudi Arabia and Kuwait [24],Canada and Brazil [25] and South Korea[26]. This high resistance of *P. aeruginosa* is believed to be as a result of efflux systems and the ability of the organism to undergo mutation and acquire resistant genes at a faster rate compared to *Enterobacteriaceae* [27].

After irradiation with diod laser for 1 minute exposure slight changes in sensitivity to antibiotics was shown .While for biochemical tests slight decrease in the ability of bacterial haemolysin production. After 3 minutes exposure observable reduction in MICs values of (amikacin and piperacillin) and strong decreasing in the ability of bacterial growth on cetrimide agar, haemolysin production, reduce the production of alkaline protease and pyocyanine, no changes were observed regarding oxidase,catalase and methyl red as shown in table (1). While complete killing of bacteria was achieved at 5 minutes exposure time.

Table 1: Effect of Diod laser at different exposure times on biochemical characteristics of P.aeruginosa

Test	control	Laser treatment		
		1min	3 min	5min
Blood haemolysis	+	+-	+--	No growth
Alkaline protease	+	+	+--	No growth
Pyocyanine	+	+	+--	No growth
Growth on Cetrimide agar	+	+	--+	No growth
Oxidase	+	+	+	No growth
catalase	+	+	+	No growth
Methyl red	+	+	+	No growth

+=positive results
+-=weak positive results
+--=very weak positive results.
-=negative results.

Changes in sensitivity of bacterial isolates to the antimicrobial agents after treatment with diode laser is may be due to the combination effect of laser and antimicrobial agent making the bacterial cell more sensitive to the antimicrobial agents. The sensitivity of bacteria to amikacin may be due to the changing in bacterial pumping system (efflux pump) that mainly responsible on bacterial resistance to antibiotics such as (B-lactams, aminoglycoside). Failure of bacteria to produce specific enzymes that chemically modify specific antibiotic also may be increased the bacterial sensitivity to the antibiotics.The terminal enzyme of the respiratory chain in eukaryotic cell (cytochrom c oxidase) that plays a central role in the bioenergetics of the cell and the cytochrom bd and bo complexes in prokaryotic cells are believed to be the photo acceptor molecules for red-to-near IR radiation [28]. Different molecular mechanisms may be explain the effect of diode laser on the metabolism of *P.aeruginosa*, first is due to the absorption of laser light λ by certain chromophore (CuA) that has a rang of absorption in the IR region. CuA play an important role in metabolism and production of ATP[29] ,second possible mechanism is due to converting a fraction of the excitation energy to heat, in which a local transient increase in the temperature of the absorbing chromophores . Any appreciable time-or space-averaged heating of the sample can be prevented by controlling the irradiation intensity and dose appropriately .However, there is still the possibility of localized transient heating of absorbing chromophores. The local transient rise in temperature of absorbing bio molecules may cause structural (e.g. conformational) changes and trigger biochemical activity (second dark reactions) such as activation or inhibition of enzymes[30-31].

Conclusion: The more effect of 805nm diode laser that reduced the MIC values and increase the susceptibility of bacteria to antibiotics and change their biochemical properties was observed with 7.07W/cm^2 power density at 3 minutes exposure time, that mean the values of MIC decreasing and the susceptibility of the bacterial

isolates to the antibiotics increasing with increasing the laser radiation exposure times and laser dose.

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