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# Effects of serum, pH and growth phase on Laser photosensitization death time values of multi-drug resistant *Staphylococcus aureus*

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**Abstract**

**Background**: *Staphyllococcus aureus* is a common pathogen in hospitals infections; it causes skin lesions, urinary tract infection (UTI) and meningitis. Previous studies reported that laser irradiation with photosensitizer can have bactericidal effect. Also, photosensitization has been better alternative to antibiotics because of its ability to kill gram-negative and gram positive bacteria. Therefore, the aim of this study was to determine the effects of 50% Human serum, pH value and growth phase on Laser Death Time(LDT) against multi-drug resistant *S. aureus* in presence of 30 µg/ml of toluidine blue O (TBO) using different laser powers (635nm) in different exposure times.

**Methods**: *S. aureus* isolates were collected from patients with urinary tract infection. Antibiotic susceptibility test carried out. Sensitivity to photosensitization against TBO. The effect of 50% human% human serum on laser photosensitization death time after preparation of mannitol broth culture 18-24h incubation was studied. Study the effect of different pH values ranging from (5 to -8.5) on laser photosensitization. The effect of bacterial growth phase on laser photosensitization using 30µg /ml of TBO, 10 mw/cm$^2$ laser density and 60 sec exposure time.

**Results**: the results proved that the survival of bacteria decreased with increasing laser power in presence of TBO. In addition, the killing of the bacteria is possible in presence of 50% human serum. The best results were at 50mw laser power and the LDT was 250 sec, but in absence of human serum the LDT was 60 sec. On the other hand, we found that the survival of bacteria decreased in presence of laser and TBO with increasing pH value. However, the growth phase did not affect photosensitization and LDT

**Keywords**: photosensitizer, toluidine blue O; Diode laser S. aureus, human serum.

## INTRODUCTION

*Staphyllococcus aureus* is a common pathogen in hospitals infections; it causes skin lesions, urinary tract infection (UTI) and meningitis [1]. The infections caused by these bacteria became difficult to treat, because the ability of the bacteria to resist a wide spectrum of antibiotics.

In the United Kingdom,40 to 60% of *S. aureus* are multi-drug resistant [2], so it is necessary to develop alternative ways to treat these bacteria. Some researchers reported that laser irradiation with photosensitizer can have bactericidal effect [3]. Also, photosensitization has been better alternative to antibiotics because of its ability to kill gram-negative and gram positive bacteria [4]. Low-power laser can kill bacteria in the presence of dyes [5].

The aim of this study was to investigate the effects of pH, human serum and growth phase on laser photosensitization in different parameters using toluidine Blue O (TBO) against *S. aureus* isolated from patients with UTI.

## MATERIALS AND METHODS

1- *S. aureus* isolates were collected from patients with UTI by smearing on sterile Petri dishes with mannitol-salt agar, identification of the isolates carried out by traditional methods.
2- Antibiotic susceptibility test of the isolates was carried out using tetracycline, Augmentin and Penicillin by disk diffusion method.(data not shown).
3- Sensitivity to photosensitization against TBO by irradiation of the sample (known number of *S. aureus* cells) with red laser light (wave length 635 nm) with different exposure time.
4- The effect of 50% human serum on laser photosensitization death time after preparation of mannitol broth culture 18-24h incubation. We washed with phosphate buffer saline (PBS) and resuspended in human serum and exposed the sample to the red laser in presence and absence of human serum and in presence of TBO. Bacterial suspension in 50% human serum was kept in the fridge while processing a batch (for laser photosensitization keeping in dark for 15-20 min. Irradiation, dilution and plating) to prevent bacterial division while waiting for processing next batch.(pH =7.3 was chosen to run laser photosensitization death time experiment LPD in presence of 50% human serum due to the fact that the pH of human serum is 7.3±0.1.
5- Study the effect of different pH values ranging from 5 to 8.5 on laser photosensitization. The experiment was done to calculate bacterial survival without laser exposure in absence of TBO (L-D-), without laser exposure in presence 30µg of TBO(L-D+), with laser exposure in presence 30µg of TBO(L+D+) and the resultant laser photosensitization influence factor (LPIF) in terms of survival.
6- Investigate the effect of bacterial growth phase on laser photosensitization using 30µg /ml of TBO, 10 mw/cm$^2$ laser density and 60 sec exposure time, depending on growth curves by measuring the optical density at 600nm, suspension of cells from the growth phase (lag, logarithmic and stationary phase ) were prepared .

## RESULT AND DISCUSSION

The experiments were performed on the most resistant isolates of 18 samples depending on the results of sensitivity to the antibiotics. After exposure to laser light at 635nm in different laser powers (3,6, and12 mw) in presence of 30µg/ml of TBO in absence of human serum, the killing of bacteria reached up to 99.9% at 6, 5, 4 minutes, respectively. This means that bacterial survival decreases with increasing laser power while in the presence of 50% human serum in the same laser power, the survival proportions of bacteria were 53%, 37.7% and 23.1%, respectively, in the same time of the LDT in absence of serum (Tables 1, 2 & 3). On the other hand, when we used laser powers of 20, 30, 40 and 50 mw, the LDT in absence of human serum were 99.9% at times of 120, 90, 75 and 60 sec. Moreover, the same experiments in presence of 50% human serum and same laser powers recorded that the proportions of bacterial survivals within same times of LDT were 17.1%, 20.9%, 14.2%, and 11.9%, respectively(Tables 4, 5, 6 and 7).

It is clear from Figures 1 and 2 that bacteria can be killed in the presence of 50% human serum, but the survival decreased in the presence of high laser power. These observations are in agreement with those reported in a previous study [6].

Figures 3 and 4 show the effects of different pH values (5-9) on laser photosensitization using TBO at laser power 5mw. The survival of bacteria decreased in presence of laser and TBO with increasing the pH value. These finding are supported by those reported by a previous study [7] which revealed that survival of *E. coli* was decreased at pH values exceeding 7 in photosensitization. Moreover, Figure 5 shows the effect of growth phase on the survival of bacteria in case of exposure to laser power,10mw and 30µg/ml of TBO. It is clear that the killing of bacteria is not depending on growth phase of bacteria and this is supported by previous studies [6,8]. On the other hand, the effect of TBO in presence of laser light in killing bacteria is very clear this due to the fact that the photosensitizer absorbs the laser light which may generate singlet oxygen which may lead to damage the cell membrane [9,10,11]. Furthermore, these effects are depending on wave length of the laser irradiation, time of irradiation and power density [12] while the laser alone and TBO alone were not able to kill *S. aureus* bacteria [13].

**Table 1 Laser photosensitization in presence of 50% Human serum with laser power 3mw**

| Exposure time (min) | Survival in absence of serum (%) | Survival in presence of 50% Human serum |
|---|---|---|
| 0 | 100 | 100 |
| 0.5 | 82.6 | 79.1 |
| 1 | 53.5 | 69.9 |
| 2 | 33 | 64 |
| 3 | 12.6 | 58.4 |
| 4 | 4.2 | 58 |
| 5 | 0.82 | 53 |
| 6 | 0 | 51.4 |
| 8 | | 42 |
| 10 | | 33.7 |

**Table 2 Laser photosensitization in presence of 50% Human serum with laser power 6mw**

| Exposure time (min) | Survival in absence of serum (%) | Survival in presence of 50% Human serum |
|---|---|---|
| 0 | 100 | 100 |
| 0.5 | 46 | 50.1 |
| 1 | 19.6 | 44.3 |
| 2 | 5.4 | 42.1 |
| 3 | 1.5 | 39.9 |
| 4 | 0.3 | 39.3 |
| 5 | 0 | 37.7 |
| 6 | | 36.2 |
| 8 | | 27.5 |
| 10 | | 19.5 |

**Table 3 Laser photosensitization in presence of 50% Human serum with laser power 12mw**

| Exposure time (min) | Survival in absence of serum (%) | Survival in presence of 50% Human serum |
|---|---|---|
| 0 | 100 | 100 |
| 0.5 | 34.9 | 37.7 |
| 1 | 12.6 | 31.3 |
| 2 | 2.3 | 27.7 |
| 3 | 0.93 | 25 |
| 4 | 0 | 23.1 |
| 5 | | 21 |
| 6 | | 18.1 |
| 8 | | 10.8 |
| 10 | | 5.1 |

**Table 4 Laser photosensitization in presence of 50% Human serum with laser power 20mw**

| Exposure time (sec) | Survival in absence of serum (%) | Survival in presence of 50% Human serum |
|---|---|---|
| 0 | 100 | 100 |
| 15 | 51.9 | 49.3 |
| 30 | 20.9 | 36.6 |
| 45 | 9,9 | 33 |
| 60 | 4 | 29.7 |
| 75 | 2 | 28 |
| 90 | 0.8 | 25.3 |
| 105 | 0.3 | 21 |
| 120 | 0 | 17.1 |
| 180 | | 14.3 |
| 240 | | 9.8 |

**Table 5 Laser photosensitization in presence of 50% Human serum with laser power 30mw**

| Exposure time (sec) | Survival in absence of serum (%) | Survival in presence of 50% Human serum |
|---|---|---|
| 0 | 100 | 100 |
| 15 | 44.6 | 45.2 |
| 30 | 12.8 | 33.4 |
| 45 | 5.4 | 31 |
| 60 | 2.1 | 27.2 |
| 75 | 0.7 | 26 |
| 90 | 0 | 20.9 |
| 105 | | 18 |
| 120 | | 11.7 |
| 180 | | 8.3 |
| 240 | | 5.3 |

**Table 6 Laser photosensitization in presence of 50%Human serum with laser power 40mw**

| Exposure time (sec) | Survival in absence of serum (%) | Survival in presence of 50% Human serum |
|---|---|---|
| 0 | 100 | 100 |
| 15 | 20.3 | 30.6 |
| 30 | 4.4 | 25.8 |
| 45 | 0.9 | 21.2 |
| 60 | 0.5 | 16.9 |
| 75 | 0 | 14.2 |
| 90 | | 11.9 |
| 105 | | 11.1 |
| 120 | | 7.3 |
| 180 | | 3.6 |
| 240 | | 1.7 |

**Table 7 Laser photosensitization in absence and presence of 50% Human serum with laser power 50mw**

| Exposure time (sec) | Survival in absence of serum (%) | Survival in presence of 50% Human serum |
|---|---|---|
| 0 | 100 | 100 |
| 15 | 12.6 | 19.8 |
| 30 | 4.7 | 16.2 |
| 45 | 1.4 | 15 |
| 60 | 0 | 11.9 |
| 75 | | 9.8 |
| 90 | | 7.3 |
| 105 | | 5.8 |
| 120 | | 2.9 |
| 180 | | 1.7 |
| 240 | | 0 |

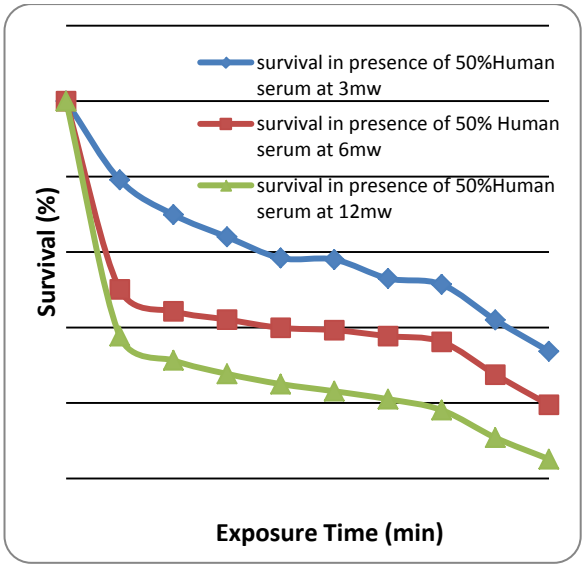


**Figure 1 Laser photosensitization in presence of 50% Human serum irradiated by 3, 6 and 12 mw at different exposure times**

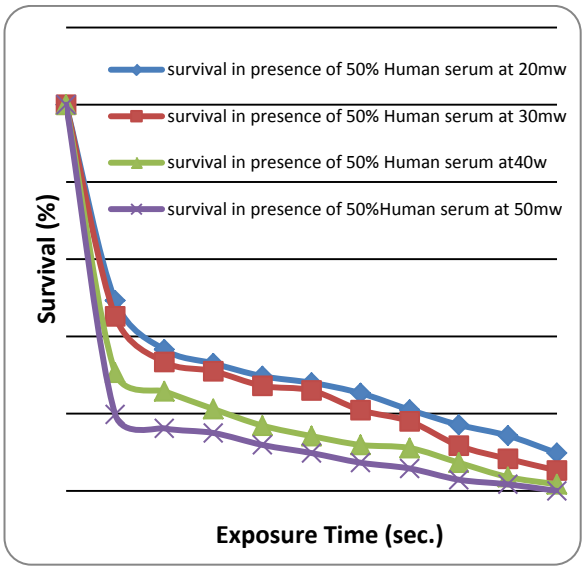


**Figure 2 Laser photosensitization in presence of 50% Human serum irradiated by 20, 30, 40 and 50 mw at different exposure times**

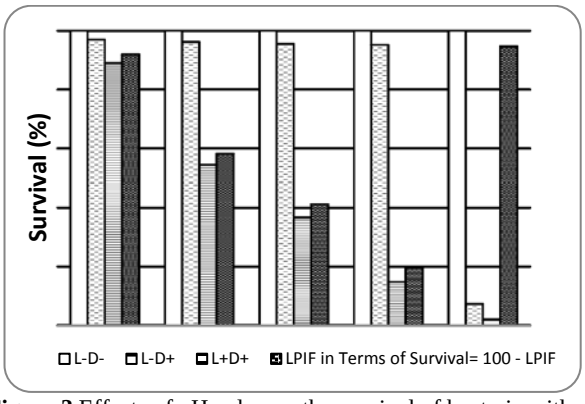


**Figure 3** Effects of pH value on the survival of bacteria without laser exposure in absence of TBO (L-D-), without laser exposure in presence of 30µg of TBO, with laser exposure in presence of 30µg of TBO, and the resultant laser photosensitization influence factor (LPIF) in terms of survival.

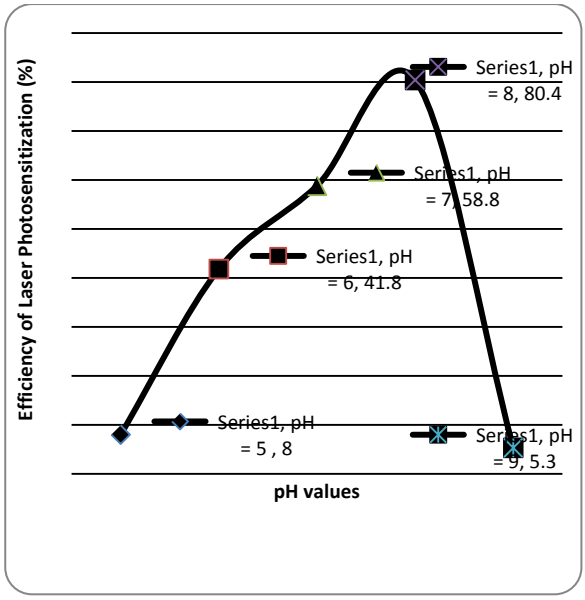


**Figure 4 Efficiency of laser photosensitization in bacterial killing against different pH values**

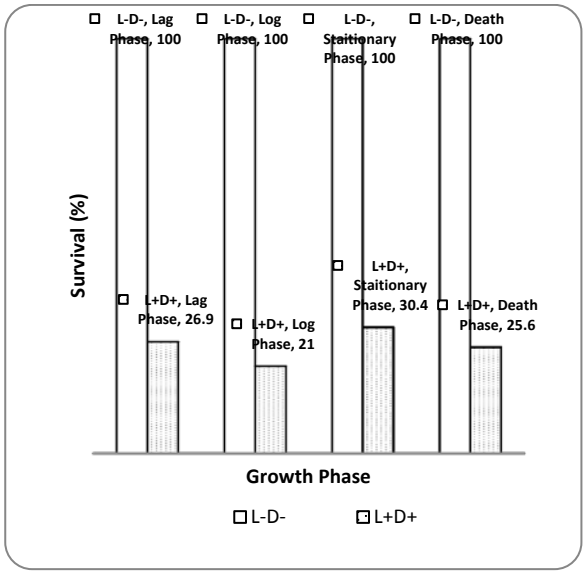


**Figure 5 Effect of growth phase on laser photosensitization using 30µg/ml of TBO, 10mW/cm² power density and 60sec exposure time**

**Ethical Clearance:** It was obtained from the Scientific Research Committee at College of Science /Mustansiriyah University, Iraq

**Financial Disclosure**: There is no financial disclosure.

**Conflict of Interest**: None to declare.

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