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Effect of blue laser on viability of *Proteus mirabilis*

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Abstract:

The usage of blue laser has been considered as a therapeutic approach to prohibit the viability of bacterial species, but there is no agreement about optimum parameters to be used. The aim of this project is to study the influence of blue laser (450 nm) on the viability of the gram-negative bacteria *Proteus mirabilis* isolated from burn wounds, using different exposure times (i.e. doses) *in vitro*. Seventy swab samples were collected from burn wounds of patients admitted to the burns unit in AL-Yarmouk teaching hospital in Baghdad, during the period from June to August 2019. The Bacteria were isolated and identified depending on their culture characteristics, biochemical tests, gram staining, and morphology, being finally confirmed by API 20E Test System. By using the disk diffusion method, susceptibility of the isolates to 12 different antibiotics was examined. One isolate of *P. mirabilis* was elected according to susceptibility to all antibiotics used. To prepare bacterial solution, *P. mirabilis* was mixed with normal saline solution. Dilution of 10^{-6} cell/ml for *p. mirabilis* was selected from other serial dilutions. A number of colonies and colony forming units (CFUs/ml) were achieved and correlated to controls. *P. mirabilis* was irradiated by blue diode laser (450 nm, 500mw) and exposed to different doses (24, 48, 72, 96, 120J/cm^2) corresponding to respective exposure times (4, 8,12,16,20 minutes). The results of antibiotic susceptibility test indicate that the entire isolates of *P. mirabilis* were multidrug resistant. With the increase in laser dose (exposure times), the number of colonies and CFUs/ml were reduced, reaching a highest inhibition in CFU/ml at exposure time of 20 minutes, i.e. a dose of 120J/cm^2 , with irradiance of 0.1 watt/ cm^2. No significant reduction was recorded in CFU/ml at exposure time of 4 min (a dose of 24J/cm^2). As a conclusion, the blue laser irradiation at wavelength of 450 nm and 500mw had antibacterial effects on *P. mirabilis* isolated from burn wounds with irradiance of 0.1watt/cm^2 *in vitro*, as evidenced by the effective reduction in the viability of bacteria at a dose of 120J/cm^2 corresponding to exposure time of 20 minutes.

Keywords: blue laser, proteus mirabilis, phototherapy.

تـأثير الليـزر الازرق على حيـويه بكتيـرياProteus mirabilis

مـــوده موســـى فنجـــان

فـرع الفزيولوجي– كليه الطـب – جامعه بغداد– بغـداد – العـراق

الخلاصه

يمكن استخدام الليزر الازرق لتثبيط نمو وقتل انواع مختلفه من البكتريا لكن لايوجد اتفاق حول افضل العوامل التي يمكن استخدامها ولذلك كان هدف هذا البحث هو دراسه تأثير الليزر الازرق 450nmعلى حيويه

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البكتريا السالبه الغرام (p. mirabilis) باستخدام ازمان تعريض مختلفة (جرع مختلفة). حيث تم جمع 70مسحه من جروح الحروق لكلا الجنسين ولجميع الاعمار في مستشفى اليرموك التعليمي وحده الحروق . خلال الفتره من شهر حزيران الى شهر اب 2019. تم عزل وتشخيص البكتريا في المختبرات التعليميه للمستشفى اعتمادا على دراسه الخصائص المجهريه والاختبارات الكيمياويه والمظهريه والفحوصات البايوكيمياءيه حيث تم استخدام نظام API 20E وباستخدام طريقه Disk diffusion methodتم قياس حساسيه العزلات البكتيريه الى 12 نوع مختلف من المضادات الحيويه . ثم تم اختيار العزله الاكثر مقاومه للمضادات الحيويه و تم وضع العزله المقاومه في سائل normal saline وأجراء عمليه التخفيف لاختيار التخفيف المناسب حيث كان10 6− cell/mlحيث وضع 4 mlمن هذا التخفيف في انبوبه اختبار معقمه و تم تعريضها لليزر بالازمان المختاره . وكذلك تم أخذ 4mlووضعها في انبوبة اختبار حيث انها غير معرضه لليزر .((controlوتم اخذ 4 mlاخرى من التخفيف 10 6− cell/ml ووضعت في microtiter plate وتم التشعيع بالازمان 4 و 8 و 12و 16و 20 دقيقه والتي توافق الجرعات 24و 48و 72و 96و J/cm2120وتم بعدها حساب عدد المستعمرات البكتيرية وحيوية البكتريا .(CFU/ml) تبين من اختبار الحساسيه للمضادات الحيويه ان كل العزلات كانت مقاومه للمضادات الحيويه بنسب مختلفه وتم اعتماد العزله الاكثر مقاومه للمضادات الحيويه . حيث اظهرت التجارب ان عدد المستعمرات البكتيريه و حيويه البكتيريا (CFU/ml)تقل بزياده جرعه الليزر وزياده وقت التشعيع حيث ; كان الانخفاض الاكبر في حيويه البكتريا عند الزمن 20 دقيقه)J/cm2120(ولم نحصل على تأثير ملموس عند الجرعه J/cm224 بزمن 4. min نستنتج ان الليزر الازرق ذو الطول الموجي ,500mw 450 nmله تأثير قاتل على بكتريا p. mirabilis المعزوله من الحروق والجرعه الاكثر تاثيرا كانت 120 J/cm2 عند زمن تعريض 20 دقيقه.

Introduction

Proteus mirabilis is a member of Enterobacteriaceae family of bacilli. It is a gram- negative bacterium with inability to ferment lactose and ability to ferment maltose. When *p. mirabilis* contacts with solid surfaces, it has the ability to secrete polysaccharides and self – elongate, which permits an action of obvious motility along surfaces of medical apparatus. It also has swarm motility, where the flagella of *proteus mirabilis* are responsible for its motility that is correlated with the capability to secrete biofilms. This motility is utilized to support colonization and implied to contribute to resistance to definitive antibiotics [1- 3].

P. mirabilis is associated with infections of ulcers and wounds [4-7] . Over years , the bacterium has been recognized as a common pathogen isolated from infected wounds. Furthermore, it expresses multidrug resistance (MDR) , which evolved to represent an essential clinical issue. Therefore, there is a necessity to develop strategies that counteract the increase of MDR [8].

Following the invention of laser in the past century, there was continuous development in applying laser radiation on microorganisms and biological tissues. In recent years, the visible light has been employed to investigate its bactericidal influence, and the blue part (430- 480nm) has been concluded to be effective in killing different microorganisms [9-12]. Kazemikhoo *et al*. [13 used low intensity laser in the treatment of burn wounds and cutaneous ulcers to enhance the process of healing. Another *in vitro* study focused on using laser radiation of 425 nm, which showed bactericidal influences on the growth of *Staphylococcus aurus* and *Escherichia coli*. [14].

The effects of laser radiation and its associated parameters, such as wavelength, irradiance, and dose, on the growth of microorganisms are inadequately understood. A number of previous studies demonstrated that laser effects on bacteria were strongly dependent on wavelength, irradiance, and species, which are most important parameters for prohibiting bacterial growth [15-17].

Since there are no studies that describe the influence of blue laser on *P. mirabilis* growth, the aim of the current study is to examine the effects of blue laser on the viability of *P. mirabilis* isolated from burn wounds in *vitro* at different exposure times (doses).

Methods

Isolation and identification of bacteria

Through the period extending from June to August 2019, seventy swab samples were collected from burn patients (burn wounds) from the burns unit at Al-Yarmouk Teaching Hospital in Baghdad,

using sterile cotton swabs in transport media . Patients on antibiotic treatment were eliminated from the study. Swabs were obtained from areas that showed deep-seated wounds with discharge. Isolation and biochemical analysis of *Proteus mirabilis* were performed at Al-Yarmouk Teaching Hospital Laboratories. Burn wound swabs were directly streaked on blood and macconkey agars and then incubated aerobically for 24 hours at 37°C. Identification of the isolates was accomplished depending on the biochemical and culture characteristics, according to the API 20E Test System [18].

Antibiotic susceptibility test

Kirby–Bauer method was used, as reported by the laboratory standards institute [19], to achieve the antibiotic susceptibility test for 12 different antibiotics that are commonly used to treat burn wounds: Gentamicin, Ampicillin, Ampiclox, Amikacin, Tetracycline, Erythromycin, Ciprofloxacin, Imipenem, Meropenem, Cefotaxime, Ceftazidime, and Trimethoprim/sulphamethoxazole. The isolates were classified as resistant, intermediate, or sensitive to antibiotics according to the values obtained by the National Committee for Clinical Laboratory (NCLS) [20].

Blue laser experiments

A portable continuous wave diode laser (UK -scientific Ltd) was used in all experiments of wavelength (450 nm , 500 mw). The treatment parameters of laser used in this study are shown in table 1. A readjustment of laser equipment was made at the beginning of each experiment to verify precise doses during the experiments. A laser power meter (SoLo PEgenetc -Eoinc Canada) was used to measure the output power of the laser used.

Table 1- Treatment parameters of blue laser used in this study.

Parameter	Specification				
Wavelength	450nm				
Output power of laser (mw)	500 mw				
Spot size of laser beam (cm^2)	$5cm^2$				
Irradiance measured at target area ($watt/cm^2$)	$0.1watt/cm^2$				
Dose= Irradiance ($watt/cm^2$) × exposure time. Dose= (watt × sec)/ cm^2 Dose= J/ cm^2					
Exposure time(min)	4	8	12	16	20
Dose J/cm^2	24	48	72	96	120

The laser was fitted with a convex lens which is used as a beam expander at the end to illuminate a circular area. Laser spot size of 5 cm^2 was produced by using a convex lens that is keeping the distance between the microtiter plate and the lens definitive. The value of irradiance measured at the $5cm^2$ target area (microtiter plate) was 0.1 $watt/cm^2$.

One isolate of *P. mirabilis* was elected according to virulence factors, including urease production, adherence capability, having flagella, and susceptibility to all antibiotics used [21]. To prepare the bacterial solution, the selected isolate was cultured on Macconkey agar, from which few colonies were transferred and mixed with sterile saline solution. The turbidity of bacterial suspension was determined to an optical density of 0.5 by using spectrophotometer at a wavelength of 530 nm. After that, serial dilutions were prepared, from 10^{-1} to 10^{-10} **,** and the dilution of 10^{-6} was selected , which contained 30-300 colonies [22]. Four millilitres of bacterial suspension, with 10^{-6} cell/ml, was placed in a sterilized test tube and kept unirradiated by laser (control group). By using L-shape spreader, an

aliquot of 100µl out of 4ml (bacterial dilution of 10^{-6} cell/ml) was spread on nutrient agar. Eight plates were prepared as control plates for each assay and incubated aerobically at 37 ºC for 24 hours, and then CFU/ml was calculated after incubation (control group). Another four millilitre**s** of bacterial suspension, with 10^{-6} cell/ml, was placed in wells of microtiter plate, distributed at an area of $5cm^2$ in accordance with laser spot size of $5cm^2$ and exposed to different doses (24, 48, 72,96, 120 J/cm^2) of blue laser, corresponding to 4,8,12,16,20 minutes. After irradiation by laser light, 100µl out of 4ml bacterial suspension, with 10^{-6} cell/ml, was spread on nutrient agar using L-shape spreader. Eight plates were prepared for each assay and incubated aerobically at 37 ºC for 24 hours (Irradiated groups) and CFU/ml was then calculated. Manual colony counter (colony counter, SC6, Fisher scientific) was used to count bacterial colonies on agar plate.

For each experiment, bacterial suspension was freshly prepared and each experiment had its control plates, to reduce the variability. All apparatus were adjusted at the beginning of the study to confirm that they delivered definite doses at the time of each experiment. The method of irradiation was systematized before the experiments. To improve data precision, each experiment for the irradiated dose was repeated 3 times. CFU is a measure of viable cells of bacteria. CFU/ml can be calculated using the following formula [22]:

$$\frac{\text{CFU}}{\text{ml}} = (\text{Colony number}) \times \text{ dilution factor } \times \frac{1}{\text{volume plated}}$$

Volume plated = 100µl = 0.1 ml

Statistical analysis

Statistical analysis was achieved by using the statistical package for social sciences (SPSS, version 20.0 for windows, SPSS, Chicago, IL, USA).. Data were presented as mean ± standard deviation SD (95% Confidence Interval). Data normal distribution was examined using the Shapiro-Wilk test, where colony number and CFU were not normally distributed (0.885, P=0.004). Comparisons between control and irradiated groups were performed using the Wilcoxon signed ranks test. Comparisons between irradiation times were conducted using the Kruskal-Wallius test. Statistically significant differences were considered at $p< 0.05$.

Results

Bacterial isolates

The outcomes of isolation and identification of bacteria showed that 70 different isolates from burn wounds (25.714 %, 18 isolates) were *P. mirabilis*. Each isolate was tested for susceptibility to 12 different disks of antimicrobial agents. The outcomes exhibited that all the isolates of *P. mirabilis* were resistant to 4 antibiotics, namely Ampiclox, Ampicillin, Tetracycline, and Erythromycin (table 2), while they showed lower resistance for Gentamicin (77.77%), Ciprofloxacin (77.77%), Cefotaxime (65%), Meropenem (61%), Ceftazidime (60%), Amikacin (50%), Trimethoprim/ sulphamethoxazole (44%), and Imipenem (38%) (Table 2).

Table 2- Percentage of resistance of *P. mirabilis* against various types of antimicrobial agents**.**

Microbial agents	No of all isolates	Resistance		Sensitive		intermediate	
		no	%	no	%	no	%
Gentamicin (30 µg)	18	14	77.77	3	16.66	1	5.5
Ampicillin (30 µg)	18	18	100	-	-	-	-
Ampiclox (10 µg)	18	18	100	-	-	-	-
Amikacin (30 µg)	18	9	50	7	38.88	2	11.11
Tetracycline (10 µg)	18	18	100	-	-	-	-
Erythromycin (10 µg),	18	18	100	-	-	-	-
Ciprofloxacin(10 µg)	18	14	77.77	-	-	4	22.22
Imipenem (10 µg)	18	7	38.88	8	44.44	3	16.66
Meropenem (10 µg)	18	11	61.11	5	27.77	2	11.11
Cefotaxime (30 µg)	18	12	65	6	35	-	-
Ceftazidime (30 µg)	18	11	60	7	40	-	-
Trimethoprim /sulphamethoxazole.(10 µg)	18	8	44.44	6	33.33	4	22.22

The influence of laser radiation on viability of *p. mirabilis*

The results of the influence of blue laser on colony number (colony count) of *p. mirabilis* at irradiance of 0.1watt/cm^2 and various exposure times are shown in table 3. The mean **colony** number and viability of *P. mirabilis* (CFU/ml) were significantly reduced at exposure times of 8, 12, 16, and 20 min (doses (48, 72, 96, 120J/cm^2, respectively) as compared with control groups (tables 3 and 4) . However, effective reductions in colony number and CFU/ml of *P. mirabilis* were obtained after 20 min, i.e. dose of 120J/cm^2 (Tables 3 and 4).

Figure 1 shows the relationships of different exposure times of laser (4, 8, 12, 16,and 20 minutes) corresponding to their respective doses (24,48,72,96, and 120J/cm^2) on viability of *P. mirabilis* (CFU/ml) . Wavelength of 450 nm laser radiation produced a statistically significant decline in the colony number and CFU/ml, which was parallel to increasing the dose (Tables 3 and 4; figure1).

Table 3- Mean and standard deviation values of colony number (colony count) of *P. mirabilis* irradiated by 450 nm laser light at irradiance of 0.1 watt/cm^2 and different exposure times (doses) .

Exposure time (min)	Dose J/cm^2	Control colony number		Irradiated colony number		P value
		Mean±SD	CI 5-95%	Mean± SD	CI 5-95%	
4	24a	105± 35	69-159	106± 36	77-166	0.893
8	48 b	88± 15	67-109	65± 11	48-75	0.043*
12	72 c	106± 9	96-117	57± 7	49-64	0.043*
16	96 d	111± 10	98-120	25± 4	21-30	0.043*
20	120	102± 13	88-121	6± 2	4-7	0.043*
Dose (J/cm^2) = Irradiance (watt/cm^2)× exposure time.						

* = significant difference between control and irradiated groups using Wilcoxon Signed Ranks test.
Comparing irradiated colony numbers at different irradiation times using Kruskal-Wallis test (X^2=27.473, P<0.005):
a: significant difference between 24 J/cm2 and 48, 72, 96, and 120 J/cm^2 irradiation (P<0.005).
b: significant difference between 48 and 96 J/cm^2, and 120 J/cm^2 irradiation (P<0.005).
c: significant difference between 72 J/cm^2 and 96 J/cm^2 , and 120 J/cm^2 irradiation (P<0.005).
d: significant difference between 96 J/cm^2 and 120 J/cm^2 irradiation (P<0.005).

Table 4 - Mean and standard deviation values of viability of *P. mirabilis* CFU/ml irradiated by 450nm laser light at irradiance of 0.1watt/cm^2 and different doses.

Exposure Time (min)	Dose J/cm^2	Control CFU/ml			Irradiated CFU/ml			P value
		Mean	SD	CI 5-95%	Mean	SD	CI 5-95%	
4	24 a	1,048X10^6	353X10^6	690X10^6-1,590X10^6	1,056X10^6	356X10^6	770X10^6-1,660X10^6	0.893
8	48 b	882X10^6	148X10^6	670X10^6-1,090X10^6	648X10^6	107X10^6	480X10^6-750X10^6	0.043*
12	72 c	1,058X10^6	92X10^6	960X10^6-1,170X10^6	574X10^6	73X10^6	490X10^6-640X10^6	0.043*
16	96 d	898X10^6	443X10^6	120X10^6-1,180X10^6	248X10^6	35X10^6	210X10^6-300X10^6	0.080*
20	120	1,024X10^6	128X10^6	880X10^6-1,210X10^6	56X10^6	15X10^6	40X10^6-70X10^6	0.043*
Dose (J/cm^2) = Irradiance (watt/cm^2) × exposure time.								

* = significant difference between control and irradiated groups using Wilcoxon Signed Ranks test. Comparing CFU values at different irradiation times using Kruskal-Wallis test (X^2=27.473, P<0.005):

a: significant difference between 24 J/cm^2 and 48, 72, 96, and 120 J/cm^2 irradiation (P<0.005).
b: significant difference between 48 J/cm^2 and 96 and 120 J/cm^2 irradiation (P<0.005) .
c: significant difference between 72and 96, and 120 J/cm^2 irradiation (P<0.005) .
d: significant difference between 96 J/cm^2 and 120 J/cm^2 irradiation (P<0.005)

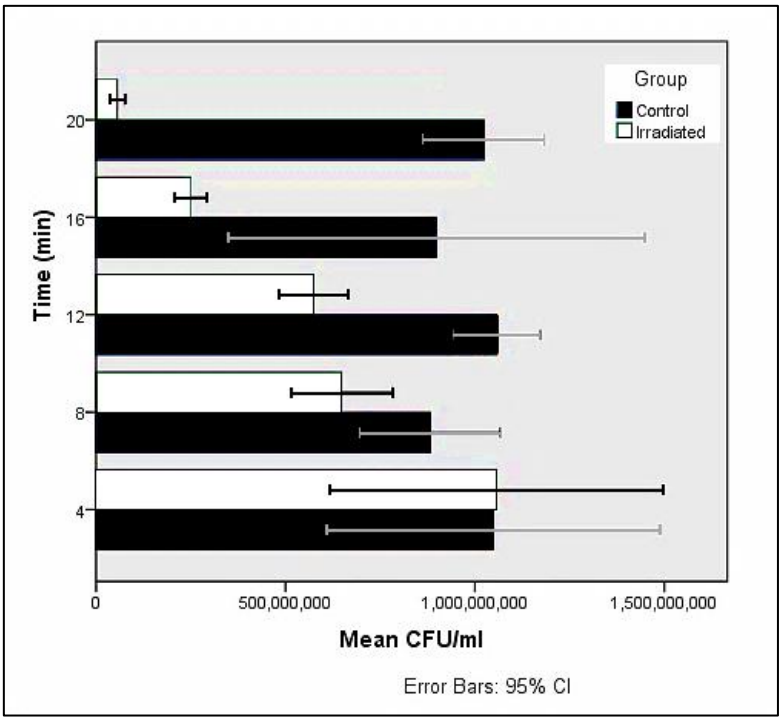

Figure 1-The relation between blue laser radiation at different exposure times and viability of *P. mirabilis* (the mean of CFU/ml with its 95% confidence interval). Dose = Irradiance (watt/cm^2) × exposure time.

Discussion

The results of this study showed that the blue laser at a wavelength of 450 nm had a bactericidal effect on the multidrug resistant *P. mirabilis* bacteria at exposure times of 8, 12, 16, and 20 minutes, corresponding to doses of 48, 72,96, and 120 J/cm^2), at irradiance of 0.1 watt/ cm^2. These results are in accordance to an earlier study conducted by Rupel *et al*. [23] who found that the blue laser light at a wavelength of 445 nm had a bactericidal effect on gram negative bacteria *Pseudomonas aeruginosa* in planktonic growth at doses of 40, 60, and 120 J/cm^2 and irradiance ≤ 0.30watt/ cm^2. In the present study, the inhibiting rate of *P. mirabilis* started at a dose of 48J/cm^2, which is relatively compatible with the results of Enwemeka *et al*. [24], who used blue laser (150mw, 470nm) at various doses (1,3,5,9,7,13,11,15,17,19,25,35,30,40,45,50,55,60 J/cm^2) to determine the bacterial inhibition of two strains of methicillin-resistant *S. aureus* (MRSA). Irradiation with blue laser produced a statistically significant dose-dependent inhibition in the number of colonies formed by the two strains. More bacterial inhibition was achieved as the dose was increased, but the influence was non- linear and better notable at lower doses. Also, the results of Enwemeka [25] are consistent with those of the present study, as he noticed that using 405nm laser (500mw) can prohibit the viability of two strains of MRSA at the same doses used in his aforementioned study [24]. He concluded that blue laser irradiation resulted in a statistically significant dose –dependent inhibition in the number of colonies in both strains of MRSA; as irradiation time was increased the viability of bacteria was decreased.

Another study by Guffey and Wilborn [26] demonstrated that exposure to 407 nm laser light completely killed *P. aeruginosa* at doses of 1, 3,5,10, and 15 J/cm^2, but inhibited *S. aureus* at 10 and 15 J/cm^2**.** The 405 nm light at doses of 1, 3, 5, 10, and 15 J/cm^2 provided a dose- dependent antimicrobial influence on *S. aureus* and *P. aeruginosa*. de Sousa and co-workers [27] demonstrated that the blue light had an inhibition effect on the growth of *P. aruginosa*, *Escherichia coli*, and *S. saureus* at doses higher than 6 J/cm2 , but *Escherichia coli* was inhibited at all doses used, except the dose of 24J/cm^2. In the current study, the maximum inhibition of *P. mirabilis* was achieved at the dose of 120 J/cm^2. However , the results of the present and the previous studies support the assumption that irradiation parameters (output power of laser, irradiance, exposure time) can affect the results definitely [23- 29].

The optimum effect of blue laser on *P. mirabilis* has not been yet fully investigated, and more studies are needed to determine the irradiation parameters used to inhibit this pathogenic bacterium.
The bactericidal impact of the blue light may be explained according to the outcomes of a previous study by Yin *et al.* [30], which showed that higher doses of light with wavelengths longer than 400 nm are capable of destroying bacteria. Treatment with UV kills gram negative and positive bacteria due to the absorption of the energy of light by cytosine and thymidine, the pyrimidine bases of DNA. The energy absorbed separates the bonds and allow the UV radiation to alter the base which will communicate with adjacent bases through modifying the architectural arrangement. The irradiation dose necessary for killing cells is achieved when the rate of DNA destruction is increased to limits above the rate of repair [31]. It is apparent that photo-destruction of DNA by the blue light causes antibacterial effects on *P. mirabilis* and other gram positive and negative bacteria [32-36] . More researches are needed to understand the actual mechanisms concerned with the influences of blue light on bacteria. As shown in tables 3 and 4, *P. mirabilis* was inhibited at doses of 48, 72, 96, and 120 J/cm^2. These significant inactivations of bacteria at these doses indicate that laser light at 450nm with irradiance of 0.1watts/cm^2 may be a practical alternative to treatment with antibiotics, particularly in subcutaneous and cutaneous *P. mirabilis* infections. The possible clinical application of these outcomes can be achieved by the transmission of laser light through the structure of a probe with handle use. This design would perfectly adjust to an open wound infection.

Conclusion

The results demonstrate that *in vitro* 450nm blue laser light at doses of 48, 72, 96, and 120 J/cm^2 produces a bactericidal influence on *P. mirabilis* isolated from infected burn wounds. This approach of killing *P. mirabilis* may suggest original method to heal wounds infected by this colonized bacteria.

References

1. Wang, S., Zhang, Y., Zhang, X. and Li, J. **2020**. An evaluation of multidrug-resistant (MDR) bacteria in patients with urinary stone disease: data from a high-volume stone management center. *World journal of urology*, ***38***(2), 425–432.
2. Marcon, J., Schubert, S., Stief, C. G., & Magistro, G. **2019**. In vitro efficacy of phytotherapeutics suggested for prevention and therapy of urinary tract infections. *Infection*, **47**(6), 937–944.
3. Pavez, M., Troncoso, C., Osses, I., Salazar, R., Illesca, V., Reydet, P., Rodríguez, C., Chahin, C., Concha, C., &Barrientos, L. **2019**. High prevalence of CTX-M-1 group in ESBL-producing enterobacteriaceae infection in intensive care units in southern Chile. *The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases*, **23**(2), 102–110.
4. Bowler, P. G., Duerden, B.I.,& Armstrong, D. G. 2**001**. Wound microbiology and associated approaches to wound management. *Clinical microbiology reviews*, ***14***(2), 244–269.
5. Libertucci, J., Bassis, C. M., Cassone, M., Gibson, K., Lansing, B., Mody, L., Young, V. B., & Meddings, J. **2019**. Bacteria Detected in both Urine and Open Wounds in Nursing Home Residents: a Pilot Study. *mSphere*, **4**(4), e00463-19.
6. Church, D., Elsayed, S., Reid, O., Winston, B., & Lindsay, R. **2006**. Burn wound infections. *Clinical microbiology reviews*, **19**(2), 403–434.
7. Hegazy, W. A. H. **2016**. Diclofenac inhibits virulence of Proteus mirabilis isolated from diabetic foot ulcer. African Journal of Microbiology Research, **10**(21), 733-743.

8. Castano, A. P., Demidova, T. N., & Hamblin, M. R. **2004**. Mechanisms in photodynamic therapy: part one-photosensitizers, photochemistry and cellular localization. *Photodiagnosis and photodynamic therapy*, **1**(4), 279–293.

9. Hamblin M. R. **2016**. Photobiomodulation or low-level laser therapy. *Journal of biophotonics*, **9** (11-12), 1122–1124.

10. Zwinkels, J. **2015** . Light, Electromagnetic Spectrum. In L. R (Ed.), Encyclopedia of Color Science and Technology (pp. 1–8). Berlin, Heidelberg: Springer.

11. Libertucci, J., Bassis, C. M., Cassone, M., Gibson, K., Lansing, B., Mody, L., Young, V. B., &Meddings, J. **2019** . Bacteria Detected in both Urine and Open Wounds in Nursing Home Residents: a Pilot Study. *mSphere*, **4**(4), e00463-19.

12. Dai, T., Gupta, A., Murray, C. K., Vrahas, M. S., Tegos, G. P., & Hamblin, M. R. **2012**. Blue light for infectious diseases: Propionibacterium acnes, Helicobacter pylori, *and beyond?. Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy*, **15**(4), 223–236.

13. Kazemikhoo, N., Vaghardoost, R., Dahmardehei, M., Mokmeli, S., Momeni, M., Nilforoushzadeh, M. A., Ansari, F., Razagi, M. R., Razagi, Z., Amirkhani, M. A., & Masjedi, M. R. **2018** . Evaluation of the Effects of Low Level Laser Therapy on the Healing Process After Skin Graft Surgery in Burned Patients (A Randomized Clinical Trial). *Journal of lasers in medical sciences*, **9**(2), 139–143.

14. Kim S, Kim J, Lim W, et al. In vitro bactericidal effects of 625, 525, and 425 nm wavelength (red, green, and blue) light-emitting diode irradiation. *Photomed Laser Surg*. 2013;31(11):554-562.

15. Chung, H., Dai, T., Sharma, S. K., Huang, Y. Y., Carroll, J. D., & Hamblin, M. R. **2012**. The nuts and bolts of low-level laser (light) therapy. *Annals of biomedical engineering*, **40**(2), 516–533.

16. Nussbaum EL, Lilge L, Mazzulli T. **2002**. The effects of 630, 660, 810, and 905 nm laser irradiation delivering radiant exposures of 1–50 J/cm2 on three species of bacteria in vitro. *J Clin Laser Med Surg.* **3**, pp: 245- 51.

17. Nussbaum, E. L., Lilge, L., & Mazzulli, T. **2002**. Effects of 630-, 660-, 810-, and 905-nm laser irradiation delivering radiant exposure of 1-50 J/cm2 on three species of bacteria in vitro. *Journal of clinical laser medicine & surgery*, **20**(6), 325–333.

18. Macfaddin J. F. **2000**. Biochemical test for identification of medical Bacteria. 3rd ed. The Williams and Wilkins. Baltimore USA. Pp: 214-289.

19. Vandepitte, Jozef, Engbaek, Kraesten, Rohner, P, Piot, Peter, Heuck, Claus C. et al. **2003**. Basic laboratory procedures in clinical bacteriology / J. Vandepitte ... [et al.], 2nd ed. World Health Organization.

20. National Committee for Clinical Laboratory. *Performance Standard for Antimicrobial Disk Susceptibility Test for Bacteria*. 6th Edition. NCCLS.

21. Burall, L. S., Harro, J. M., Li, X., Lockatell, C. V., Himpsl, S. D., Hebel, J. R., Johnson, D. E., & Mobley, H. L. **2004** . Proteus mirabilis genes that contribute to pathogenesis of urinary tract infection: identification of 25 signature-tagged mutants attenuated at least 100-fold. *Infection and immunity*, **72**(5), 2922–2938.

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

23. Rupel, K., Zupin, L., Ottaviani, G., Bertani, I., Martinelli, V., Porrelli, D., Vodret, S., Vuerich, R., Passos da Silva, D., Bussani, R., Crovella, S., Parsek, M., Venturi, V., Di Lenarda, R., Biasotto, M., & Zacchigna, S. **2019**. Blue laser light inhibits biofilm formation in vitro and in vivo by inducing oxidative stress. *NPJ biofilms and microbiomes*, **5**, 29.

24. Enwemeka CS, Williams D, Enwemeka SK, Hollosi S, Yens D. **2009** Blue 470-nm light kills methicillin-resistant Staphylococcus aureus (MRSA) in vitro. *Photomed Laser Surg*.;**27**(2):221-226

25. Enwemeka, C. S., Williams, D., Hollosi, S., Yens, D., & Enwemeka, S. K. **2008** . Visible 405 nm SLD light photo-destroys methicillin-resistant Staphylococcus aureus (MRSA) in vitro. *Lasers in surgery and medicine*, **40** (10), 734–737.

26. Guffey, J. S., & Wilborn, J. **2006** . In vitro bactericidal effects of 405-nm and 470-nm blue light. *Photomedicine and laser surgery*, **24** (6), 684–688.

27. 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*(5), 278–282. https://doi.org/10.1089/pho.2014.3854

28. Guffey, J. S., &Wilborn, J. **2006** . Effects of combined 405-nm and 880-nm light on Staphylococcus aureus and Pseudomonas aeruginosa in vitro. *Photomedicine and laser surgery*, **24(**6), 680–683.

29. Nussbaum, E. L., Lilge, L., &Mazzulli, T. **2002**. Effects of 810 nm laser irradiation on in vitro growth of bacteria: comparison of continuous wave and frequency modulated light. *Lasers in surgery and medicine*, **31**(5), 343–351

30. Yin, R., Dai, T., Avci, P., Jorge, A. E., de Melo, W. C., Vecchio, D., Huang, Y. Y., Gupta, A., & Hamblin, M. R. **2013** . Light based anti-infectives: ultraviolet C irradiation, photodynamic therapy, blue light, and beyond. *Current opinion in pharmacology*, **13**(5), 731–762.

31. Gad, F., Zahra, T., Hasan, T., & Hamblin, M. R. **2004**. Effects of growth phase and extracellular slime on photodynamic inactivation of gram-positive pathogenic bacteria. *Antimicrobial agents and chemotherapy*, **48**(6), 2173–2178.

32. Papageorgiou, P., Katsambas, A., & Chu, A. **2000**. Phototherapy with blue (415 nm) and red (660 nm) light in the treatment of acne vulgaris. *The British journal of dermatology*, **142**(5), 973–978.

33. Kawada, A., Aragane, Y., Kameyama, H., Sangen, Y., & Tezuka, T. **2002** . Acne phototherapy with a high-intensity, enhanced, narrow-band, blue light source: an open study and in vitro investigation. *Journal of dermatological science*, **30**(2), 129–135.

34. Maclean, M., Macgregor, S. J., Anderson, J. G., & Woolsey, G. A. 2008. The role of oxygen in the visible-light inactivation of Staphylococcus aureus. *Journal of photochemistry and photobiology. B, Biology*, **92**(3), 180–184.

35. Ayat M. Ali .**2015**. The effect of 650 nm diode Laser on growth curve of gram-negative bacteria and their phagocytic killing Assay by PMN cells. *Iraqi Journal of Science*, **56**,(3B) , 2264-2274.

36. Munira CH. Ismail, Sinai Waleed, Faheema Jabbar, Khawla Ibrahim **2012.** Effect of Diode Laser (805) nm on alpha-toxin production and antibiotic sensitivity of Staphylococcus aureus. *Iraqi Journal of Science*, **53** (2) , 755-759