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Effect of 410 nm Diode Laser Irradiation on the Growth of Burn Wounds-associated Bacteria, *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*

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Abstract: The effect of 410nm with 100 mW output power and one centimetre spot size (0.128 W/cm^2 power density) Diode laser irradiation at different exposure times on the growth of Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus* was evaluated. Seventy swap samples were collected from burn and infected wounds of 35 patients admitted to the burn-wound unit in Al-Yarmouk Teaching Hospital in Baghdad during the period from December 2014 to February 2015. These bacteria were isolated and identified depending on their growth on selective media, cultural characteristics, Gram stain morphology and biochemical tests and finally were confirmed by Vitek 2 compact system test .Susceptibility of bacterial isolates to 15antibiotics was tested using the disk diffusion method. Bacterial standard suspension of 10^8 cell/ml was prepared for *P. aeruginosa* and *S. aureus*. Dilutions of 10^{-6} cell/ml for *P. aeruginosa* and 10^{-5} cell/ml for *S. aureus* were selected. Ten replicates were used for each experimental group. Following irradiation, CFU/ml was calculated, and antibiotic susceptibility test was performed for the most resistant isolate for each bacterial species. From the results, it was found that out of the 70 samples, 17 isolates (24.3%) were *P. aeruginosa* and 9 isolates (12.9%) were *S. aureus*. Antibiotic susceptibility test showed that all isolates of *P. aeruginosa* and *S. aureus* were multidrug resistant. It was shown that laser irradiation did not affect the susceptibility of *P. aeruginosa* isolate to all antibiotics tested. However, a slight increase in the susceptibility of *S. aureus* isolate to Ampicillin/Cloxacillin, Tetracycline and Vancomycin was observed. Laser Irradiation experiments showed that the number of CFU/ml of *P. aeruginosa* and *S. aureus* was significantly reduced with increasing exposure times, reaching a100% bacterial mortality at 13 minutes for *S. aureus* and 19 minutes for *P. aeruginosa*. In conclusion, the blue laser irradiation seems to have more bactericidal effect on Gram-positive bacteria (*S. aureus*) than on Gram-negative (*P. aeruginosa*).

Keywords: *Diode laser (410 nm), burn wound, Pseudomonas aeruginosa, Staphylococcus aureus, antibiotics*

Introduction

Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus* are the main bacterial species that infect cutaneous burn wound and ulcers (Lesseva *et al.*, 1994; Bang *et al.*, 1999; Alwan *et al.*, 2011). Both often express multidrug resistance. *Staphylococcus aureus* became the causative agent of burn wound infections (Lilly *et al.*, 1979; Phillips *et al.*, 1989) shortly after the introduction of penicillin G in the early 1950s. Over the years, *S. aureus* and *P. aeruginosa* became the most common pathogens isolated from burn wound (Nasser *et al.*, 2003; Agnihorti *et al.*, 2004).

The growing resistance of pathogenic microorganisms against several antimicrobial agents has generated a search for alternative

treatments for infections (Wyatt *et al.,* 1977; Coates *et al.* 1997; Ross *et al*., 2003).

Since the invention of the laser in the early sixties of the previous century there was a gradual growing interest in applying laser radiation on biological tissue and microorganisms. Light based antimicrobial treatments, such as photodynamic therapy (PDT) (Castano *et al.,* 2004) and ultraviolet (UV) irradiation therapy (Qualls and Johnson, 1983; Zemke *et al*., 1990; Rames *et al*., 1997; Warriner *et al*., 2000; Lin and Blatchley, 2001; Favier *et al*., 2001) , have been extensively investigated. However, the use of photosensitizers in PDT and UV irradiation may cause damage to the infected host tissue (Dai *et al.,* 2012a). Recently there have been several reports on the bactericidal effect of visible light. In most of those reports, the blue part (400–500 nm) is found to be responsible for killing various pathogens without using exogenous photosensitizers (Feuerstein *et al*.,2004; Maclean *et al*.,2008; Dai *et al.,* 2012b; de Sousa *et al.,* 2015).

The present work aims to investigate the bactericidal effect of safe low level diode laser light at 410nm wavelength on the growth of Gram-negative bacteria (*P. aeruginosa*) and Gram positive (*S. aureus*) at different exposure times. In addition, the study of the effect of the same laser on the susceptibility of both bacteria to antibiotics is also sought.

Materials and Methods

Microorganisms and Culture

Pseudomonas aeruginosa and *Staphylococcus aureus* strains were isolated from patients admitted to the burn-wound unit in Al-Yarmouk Teaching Hospital in Baghdad during the period from December 2014 to February 2015. These patients were treated previously with antibiotics such as gentamicin, fluomizin, meropenem, ceftriaxone and flagyl. A total of 70 swap samples were collected from burn wound infected areas. These swab samples were cultured aerobically overnight in selective media (Cetrimide agar and Mannitol Salt agar for *p. aeruginosa* and *S. aureus* respectively) at 37 ºC. Bacterial isolates were identified using gram staining, microscopic examination and biochemical methods, and were finally confirmed by Vitek2 test (MacFaddin, 2000; BioMerieux, 2004; Murray *et al.,* 2011; Tang and Stratton, 2013).

Antibiotic Susceptibility Test

A total of 15 commonly used antibiotic disks were used to determine the susceptibility of both *P. aeruginosa* and *S. aureus* isolates according to Kirby-Bauer Disk Diffusion method (Bauer *et al.,* 1966). These antibiotic disks included: Amikacin (10 µg), Gentamicin (30 µg), Tobramycin (10 µg), Ampicillin (30 µg), Ceftazidime (30 µg), Cefotaxime (10 µg), Methicillin (10 µg), Oxacillin (10 µg), Cephalexin (30 µg), Ciprofloxacin (10 µg), Vancomycin (10 µg), Clindamycin (2 µg), Erythromycin (15 µg), Amoxicillin (25 µg), and Tetracycline (10 µg). In this test, the bacteria were cultured on Mueller-Hinton agar and incubated at 37^{o}C for 18-24 hrs. The most resistant isolate of each bacterium was then chosen for laser irradiation experiments.

Laser Irradiation Experiments

A standard bacterial suspension was prepared for each selected isolate by mixing few bacterial colonies with sterile normal saline (0.85 %). The turbidity of the suspension was then adjusted to an optical density of 0.05 at 532 nm using a spectrophotometer. Serial dilutions from 10^{-1}-10^{-8} were performed, and dilutions of 10^{-6} and 10^{-5} were selected for *P. aeruginosa* and *S. aureus,* respectively according the colony forming units per ml (CFU/ml^{-1}).

A continuous wave 410 nm diode Laser with 100 mW output power and one centimetre spot size (0.128 W/cm^2 power density) was used in the irradiation of the bacteria under study. An amount of 1.5 ml of each bacterial suspension was transferred into a sterile Eppendorf tube and subjected to laser irradiation. The laser beam was focused on the surface of the suspension at a distance of 21 cm and a beam diameter of 1 cm using a convex lens. The experiments were designed so that the main variable was the exposure time. This parameter was set with a step of one minute in each irradiation from 1 to 19 minutes (19 exposures) for *P. aeruginosa* and from 1 to 13 minutes (13 exposures) for *S. aureus*. After irradiation, aliquot of 100 µl of the irradiated suspension was spread evenly over a plate surface of the selective media (Cetrimide agar for *P. aeruginosa* and Mannitol salt agar for *S. aureus*). Ten replicates were used for each exposure time**.** The same procedure was performed without laser irradiation which was considered as a control. The inoculated plates were then incubated aerobically at 37 °C for 24 hrs. The number of colonies was counted and

the colony forming units (CFUs) were then calculated using the following formula (Cappuccino, and Sherman, 2002).

*Colony forming unit (CFU/ml) = (No. of colonies * dilution factor) / volume inoculation*

All irradiation experiments were performed in the dark. The data were log-transformed and analysed using one way ANOVA followed by Tukey's post hoc test. A value of $P<0.05$ was considered significant.

Results

Bacterial Isolates

The results of isolation and identification showed that *P. aeruginosa* (24.3%) was the predominant pathogen in burn wound infection, while *S. aureus* accounted for only 12.9%.

Antibiotic Susceptibility Tests

In this test, 17 isolates of *P. aeruginosa* and 9 isolates of *S. aureus* were used to determine their susceptibility to the 15 antibiotic disks. The results showed that all isolates (100%) of *P. aeruginosa* were resistant to 7 antibiotics, Ampicillin/Cloxacillin, Cephalexin, Cefotaxime, Methicillin, Oxacillin, Vancomycin, and Ceftazidime Figure (1). While the number of resistant isolates were lower for Gentamicin 16 (94.12%), Tetracycline 15 (88.24%), Tobramycin 13 (76.47%), Amikacin 12 (70.59%) and Ciprofloxacin 9 (52.94%).

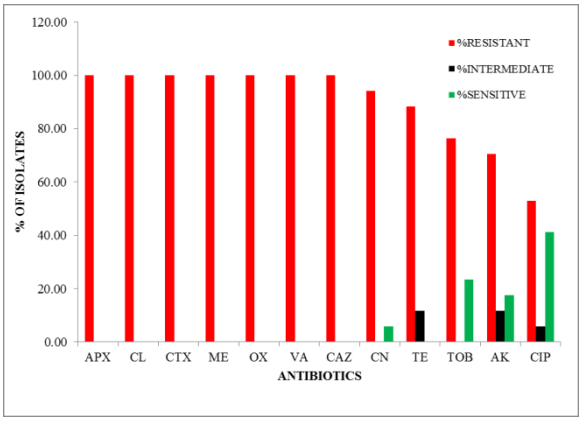

Fig. (1): Susceptibility of p. aeruginosa to different antibiotics

The results of antibiotic susceptibility test for *S. aureus* showed that most of *S. aureus* isolates were resistant to all antibiotics tested except for Vancomycin Figure (2). All isolates (100%) were resistant to Ciprofloxacin, Erythromycin, Ampicillin/Cloxacillin, Amoxicillin, Cefotaxime Methicillin and Oxacillin. On other hand, the number of resistant isolates was lower for Clindamycin 8 (88.89%), Gentamicin 5 (55.56%) and Tetracycline 4(44.44%) Figure(2).

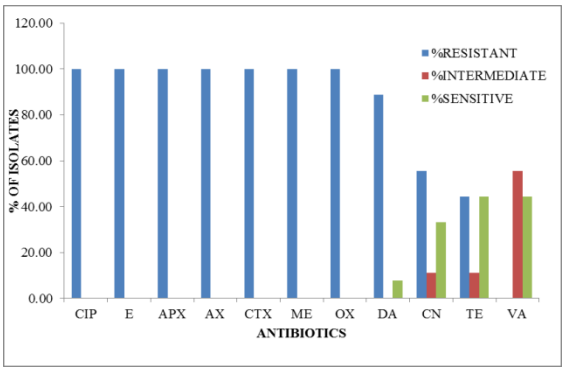

Fig. (2): Susceptibility of S. aureus against different antibiotics

Laser Irradiation

The effect of diode laser, with the specified parameters mentioned above, on the growth of *P. aeruginosa* and *S. aureus* at different exposure times is shown in Table (1) and Table (2). The mean number of log CFU/ml of *P. aeruginosa* was significantly ($p<0.0001$) reduced at 13 min exposure time (99.53%) and above compared with the control group (7.44). However, a 100% reduction in the number of CFU/ml for *P. aeruginosa* was achieved after 19 minutes exposure Table (1). Similarly, the diode Laser (410nm) irradiation also inhibited the growth of *S. aureus* at different doses Table (2). Significant reduction ($P<0.0001$) in the number of log CFU/ml (4.52) was observed following exposure to laser light for 6 minutes compared with the control group (6.03). The number of log CFU/ml decreased as the time increased, reaching a 100% bacterial reduction after 13 minutes exposure time Table (2).The percentage of reduction in the growth of *P. aeruginosa* and *S. aureus* is shown in Figure (3). In general, 19 minutes exposure was needed to achieve a 100% reduction in the number of CFU/ml for Gram-negative *P. aeruginosa*, while less exposure time (13 minutes) was required for Gram-positive *S.aureus* to achieve 100% reduction Figure (3). Table (3) shows the results of antibiotic sensitivity test for the most resistant isolates of *P. aeruginoa* and *S. aureus* to antibiotics before and after irradiation. No effect was observed on the susceptibility of *P. aeruginoa* isolate to all antibiotics tested. This resistant isolate remained resistant to these antibiotics after laser irradiation for 19 minutes Table (3).

Table (1): Mean, standard d eviation and Tukey's post hoc test of log CFU/ml obtained for *P. aeruginosa* irradiated with 410 nm diode laser at 0.128 W/cm^2 power density and different exposure times. (F value= 44.15, $p<0.0001$).

Exposure time (min.)	**Mean ± S. D.**	**P value for means compared with control mean ***
0 (Control)	7.44 ± 0.03	
1	7.26 ± 0.04	NS
2	7.23 ± 0.03	NS
3	7.13 ± 0.07	NS
4	6.87 ± 0.04	NS
5	6.69 ± 0.06	NS
6	6.57 ± 0.06	NS
7	6.38 ± 0.07	NS
8	6.58 ± 0.07	NS
9	6.75 ± 0.08	NS
10	5.95 ± 0.08	NS
11	5.65 ± 0.16	P<0.01
12	6.29 ± 0.08	NS
13	4.62 ± 1.63	P<0.0001
14	5.34 ± 0.15	P<0.0001
15	4.86 ± 1.72	P<0.0001
16	3.03 ± 2.61	P<0.0001
17	5.38 ± 0.11	P<0.001
18	1.53 ± 2.4	P<0.0001
19	0 ± 0	P<0.0001

*NS= NOT SIGNIFICANT

Table (2): Mean, standard deviation and Tukey's post hoc test of log CFU/ml obtained for *S.aureus* irradiated with 410 nm diode laser at 0.128 W/cm^2 power density and different exposure times. (F value= 65.69, p<0.0001).

Exposure time (min.)	Mean ±S. D.	P value for means compared with control mean *
0 (Control)	6.03 ± 0.03	
1	5.61 ± 0.11	NS
2	5.49 ± 0.07	NS
3	5.18 ± 0.08	NS
4	5.09 ± 0.06	NS
5	5.42 ± 0.11	NS
6	4.52 ± 0.23	P<0.0001
7	4.32 ± 0.21	P<0.0001
8	4.70 ± 0.08	P<0.01
9	4.56 ± 0.20	P<0.001
10	2.83 ± 1.96	P<0.0001
11	4.54 ± 0.15	P<0.0001
12	0.8 ± 1.69	P<0.0001
13	0 ± 0	P<0.0001

*NS= NOT SIGNIFICANT

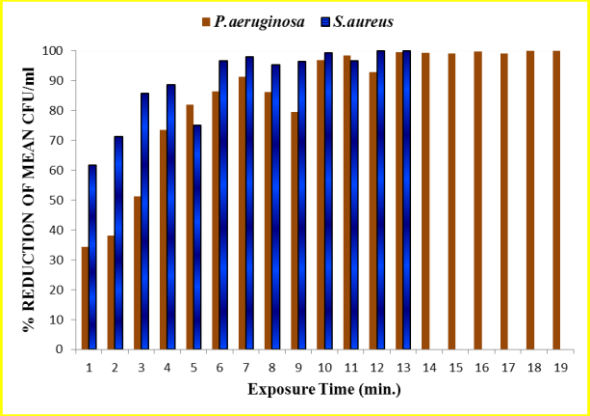

Fig. (3): Percentage of reduction of Mean values of CFU/ml obtained for P.aeruginosa and S.aureus irradiated with 410 nm diode laser at 0.128 W/cm2 power density and different exposure time.

On the other hand, the results showed that for only 13 minutes exposure time the sensitivity of *S. aureus* was increased to Ampicillin/Cloxacillin, Oxacillin and Vancomycin antibiotics Table (3). For Ampicillin/ Cloxacillin, the laser irradiation increased the diameter of inhibition zone from 0 mm to 17.5 mm, leading to a remarkable change in the susceptibility of *S. aureus* isolate to this antibiotic from resistant to sensitive isolate Table (3). A less increase in the diameter of inhibition zone was noticed for Oxacillin and Vancomycin antibiotics.

Table (3): Susceptibility of the most resistant isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* to various antibiotics before and after irradiation with 410 nm diode laser*

Antibiotics	Zone of Inhibition (mm)			
	Pseudomonas aeruginosa		*Staphylococcus aureus*	
	Before irradiation (Control)	After irradiation for 19 min	Before irradiation (Control)	After irradiation for 13 min
Ciprofloxacin	0 (R)	12.5 (R)	0 (R)	15(R)
Erythromycin			0 (R)	0 (R)
Ampicillin/ Cloxacillin	0 (R)	0 (R)	0 (R)	17.5(S)
Amoxicillin			10(R)	15(R)
Cefotaxime	0 (R)	0 (R)	0 (R)	0 (R)
Tetracycline	0 (R)	0 (R)	15(S)	25(S)
Methicillin	0 (R)	0 (R)	0 (R)	0 (R)
Oxacillin	0 (R)	0 (R)	0 (R)	12.5(In)
Gentamicin	0 (R)	0 (R)	15(S)	15(S)
Vancomycin	0 (R)	0 (R)	15(In)	17.5(S)
Clindamycin			0 (R)	10(R)
Amikacin	0 (R)	0 (R)		
Tobramycin	0 (R)	0 (R)		
Cephalexin	0 (R)	0 (R)		

Discussion

The results of our study showed that 410 nm, 0.128 W/cm^2 power density blue diode laser irradiation significantly inhibited the growth of bacteria, *P. aeruginosa* and *S. aureus*, with complete reduction (100%) at 19 and 13 minutes exposure time respectively as shown in Figure (3). This agrees with other researchers' findings regarding the bactericidal effect of blue light, especially in the wavelength range of 405-470 nm without using exogenous photosensitizers (Guffey and Wilborn, 2006; Maclean *et al.*, 2008; Enwemeka *et al.*, 2009; De Lucca *et al.*, 2012; de Sousa *et al.*, 2015).

The mechanism behind this bactericidal effect of blue light may be due to photo-excitation of some endogenous photosensitizers, such as cytochromes, porphyrins, flavins and NADH (Lavi, *et al.,* 2004; Maclean *et al.,* 2008). These may absorb light and increase the production of free radicals (Fischer, 2004), which may affect cell membrane proteins and DNA (Bertoloni *et al.,* 2000). Also it is possible that those bacterial endogenous photosensitizers may have a direct effect on photo labile pigments in bacteria (Eraso and Albesa, 1998). High amount of reactive oxygen species (ROS) could also be generated by high intensity visible light thus leading to bacteria killing (Kotelevets *et al.,* 1988; Lipovsky *et al*., 2009).

The results of our work also revealed that *P. aeruginosa* is more resistant to blue light (410 nm) than *S.aureus.* This difference in bacterial photo-response between *P. aeruginosa* and *S. aureus* may be due to the amount of various porphyrins, which is responsible for cell inactivation. Nitzan *et al.* (2004) found that the amount of the predominant porphyrin (coproporphyrin) produced by Gram-positive (Staphylococcal) strains was twice to three times higher than that in the Gram-negative strains. Moreover, Nitzan *et al.* (2004) reported that Gram-negative bacteria do not have predominant porphyrins for the inactivation of cells although high amount of porphyrins were detected. Another possibility for explaining the difference in the susceptibility of both bacteria to this 410nm laser light irradiation may be attributed to their structural differences. The cell envelope of Gram-negative bacterial is thin and surrounded by outer membrane, while Gram-positive bacteria have thick cell envelope composed of peptidoglycan (Silhavy *et al.*, 2010). This thick cell wall can prevent light from reaching the inner layers of the cell to be absorbed by endogenous photosensitizers and consequently less unwanted singlet oxygen is formed. The latter is the main cause of suffocating any living cell by inhibiting transmembrane activity. Our finding in regard to the changes in the susceptibility of bacterial isolates to many antibiotics after laser irradiation may be explained as due to changes in some structural molecules (i.e. chemical) that are responsible for bacterial resistance to antibiotics. For example, efflux pumps are proteins involved in bacterial resistance to antibiotics (aminoglycoside, fluoroquinolones, β-lactams, chloramphenicol and trimethoprim) (Webber and Piddock, 2003). Another possible explanation for these changes may be attributed to a possible alteration in the target site of the antibiotic, modification of metabolic pathways, and an increase or decrease in the permeability of cell membrane (Schmieder and Edwards, 2012).

Conclusion

We expect a bactericidal effect in using even though low level short wavelength (410nm) laser radiation compared with low level long wavelength laser radiation (above 700 nm infrared light). This may be interpreted on the fact that the energy per photon at the 410 nm causes electronic transitions leading to possible chemical changes while the energy per photon at the infrared region causes vibrational transitions leading to just moderate temperature elevation of the temperature of the targeted species.

References

Agnihotri, N., Gupta, V. and Joshi, R. M. (2004). Aerobic bacterial isolate from burn wound infections and their antibiograms- a five year study. Burns.**30**: 241-243.

Alwan, M. J., Lafta, I.J., Hamzah, A. M. (2011).Bacterial isolation from burn wound infections and studying their antimicrobial susceptibility. Kufa Journal For Veterinary Medical Sciences. **2**:121-131.

Bang, R. L., R. K. Gang, S. C. Sanyal, E. M. Mokaddas, and A. R. Lari.(1999). Beta-haemolytic Streptococcus infection in burns. Burns **25**:242-246.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. **36**:493-496.

Bertoloni, G., Lauro, F.M., Cortella, G., et al. (2000.) Photosensitizing activity of hematoporphyrin on *Staphylococcus aureus* cells. Biochim. Biophys. Acta.**1475**: 169–174.

BioMerieux Inc. (2004). http://www.biomerieux-usa.com

Cappuccino, J. G., Sherman, N. (2002). Microbiology a laboratory manual, 8th Edition. Benjamin Cummings, New York. pp: 335-337.

Castano, A.P., Demidova, T.N., Hamblin, M.R. (2004). Mechanisms in photodynamic therapy: part onephotosensitizers,

photochemistry and cellular localization. Photodiagn. Photodyn. Ther.**1**:279–293.

Coates, P, Adams, C.A., Cunliffe, W.J., McGinley, K.T., Eady, E.A., Leyden, J.J., Ravenscroft, J., Vyakrnam, S., Vowels, B. (1997). Does oral isotretinoin prevent Propionibacterium acnes resistance? Dermatology, **195**(Suppl.1):4–9.

Dai T., Vrahas M.S., Murray C. K, Hamblin M. R. (2012a). Ultraviolet C irradiation: an alternative antimicrobial approach to localized infections? Expert Rev Anti Infect Ther. **10**:185–195.

Dai, T, Gupta A., Murray, C. K., Vrahas, M. S., Tegos G. P., Hamblin, M. R. (2012b). Blue light for infectious diseases: *Propionibacterium acnes*, *Helicobacter pylori*, and beyond? Drug Resist Updat. **15**: 223-236.

De Lucca, A.J., Carter-Wientjes, C., Williams, K . A., Bhatnagar, D. (2012). Blue light (470 nm) effectively inhibits bacterial and fungalGrowth. Letters in Applied Microbiology. **55:** 460-466.

De Sousa, N.T., Santos, M.F., Gomes, R.C., Brandino, H.E., Martinez, R., de Jesus Guirro, R.R.(2015).Blue Laser Inhibits Bacterial Growth of Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Photomedicine and Laser Surgery. **33**:278-282.

Enwemeka, C. S., Williams, D., Enwemeka S. K., Hollosi, S., Yens, D. (2009). Blue 470-nm light kills methicillin-resistant *Staphylococcus aureus* (MRSA) in vitro. Photomed Laser Surg.**27**:221-226.

Eraso, A., and Albesa, I. (1998). Elevation of alanine minotransferase and aspartate aminotransferase produced by pyoverdin, a photolablile pigment of *Pseudomonas fluorescens.* Natural Toxins. **6**: 61–65.

Favier, G.I., Escudero, M.E., Guzman,M.S. (2001). Effect of chlorine, sodium chloride, trisodium phosphate and ultraviolet radiation on the reduction of *Yersinia enterocolitica* and mesophilic aerobic bacteria from eggshell surface. J. Food Prot. **64**: 1621-1623.

Feuerstein, O., Persman, N., Weiss E.I. (2004). Phototoxic Effect of Visible Light on Porphyromonas gingivalis and Fusobacterium nucleatum: An In Vitro Study. Photochemistry and Photobiology. **80**:412–415.

Fischer, A. (2004). Light treatments relieve acne. Biophotonics Int. **11**:48–51.

Guffey, J.S. and Wilborn, J. (2006). In Vitro Bactericidal Effects of 405 nm and 470 nm blue light. Photomedicine and Laser Surgery. **24:** 684-688.

Kotelevets, L.M., BabenkoIu, S., Lukoianova, M.A.(1988). Spectral properties of cytochromes from Staphylococcus aureus. PriklBiokhim Mikrobiol.**24(1):**68-75.

Lavi, R., Sinyakov, M., Samuni, A., Shatz, S., Friedmann, H., Shainberg A., Breitbart H., Lubart R.(2004). ESR detection of 1O2 reveals enhanced redox activity in illuminated cell cultures. Free Radic Res**.38(9):**893-902.

Lesseva, M., Girgitzova, B. P., Bojadjiev, C. (1994). Beta-haemolytic streptococcal infections in burned patients. Burns. **20**:422-425.

Lilly, H. A., Lowbury, E. J., Wilkins, M. D., Cason, J. S. (1979). Staphylococcal sepsis in a burns unit. J. Hyg. (London) **83**:429-435.

Lin, L.S. and Blatchley E.R. (2001). UV dose distribution characterization using fractal concepts for system performance evaluation. Water Sci. Technol. **43**: 181-188.

Lipovsky, A., Nitzan, Y., Friedmann, H., Lubart, R. (2009).Sensitivity of Staphylococcus aureus strains to broadband visible light. Photochem Photobiol. **85:**255-260.

MacFaddin, J. F. (2000). Biochemical Tests for Identification of Medical Bacteria. 3rd Edition. Lippincott Williams and Wilkins. USA. Pp (239-253).

Maclean, M., MacGregor ,S. J., Anderson, J. G., Woolsey, G. (2008). High-intensity narrow-spectrum light inactivation and wavelengthSensitivity of *Staphylococcus aureus*. FEMS Microbiol Lett. **285**: 227–232.

Murray, P.R., Baron, E.J., Pfaller, M., Tenover, F.C., Yolken, R.H. (2011). Manual of Clinical Microbiology, 10th ed. American Society for Microbiology, Washington, D.C.

Nasser, S., Mabrouk, A., Maher, A. (2003): Colonization of burn wounds in Ain Shams University burn unit. Burns **29**: 229-233.

Nitzan, Y., Salmon-Divon, M., Shporen, E., Malik, Z. (2004). ALA induced photodynamic effects on gram positive and negative bacteria.PhotochemPhotobiol Sci. **3**:430-435.

Phillips, L. G., Heggers J. P., Robson M. C., Boertman J. A., Meltzer T., and Smith D.

J.(1989). The effect of endogenous skin bacteria on burn wound infection. Ann. Plast. Surg. **23**:35-38.

Qualls, R.G. and Johnson, J. D. (1983). Bioassay and dose measurement in UV disinfection. Applied Environ. Microbiol. **45**: 872-877.

Rames, J., Chaloupecky V., Sojkova N., Bencko V. (1997). An attempt to demonstrate the increased resistance of selected bacterial strains during repeated exposure to UV radiation at 254 nm. Cent. Eur. J. Health. **5**: 30-31.

Ross, J.I., Snelling ,A.M., Carnegie, E., Coates, P., Cunliffe, W.J., Bettoli, V., Tosti, G., Katsambas, A., Galvan Perez Del Pulgar, J.I., Rollman, O., Torok, L., Eady, E.A., Cove, J.H.(2003)Antibiotic-resistant acne: lessons from Europe. Br. J. Dermatol.; **148**:467–478.

Schmieder, R. and Edwards, R. (2012). Insights into Antibiotic Resistance ThroughMetagenomic Approaches. Future Microbiol. **7**:73-89.

Silhavy, T. J., Kahne, D., Walker, S. (2010). The Bacterial Cell Envelope. Cold Spring HarbPerspect Biol. **2**: a000414.

Tang, Y and Straton, C.W. (2013). Advanced techniques in diagnostic microbiology. 2nd ed. Springer Science & Business Media. New York. pp. 817.

Warriner, K., Rysstad, G., Murden , A., Rumsby, P., Thomas, D., Waites, W.M. (2000). Inactivation of *Bacillus subtilis* spores on aluminum and polyethylene performed cartons by UV-excimer laser irradiation. J. Food Prot., **63**: 753-757.

Webber, M. A. and Piddock, L. J. V. (2003). The importance of efflux pumps in bacterial antibiotic resistance.J. Antimicrob. Chemother. **51**: 9-11.

Wyatt T.D., Ferguson W.P., Wilson T.S., McCormick E.(1977) Gentamicin resistant *Staphylococcus aureus* associated with the use of topical gentamicin. J. Antimicrob. Chemother. **3**: 213–217.

Zemke, V., Podgorsek, L., Schoenen, D. (1990). Ultraviolet disinfection of drinking water 1, communication: Inactivation of *E. coli* and coliform bacteria. ZentralblHyg. Umweltmed. **190**: 51-61.

تأثير أشعة ليزر الدايود (410 نانومتر) على نمو بكتريا *Pseudomonas aeruginosa* و *Staphylococcus aureus* المرتبطة بجروح الحروق

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الخلاصة: قُيِّم تأثير اشعة دايود ليزر ذو طول موجي 410 نانوميتروقدرة 100 ميلي واط بقطربقعة واحد سنتمتر (كثافة قدرة 0.128 واط/ سم^2) بأزمنة تعريض مختلفة على نمو بكتريا الزوائف الزنجارية السالبة لصبغة غرام*Pseudomonas aeruginosa* وعلى المكورات العنقودية الذهبية الموجبة لصبغة غرام *Staphylococcus aureus*. جمعت سبعين عينة مسحة من الحروق والجروح الملوثة ل 35 مريض ادخلو الى وحدة الحروق والجروح في مستشفى اليرموك التعليمي في بغداد اثناء الفترة من كانون الاول 2014 الى شباط 2015. عزلت وشخصّت هذه البكتيريا اعتمادا على نموها على اوساط اختيارية، وخصائصها الزرعية، ومظهرها الخارجي في صبغة غرام والاختبارات الكيموحيوية وأكدت في النهاية بواسطة اختبار نظام فايتك 2 المدمج. اختبرت حساسية العزلات البكتيرية ل15مضاد حيوي باستخدام طريقة انتشار القرص. حُضر العالق البكتيري القياسي للزائفة الزنجارية والمكورات العنقودية الذهبية بتركيز10^8خلية /مل . اختيرت التخفيفات 10^6 خلية/مل للزوائف الزنجارية و10^5 خلية/مل للمكورات العنقودية الذهبية. استخدمت عشر مكررات لكل مجموعة تجريبية. وبعد التشعيع، حسبت اعداد وحدات تكوين المستعمرة في الملليتر(CFU/ml)، وأجري اختبار الحساسية للمضادات الحيوية للعزلة الأكثر مقاومة لكل نوع من البكتيريا. من النتائج تبين انه من أصل 70 عينة، 17 عزلة (24.3 %) كانت لبكتريا الزوائف الزنجارية *P. aeruginosa* و 9 عزلات (12.9 %) للمكورات العنقودية الذهبية *S. aureus*. أظهر اختبار الحساسية للمضادات الحيوية أن جميع عزلات الزائفة الزنجارية والمكورات العنقودية الذهبية كانت مقاومة لعقاقير متعددة. و تبين أن أشعة الليزر لم تؤثر على حساسية عزلات الزائفة الزنجارية *P. aeruginosa* لجميع المضادات الحيوية التي تم اختبارها. غير أن زيادة طفيفة لوحظت في حساسية المكورات العنقودية الذهبية *S. aureus* للأمبيسلين/ كلوكساسيللين و التتراسيكلين وفانكومايسين . أظهرت تجارب التشعيع أن عدد وحدات تكوين المستعمرة في الملليتر للزائفة الزنجارية *P. aeruginosa* والمكورات العنقودية الذهبية *S. aureus* انخفض معنويا مع زيادة الوقت، حيث بلغ 100٪ قتل للبكتيريا عند الدقيقة 13 للزوائف الزنجارية والدقيقة 19 للمكورات العنقودية الذهبية. كأستنتاج، يبدو ان اشعة الليزر الزرقاء لها تأثير قاتل عل البكتريا الموجبة لصبغة غرام المكورات العنقودية الذهبية *S. aureus* اكثر من البكتريا السالبة لصبغة غرام الزوائف الزنجارية *P. aeruginosa* .