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Antibiotic Resistance and Biochemical Characteristics Co- Existence among *Staphylococcus aureus* Exposed to Disinfectants, Photosensitizer and Laser Irradiation

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Abstract: ***Background*:** Laser biostimulation is potentially a useful tool in the treatment of wounds, particularly those cutaneous and subcutaneous wounds that are either complicated by infection by resistant bacteria or inherently require a prolonged period of time to heal. These resistance variants which multiply in hospital environment could lead to serious epidemic outbreaks. Also, the consequence changes in phage susceptibility patterns might be affect the epidemiological reporting. ***Methodology*:** 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 isolated *Staphylococcus aureus* isolates were subjected to susceptibility diffusion discs of penicillin, benzathine penicillin, chloramphenicol, streptomycin, gentamicin, tetracycline, erythromycin, colistin, nitrofurantoin, Fucidin and clindamycin. All strains were exposed to series of disinfectants exposure was as follow: hibitane, Dettol, cetavlon, savlon and providine-iodine. Biostimulation was carried out utilizing the laser light of Helium-Neon (He/Ne) gas type. ***Results*:** The biochemical characters were changed after disinfectants exposure and the negativity increased to urease, arginine decarboxylase and mannitol fermentation. Laser light exposure lead to an increase in negativity to certain biochemical characters like urease and Voges-Proskauer. The resistance frequency to antibiotics was decreased e.g. resistance number to chloramphenicol, benzathine penicillin and gentamicin was, and the 8, 8 and 3 respectively before exposure and then became 7, 6 and 2 respectively after laser light exposure. ***Conclusions*:** three strains were resistant to gentamicin before exposure and the number was increased to ten after second exposure to various disinfectants. Susceptibility of the ten strains to nitrofurantoin did not change after exposure. Laser light exposure led to an increase in negativity to certain biochemical characters like urease and Voges-Proskauer. The resistance of *S. aureus* frequency to antibiotics was decreased. The combination of laser with providine-iodine resulted in a marked reduction in both the frequency of resistant strains and the number of resistance determinants, indicating a strong synergistic antimicrobial effect.

Keywords: Wounds, *S. Aureus*, Disinfectants Exposure, Photosensitizer, Laser, Antibiotics, Biochemical Characteristics.

Research Paper

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INTRODUCTION

Contact with disinfectants in sublethal condition allows survival and multiplication 0f bacteria. It has been found that serial passage of bacteria through diluted disinfectants not only increase minimal inhibitory concentrations, but also brings about phenotypic changes in their antibiogram [1-3]. In a study conducted by Sivaji *et al*., [4], on the effect of disinfectant exposure, they found that *S. aureus* strains become resistant to many antibiotics aftergrowth in series of diluted disinfectants [5, 6]. The mechanisms have not been fully studied and suggested that disinfectants probably altered target sites in bacterial ribosomes making it selectively less susceptible to certain antibiotics [6]. They suggested that enzymes involved in peptidoglycan synthesis might be destroyed causing resistance to penicillin's and cephalosporins. Destruction of periplasmic enzymes by groups of disinfectants is also another contributing factors [7]. The most commonly used disinfectant in microbiology laboratory and hospitals are ethanol, Dettol, chlorohexidine and soap. Ethanol, as a dehydrating agent causes cell membrane damage, denaturalization of protein and cell lyses [4]. Dettol affect bacterial cells by denaturation of protein and also act on the cytoplasmic membrane of microorganisms. Bleach with a main constituent of sodium hypochlorite effect can be achieved by oxidizing of the cell of microorganism of attaching essential cell component including protein, lipid and DNA, while hibitane (chlorohexidine) act by disruption of membranes, precipitation of proteins and inactivation of enzymes [4]. The role of antibiotics in propagating of antibiotic resistance has been extensively studied, other emerging evidence demonstrates the contribution of environmental pollutants, such as microplastics and heavy metals, to this phenomenon. It was previously thought that these pollutants, to be passive contaminants, were playing an active role in driving the evolution of microbial resistance dynamics and amplifying the major threats posed by antimicrobial resistance toward public and environmental health. Effects of co-selection of antibiotic-resistance and metal-resistance genes on antibiotic-resistance potency of environmental bacteria and related ecological risk factors [8]. Resistance genes codifying to Co, Ni (e.g., *rcn*A), and Co, Zn, Cd (*czc*A) have been already identified as dominant in metal-polluted samples, mostly co-existed with resistance to beta-lactams (e.g., *bla*CTX-M, *bla*NDM-1), macrolides (*erm*A, *mef*A, *mph*A), tetracyclines (*tet*A, *tet*M, *tet*W), and other antibiotics [9,10]. Resistance transfer studies and plasmid screening experiments demonstrated that multi-resistance phenotype was due to transmissible plasmid and/or transposons. These strains also shown resistance to cephalosporins which is chromosomaly encoded [11]. Most of hospital infections caused by multiple resistant organisms [12-14], especially multiple resistant *S. aureus* which has been frequently isolated from patients. The literature reviewed suggests that biostimulation with lasers: 1) accelerates the inflammatory phase of wound healing by altering the levels of various prostaglandins, 2) increases ATP synthesis by enhancing electron transfer in the inner membrane of mitochondria, 3) quickens protein (collagen) synthesis by quickening DNA and RNA synthesis, 4) augments fibroplasia by a mechanism that is still being explored, and 5) enhances the ability of immune cells to combat invading pathogens. Although these findings were made in vitro and in vivo in various animal models, their clinical implications are quite clear. Laser biostimulation is potentially a useful tool in the treatment of wounds, particularly those cutaneous and subcutaneous wounds that are either complicated by infection or inherently require a prolonged period of time to heal [6]. These resistance variants which multiply in hospital environment could lead to serious epidemic outbreaks. Also the consequence changes in phage susceptibility patterns might be affect the epidemiological reporting [5, 6]. Low-power lasers deliver low doses of light over long time and cause photochemical effects on irradiated cells. The present study was performed to determine the effect of disinfectants exposure on the antibiotics resistance patterns and on selected biochemical characters of *S. aureus* exposed to laser irradiation with photosensitizer.

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 [4]. 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.

Antibiotic Susceptibility Testing

A loopful growth from 10 isolates of *Staphylococcus aureus* were inoculated into nutrient broth and incubated at 37 ^{0}C for 18 hours. The bacterial suspensions were diluted with ringer solution. The proportion of dilution was 1:1000 [6]. Diluted bacterial suspension were poured onto the surface of the Muller-Hinton agar plates. The excess of bacterial suspensions

were discarded using Pasteur pipette and plates were left for one hour at room temperature to dry. The antibiotic discs which are shown in Table 1 were selectively applied by using sterile forceps which was flamed after being cleansed with alcohol. The plates were incubated at 37 ^{0}C for overnight. The size of zones of inhibition were measured from edge of disc to the edge of inhibition of growth and the result was compared with standard diameter of inhibition zones for each antibiotic utilizing the method of Bauer *et al*., [15]. The following standard strain *Staphylococcus aureus* ATCC25923 was used as a reference strain.

Table 1: Antibiotics used in susceptibility testing

Antibiotic	Code	Concentration (µg/disc)	Manufacturer
Chloramphenicol	C	30	Oxoid *
Gentamicin	CN	10	Oxoid
Tetracycline	TE	30	Oxoid
Erythromycin	E	15	Oxoid
Penicillin G	P	10	Oxoid
Nitrofurantoin	F	300	Oxoid
Fucidin	FU	30	Leo**
Clindamycin	CL	2	SDI
Trimethoprim	TMP	5	SDI
Benzathine penicillin	Bp	6	SDI

*= Oxoid Ltd., Britain; **= Leo Pharmaceutical Products, Denmark; ***= SDI, Samarra Drug Industry, Samarra, Iraq.

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 2. 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 ^{0}C for three hours producing a growth yield of 10^6 – 10^7 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 ^{0}C for 18 hours. Colony from subminimal inhibitory concentration (SMIC) was picked up and inoculated into nutrient broth and incubated at 37 ^{0}C 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 [6]. The strains then kept on nutrient agar slant at 4 ^{0}C for performing biochemical and antibiotic susceptibility testing. The second exposure to the same disinfectants was repeated for the same strains as it was done in the first exposure. The strains after second exposure were kept on nutrient agar slants at 4 ^{0}C for performing the second biochemical and antibiotic susceptibility testing.

Table 2: 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)

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 [14-16].

RESULTS

Effect of Laser Light

The present study shows the biochemical characters and antibiotic resistance before and after second exposure to of disinfectants for the 10 strains of *Staphylococcus aureus* isolated from wounds (Table 3). The biochemical characters were changed after disinfectants exposure and the negativity increased to

urease, arginine decarboxylase and mannitol fermentation. Disinfectants exposure also changed their antibiograms. Generally, resistance was increased after second exposure, e.g. three strains were resistant to gentamicin before exposure and the number was increased to ten after second exposure to various disinfectants. Susceptibility of the ten strains to nitrofurantoin did not change after exposure. Mann–Whitney results revealed that the Overall U was 91.00 and the p = 0.1412 which indicating statistically significant difference was concluded with respect to biochemical characters change after disinfectant exposure. For antibiotics resistance the U was equal to 11.00 with p value = 0.0077, indicating a significant increase after exposure (Figure1).

Table 3: The effect of disinfectant exposure for 10 strains 0f *Staphylococcus aureus* on biochemical and antibiotic susceptibility profiles

Before exposure	After exposure
Biochemical characters	
Urease +(9)*	Urease+ (4)
Coagulase+ (10)	Coagulase+ (10)
Deoxynuclease+ (10)	Deoxynuclease+ (10)
Voges- Poskauer+(10)	Voges- Poskauer+(10)
Mannitol+(10)	Mannitol+(9)
Lactose+(10)	Lactose+(10)
Arginine+(9)	Arginine+(7)
Antibiotics resistance	
Penicillin(9)	Penicillin(10)
Tetracycline(9)	Tetracycline(10)
Trimethprim(8)	Trimethprim(10)
Chloramphenicol(8)	Chloramphenicol(9)
Erythromycin (9)	Erythromycin (10)
Clindamycin(9)	Clindamycin(10)
Gentamicin(3)	Gentamicin(10)
Fucidin(3)	Fucidin(4)
Benzathine penicillin(8)	Benzathine penicillin(9)

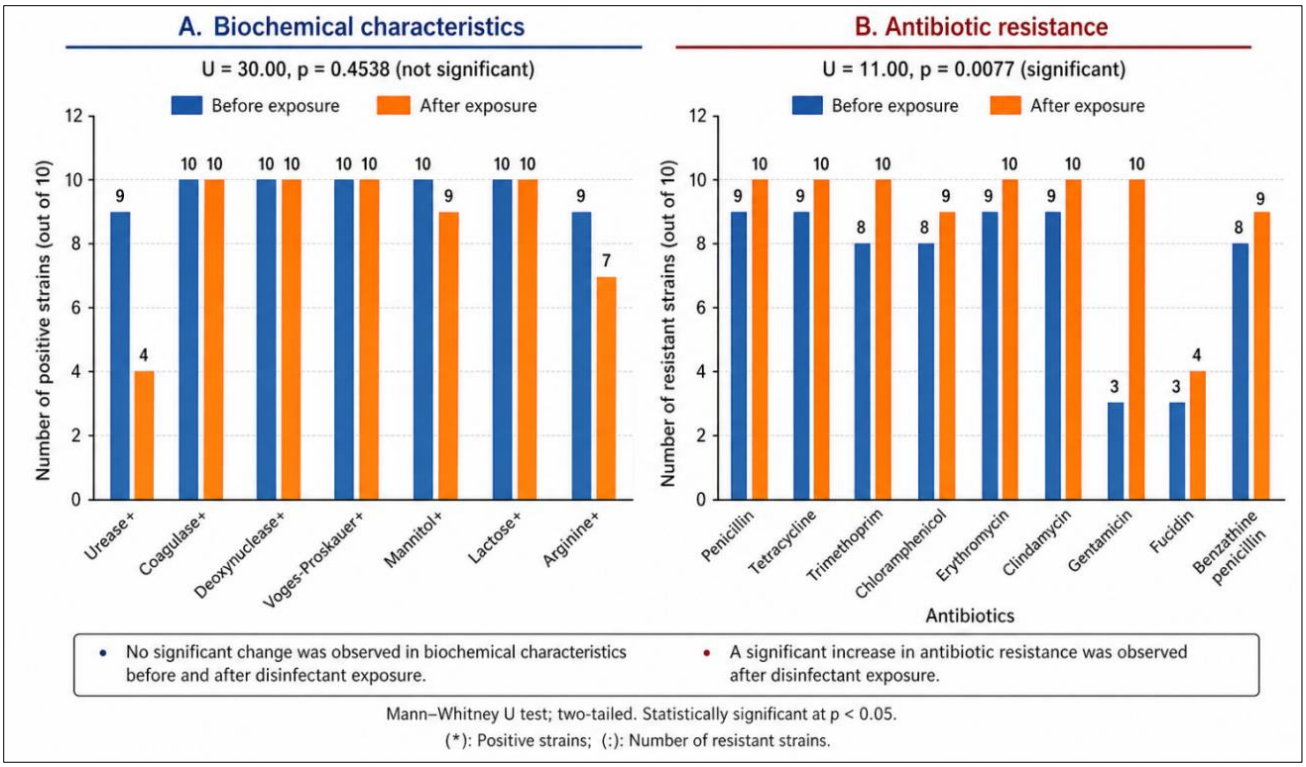

Figure 1: Mann–Whitney U Analysis of Biochemical Characteristics and Antibiotic Resistance in *Staphylococcus aureus* before and After Disinfectant Exposure

The present study also shows the effect of laser light exposure on biochemical characters and antibiogram for the same 10 strains of *S. aureus* under estimation (Table 4). Laser light exposure lead to an increase in negativity to certain biochemical characters like urease and Voges-Proskauer, The resistance frequency to antibiotics was decreased e.g. resistance number to chloramphenicol, benzathine penicillin and gentamicin was, and the 8, 8 and 3 respectively before exposure and then became 7, 6 and 2 respectively after laser light exposure. Disinfectant exposure did not significantly alter biochemical profiles of *S. aureus*. There was a statistically significant increase in antibiotic resistance suggesting possible selection pressure and potential adaptive resistance mechanisms the present analysis supports the hypothesis that disinfectant exposure may contribute to cross-resistance development (Figure 2).

Table 4: The effect of laser exposure for 10 strains of *Staphylococcus aureus* on biochemical and antibiotic susceptibility profiles

Before exposure	After exposure
Biochemical characters	
Urease +(9)*	Urease+ (7)
Coagulase+ (10)	Coagulase+ (10)
Deoxynuclease+ (10)	Deoxynuclease+ (10)
Voges- Poskauer+(10)	Voges- Poskauer+(7)
Mannitol+(10)	Mannitol+(10)
Lactose+(10)	Lactose+(10)
Arginine+(9)	Arginine+(9)
Antibiotics resistance	
Penicillin(9)	Penicillin(9)
Tetracycline(9)	Tetracycline(9)
Trimethprim(8)	Trimethprim(8)
Chloramphenicol(8)	Chloramphenicol(7)
Erythromycin (9)	Erythromycin (9)
Clindamycin(9)	Clindamycin(10)
Gentamicin(3)	Gentamicin(2)
Fucidin(3)	Fucidin(3)
Benzathine penicillin(8)	Benzathine penicillin(6)

( )*, positive strains; ( )**, number of resistant strains

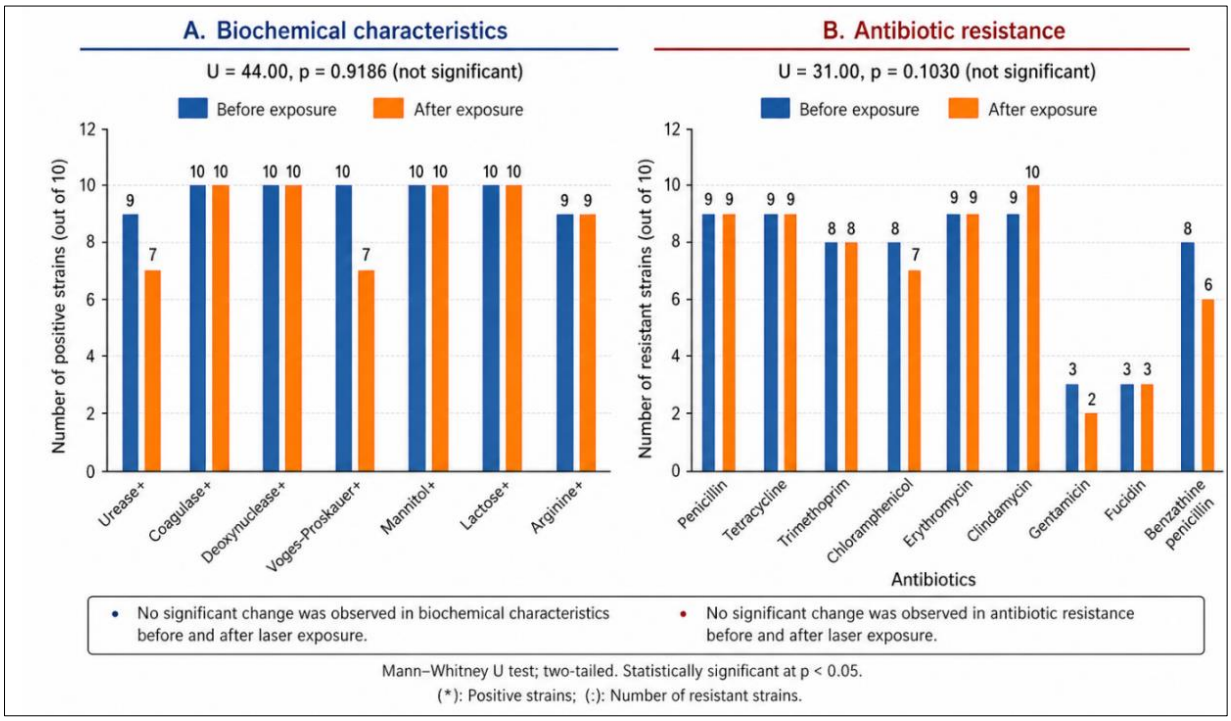

Figure 2: Mann–Whitney U Analysis of the Effect of Laser Exposure on Biochemical Characteristics and Antibiotic Resistance in *Staphylococcus aureus*

Table 5 shows the effect of laser light on the biochemical characters and antibiogram for 10 strains of second disinfectant-exposed strains of S. aureus. It was found that the negativity increased for Voges-Proskauer only and other biochemical characters and antibiotic susceptibility test results did not change after exposure to laser light. Duncan's multiple range test revealed no statistically significant differences ($p > 0.05$) between pre- and post-laser exposure groups in both biochemical characteristics and antibiotic resistance profiles of *Staphylococcus aureus*. Minor variations observed in Voges–Proskauer reaction and benzathine penicillin resistance did not reach statistical significance, indicating that laser exposure has negligible impact on phenotypic and antimicrobial susceptibility patterns (Figure 3).

Table 5: The effect of laser exposure for 10 strains of second disinfectant-exposed *Staphylococcus aureus* on biochemical and antibiotic susceptibility profiles

Before exposure	After exposure
Biochemical characters	
Urease +(4)*	Urease+ (4)
Coagulase+ (10)	Coagulase+ (10)
Deoxynuclease+ (10)	Deoxynuclease+ (10)
Voges- Poskauer+(10)	Voges- Poskauer+(8)
Mannitol+(9)	Mannitol+(9)
Lactose+(10)	Lactose+(10)
Arginine+(7)	Arginine+(7)
Antibiotics resistance	
Penicillin(10)	Penicillin(10)
Tetracycline(10)	Tetracycline(10)
Trimethprim(10)	Trimethprim(10)
Chloramphenicol(9)	Chloramphenicol(9)
Erythromycin (10)	Erythromycin (10)
Clindamycin(10)	Clindamycin(10)
Gentamicin(10)	Gentamicin(10)
Fucidin(4)	Fucidin(4)
Benzathine penicillin(8)	Benzathine penicillin(9)

( )*, positive strains; ( )**, number of resistant strains.

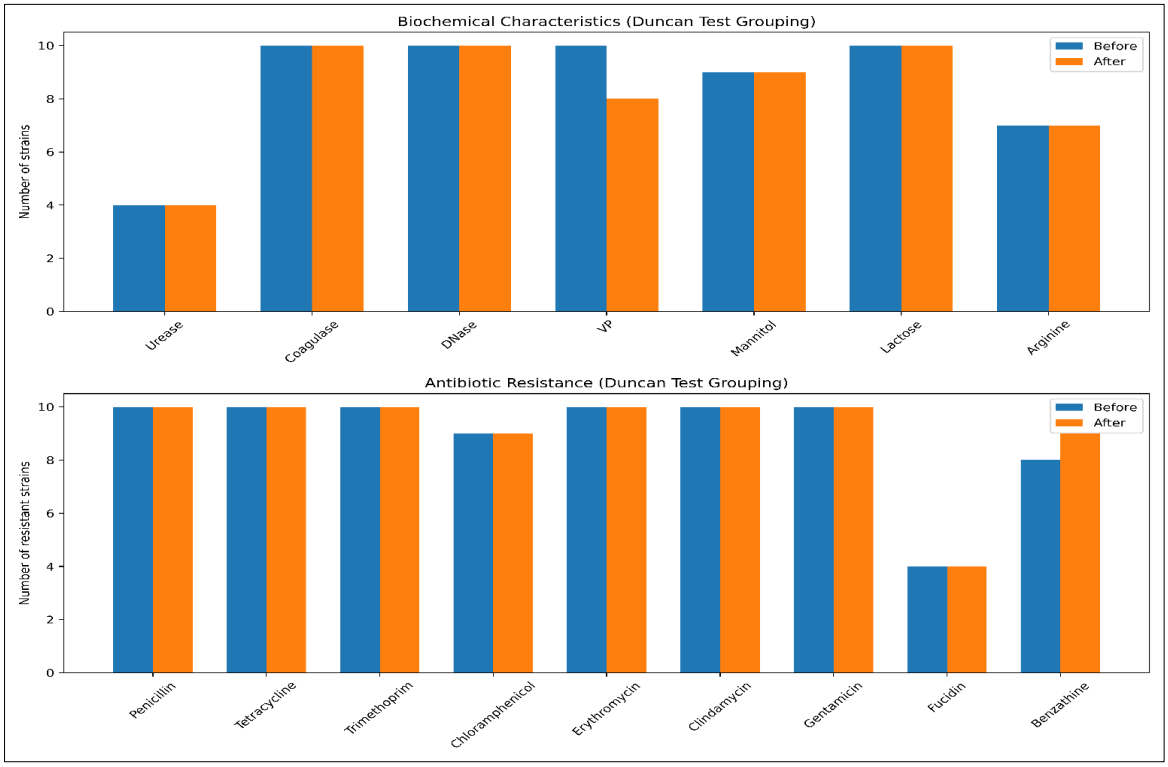

Figure 3: Duncan's Multiple Range Test Showing the Effect of Laser Exposure on Biochemical Characteristics and Antibiotic Resistance of *Staphylococcus aureus* Strains

Effect of Laser and Providine-Iodine Combination

The present study shows the effect of laser-providine-iodine combination on biochemical characters and antibiotic resistance patterns for the 10 strains of second disinfectant-exposed strains of *S. aureus* (Table 6). It was found that the negativity increased for urease, arginine decarboxylase and fermentation of mannitol. The antibiotic susceptibility also changed after exposure to laser-providine-iodine. Generally, there was no increase in the sensitivity to antibiotics after exposure e.g. there was only one strain sensitive to chloramphenicol before exposure to same combination and became resistant to exposure to this combination. Also the sensitivity to tetracycline, penicillin, Fucidin, clindamycin, trimethoprim, benzathine penicillin and colistin before exposure to laser-providine-iodine combination was 0, 0, 4, 0, 0,1 and 0 respectively and the number become 8, 4, 4, 5, 4, 7 and 10 respectively after exposure. Laser + providine-iodine caused moderate reduction in metabolic activity, particularly affecting carbohydrate metabolism (mannitol) and amino acid utilization (arginine). Combined laser and providine-iodine exposure significantly reduced antibiotic resistance in *S. aureus*, with complete elimination of resistance observed for gentamicin, fucidin, and benzathine penicillin. This suggests a strong synergistic antimicrobial effect, likely mediated through disruption of bacterial cell integrity and resistance mechanisms (Figure 4).

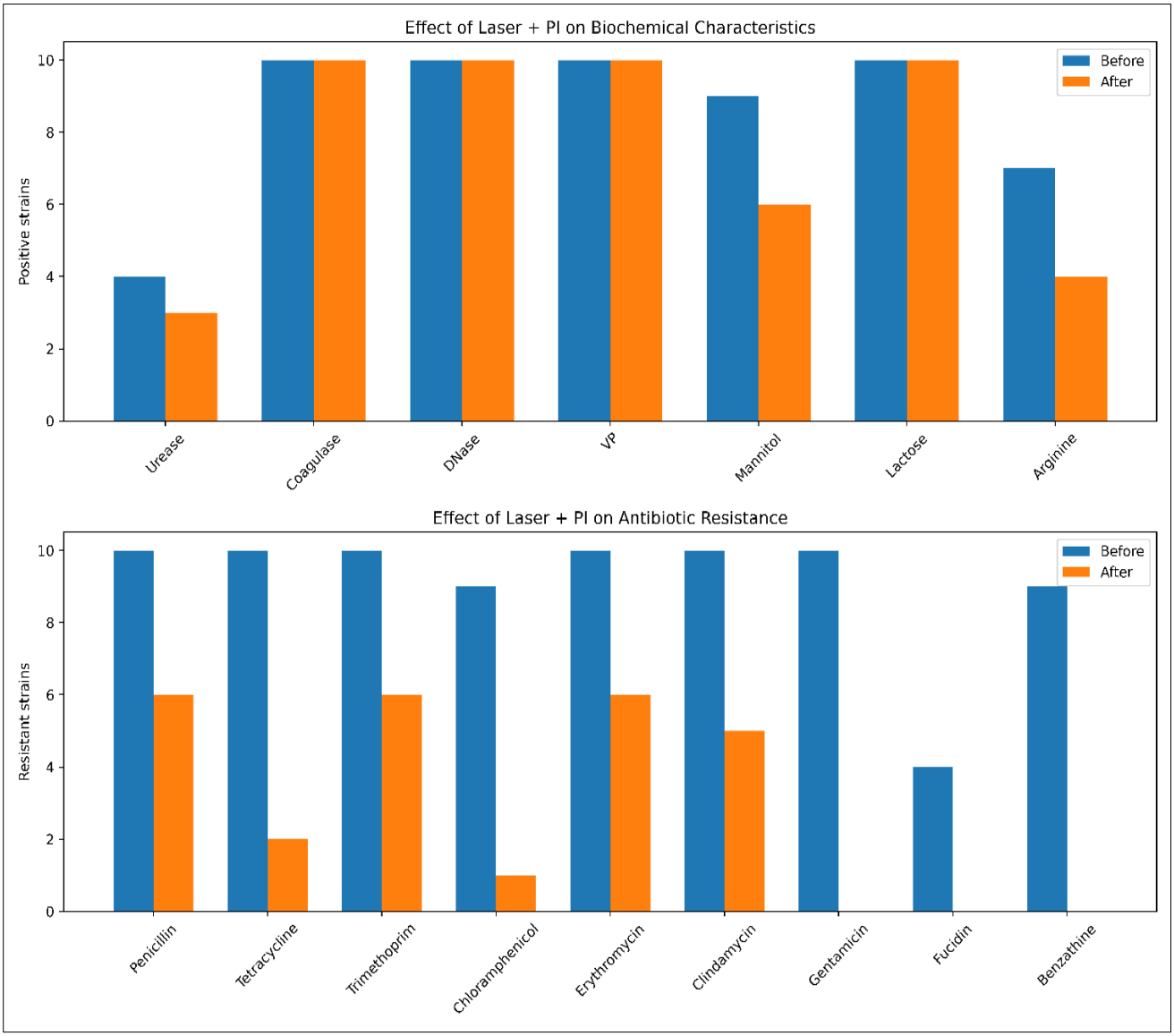

Figure 4: Effect of Combined Laser and Providine-Iodine Exposure on Biochemical Characteristics and Antibiotic Resistance of *Staphylococcus aureus*

Table 6: The effect of laser providine-iodine combination exposure for 10 strains of second disinfectant-exposed *Staphylococcus aureus* on biochemical and antibiotic susceptibility profiles

Before exposure	After exposure
Biochemical characters	
Urease +(4)*	Urease+ (3)
Coagulase+ (10)	Coagulase+ (10)
Deoxynuclease+ (10)	Deoxynuclease+ (10)
Voges- Poskauer+(10)	Voges- Poskauer+(10)
Mannitol+(9)	Mannitol+(6)
Lactose+(10)	Lactose+(10)
Arginine+(7)	Arginine+(4)
Antibiotics resistance	
Penicillin(10)	Penicillin(6)
Tetracycline(10)	Tetracycline(2)
Trimethprim(10)	Trimethprim(6)
Chloramphenicol(9)	Chloramphenicol(1)
Erythromycin (10)	Erythromycin (6)
Clindamycin(10)	Clindamycin(5)
Gentamicin(10)	Gentamicin(0)
Fucidin(4)	Fucidin(0)
Benzathine penicillin(9)	Benzathine penicillin(0)

( )*, positive strains; ( )**, number of resistant strains

Table 7 shows the frequency of antibiotic resistance for the 10 strains of second disinfectant-exposed *S. aureus* before and after exposure to laser and laser-providine-iodine combination. It was found that a significant decrease in resistance after exposure to laser-providine-iodine combination e,g. resistance to penicillin, tetracycline and chloramphenicol was 100, 100, and 90 to be 60, 20 and 10% respectively after exposure. There was a significant difference between groups before exposure and after laser and laser-providine-iodine combination treatment which showed a strong reduction in resistance across most antibiotics while laser alone showed almost no change compared to baseline which indicated that combined laser-providine-iodine was a significantly stronger antibacterial effect (Figure 5).

Table 7: Frequency of antibiotic resistance for 10 strains of second disinfectant-exposed *Staphylococcus aureus* strains isolated from wounds before and after exposure to laser and providine-iodine combinations

Type of exposure	No. of strains resistant to*:										No.(%) of multiple resistant strains**
	P	TE	C	E	CL	CN	FU	BP	F	TMP	
Before e=exposure to L+PI***	10	10	9	10	10	10	4	9	0	10	10
After exposure to laser	10	10	9	10	10	10	4	9	0	10	10
After exposure to L+PI	6	2	1	6	5	0	0	0	0	6	8

*, Penicillin; TE, Tetracycline; TMP, Trimethprim; C, Chloramphenicol; E, Erythromycin; Cl, Clindamycin; CN, Gentamicin; FU, Fucidin; F, Nitrofurantoin. **= Strains resistant to more than one antibiotic at the same time. ***= L, Laser; PI, Providine-iodine.

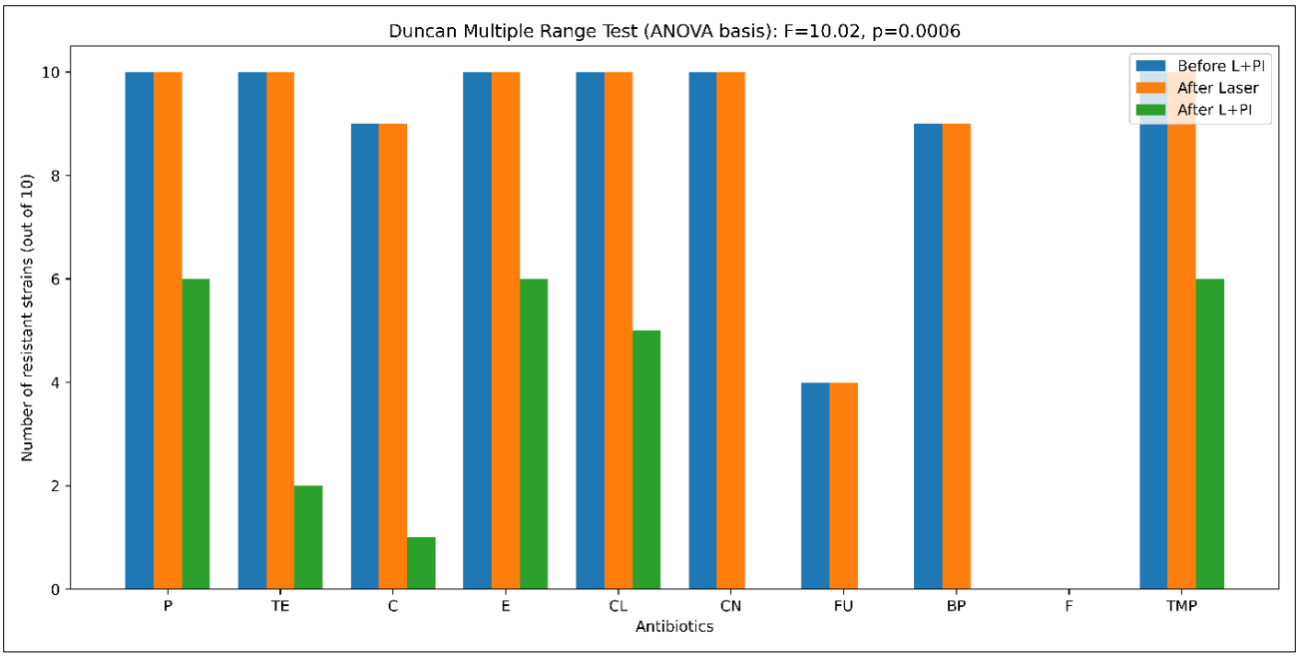

Figure 5: Duncan Multiple Range Test of Antibiotic Resistance in *Staphylococcus aureus* Following Laser and Laser–Providine Iodine Exposure

Table 8 shows the frequency of antibiotic resistance for 10 strains of non-disinfectant exposed *S. aureus* before and after exposure. It was found that resistance to chloramphenicol, gentamicin and penicillin was decreased from 80, 30 and 80 to 70, 20 and 60% respectively. Susceptibility of all strains tested to penicillin, tetracycline, erythromycin, clindamycin, fucidin and trimethoprim did not change after exposure. Laser exposure resulted in a slight reduction in antibiotic resistance frequencies, particularly for chloramphenicol, gentamicin, and bacitracin/penicillin group (BP. Wilcoxon signed-rank test showed no statistically significant difference ($p > 0.05$), indicating that laser exposure did not produce a significant overall change in resistance patterns**.** McNemar test (paired data) did not show statistically significant differences ($p > 0.05$) were observed in antibiotic resistance profiles before and after laser exposure, confirming that laser treatment alone does not significantly alter antimicrobial resistance in *S. aureus* (Figure 6).

Table 8: Frequency of antibiotic resistance for 10 strains of non-disinfectant exposed *Staphylococcus aureus* isolated from wounds before and after exposure to laser light

Type of exposure	No. of strains resistant to*:										No. of multiple resistant strains
	P	TE	C	E	CL	CN	FU	BP	F	TMP	
Before exposure	9	9	8	9	9	3	3	8	0	8	9
After exposure	9	9	7	9	9	2	3	6	0	8	9

*, Penicillin; TE, Tetracycline; TMP, Trimethprim; C, Chloramphenicol; E, Erythromycin; Cl, Clindamycin; CN, Gentamicin; FU, Fucidin; F, Nitrofurantoin. **= Strains resistant to more than one antibiotic at the same time.

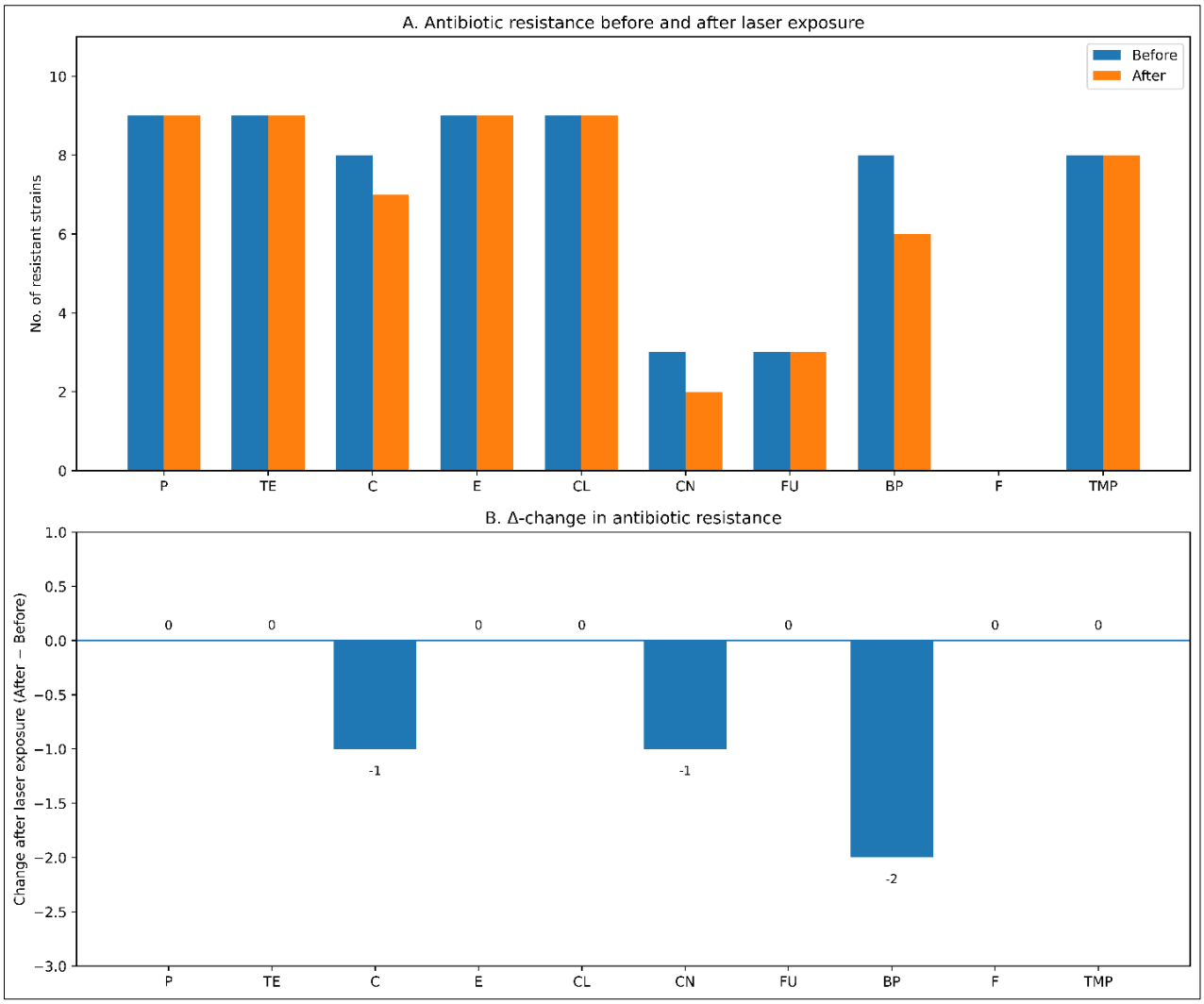

Figure 6: Graphical representation of antibiotic resistance profiles before and after laser exposure, showing minimal changes across most antibiotics with slight reductions in selected agents

The present study revealed the antibiotic resistance patterns for the 10 strains of *S. aureus* tested before and after second disinfectants exposure treatment of these bacteria (Table 9). There were three different resistance patterns seen before exposure to laser and most frequent pattern was chloramphenicol, tetracycline, erythromycin, penicillin, clindamycin, trimethoprim and benzathine penicillin, while no pattern concluded after exposure to laser light only. The antibiotic resistance pattern for the 10 strains secondly-exposed to disinfectants were listed in Table 9. It was found that there were two patterns before exposure to laser light, and the most frequent pattern was chloramphenicol, gentamicin, tetracycline, erythromycin, penicillin, clindamycin, trimethoprim and benzathine penicillin. These patterns did not change after exposure to laser light, while only one pattern concluded after exposure to laser-providine-iodine combination and this pattern was penicillin, clindamycin and trimethoprim. Kruskal–Wallis test (for R-determinants) was utilized which showed that laser exposure alone did not reduce multidrug resistance and may even select for higher resistance complexity. However, the combination of laser with providine-iodine resulted in a marked reduction in both the frequency of resistant strains and the number of resistance determinants, indicating a strong synergistic antimicrobial effect (Figure 7).

Table 9: Frequency of antibiotic resistance patterns occurred twice or more among 10 strains of non-disinfectant and second disinfectant exposed *Staphylococcus aureus* isolated from wounds before and after exposure to laser and laser-providine-iodine combinations

Type of exposure	No. of R-determinants	Resistant patterns to*:	No. of resistant strains	Total
Before exposure to disinfectants	8	C, CN,TE, E, P, CL, TMP, BP	2	6
	8	C, TE, E, P, FU, CL, TMP, BP	2	

	7	C, TE, E, P, CL, TMP, BP	2	
After 2nd disinfectant exposure only	8	C, CN, TE, E, P, CL, TMP	5	9
	8	C, CN, TE, E, P, CL, TMP	4	
After exposure to laser	8	C, CN, TE, P, E, CL, TMP, BP	5	9
	9	C, TE, E, CN, P, FU, CL, TMP, BP	4	
After exposure to Laser+Providine-iodine	3	P, CL,TMP	2	2

*, Penicillin; TE, Tetracycline; TMP, Trimethprim; C, Chloramphenicol; E, Erythromycin; Cl, Clindamycin; CN, Gentamicin; FU, Fucidin; F, Nitrofurantoin. **= Strains resistant to more than one antibiotic at the same time.

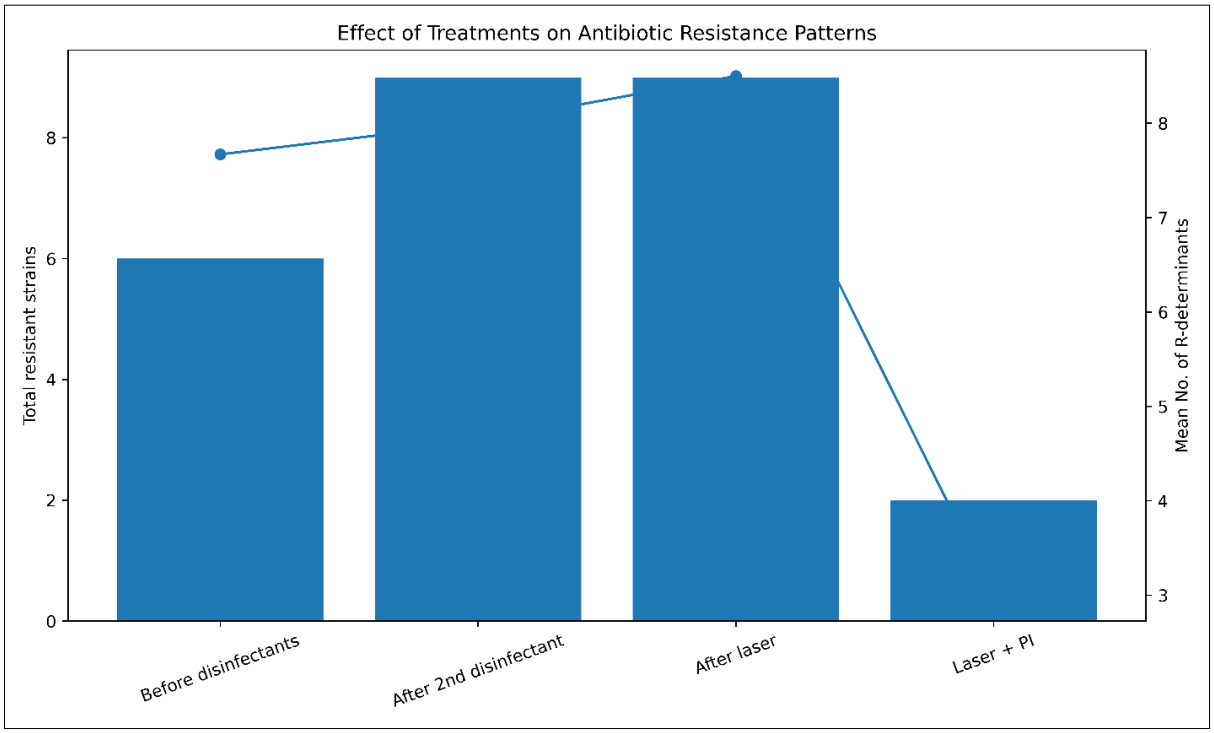

Figure 7: Impact of Laser and Laser–Providine-Iodine Combination on Multidrug Resistance Patterns in *Staphylococcus aureus*

DISCUSSION

Biochemical characters of ten selected strains of *S. aureus* changed markedly after exposure to series of various disinfectants e. g. there were nine strains of staphylococci which showed urease enzyme reduced to be four after exposure to disinfectants i.e. five strains lost their ability to produce this enzyme. Mannitol fermentation and arginine decarboxylation characters were also changed among 1 and 2 strains respectively (Table 3). It was found by other workers that nine strains of *S. aureus* were positive for urease enzyme and then the number of urease-producing strains reduced to be three after second exposure to disinfectants i.e 6 strains lost their ability to produce this enzyme[17, 18]. The present study revealed that most of *S. aureus* strains became resistant to antibiotics after exposure to disinfectants. For example, number of strains resistant to penicillin and gentamicin were 9 and 3 respectively before exposure to disinfectants and resistant strains elevated to 10 for both of antibiotics. Savaji *et al*., found almost the same result in India who showed that serial passages of strains of *S. aureus* through disinfectants at subminimal inhibitory concentration (SMICs) induced antibiotic resistance in almost 30% of the bacteria studied [4]. Al-Jebouri also found almost the same effect on the pathogen tested [5-19]. The increase in antibiotic resistance could be explained by the fact that the disinfectant cause destruction of periplasmic enzymes and cause decrease in the uptake of antibiotic [4]. Moreover, the disinfectants probably altered the target site in bacterial ribosome making it less susceptible to some of these antibiotics [11].

The present study also shows the effect of laser light exposure on biochemical characters and antibiogram for the same 10 strains of *S. aureus* under estimation (Table 4). Laser light exposure lead to an increase in negativity to certain biochemical characters like urease and Voges-Proskauer, The resistance frequency to antibiotics was decreased e.g. resistance number to chloramphenicol, benzathine penicillin and

gentamicin was, and the 8, 8 and 3 respectively before exposure and then became 7, 6 and 2 respectively after laser light exposure. Disinfectant exposure did not significantly alter biochemical profiles of *S. aureus*. There was a statistically significant increase in antibiotic resistance suggesting possible selection pressure and potential adaptive resistance mechanisms The present analysis supports the hypothesis that disinfectant exposure may contribute to cross-resistance development. Moreover, Al-Jebouri and Al-Bayati found that biochemical characters of eleven selected strains of *S. aureus* were changed markedly after exposure to disinfectants e.g. there were nine strains of *S. aureus* showed urease activity before exposure to disinfectants, then the number of urease -producing strains became seven and then three after the first and after the second exposure respectively [17]. The present study showed that disinfectant exposure caused progressive and multidimensional changes in both biochemical traits and antibiotic resistance, with the biggest jump was clearly seen between after first and after second exposures [18].The same change was almost found by other workers who found that the number of urease positive strains were six before the first disinfectant exposure and all strains became urease negative after the first and second exposure to a series of disinfectants [6]. These changes might be due to disturbance in the transport mechanism from and to the bacterial cell as reported by Bergman who found that exposure of *E.coli* strains to thiolutine lead to an inhibition of carbon utilization by exposed strains [19]. Furthermore, it was found elsewhere that disinfectants exposure lead to destruction of periplasmic enzymes and consequently changes in the biochemical characters of the exposed strains [20-27]. 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 clinical bacterial isolates 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 [28-29].

It was found that there were two patterns before exposure to laser light, and the most frequent pattern was chloramphenicol, gentamicin, tetracycline, erythromycin, penicillin, clindamycin, trimethoprim and benzathine penicillin. These patterns did not change after exposure to laser light, while only one pattern concluded after exposure to laser-providine-iodine combination and this pattern was penicillin, clindamycin and trimethoprim. Kruskal–Wallis test (for R-determinants) was utilized which showed that Laser exposure alone did not reduce multidrug resistance and may even select for higher resistance complexity. However, the combination of laser with providine-iodine resulted in a marked reduction in both the frequency of resistant strains and the number of resistance determinants, indicating a strong synergistic antimicrobial effect. Bacterial resistance to antibiotics and other chemotherapeutic agents is a phenomenon that has been known for many years [11-18]. In the present study, strains of *S. aureus* frequently showed shared resistance to pairs of antibiotics such as ampicillin and erythromycin, ampicillin and trimethoprim. The same resistance association was found by Macnil and his colleagues who found that strains of methicillin-resistant *S. aureus* isolated from Australian teaching hospitals frequently showed resistance to many other antibiotics [30]. The result of present study showed that resistance is frequently multiple and that cross-infection in the hospital plays an important role in the resistance of microorganisms to antibiotics. Furthermore, nitrofurantoin and Fucidin were two effective drugs and showed no shared resistance with any antibiotic used. This is possibly due to absence of genetic markers to be combined with those responsible for resistance of other antibiotics [31]. The percent of resistance to pairs of antibiotics was not symmetrical as shown in Table 4, e.g. resistance between penicillin G and streptomycin. In general, other similar findings were found elsewhere [33-35]. Richardson and Marples found the same finding among isolates of *S. epidermidis* [32]. The common mechanism by which bacteria become resistant to antibiotics is the modification of the antibiotic's target like penicillin-binding proteins (PBPs) which leads to resistance to β-lactam drugs [24,-38]. The mechanism of resistance reflects the amount of drug that can combine to the target is affected by changes in the number of PBPs [24]. A structural modification e.g. the development of the mecA gene in *S. aureus* will decrease or might completely prevent drug binding to the target [25-40]. The methylase of the erythromycin ribosome (erm) gene family, will methylate 16S rRNA and changes the drug-binding site leads to resistance to macrolides, streptogramins, and lincosamines due binding prevention with them [26-42]. Resistance mediated by changes in DNA gyrase or topoisomerase IV leads to inactivation of fluoroquinolones by inhibit nucleic acid synthesis. These mutations lead to the composition change of gyrase and topoisomerase to reduce or exclude the drug's ability to link to these components [27-46]. However, the present findings revealed that exposure of some hospital *Staphylococcus* to disinfectants could change the antibiotic sensitivity pattern, and this might lead to the erroneous conclusion that strains are unrelated. In these circumstances other typing methods are required. Antimicrobial photodynamic inactivation (aPDI) is starting to be considered promising in the fight against antibiotic resistant microorganism [47-49]. In contrast to conventional antibiotics, the broad spectrum of action of aPDI reduces the likelihood of bacterial resistance as it acts simultaneously on multiple cellular components [50-52]. In addition, the localized and selective properties of aPDI are particularly interesting for the treatment of topical and superficial bacterial infections, including acute *S. aureus* skin wound infections [53-56]. Topical antibiotics have been found

to accelerate the development of resistance compared to these taken by oral or intravenous routes of administration, further emphasizing the promising role of aPDI in the treatment of skin wound infections [57-60]. During the aPDI process, it is crucial to employ light of a specific wavelength that aligns with the absorption peak of the photosensitiser molecules in the presence of oxygen [61-65]. The adequate wavelength selection ensures optimal excitation of the photosensitiser, thereby maximising its antimicrobial activity. Despite the wide range of known photosensitisers, natural molecules have recently garnered significant attention [66-69]. Their use holds potential for reducing toxicity to healthy tissues and minimizing side effects, making them a sustainable and environmentally friendly way to fight a broad spectrum of microbial pathogens, including those multi-resistant [70-74]. In particular, phytochemicals (molecules from the secondary metabolism of plants) with photosensitising properties have been studied in aPDI [75-78]. Additionally, these molecules can act as antibiotic adjuvants helping to circumvent resistance mechanisms. This effect may be attributed to functional groups such as electrophiles (*e.g.,* epoxides, Michael acceptors and strained β-rings), which allow phytochemicals to exhibit a differentiated profile of activity with multiple cellular targets [79-81]. This means that they can simultaneously act on different structural and functional cellular targets and thus sensitize the bacteria to the effect of antibiotics [82-86]. Therefore, phytochemicals are starting to be recognized as relevant for restoring antibiotic activity and expanding their spectrum of activity [87-92]. Furthermore, wavelength is crucial for both absorption by chromophores and the depth of tissue action. Red light (600–700 nm) exhibits strong absorption by cytochrome c oxidase and mitochondrial flavoproteins, favoring ATP synthesis and cell proliferation in epidermal and superficial dermal layers. Near-infrared light (780–950 nm) has greater penetration capacity (5–10 mm), reaching fibroblasts, endothelial cells, and vascular structures located in the dermis and hypodermis [93-97]. Chronic wounds represent a major clinical challenge due to delayed healing, susceptibility to infections, and substantial functional and economic burdens [98, 99]. Although this review presents consistent preclinical evidence, the relevance of these cellular responses to the context of chronic wounds is still uncertain, given that these models exhibit persistent inflammation, hypoxia, phenotypic alterations of fibroblasts, and vascular compromise [100-103]. Therefore, the next translational step should focus on rigorously characterized chronic animal models capable of reproducing this pathophysiological complexity.

CONCLUSIONS

Three strains were resistant to gentamicin before exposure and the number was increased to ten after second exposure to various disinfectants. Susceptibility of the ten strains to nitrofurantoin did not change after exposure. Laser light exposure lead to an increase in negativity to certain biochemical characters like urease and Voges-Proskauer. The resistance frequency to antibiotics was decreased. The combination of laser with providine-iodine resulted in a marked reduction in both the frequency of resistant strains and the number of resistance determinants, indicating a strong synergistic antimicrobial effect. Laser + providine-iodine caused moderate reduction in metabolic activity, particularly affecting carbohydrate metabolism (mannitol) and amino acid utilization (arginine). Combined laser and providine-iodine exposure significantly reduced antibiotic resistance in *Staphylococcus aureus*, with complete elimination of resistance observed for gentamicin, fucidin, and benzathine penicillin. This suggests a strong synergistic antimicrobial effect, likely mediated through disruption of bacterial cell integrity and resistance mechanisms.

Consent: Consent was obtained from patients who participated in the present study.

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

Conflicts of Interest: The authors declare that they have no conflicts of interest, financial or otherwise.

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