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Combination Effect of Laser, Antibiotics and Different Temperature on Locally Isolated Pseudomonas aeruginosa

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Abstract: In humans, Pseudomonas aeruginosa is the second most frequent gram negative nosocomial pathogen in hospitals and has the highest case-fatality rate of all hospital-acquired bacteremia because of the hardy resistance of these bacteria to mechanical cleansing as well as to disinfectant, and many antibiotics. The susceptibility of bacteria against the antibiotics is modulated by several local factors such as temperature which modified drug efficacy, so this study was carried out to evaluate the effect of different temperature (20,42,45)Ċon the susceptibility of Pseudomonas aeruginosa to the minimum inhibitory concentrations (MIC) of the antimicrobial agents before and after irradiation. The samples collected from 150 persons suffering from burns-wounds infections, thirty-five isolates of pseudomonas aeruginosa bacteria were obtained depending on morphological and biochemical tests. Following exposure of Pseudomonas aeruginosa isolates to the diode laser with 805nm wavelength,3W output power and (5,10,15) minutes exposure times in combination with different temperature and different concentrations of (cefotaxim, amikacin, chloramphenicol) antibiotics, highly observable change in the MIC value was achieved , the bacterial isolates became sensitive to chloramphenicol at the three exposure times and 100% killing of the cells was observed at 15 minutes exposure time at temperature 45Ċ in absence of the antibiotics. In conclusion, 3W diode laser in combination with temperature 45Ċ was the best condition that reduces the MIC value, and killing bacteria at 10 minutes exposure time.

Introduction

Since the last century, the laser occupied a large degree of attention in the scientific and technological fields. Invention of laser causes a chain of important changes in the science development especially in physics, chemistry, biology and electronics, in addition to its industrial and medical applications. For this reason the laser enters in many fields and introduces solution to many problems.

Many lasers have been used successfully for treating many cases of infection that caused by bacteria such as E.coli, Staphylococcus aureus and Pseudomonas aeruginosa. The resistance of Pseudomonas aeruginosa to many antibiotics form a big problem, especially in burns and wounds infections. Many studies have been introduced to investigate the effect of laser on microorganisms,a lotof their were related to the susceptibility of bacteria to antibiotics. This work is a trial in this regard.

Pseudomonas aeruginosa

One of the most common microorganisms encounted in hospital infection. It was isolated from various sources like air, floor, sinks and even disinfectant (Iglewski, 1980).

Pseudomonas aeruginosa is frequently present in small numbers as normal intestinal flora and on the skin of humans (Bradshaw, 1973). The most notable properties of Pseudomonas aeruginosa are its natural resistance to antibiotics and disinfectants and its

ability to inflict serious injury to immuno comprised patients (Moore, 1997).

Morphology

Pseudomonas aeruginosa is motile and rod shaped measuring about 0.6x2µm. It is gram negative and occurs as single bacteria, in Paris, or in short chains. Colonies are flat with feathered edge and its diameters reach 3 to 5mm within 48h incubation at 37Ċ (Sneath *et al*., 1986; Jawetz *et al*., 1991).

Pseudomonas aeruginosa is the only species known to excrete pyocyanin, so it is sufficient for identification such species (Iglewski, 1980; Singleton, 1997).

Culture Characters

Pseudomonas aeruginosa grows readily on most common diagnostic media (e.g MacConkey agar). Growth on blood agar may produce diffuse haemolysis.

Culture produce grap–like odor of aminoacetophenone. Temperature range 5-42Ċ optimum 37Ċ. Unlike other pseudomonas, pseudomonas aeruginosa has the ability to grow at 42Ċ .The optimum pH for growth is ranging from 7.4 to 7.6 (Collee *et al*., 1996; Forbes *et al*., 1998). On MacConky agar, the colonies appear greish yellow without fermentation of lactose sugar (El-Ghoroury *et al*., 1970).

Biochemical Reactions

In general, Pseudomonas aeruginosa appears inert in the typical tests that were used for fermentation tests of gram negative bacilli, e.g. Indol and H2S are not produced. Voges–proskauer is negative and methyl red reaction is positive (Collee *et al*., 1996).

Acids are produced only from catalyzed glucose, the other four sugars (lactose, sucrose, maltose, and mannite) are not attacked by the bacteria. Gelatin is liquefied and the growth is well on citrate media (Sneath *et al*., 1986; El-Ghoroury *et al*., 1970).

Pseudomonas aeruginosa is oxidase positive, although a few gram negative bacilli are also oxidase positive but can't react as swiftly as Pseudomonas aeruginosa which gives a positive reaction in less than 30 seconds (Bhatia *et al*., 1994).

Effect of Temperature on Microorganisms

Temperature is one of the most important factors that influence growth of cells. Cells grow within a well defined temperature growth range, this growth range is defined by a minimum temperature below which cells are metabolically inactive and a maximum temperature above which cells do not grow. Within this range of extreme is an optimal growth temperature at which cells exhibit their highest rates of growth and reproduction (Atlas, 1995). The optimum temperature for growth of parasitic bacterial to man is 37Ċ which is the temperature provided by the incubator (El-Ghoroury *et al*., 1970).

Although some heat is required for the growth of microorganisms, excessive heat will cause their destruction and low temperature (3-5Ċ) will stop their multiplication and allow them to survive for a relatively long time (Todar, 1997; Todar, 2004).

Bacteria that grow best at low temperatures (<20Ċ) are called sychrophiles, those that reproduce fastest at moderate temperature (20 to 40Ċ) are called mesophiles, and those with fastest growth rates at high temperature (>40Ċ) are called thermophiles (Pirt, 1983). Above maximum growth temperature, biochemical changes in the cell's organic molecules or chemical break downs of structural molecules, especially in the cell membrane. Heat also drives off water, and since all organisms defend on water, this loss may be lethal (Alcamo, 1998).

Also the heat kills microorganisms by denaturing their proteins which involves changes in the chemical or physical properties of protein. Denaturation includes structural alterations due to destruction of the chemical bonds holding proteins in a three-dimensional form. As proteins revert to a two-dimensional structure they coagulate (denature) and been nonfunctional (Pirt, 1983).

Materials and Methods

The solutions and reagents:- oxidase test reagent, catalase indicator, Kovac's reagent, methyl red reagent, Voges – Proskauer reagent Physiological saline solution, Standard McFarland solution, stock solutions of the used antibiotics (Cefotaxim: by dissolving 1mg of cefotaxim in 1ml of sterile distilled water to get 1mg/ml concentration), (Chloramphenicol: by dissolving 1mg of chloramphenicol in 1ml of 95% ethanol to get 1mg/ml concentration) (Todar, 2004), (Amikacin: by dissolving 500mg of amikacin in 2ml of physiological saline solution to get 250 mg/ml concentration).

Culture Media

Blood agar, Brain – heart infusion broth , Kligler iron agar, MacConky agar, Muller – hinton broth, Nutrient agar, Nutrient broth, Pepton, SimmonCitrate agar, Cetrimide Agar.

Samples Collection

150 samples were collected from patients suffering from wounds – burns infections by using sterile swabs. Samples were collected from Al-Kendy Hospital (burns unit).

Isolation and Identification of Pseudomonas aeruginosa

After collection the samples from infected wounds and burns, samples were identified according to Bergeys manual using different morphological and biochemical tests. First the samples were grown on blood agar and MacConky agar, incubated at 37Ċ for 24h.

The colonies that give pale green were picked and cultivated on the cetrimid medium to observe the pigment production from the bacteria. The growing bacteria on the latter medium were cultivated on nutrient agar and incubated for 24h once at 4Ċ and at 42Ċ once more to differentiate Pseudomonas aeruginosa from other species (Bhatia *et al.*, 1994).

The bacterial isolates identified by using microscope examination, Cultural characteristics and Biochemical tests.

Growth at Different Temperatures

Few colonies of bacterial growth were transport to a test tube containing 5ml of Muller-Hinton broth .The tube incubated at different temperature (20, 42, 45) Ċ for 24-48h. Growth was examined after incubation (Wistreich, 1997), then the (MIC) value of growing bacteria was determined.

Minimum Inhibitory Concentration (MIC) of the antibiotics (cefotaxim, amikacin, chloramphenicol)

The broth dilution method was used to determine the MIC of the three antibiotics against Psendomonas aeruginosa as follow:

a. Stock solutions of the three antibiotics (cefotaxim, amikacin and chloramphenicol) were prepared as explained above.

b. Serial dilutions of each antibiotics (0.5,1,2,4,8,16,32,64,128 and 256) µg/ml were prepared using Mueller hinton broth medium.

c. 0.1 ml of the bacterial suspension containing approximately $1.5x10^8$ CFU/ml (by comparing to 0.5 McFarland turbidity) was added to each tubes containing the dilutions of the antibiotics, these tubes were incubated at 37 Ċ for 16-18h (Iglewski, 1980).

d. A test tube containing the broth and the test bacteria without antibiotics was incubated at 37 Ċ for 16-18h as a control.

e. After incubation, the turbidity of the tubes was observed and the (MIC) was determined as the minimum amount of the antibacterial agent that will inhibit the visible growth (turbidity) after over night incubation (Iglewski, 1980).

f. The resultant MIC values were compared with NCCLS to determine whether the bacteria are sensitive or resistant to the used antibiotics (El-Ghoroury *et al.*, 1970).

Irradiation Procedure

Bacterial Samples Preparation

A loopful of the culture was transferred from the nutrient agar slants to a test tube containing brain – heart infusion broth and incubated at 37Ċ for 18h. The suspension was centrifuged at (3500 r.p.m) for 10 minutes, supernatant was removed and the bacterial pellete was resuspended using physiological saline. The suspension was mixed using vortex to get homogenous suspension, that compared with the McFarland solution to get suspension of $1.5 x10^8$ CFU/ml concentration (Gorbach *et al.*, 1998).

Laser Parameters

The laser that use in this study was the Diode laser which was considered as CW laser and had the following parameters:

The wavelength was 805nm, the output power was (3W), the beam diameter was (8mm), the exposure times varied (5, 10, 15) minutes, power density (PD) = P/A = 5.9 W/cm^2, where P is the output power of the laser (watt), A is the exposed area to laser beam (cm^2).

Irradiation of Pseudomonas aeruginosa by Diode Laser

The irradiation procedure of pseudomonas aeruginosa in this study includes three groups as follow: Group A which includes the growing bacteria at temperature 37Ċ that exposed to the laser radiation at (5,10,15) minutes with presence of (0.5,1,2,4,8,16,32,64,128,256)µg/ml concentrations of Cefotaxim, Amikacin and

Chloramphenicol. Group B include the growing bacteria at temperature (20, 42, 45) Ċ which was treated with laser light at (5,10,15) minutes in the presence of (0.5,1,2,4,8,16,32,64,128,256) µg/ml concentrations of Cefotaxim, Amikacin and Chloramphenicol.

Laser Treatment

One milliliter of the diluted bacterial suspension from each group was transferred to sterial Eppendroff tube and exposed to laser light at different exposure times, another Eppendroff tube also contains one milliliter of the diluted bacterial suspension did not exposed to laser light in order to keep it as a control, then the irradiation and non irradiation suspension in Eppendroff tubes was added to the serial dilutions of the antibiotics and incubated at 37 Ċ for 16-18 h, after incubation the minimum inhibitory concentration (MIC) for the antibiotics was determined.

Results and Discussion

Results of isolation and identification of pseudomonas aeruginosa: 35 isolates were identified according to the following results of cultural characteristics, microscopic and biochemical examination.

Cultural Characteristics

The colonies of this bacteria were feathered edges giving characteristics overripe grapes oder. The colonies looked pale on MacConkey agar due to the inability of these bacteria to ferment lactose. In most cases the isolates produced pyocyanin which is blue-green in color Such property was observed on uncolored media (nutrient and cetrimide agar). On blood agar the isolates were small brown to black in color, most of them produced clear zones around the colonies representing beta hemolysis. The bacteria were able to grow on nutrient agar at 42Ċ and 4 Ċ.

Microscopic Examination

Under the compound light microscope after staining with gram stain, smears of these bacteria showed small single gram-negative rods, occurred singly, but in few cases in group.

Biochemical Tests

The results of the biochemical tests for pseudomonas aeruginosa isolates are shown in Table (1).

Table (1) The results of the biochemical tests for pseudomonas aeruginosa isolates.

Biochemical test	Results
Oxidase	+
Catalase	+
Lactose fermentation	-
Growth at 42Ċ	+
Kligler test	A/K/-/-
H2S formation	-
Indole	-
Methyl red	+
Voges-proskaure	-
Citrate utilization	+

Key (+): positive reaction, (-) negative reaction.

Results of the susceptibility test of Pseudomonas aeruginosa to antimicrobial agentsusing MIC method

Table (2) illustrates the results of susceptibility of pseudomonas aeruginosa isolates against three antimicrobial agents.

The results showed that all the isolates were sensitive to cefotaxim and amikacin, and resistant to chloramphenicol in comparing with NCCLS (National Committee, 2000). Cefotaxim belong to the third generation cephalosporins that have excellent activity against a wide

spectrum of gram-negative organisms (Mandell *et al*., 1979).

Many studies illustrated that cefotaxim is useful for treating gram-negative nosocomial infections such as pneumonia and wound infections (Harries, 1974). Many researches have been reported that the amikacin was most active against gram-negative bacteria than gentamicin and tobramycin because the amikacin has a modification that confers resistance to enzymes that inactivate the gentamicin and tobramycin (Mandell *et al.*, 1979). Among gram negative bacteria pseudomonas aeruginosa was most sensitive to amikacin (Saini *et al.*, 2004)

Also the results showed that all the isolates were resistant to chloramphenicol such resistance may be due to the ability of this bacteria to produce the enzyme chloramphenicol transacetylase (Harries, 1974) . This enzyme acetylates the hydroxyl groups on the chloramphenicol structure. Acetylated chloramphenicol binds less well to the 50S ribosome, allowing the bacteria to continue their protein synthesis (Clarck *et al.*, 1992).

Table (2) Antibiotics susceptibility test for Pseudomonas aeruginosa

Isolate No.	antibiotics	Break point			MIC Value(µg/ml)	Susceptibility
1	Ce	Cefotaxim			2	S
	Am	S	I	R	4	S
	Ch	≤8	16-32	≥64	256	R
2	Ce				2	S
	Am				4	S
	Ch	Amikacin			128	R
		S	I	R		
3	Ce	≤16	32	≥64	1	S
	Am				2	S
	Ch				256	R
4	Ce				1	S
	Am	Chloramphenicol			1	S
	Ch	S	I	R	256	R
5	Ce	≤8	16	≥32	2	S
	Am				4	S
	Ch				256	R

Key (Ce):Cefotaxim , (Am):Amikacin , (Ch):Chloramphenicol , (S):sensitive ,(I) Intermediat, (R):resistant.

The effect of Different Temperature on the Susceptibility of bacteria to the antimicrobial agents

The effect of different temperature (20,42,45Ċ) on the susceptibility of pseudomonas aeruginosa to antibiotics was shown in Table (3). The results showed that at temperature 20 Ċ there was no observable change neither in the sensitivity and resistance percentage of pseudomonas aeruginosa nor in the MIC values of the antibiotics to all isolates. At temperature 42 Ċ there is no change in the percentage of sensitivity and resistance against the antibiotic for all isolates. For cefotaxim there is no change in the MIC values for isolates (3,4,5) but there is a remarkable change for the isolates (1,2) . Observable change in the MIC values for chloramphenicol to all isolates except

the isolate (5). Also the MIC values for amikacin was changed for all isolates except the isolates (3,4). The results showed that at 45Ċ there is an observable change in the MIC values for cefotaxim in all isolates except the isolates (3,4). For amikacin the isolate (4) has no change in the MIC value while the other isolates have remarkable changes in the MIC values. Remark changes in the MIC values for chloramphenicol were observed for all isolates, and no change in the percentage of sensitivity and resistance of pseudomonas aeruginosa against the antibiotics. Many researchs found that at 42Ċ the gram – negative bacteria have no ability to synthesize their outer layers (LPS), which would act as a sort of armor that protect the bacteria from being perforated and killing by many antibiotics.

At 45Ċ which is above the maximum growth temperature of Pseudomonas aeruginosa, biochemical changes in the organic molecules of the cell that arise from alteration in enzyme molecules or chemical break downs of structural molecules, especially in the cell membrane may be lead to increasing the sensitivity of the bacteria to the antibiotics (Alcamo, 1998). The antibiotics susceptibility of bacteria is modulated by several local factors that may modify drug efficacy. Many studies found that the two most important environmental factors that affect the susceptibility of the microorganism to the antibiotics, their growth and multiplication are the pH and temperature (Estrela, 1995; Segun 2004).

Table (3) The effect of temperature on the susceptibility of Pseudomonas aeruginosa to the antibiotics

Isolates No.	Antibiotics	MIC value (µg/ml)			
		Control 37Ċ	Temperature 20Ċ	Temperature 42Ċ	Temperature 45Ċ
1	Ce	2 (S)	2 (S)	1(S)	1(S)
	Am	4 (S)	4 (S)	2(S)	1(S)
	Ch	256 (R)	256 (R)	64(R)	64(R)
2	Ce	2 (S)	2 (S)	1(S)	1(S)
	Am	4 (S)	4 (S)	1(S)	1(S)
	Ch	128 (R)	128 (R)	64(R)	64(R)
3	Ce	1 (S)	1 (S)	1(S)	1(S)
	Am	2 (S)	2 (S)	2(S)	1(S)
	Ch	256 (R)	128 (R)	128(R)	128(R)
4	Ce	1 (S)	1 (S)	1(S)	1(S)
	Am	1 (S)	1 (S)	1(S)	1(S)
	Ch	256(R)	256(R)	64(R)	64(R)
5	Ce	2 (S)	2 (S)	2(S)	1(S)
	Am	4 (S)	4 (S)	2(S)	1(S)
	Ch	256 (R)	256(R)	256(R)	32(R)

Key (Ce):Cefotaxim , (Am):Amikacin , (Ch):Chloramphenicol , (S):Sensitive , (R):Resistant.

The combination effect of 3W diode laser and different temperature on the susceptibility of bacteria to the antimicrobial gents

The effect of laser light and 20Ċ in combination was shown in table (4) the results of this table shows that there is no observable change in the sensitivity and resistance percentage of pseudomonas aeruginosa against the antibiotics at (5) minutes exposure time. Also with this exposure time there is no remarkable change in the MIC value for all isolates except the isolates (1,2,3) for cefotaxim, isolate 5 for amikacin.

At 10 min exposure time there was noticeable change in the MIC value for all antibiotics to all isolates except the isolates (3,4) for cefotaxim,

isolates (1,4) for amikacin. With this exposure time 20% of the isolate became intermediate to the chloramphenicol that were 100% resistant to it before irradiation. At 15 minutes exposure time 60% of pseudomonas aeruginosa isolates became intermediate and 20% sensitive to chloramphenicol. While all the isolates still sensitive to the other antibiotics.

There are noticeable changes in the value of the MIC, for all antibiotics in all isolates except the isolate (3) that was killed at 0.5µg/ml concentration of cefotaxim and amikacin. The same thing to the cefotaxim in the isolate (4), the isolate (1) that killed at this exposure time without presence of the antibiotics.

Table (4) Combination effect of 3W diode laser and tempreture (20Ċ) on the susceptibility of Pseudomonas aeruginosa to the antibiotics

Isolates No.	Antibiotics	MIC values(µg/ml)				
		Control 1 37Ċ 0 minutes	Control 2 20Ċ 0 minutes	5 minuts 20Ċ	10 minutes 20Ċ	15 minutes 20Ċ
1	Ce	2(S)	2(S)	1(S)	1(S)	-
	Am	4(S)	4(S)	4(S)	4(S)	-
	Ch	256(R)	256(R)	256(R)	64(R)	-
2	Ce	2(S)	2(S)	1(S)	1(S)	1(S)
	Am	4(S)	4(S)	4(S)	1(S)	1(S)
	Ch	128(R)	128(R)	128(R)	64(R)	16(I)
3	Ce	1(S)	1(S)	1(S)	1(S)	*
	Am	2(S)	2(S)	2(S)	1(S)	*
	Ch	256(R)	128(R)	128(R)	32(R)	16(I)
4	Ce	1(S)	1(S)	1(S)	1(S)	*
	Am	1(S)	1(S)	1(S)	1(S)	1(S)
	Ch	256(R)	256(R)	256(R)	16(I)	2(S)
5	Ce	2(S)	2(S)	1(S)	1(S)	1(S)
	Am	4(S)	4(S)	2(S)	1(S)	1(S)
	Ch	256(R)	256(R)	256(R)	64(R)	16(I)

Key (Ce): Cefotaxim, (Am):Amikacin, (Ch):Chloramphenicol, (S):Sensitve, (R):Resistant, (I):Intermediate, (-): bacteria was killed after irradiation in the absence of the antibiotics, (*): no growth at 0.5µg/ml antibiotic concentration.

Table (5) illustrated the combination effect of laser and temperature 42Ċ on the susceptibility of bacteria to the antibiotics.

As shown in this table the values of the MIC for all antibiotics was not differ from the control (2) values at exposure time 5 minutes except in the isolate (5) for cefotaxim, (4,5) for chloramphenicol. 20% of the isolates became intermediate against chloramphenicol .

At 10 min exposure time 40% of the isolate became intermediate against chloramphenicol while there is remarkable changes in the value of the MIC for all antibiotics to all isolates except the isolate (1,3,4) for cefotaxim. At 15 minutes exposure time 20% of the isolates that were intermediate to chloramphenicol became sensitive to it and 40% of the isolates became intermediate against chloramphenicol. The isolate (3,4) were killed with 0.5 µg/ml cefotaxim, amikacin concen-tration while the isolate (5) has observable change in the MIC value for the three antibiotics. The isolates (1,2) were killed after irradiation in the absence of the antibiotics

Table (5) Combination effect of 3W diode laser and tempreture (42Ċ) on the susceptibility of Pseudomonas aeruginosa to the antibiotics

Isolates No.	Antibiotics	MIC values(µg/ml)				
		Control1 37Ċ 0 minutes	Control 2 42Ċ 0 minutes	5 minuts 42Ċ	10 minutes 42Ċ	15 minutes 42Ċ
1	Ce	2(S)	1(S)	1(S)	1(S)	-
	Am	4(S)	2(S)	2(S)	1(S)	-
	Ch	256(R)	64(R)	64(R)	32(R)	-
2	Ce	2(S)	1(S)	1(S)	*	-
	Am	4(S)	1(S)	1(S)	*	-
	Ch	128(R)	64(R)	64(R)	16(I)	-
3	Ce	1(S)	1(S)	1(S)	1(S)	*
	Am	2(S)	2(S)	2(S)	1(S)	*
	Ch	256(R)	128(R)	128(R)	32(R)	16(I)
4	Ce	1(S)	1(S)	1(S)	1(S)	*
	Am	1(S)	1(S)	1(S)	*	*
	Ch	256(R)	64(R)	16(I)	16(I)	2(S)
5	Ce	2(S)	2(S)	1(S)	1(S)	1(S)
	Am	4(S)	2(S)	2(S)	1(S)	1(S)
	Ch	256(R)	256(R)	128(R)	64(R)	16(I)

Key (Ce): Cefotaxim , (Am):Amikacin , (Ch):Chloramphenicol , (S):Sensitve , (R):Resistant , (I):Intermediate , (-):bacteria was killed after irradiation in the absence of the antibiotics, (*): no growth at 0.5µg/ml antibiotic concentration.

Table (6) shows the combination effect of the laser and temperature 45Ċ on the sensitivity of bacteria to the antibiotics. The results show that at (5) minutes exposure time 60% of the isolates became sensitive and 20% became intermediate to chloramphenicol, another isolates still resistant against it. The value of MIC for chloramphenicol was changed to all isolates in comparing with the control (2) except the isolate (2), but there is no change in the MIC value for the rest antibiotics except the isolates (5) for cefotaxim, the isolates (1,3) for amikacin. At 10 min exposure time all the isolates were killed after treatment with laser light without presence of antibiotics except the isolate (4) that killed with 0.5 µg/ml concentration of cefotaxim and has observable change in the MIC value for amikacin and chloramphenicol.20% of the isolates became sensitive to chloramphenicol with this exposure time. Finally at 15 min exposure time all the isolates killed after treatment and with absence of the antibiotics.

At 20 Ċ there was an observable change in the MIC value and the resistance percentage of the bacteria after laser irradiation, this may be due to the direct effect of the laser radiation on the cytoplasmic membrane enzyme. At 42 Ċ and 45 Ċ there was remarkable change in the value of the MICs and in the sensitivity and resistance percentage of the bacteria against the antibiotics. Also most of the isolates were killed at 10 and 15 min exposure time, this may be due to the accumulative effect of the laser light and the temperature that affected the enzymatic system in the cytoplasmic membrane and some other protein in the outer membrane of gram negative bacteria which play important role in the resistance of bacteria to the antibiotics The terminal enzyme of the respiratory chain in eukaryotic cell (cytochrom c oxidase) that plays a central role in the bioenergetics of the cell and the cytochrom β complexes in prokaryotic cells are believed to be the photoacceptor molecules for red-to-near IR radiation (Karu, 1993).

Table (6) Combination effect of 3W diode laser and tempreture (45Ċ) on the susceptibility of Pseudomonas aeruginosa to the antibiotics

Isolates No.	Antibiotics	MIC values(µg/ml)				
		Control 1 37Ċ 0 minutes	Control 2 45Ċ 0 minutes	5 minuts 45Ċ	10 Minutes 45Ċ	15 minutes 45Ċ
1	Ce	2(S)	1(S)	1(S)	-	-
	Am	4(S)	4(S)	1(S)	-	-
	Ch	256(R)	64(R)	16(I)	-	-
2	Ce	2(S)	1(S)	1(S)	-	-
	Am	4(S)	1(S)	1(S)	-	-
	Ch	128(R)	64(R)	64(R)	-	-
3	Ce	1(S)	1(S)	1(S)	-	-
	Am	2(S)	1(S)	*	-	-
	Ch	256(R)	128(R)	8(S)	-	-
4	Ce	1(S)	1(S)	1(S)	*	-
	Am	1(S)	1(S)	1(S)	1(S)	-
	Ch	256(R)	64(R)	8(S)	4(S)	-
5	Ce	2(S)	1(S)	*	-	-
	Am	4(S)	1(S)	1(S)	-	-
	Ch	256(R)	32(R)	8(S)	-	-

Key (Ce): Cefotaxim, (Am): Amikacin, (Ch):Chloramphenicol, (S):Sensitve, (R):Resistant, (I):Intermediate, (-): bacteria was killed after irradiation in the absence of the antibiotics, (*): no growth at 0.5µg/ml antibiotic concentration.

Conclusion

The more effective combination between 805 nm diode laser and 45Ċ temperature which resulted in best reduction to the MIC values and increase the susceptibility of bacteria to antibiotics, is observed with 5.9 W/cm^2 power density at 10 minutes exposure time, that mean the values of MIC are changed with changing the microenvironmental conditions such as temperature, so this point must be taken in to account when the antibiotics are demonstrated in vivo.

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التأثير الجمعي لليزر والمضادات الحياتية ودرجات الحرارة المختلفة على بكتريا الزوائف الزنجارية المعزولة محلياً

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الخلاصة

تعتبر بكتريا الزوائف الزنجارية ثاني اهم البكتريا السالبة لصبغة كرام الممرضة للانسان حيث تسبب نسبة وفيات عالية في المستشفيات بسبب مقاومتها للمنظفات وعدد كبير من المواد الكيميائية والمضادات الحياتية .ممكن ان تتأثر استجابة البكتريا للمضادات الحياتية بعدد من العوامل مثل الحرارة التي يمكن ان تؤثر على فعالية الادوية. تم اجراء هذه الدراسة لتقيم تأثير درجات حرارة مختلفة (45,42,20) درجة مئوية على استجابة بكتريا الزوائف الزنجارية للتركيز المثبط الادنى للمضادات المايكروبية قبل وبعد التشعيع .تم جمع النماذج من مائة وخمسون مريضا مصابين بتلوثات جروح الحروق. تم عزل خمس وثلاثون عزلة من بكتريا الزوائف الزنجارية اعتماداً على الصفات المظهرية والكيميائية الحياتية. بعد تشعيع العزلات بليزر الدايود بالطول الموجي 805 نانومتر وقدرة 3 واط ولمدد التشعيع(15,10,5) دقيقة وبوجود درجات حرارة مختلفة وتراكيز مختلفة من المضادات الحياتية(السيفوتاكسيم، الاميكاسين، الكلورامفنكول). وجد تأثير ملحوظ على تغيير قيم تركيز المضاد المثبت الادنى حيث اصبحت عزلات البكتريا حساسة للمضاد الحياتي الكلورامفنيكوك عند اوقات التشعيع الثلاثة.وتم الحصول على نسبة 100 % قبل لخلايا البكتريا عن مدة تشعيع 15 دقيقة ودرجة حرارة 45 م بعدم وجود المضاد .تم الاستنساج بأن التشعيع بليزر الدايود وبدرجة حرارة 45 م هو الافضل تقليل التركيز المثبط للاذى وقتل البكتريا عن زمن التشعيع 10 دقائق .