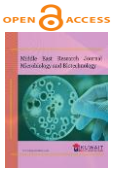

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Effects of Combined Helium/ Neon Laser Radiation and Photosensitizer on Disinfectant-Exposed *Staphylococcus aureus In vitro*

Mohemid Maddallah Al-Jebouri[1*], Hussein Saher Al-Obaidy[2]
[1]Department of Medical Laboratory Technology, Health and Medical College of Technology, Al-Qalam University, Kirkuk, Iraq
[2]Department of Microbiology, College of Medicine, University of Tikrit, Tikrit, Iraq

Abstract: ***Background*:** The mode of action of disinfectants on bacterial cells is variable. Savlon, cetrimide, and chlorhexidine are bactericidal at high concentrations and bacteriostatic at low concentrations, and cause lesions in the cell membrane and leakage of components. ***Materials and Methods*:** The present work was conducted on 200 patients with wounds. Their ages ranged from 1-40 years. Wound swab was taken on the third postoperative day from hospitalized patients and on the seventh postoperative day from patients attended outpatient clinic. The effect of low-power diode laser light with or without photochemical agents such as toluidine blue O, providine-iodine and tetracycline on total viable counts (TVC's) was determined. ***Results*:** There was a significant decrease in the viable counts, e.g. TVCs of the strain no. 1 decreased from 550 X 10^8 before exposure to 300 x 10^8 after exposure to laser light. The differences in TVCs before and after exposure was statistically significant (P <0.05) using microstat test. It was found that the laser+ providine-iodine combination was the most effective than other types of exposure especially against secondly disinfectant-exposed strains of *S. aureus*. The present study showed also differences in the TVCs between laser light effect and laser+ toluidine blue O combination at 32, 16, 8, and 4 minutes of exposure were statistically not significant (P>0.05). ***Conclusions*:** Laser irradiation for 32 minutes resulted in a significant reduction in bacterial load, with most strains showing a consistent decline. The paired analysis confirms that this reduction is not due to random variation but reflects a true antimicrobial effect of laser exposure. the combination of laser and povidine-iodine resulted in the greatest reduction, with several strains approaching complete eradication. Statistical analysis confirmed a highly significant difference among treatment conditions (Friedman test, $p < 0.001$), supporting a synergistic interaction between laser exposure and chemical disinfection. The combination of laser with povidone-iodine yielded the most pronounced bactericidal effect, achieving near-complete or complete elimination of bacterial counts within short exposure times. This indicates a strong synergistic interaction between physical (laser) and chemical (disinfectant) treatments.

Keywords: Wounds, *S. aureus*, Laser, Photosensitizer, TVCs.

Research Paper

***Corresponding Author:**
Mohemid Maddallah Al-Jebouri
Department of Medical Laboratory Technology, Health and Medical College of Technology, Al-Qalam University, Kirkuk, Iraq

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INTRODUCTION

The mode of action of disinfectants on bacterial cells is variable. Savlon, cetrimide, and chlorhexidine are bactericidal at high concentrations and bacteriostatic at low concentrations, and cause lesions in the cell membrane and leakage of components. Povidine-iodine causes disorder of protein structure, oxidation of (-SH) groups in amino acids, and membrane immobilization. Chloroxylenol (Dettol) causes poisoning of the protoplasm and disruption of the cell wall and its proteins [1-3]. Serial passage of bacteria through diluted disinfectants not only increases the minimal inhibitory concentration, but also brings about phenotypic changes in their antibiogram [4-6]. In a study conducted elsewhere [7, 8], on the effect of disinfectant exposure, it was found that *Staphylococcus aureus* strains become resistant to many antibiotics after growing in a series of diluted disinfectants. The mechanisms have not been fully studied, and it was suggested that the disinfectants probably altered sites in the bacterial ribosome, making it selectively less susceptible to certain antibiotics. Others suggested that enzymes involved in peptidoglycan synthesis might be destroyed, causing

resistance to penicillins and cephalosporins. Destruction of periplasmic enzymes by groups of disinfectants is also another contributing factor [7-9]. Unjustifiable use of disinfectants in improper, sublethal dilutions and wide usage for long periods should be avoided, since it gives a chance for survival and multiplication of bacteria due to the development of resistance to these agents as well as to many antibiotics [4]. These resistant variants, which multiply in the hospital environment, could lead to serious epidemic outbreaks. Also, the consequent changes in phage susceptibility patterns might affect the epidemiological reporting [10]. Low-power lasers deliver low doses of light over long periods and cause photochemical effects on irradiated cells [11, 12]. Helium-Neon lasers are the most common lasers used in the killing of bacteria. When its output is at 632.8 nm, the maximum power is still weak and not useful in surgery (50 - 60 nm), continuous waves penetrate up to 1.5 cm depth in tissue [13,14]. *Helicobacter pylori* could be killed by low-power laser light in the presence of a photosensitizer [15]. Non-pigmented bacteria are not affected by low-power laser light [16]. Black-pigmented anaerobic bacteria such as *Porphyromonas gingivalis* and *Prevotella intermedia* would be susceptible to killing by low-power laser light as a result of endogenous photosensitization [17, 18]. Appropriate photosensitizers can render transparent organisms susceptible to killing by low-power laser light. Gram-positive *Sarcina lutea*, *Escherichia coli*, and *Pseudomonas aeruginosa* species could be killed by He-Ne laser light after treatment with toluidine blue O, TBO [19, 20]. Martinetto *et al*., [21], used hematoporphyrin as a photosensitizer and found that *S. aureus* and *E. coli* could be killed by He-Ne laser light. Povidone-Iodine (PI) was used by Al-Jebouri and Al-Obaidy as a photosensitizer, which was more effective than toluidine blue O [22, 23].

MATERIALS AND METHODS

Patients

This study was carried out in teaching hospital of Tikrit. The present work was conducted on patients with wounds. The acceptance for participation in the present study was taken from all the participants whose native language is Arabic. They were not mentally retarded and they were completely healthy considering hearing and speaking.

Sampling

Wound swab was taken on the third postoperative day from hospitalized patients and on the seventh postoperative day from patients attended outpatient clinic. Samples were taken by using sterile cotton swabs moistened with nutrient broth carried in test tubes contained 2 ml broth liquid [24]. Wound swabs were enriched in nutrient broth at 37 °C for 18 hours. Each sample was sub-cultured on mannitol salt agar and incubated at 37°C for 24 hours. Pure cultures were obtained after isolation on appropriate selective media. The suspected colonies were purified twice then sub-cultured on nutrient agar slants and kept at 4 0C for full identification and further studies.

Determination of Disinfectants Exposure for Staphylococcus Aureus

Table 4 shows the types of disinfectants used, doubled concentrations of each disinfectant and their manufacturer. Ten strains of *S. aureus* were selectively exposed to five types of chemical disinfectants in concentrations shown in the Table 1. The series of disinfectants exposure was as follow: hibitane, Dettol, cetavlon, savlon and providine-iodine. All strains were inoculated into nutrient broth and incubated at 37 0C for three hours producing a growth yield of 106 – 107 cells/ml. Plates of Mueller-Hinton agar containing doubling dilutions of disinfectants were prepared. Strains in broth cultures were subcultured by streaking onto Mueller-Hinton agar plates containing the first disinfectant and incubated at 37 0C for 18 hours. Colony from subminimal inhibitory concentration (SMIC) was picked up and inoculated into nutrient broth and incubated at 37 0C for three hours. The identity of strains was checked by subculturing on blood agar, Gram stain and coagulase test [5] and the same was done for the remaining disinfectants. The process of exposure was repeated on media containing the second disinfectants [25].

Table 1: Disinfectants used in susceptibility testing of *Staphylococcus aureus* isolated from the caesarean wounds

Disinfectant	Scientific name	Chemical concentration	Concentration used(µg/ml)	Manufacturer
Cetrimide	Cetrimide	Pure powder	0.5-128	ICI(Britain)
Hibitane	Chlorhexidine gluconate	5%	0.5-128	ICI (Britain)
Dettol	Chloroxylenol	4.8%	0.5-128	SDI, Samarra, Iraq
Savlon	Chlorhexidine: Cetrimide	3%cetrimide:0.3% Chlorohexidine	0.3cetrimide:0.03 chlorhexidine -76.8 Cetrimide:7.68 chlohexidine	ICI (Britain)
Providine-iodine		10%	0.5-1024	ICI(Britain)

Effect of Laser Combined with Photosensitizers on Total Viable Counts (TVC's) of S. Aureus

Ten strains of non-, firstly- and secondly-disinfectant exposed isolates of *S. aureus* were used in this test. Ten-fold serial dilutions of the contents of each tube were prepared in sterile nutrient broth. Duplicated (50 µl) aliquots were spread over the surface of Mueller-Hinton plates, and incubated overnight at 37 0C, the resulting colonies were counted [25-27].

Statistical Analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics such as means, standard deviations, and frequency distributions were computed to summarize the data [28, 29].

RESULTS

Effect of Laser Irradiation on Total Viable Counts

Laser light exposure on TVCs of S. aureus strains was shown in Table 2. The 10 selected strains were exposed to laser irradiation for 32 minutes. There was a significant decrease in the viable counts, e.g. TVCs of the strain no. 1 decreased from 550 X 10^8 before exposure to 300 x 10^8 after exposure to laser light. The differences in TVCs before and after exposure was statistically significant (P <0.05) using microstat test. Laser irradiation for 32 minutes resulted in a significant reduction in bacterial load, with most strains showing a consistent decline. The paired analysis confirms that this reduction is not due to random variation but reflects a true antimicrobial effect of laser exposure (Figure 1).

Table 2: The effect of laser light exposure on total viable counts (TVCs) X 10^8 for 10 strains of *Staphylococcus aureus* isolated from wounds before exposure to disinfectants

Strain code	TVCs before exposure to laser	TVCs after exposure to laser for 32 min.
1	550	300
2	500	300
8	450	300
10	450	350
13	195	180
20	220	195
21	350	300
37	100	95
38	115	57
39	250	240
Mean ±SD	318 + 163.1	231.7 + 98.0

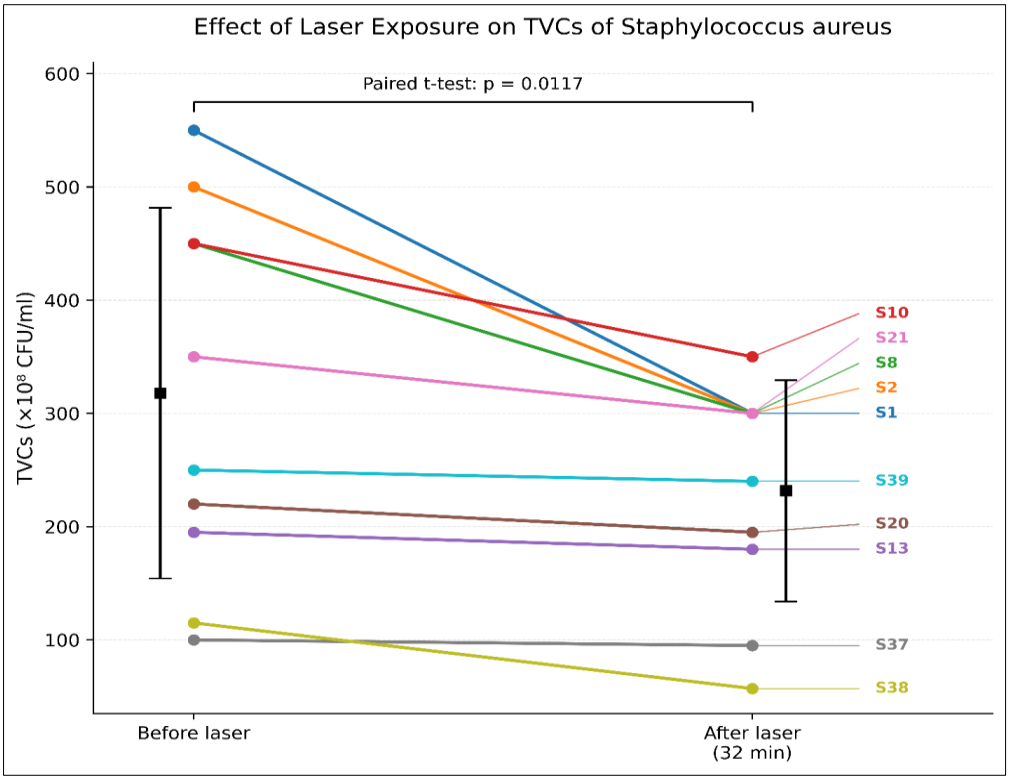

Figure 1: Paired-t test shows the effect of Laser Exposure on Total Viable Counts of *Staphylococcus aureus*

Effect of Laser and Laser+ Providine-Iodine Combination

There was a significant decreases in TVCs after exposure to laser (P <0.05) but a highly significant decrease was seen after exposure to laser +providine-iodine combination (P <0.01) using microstat test, e.g. TVCs of strain no. 1 was 216 x 10^8 before exposure and then reduced to 190 x 10^8 after exposure to laser for 32 minutes and further decreased to be 50 x 10 ^{8} after exposure to laser +providine-iodine combination for 2 minutes (Table 3). The differences in total viable numbers among all types of irradiation was statistically significant (P <0.05) using microstat test. Laser irradiation alone produced a moderate reduction in total viable counts, while povidine-iodine demonstrated a significantly stronger antimicrobial effect. The combination of laser and povidone-iodine resulted in the greatest reduction, with several strains approaching complete eradication. Statistical analysis confirmed a highly significant difference among treatment conditions (Friedman test, p < 0.001), supporting a synergistic interaction between laser exposure and chemical disinfection (Figure 2).

Table 3: The effect of laser, providine-iodine and laser+ providine-iodine combinations 0n total viable counts (TVCs) X 10^8 for 10 strains of *Staphylococcus aureus* isolated from wounds after second exposure to disinfectants

Strain code	TVC before exposure to L+PI*	TVC after exposure to L for 32 min.	TVCs after exposure to PI	TVCs after exposure to L+PI for 2 min.
1	216	190	80	50
2	400	160	43	30
8	200	190	140	80
10	550	370	120	70
13	70	70	63	15
20	375	360	84	8
21	285	270	65	13
37	420	390	17	0
38	500	380	200	14
39	240	210	190	0
Mean ±SD	325.6 + 149	259.0 + 11.5	100.2 +61	28.0 + 28.9

*= L, laser; PI, providine-iodine; SD, standard deviation

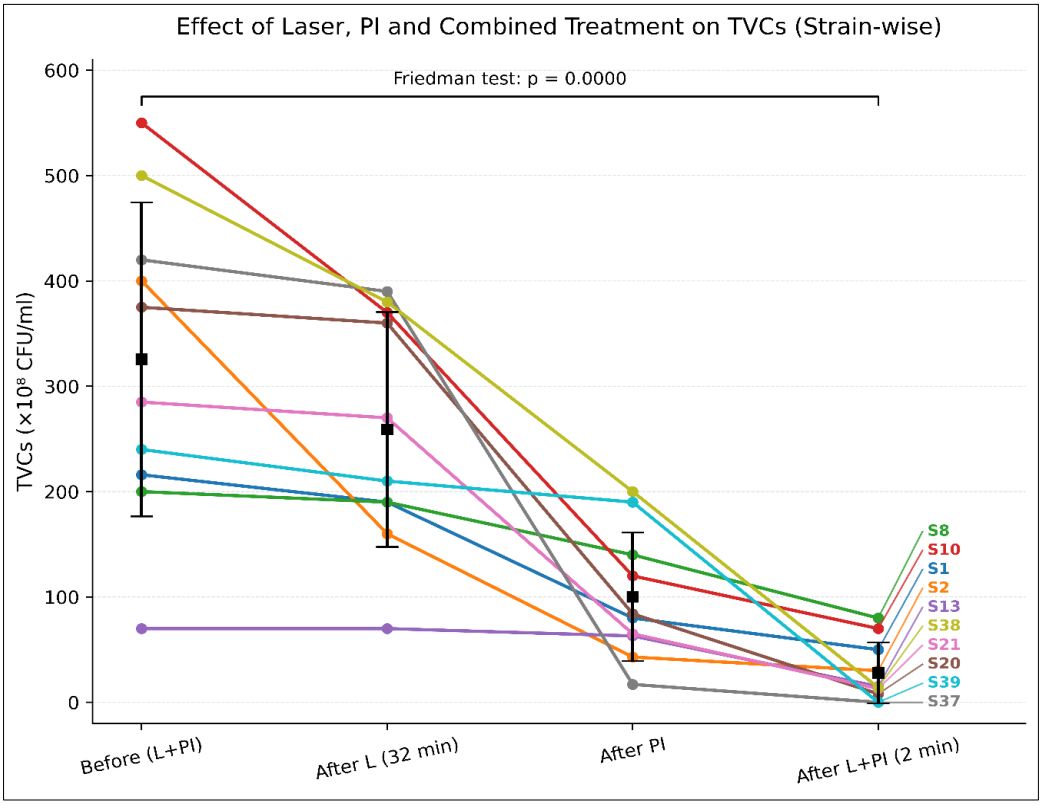

Figure 2: Paired-t test shows the effect of repeated-measures analysis of laser, povidone-iodine, and combined therapy on bacterial load reduction in *Staphylococcus aureus*

Effect of Laser, Laser+ Toluidine Blue O and Laser + Providine-Iodine Combination

It was found that the laser+ providine-iodine combination was the most effective than other types of exposure especially against secondly disinfectant-exposed strains of *S. aureus* (Table 4). The differences in the TVCs among all non-disinfectant exposed strains was statistically not significant (P >0.05) except the strain no.1 and the strain no.3 which showed a significant difference (P <0.05) using microstat test. The differences in the TVCs among all firstly-disinfectant exposed strains was statistically significant (P <o. 05), but the first and the second strains showed no significant difference (P >0.05). The present study showed also that the differences in the TVCs between laser light effect and laser+ toluidine blue O combination at 32, 16, 8, and 4 minutes of exposure were statistically not significant (P>0.05). Also there were highly significant differences between laser+toluidine blue O and laser+providine-iodine combination effects (P <0.01) at 32, 16, 8 and 4 minutes of exposure. This study shows that the *S. aureus* can be killed after exposure to laser+ providine-iodine combination for 2 minutes and a sharp decrease in TVCs was achieved, e.g. TVCs of second disinfectant-exposed strains of no.1 and no.2 was 216 and 400 x 10^8 respectively before exposure to laser+providine-iodine combination and then became 0 and 5 x 10^8 respectively after exposure to this combination for 2 minutes. There is a highly significant reduction in total viable counts across different treatments and exposure times. While laser and toluidine blue O produce moderate antimicrobial effects, povidine-iodine demonstrates a stronger reduction. However, the combination of laser with povidone-iodine yields the most pronounced bactericidal effect, achieving near-complete or complete elimination of bacterial counts within short exposure times. This indicates a strong synergistic interaction between physical (laser) and chemical (disinfectant) treatments (Figure 3).

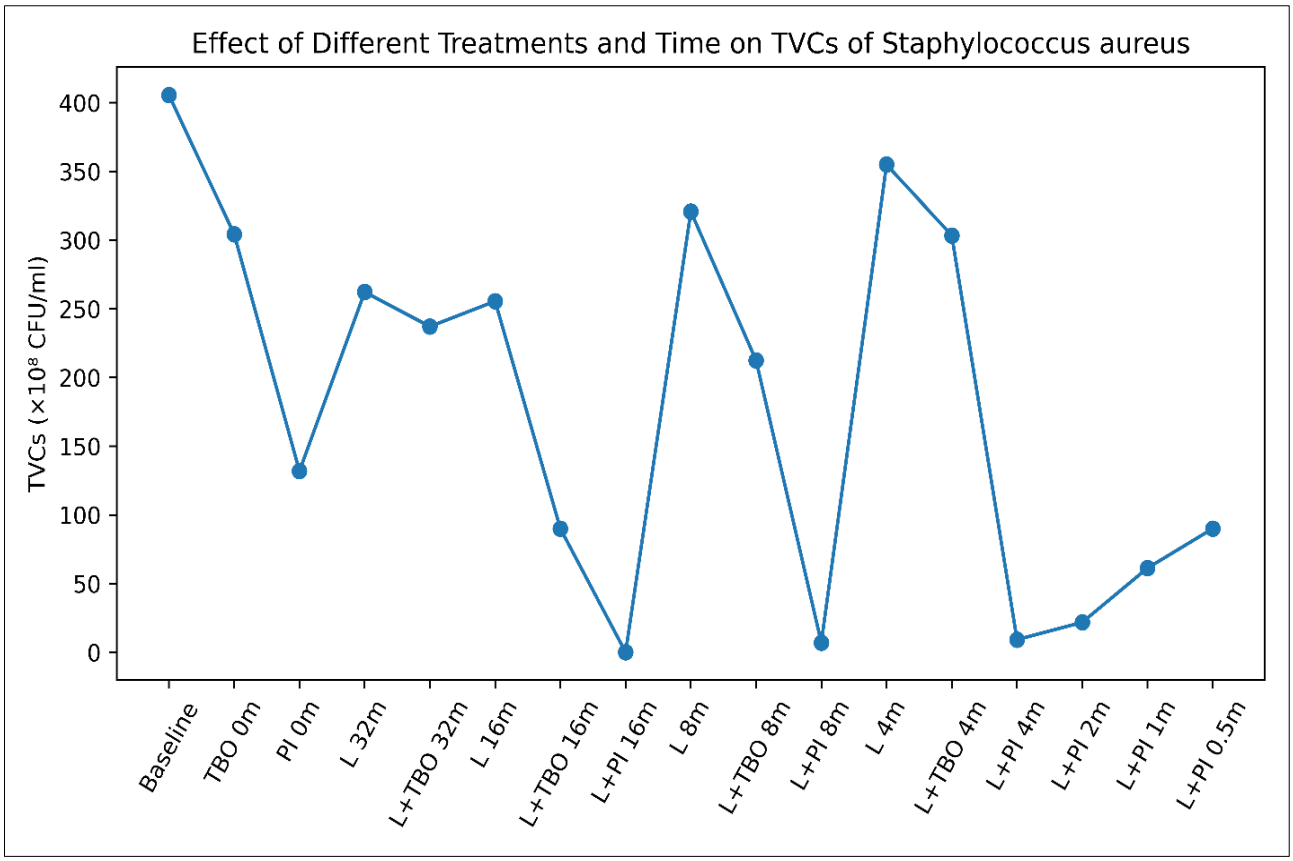

Figure 3: Combined laser and disinfectant treatments, particularly L+PI, produced the greatest reduction in bacterial counts of *Staphylococcus aureus* with near-complete elimination observed at short exposure times

Table 4: Total viable counts (TVCs) X10^8 of 4 bacterial strains of non-disinfectant, first and second disinfectant-exposed *Staphylococcus aureus* isolated from wounds before and after exposure to toluidine blue O (TBO), providine-iodine (PI), laser(L) and their combinations

Exposure	Time (Min)	Non-exposed strain				Firstly exposed strain				Secondly exposed strain				Mean	SD
		1	2	3	4	1	2	3	4	1	2	3	4		
Before L, TBO, PI	0	550	500	450	450	400	300	550	300	216	400	200	550	405.5	126.5
After TBO	0	400	350	400	350	120	280	300	250	200	350	150	500	304.2	110.8
After PI	0	80	43	140	120	100	300	290	200	75	80	60	95	131.9	86.4
After L	32	300	300	300	350	76	300	400	270	90	160	190	370	262.2	95.0
After L+TBO	32	150	300	220	400	133	54	300	270	180	380	133	320	237.1	107.9
After L	16	250	300	300	350	80	38	450	300	200	200	198	400	255.5	121.1
After L+TBO	16	45	200	75	70	65	70	95	70	36	147	7	200	90.0	61.3
After L+PI	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0
After L	8	400	400	400	400	100	300	450	300	200	250	200	450	320.8	113.7
After L+TBO	8	200	250	250	200	250	30	250	250	29	350	140	350	212.4	103.3
After L+PI	8	0	0	20	55	10	0	0	0	0	0	0	0	7.0	16.3
After L	4	420	450	400	400	300	300	500	300	200	370	200	450	355.0	96.3
After L+TBO	4	250	500	350	400	300	29	400	280	120	370	190	450	303.3	138.4
After L+PI	4	40	50	30	25	10	0	0	0	0	0	0	0	9.2	14.3
After L+PI	2	50	30	80	70	2	2	7	2	0	5	7	7	21.8	28.9
After L+PI	1	54	30	81	95	31	86	167	153	10	7	8	11	61.3	56.0
After L+PI	0.5	95	37	95	112	78	219	195	174	15	25	19	17	90.0	72.6

DISCUSSION

The present study revealed that laser irradiation for 32 minutes resulted in a significant reduction in bacterial load, with most strains showing a consistent decline. The paired analysis confirms that this reduction is not due to random variation but reflects a true antimicrobial effect of laser exposure. However, like any other electromagnetic treatment, the primary effects of laser interaction with a biological tissue are divided into thermal and nonthermal mode of action. Low level laser therapy (LLLT) is basically a non- thermal energy application. Although delivery and absorption of any energy to the body will produce heat in some extent, nonthermal in this context means no accumulative thermal energy occurs or temperature elevation in macroscopic scale is averaged zero. Photobioactivation is a common term for LLLT and indicates stimulating various biological events using light energy without significant temperature changes. There are some other alternative terms such as photobiostimulation and photobiomodulation [8]. However, It was found previously that a reduction in the TVCs was time-dependent, i.e., as long as the exposure time continues, the total viable counts are reduced. Wilson *et al*., indicated that the exposure of a bacterial suspension of *S. aureus* to the He/Ne laser light (35 mW) for 60 seconds in the absence of TBO caused a small reduction in the viable count; they also demonstrated in other studies that exposure of *S. aureus* to the same laser type (7.3 mW) in the absence of the dye resulted in a decrease in the viable count, but not statistically significant [23-26]. On the other hand, Al-Jebouri and Al-Obaidy demonstrated that the TVCs for one strain of *S. aureus* were 550 × 108 before exposure and became 300, 250, 400, and 420 × 108 after exposure to laser light for 32, 16, 8, and 4 minutes in the presence of dye respectively [30]. In contrast, Hardee *et al*., found that there were no significant differences observed in the reduction of colony-forming units among groups of *Bacillus stearothermophilus* when pulsed Nd: YAG laser radiation was used with and without 0.5% sodium hypochlorite combination [23]. Moreover, laser effect was assessed by biomass measurement, colony forming unit count and cell viability assay. It was shown that the laser treatment has not affected the biofilms biomass neither the cell viability, although a small disruptive action was observed in the structure of all biofilms tested. A reduction on cell growth was observed in *S. aureus* and in polymicrobial biofilms. This work represents an initial in vitro approach to study the influence of NIR laser treatment on bacterial biofilms in order to explain its potentially advantageous effects in the healing process of chronic infected wounds [31]. On the other hand, in a study carried out elsewhere, it was demonstrated that TBO-mediated a PDT with a 635 nm diode laser effectively reduced *Candida spp*. and *Staphylococcus aureus* under in vitro conditions. The strongest effects were achieved with 10 min incubation for *Candida* and 5 min for *S. aureus*, followed by irradiation at 400 mW for 120 s. Antimicrobial efficacy required the combined use of both photosensitizer and light, as neither alone produced significant effects. These results support the potential of TBO-mediated a PDT as an adjunct to conventional therapy, with possible application as an alternative in cases of drug resistance. The identified conditions (≥400 mW, ≥120 s) provide a baseline for efficacy, though they should not be

considered definitive optima, as higher doses were not tested. Further dose–response and clinical studies are needed to refine parameters and standardize therapeutic protocols [32].

In other study previously carried out revealed that exposure to laser + providine -iodine + tetracycline (L+PI+TE) combination for 0.5 and 1 minute led to a significant decrease in TVC's among all non-, firstly-, and secondly disinfectants exposed isolates (P <0.05) using Duncan test and other related mathematical analyses. TVC's decreased to zero at 2 minutes exposure time which suggested to be the best time needed for killing of *Pseudomonas aeruginosa* [27]. On the other hand, the present study showed a significant decreases in TVCs after exposure to laser (P <0.05) but a highly significant decrease was seen after exposure to laser +providine-iodine combination (P <0.01) using microstat test, eg. TVCs of strain no. 1 was 216 x 108 before exposure and then reduced to 190 x 108 after exposure to laser for 32 minutes and further decreased to be 50 x 10 8 after exposure to laser +providine-iodine combination for 2 minutes. When a laser beam, or- a photon, alters the energy level of an atom through shifting between e2 and e3, this event establishes a change in physicochemical cellular function which acts as a trigger agent for beginning a mimicry cascade system.[33]. A second photon that will be in phase, propagate in the same direction as the excited photon. This phenomenon, that is called stimulated emission, is the basic of laser light generation. It has been mentioned that in most of the biological tissues, photons are preferably scattered in forward direction [34]. Three photochemical agents such as toluidine blue O, providine-iodine and tetracycline were used in the present study as photosensitizers. It was observed that toluidine blue O at concentration equal to 50 µg/ml or less couldn't sensitized *Ps. aeruginosa* isolates for killing by low-power laser light with exposure time 32 minutes. This result might be due use laser with an output 5 mW and this power was not enough to photoactivate toluidine blue O to produce free radicals generated for killing of this pathogen, therefore, to kill these bacteria, laser with output higher than this laser should be used. Al-Jebouri and Al-Faham study markedly showed that the exposure of *Ps. aeruginosa* to laser with or without photosensitizers (toluidine blue O, providine-iodine and/ or tetracycline and their combinations had a significant decrease in reducing the total viable counts. Combined treatments like (L+TBO, L+PI+TE) showed steep declines, indicating strong antibacterial action over time. But single agents e.g., TBO, TE or PI had flatter curves, meaning time alone was not sufficient to reduce TVCs. Comparatively few investigations of the effect of low-power laser on *Ps. aeruginosa* viability have been published [27]. The results presented by Al-Jebouri and Al-Faham revealed that the exposure of *Ps. aeruginosa* to photochemical agents studied singly tested had significant effect in reducing the total viable counts. While exposure of the same microbe to laser+toluidine blue O, laser+providine-iodine, laser +tetracycline and laser + providine-iodine +tetracycline combination lead to a sharp decrease in total viable counts. Therefore, it was concluded that there was synergistic effect between laser and photochemical agents. The multivariate linear regression revealed that both time and agent choice significantly influenced bacterial viability, and laser + photosensitizer combinations like (L+TBO, L+TE) have strongest bactericidal effect. Combined treatments like (L+TBO, L+PI+TE) showed steep declines, indicating strong antibacterial action overtime [27]. But single agents e.g. TBO, TE or PI had flatter curves, meaning time alone was not sufficient to reduce TVC,s significantly [25-27]. However, The present study shows that the *S. aureus* can be killed after exposure to laser+ providine-iodine combination for 2 minutes and a sharp decrease in TVCs was achieved, e.g. TVCs of second disinfectant-exposed strains of no.1 and no.2 was 216 and 400 x 108 respectively before exposure to laser+providine-iodine combination and then became 0 and 5 x 108 respectively after exposure to this combination for 2 minutes. There was a highly significant reduction in total viable counts across different treatments and exposure times. While laser and toluidine blue O produce moderate antimicrobial effects, povidine-iodine demonstrates a stronger reduction. However, the combination of laser with povidine-iodine yielded the most pronounced bactericidal effect, achieving near-complete or complete elimination of bacterial counts within short exposure times. This indicates a strong synergistic interaction between physical (laser) and chemical (disinfectant) treatments [27-34]. In the case of radicals generated by sensitizer molecules bound to peripheral structures, quenching by neighboring molecules without concomitant damage to essential cell structures would be more likely eventually than their interaction with the distinct cytoplasmic membrane. Qualitative and quantitative differences in the surface components of the three target organisms may also have contributed to their varying susceptibilities to lethal photosensitization [35-39].

The growing concerns about the development of biocide resistance and cross-resistance with antibiotics among pseudomonads have been suggested. It is clear that clinical isolates particularly of *P. aeruginosa* should be under continuous surveillance and possible mechanisms associated with disinfectant-resistance should be further investigated particularly among hospitals where patients who are mostly immunocompromised are resident [40-44]. It is natural for bacteria to develop antibiotic resistance which is encoded by the antibiotic resistance genes (ARGs) which is not more than production of billions of years of evolution. It has been found that bacteria living in the environment already possess ARGs which are responsible for resistance to newly approved antibiotics before using of these drugs [13]. Inherited structural and / or physiological properties lead to intrinsic resistance to antibiotics. These functional properties including

efflux to actively eliminate antibiotics from bacterial cells which entered through porin which is the mechanism by which the antibiotics unable to pass the outer membrane and by this mechanism cannot reach the target site [45-51]. Bacterial mortality increased with longer irradiation periods because they generated more reactive oxygen species. Bacterial mortality rates for *S. aureus* and *P. aeruginosa* were 80.58% and 85.50%, respectively. Gram-positive bacteria are more vulnerable to penicillin antibiotics, which is explained by variations in the makeup of their cell walls [52-54]. However, compared to gram-positive bacteria, peptidoglycan is less prevalent in the cell walls of gram-negative bacteria [55-57]. Lipoproteins and phospholipids make up the outer membrane that surrounds the peptidoglycan layer. Due to the variations in their cell walls, Gram-positive and Gram-negative bacteria react to photodynamic inactivation in distinct ways. Gram-positive bacteria have an exterior wall that is 15-80 nm thick and composed of 100 peptidoglycan layers connected by the moderately porous, negatively charged peptidoglycan teichuronic acid and lipoprotein, which adheres to the outside membrane. Gram-negative bacteria have an outer membrane composed of lipoproteins, phospholipids, and lipopolysaccharides. The outer membrane shields the cell from the damaging effects of the surroundings and possesses permeability to certain chemicals. A robust permeability barrier is produced by the outer membrane [58-62].

CONCLUSIONS

Laser irradiation for 32 minutes resulted in a significant reduction in bacterial load, with most strains showing a consistent decline of *S. aureus*. The paired analysis confirms that this reduction is not due to random variation but reflects a true antimicrobial effect of laser exposure. The combination of laser and povidone-iodine resulted in the greatest reduction, with several strains approaching complete eradication. Statistical analysis confirmed a highly significant difference among treatment conditions (Friedman test, $p < 0.001$), supporting a synergistic interaction between laser exposure and chemical disinfection. The combination of laser with povidone-iodine yields the most pronounced bactericidal effect, achieving near-complete or complete elimination of bacterial counts within short exposure times. This indicates a strong synergistic interaction between physical (laser) and chemical (disinfectant) treatments.

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Statement of Ethics

All the procedures involving human participation were conducted in strict accordance with ethical standards of Institutional Research Committee, Department of Scientific Research, Tikrit University as well as the 1964 Helsinki Declaration and its subsequent amendments or equivalent ethical norms.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest Statement: The author declares that he has no conflicts of interest, financial or otherwise.

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