The effect of heat-shock on total viable counts of *Staphylococcus aureus* isolated from wounds and exposed to various photochemical and disinfectant agents

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Abstract

Four strains of *Staphylococcus aureus* were isolated from patients hospitalized in Saddam teaching hospital of Tikrit. The strains before and after laser irradiation were exposed to heat-shock at 44, 50, 55 and 60 C° and the total viable counts were determined. The present study showed that high populations were completely destroyed at 60 C° for 30 min. The synergistic effect of laser irradiation plus heat is might be due to the inability of cells to repair radiation damage because heating inactivates repair enzymes.

Keywords: *Staphylococcus aureus*, heat-shock, laser, disinfectants, TVC's.

Introduction

The possibility of using heat in combination with irradiation was firstly suggested in the 1950's when synergistic effect was observed with a variety of biological systems including bacteria. There is still some debates about whether irradiation should be applied simultaneously, prior to, or after heating, but it is generally agreed that a significant synergistic effect on the destruction of vegetative bacterial cell and spores can be achieved by the combination (1,2). Thayer *et al.* investigated the effect of heat and ionizing radiation on *Salmonella typhimurium,* and reported that irradiation to a dose of 0.9 KGY caused heat-sensitization in this organism (2).

Lage and Menezes suggested an antagonistic effect between heat-shock and UV radiation when they found that prior heat-shock treatment of *Escherichia coli* increases its resistance to 254 nm radiation. The same heat-shock induced resistance was observed after 280 nm radiation with UV light (3). The present study was suggested to assess the effect of heat-shock without photochemical agents for the killing of *Staphylococcus aureus* isolated from the wounds of hospitalized patients in Saddam teaching hospital of Tikrit City.

Materials and Methods

Samples were taken from patients residented in Saddam teaching hospital of Tikrit City. Swabs were taken from wounds of patients on the third postoperative day. The samples were transported in test tube containing 2 ml nutrient broth (4).

The samples were enriched in nutrient broth at 37 C° for 18 hrs. Each sample was subcultured on blood agar and mannitol salt agar. The inoculated plates were incubated at 37 C° for 24 hrs. The suspected colonies of *Staphylococcus aureus* were purified in duplicate nutrient agar plates. The purified colonies were further identified using the conventional methods (5).

Four strains of *Staphylococcus aureus* before and after laser irradiation were exposed to heat- shock according to method described elsewhere (3). For experimental purposes, each strain was grown in nutrient broth at 37 C° for 3 hrs in a shaker. Heat-shock treatment were administered by transferring 5 ml of the culture to a sterile test tube and placed in a water bath at 65 C° for 30 min. The organism then was harvested by centrifugation for 8000 rev. min for 5 min and resuspended in an equal volume of 0.9% (w/v) saline. 10-fold serial dilution of the contents of each tube was prepared in sterile nutrient broth and duplicated 50 ml aliquots

were spread over the surface of blood agar plates and incubated for overnight at 37 C°. The resulting colonies were counted. Control tubes identical to those described above, were prepared and incubated at 37 C° for 30 min. The test was repeated at 60, 55, 50 and 45 C° for all strains. The heat-shocked strains were kept on nutrient agar slants at 4 C° for further investigations.

Exposure of Staphylococcus aureus to disinfectants:

Strains of *Staphylococcus aureus* were exposed to a series of dilution of hebitane, dettol, cetrimide, savlon and providine-iodine according to methods described elsewhere (6).

Effect of laser-providine-iodine combination on Staphylococcus aureus Lethal photosensitization of *S. aureus* was done according to the method described by Al-Jebouri and Al-Obaidi (7).

Results

Tables (1,2,3 and 4) showed the effect of heat-shock on total viable counts (TVC's) of four strains of ***S. aureus*** isolated from the wounds of patients. It was found that there were no significant decreases in TVC's among all firstly and secondly-disinfectant exposed strains ($P>0.05$) using Dancan's test when these strains were exposed to 44 C°. But one strains were showed a significant decrease in TVC's ($P<0.05$) using the same statistical tast (Table 1). It was found that there was no significant decrease in TVC's ($P>0.05$) among all non-disinfectant exposed strains except for those exposed to laser-providine-iodine and laser-toluidine blue O combination which showed a significant decreases in TVC's ($P<0.05$) when exposed to 50 C° for 30 min (Table 2).

Table (3) showed a significant decrease in TVC's among all non-disinfectant, firstly and secondly disinfectant-exposed strains ($P<0.05$) when they exposed to 55 C° for 30 min. The effect of heat-shock at 60 C° for 30 min on TVC's for all strains (disinfectant exposed and photochemical agent-exposed) of *S. aureus* was statistically significant and the TVC's decreased ($P<0.05$) using Dancan's test (Table 4).

Discussion

Tables (1,2,3 and 4) showed that *S. aureus* is not a particular heat-resistant bacterial pathogen and even high populations would be completely destroyed with 60 C° for 30 min. This result was almost similar to that found by other workers, who found that high population of *S. aureus* could be completely destroyed by conventional pasteurization treatment (8). Also, it was found that exposure to photochemical agents led to radiation induced heat-sensitization in *S. aureus* strains. The same result was found by Thayer *et al.* (2) who found that ionizing radiation caused heat sensitization in *Salmonella typhimurium.*

Moreover, it was found that radiation-induced heat-sensitization was occurred among *Listeria monocytogenes* isolates (9). In contrast, Lage and Menezes found that heat-shock enhanced the survival of *Escherichia coli* after UV (254 or 280 nm) radiation, but the results indicated that thermotolerance was not achieved after UV irradiation of *E. coli* (3). The possibility of using heat in combination with irradiation was firstly suggested in the 1950's when synergistic effect were observed with a variety of biological system including bacteria. However, there is still some debates about whether irradiation should be applied simultaneously with, prior to, or after heating, but it is generally agreed that a significant synergistic effect on the destruction of bacteria can be achieved by the combination (1).

The synergistic effect of irradiation plus heat is due to the inability of cell to repair radiation induced damage because heating inactivates repair enzymes. The longer the time period which elapses between irradtion and heating, the more likely it is that the cell will be able to repair radiation damage and regain its original heat resistance (10). It was found that a low dose of gama radiation results in heat-sensitization of *Listeria monocytogenes* and this sensitization persist for up to 2 weeks at 2-3 C (9). However, the present results need further investigation particularly on molecular level.

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Table(1): The effects of heat-shock (44 C° for 30 min.) on total viable counts (TVCs) for four non- disinfectant-exposed, firstly-disinfectant-exposed and secondly-disinfectant-exposed strains of *Staphylococcus aureus* isolated from wounds.

Exposure to heat-shock*	Type of photochemical exposure **	TVCs & type of disinfectant-exposed strains											
		TVCs x 10 for non-exposed strains				TVCs x 10 for firstly-exposed strains				TVCs x 10 for secondly-exposed strains			
		1	2	3	4	1	2	3	4	1	2	3	4
BH	Before	485	490	500	505	495	500	515	495	490	510	500	490
AH	Before	480	465	505	490	475	470	410	350	495	490	495	490
BH	L	490	495	505	485	490	490	495	490	510	500	490	495
AH	L	475	470	470	460	480	470	470	435	480	475	470	475
BH	L-TBO	485	505	510	445	500	505	500	495	480	510	505	500
AH	L-TBO	450	465	470	395	480	480	475	485	390	430	460	475
BH	L-PI	510	515	505	595	470	480	500	510	545	525	515	530
AH	L-PI	450	475	460	330	460	475	420	480	475	450	455	480

*, BH = Before heat-shock; AH = After heat-shock.
**, L = Laser; TBO = Toluidine blue 0; PI = Providine-iodine.

Table (2): The effects of heat-shock (50 C° for 30 min.) on total viable counts (TVCs) for four non- disinfectant-exposed, firstly-disinfectant-exposed and secondly-disinfectant-exposed strains of *Staphylococcus aureus* isolated from wounds.

Exposure to heat-shock*	Type of photochemical exposure **	TVCs & type of disinfectant-exposed strains											
		TVCs x 10 for non-exposed strains				TVCs x 10 for firstly-exposed strains				TVCs x 10 for secondly-exposed strains			
		1	2	3	4	1	2	3	4	1	2	3	4
BH	Before	540	530	515	535	495	505	600	545	530	530	490	510
AH	Before	515	485	455	500	395	230	310	325	180	295	205	215
BH	L	515	540	540	585	530	595	550	490	515	390	485	505
AH	L	320	345	390	305	205	235	154	95	120	165	150	180
BH	L-TBO	495	510	535	530	605	590	545	530	515	490	485	500
AH	L-TBO	205	195	200	100	215	220	95	100	170	200	135	195
BH	L-PI	515	530	480	495	510	535	530	500	495	505	500	510
AH	L-PI	190	155	140	90	185	180	90	105	125	190	140	180

*, BH = Before heat-shock; AH = After heat-shock.

**, L = Laser; TBO = Toluidine blue 0; PI = Providine-iodine.

Table (3): The effects of heat-shock (55 C° for 30 min.) on total viable counts (TVCs) for four non- disinfectant-exposed, firstly-disinfectant-exposed and secondly-disinfectant-exposed strains of *Staphylococcus aureus* isolated from wounds.

Exposure to heat-shock*	Type of photochemical exposure **	TVCs & type of disinfectant-exposed strains											
		TVCs x 10 for non-exposed strains				TVCs x 10 for firstly-exposed strains				TVCs x 10 for secondly-exposed strains			
		1	2	3	4	1	2	3	4	1	2	3	4
BH	Before	520	510	515	480	485	395	500	515	485	490	515	515
AH	Before	510	490	495	445	141	123	100	138	95	180	100	82
BH	L	510	515	540	535	515	510	495	505	390	420	475	485
AH	L	155	145	310	62	15	100	4	15	19	100	5	29
BH	L-TBO	520	510	485	410	485	510	485	400	415	510	485	435
AH	L-TBO	104	136	80	0	66	53	24	0	60	100	10	0
BH	L-PI	480	390	415	410	490	485	415	410	425	395	405	415
AH	L-PI	91	30	51	0	58	37	0	0	23	100	10	0

*, BH = Before heat-shock; AH = After heat-shock.
**, L = Laser; TBO = Toluidine blue 0; PI = Providine-iodine.

Table-4. The effects of heat-shock (60 C° for 30 min.) on total viable counts (TVCs) for four non-disinfectant-exposed, firstly-disinfectant-exposed and secondly-disinfectant-exposed strains of *Staphylococcus aureus* isolated from wounds.

Exposure to heat-shock*	Type of photochemical exposure **	TVCs & type of disinfectant-exposed strains											
		TVCs x 10 for non-exposed strains				TVCs x 10 for firstly-exposed strains				TVCs x 10 for secondly-exposed strains			
		1	2	3	4	1	2	3	4	1	2	3	4
BH	Before	510	495	480	395	500	485	500	395	480	510	505	490
AH	Before	0	0	0	0	1	0	0	45	0	0	0	0
BH	L	500	480	490	415	310	435	475	450	460	480	500	485
AH	L	0	0	0	0	0	0	0	0	0	0	0	3
BH	L-TBO	495	390	480	450	445	505	480	420	415	400	490	495
AH	L-TBO	1	0	0	0	0	1	0	0	0	0	0	0
BH	L-PI	400	515	535	500	515	525	490	505	410	430	415	480
AH	L-PI	0	0	0	0	0	0	0	0	0	0	0	0

*, BH = Before heat-shock; AH = After heat-shock.
**, L = Laser; TBO = Toluidine blue 0; PI = Providine-iodine.