Validation efficiency of laser radiation on Multidrug resistance *Acinetobacter baumanii* isolated from malignant tumors

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Abstract

The goal of this study in order to treatise influence efficiency of laser radiation on Multidrug resistance *Acinetobacter baumanii* isolated from malignant tumors. In this descriptive study design of 25 MDR *A.baumanii* by cross-sectional study design collected from malignant tumors.Study populations or MDR *A.baumanii* (25) isolated from malignant tumors from leukemia, liver cancer, spleen cancer and kidney cancer from Baghdad hospitals through 2019. Identification by using vitek2-GN system. The results shown in the isolation from human inclusive: 8(32%) leukemia, 7(28%) liver cancer, 6 (24%) spleen cancer and 4(16%) kidney cancer from Baghdad hospitals in 2019.

Antimicrobial susceptibility test was done by discs diffusion method of antibiotic discs to 25 isolates for 12 antibiotics that shown in table 1 and compared with the recommendation of (CLSI,2020).The antibiotic resistance for Ciprofloxacin 500(100%), Norfloxacin 475(95%), Carbenicillin 475(95%), Ofloxacin 175(35%),Polymyxin 225(45%), Gentamycin 425(85%), Tobramycin 125(25%), Aztreonam 75(15%), Amoxcillin 75(15%), Nalidixic acid 50(10%), Lomefloxacin 25(5%) and Levofloxacin 25(5%) showed in figure(4) respectively that represent the number of *MDR A.baumanii* isolated from malignant tumors resistance antibiotics.

The results number of *Acinetobacter baumanii* isolated from malignant tumors with percentage of killing for the first dilution after exhibition to radiation emit from Semiconductor laser at (10, 20, 30)min, in power 5mW, with Wavelenghth 650+-10 nm, power 5 Mw, Max out put<100W with control. Validation efficiency of laser radiation on MDR *A.baumanii* isolated from malignant tumors with increase percentage of killing because its effect directly or indirectly on cell membrane, DNA, cytoplasmic membrane via heat generated from semiconductor laser .

Results influence or validation of semiconductor laser radiation on MDR *A. baumanii* from first dilution with fertile 95 cells in 10 min,12 cells in 20 min and 48 cell in 30 min, compared

control=400 cell, the proportion of killing was highly and the fertile cells was fewer than control .

Results validation efficiency of semiconductor laser radiation on MDR *A. baumanii* from second dilution with fertile 96 cells in 10 min,10 cells in 20 min and 40 cell in 30 min, compared control=400 cell, the proportion of killing was highly and the fertile cells was fewer than control .

Validation efficiency of laser radiation on Multidrug resistance *Acinetobacter baumanii* isolated from malignant tumors high efficiency to killing bacteria resistance antibiotics in a short time.

Keywords: Bacterial malignant tumors, Semiconductor laser, Influence killing bacteria.

Introduction

Acinetobacter baumannii is gram-negative coccobacillary rod, a non fermentive, aerobic, opportunistic, Morphological findings vary according to the phase of cell growth and exposure to antimicrobial agents[1].

Multidrug-resistant *A.baumannii* is a rapidly emerging pathogen in the health care setting, where it causes infections that include bacteremia, pneumonia, meningitis, urinary tract infection, and wound infection. The organism's ability to survive under a wide range of environmental conditions and to persist for extended periods of time on surfaces make it a frequent cause of outbreaks of infection and an endemic, health care–associated pathogen[2].

Multidrug-resistant *Acinetobacter* deep wound infections, osteomyelitis, respiratory infections, and bacteremia have been reported among military personnel with traumatic injuries during the conflicts in Iraq and Afghanistan[3,4].

Resistance mechanisms for *Acinetobacter spp.* are similar to those for *Pseudomonas* species, although *Acinetobacter spp.* have not been studied as extensively [5].The mechanisms of resistance generally fall into 3 categories: (1) antimicrobial-inactivating enzymes, (2) reduced access to bacterial targets, or (3) mutations that change targets or cellular functions [6].For the first category, *Acinetobacter* species possess a wide array of b-lactamases that hydrolyze and confer resistance to penicillins, cephalosporins, and carbapenems. AmpC cephalosporinases are chromosomally encoded and confer resistance to broad-spectrum cephalosporins [7].

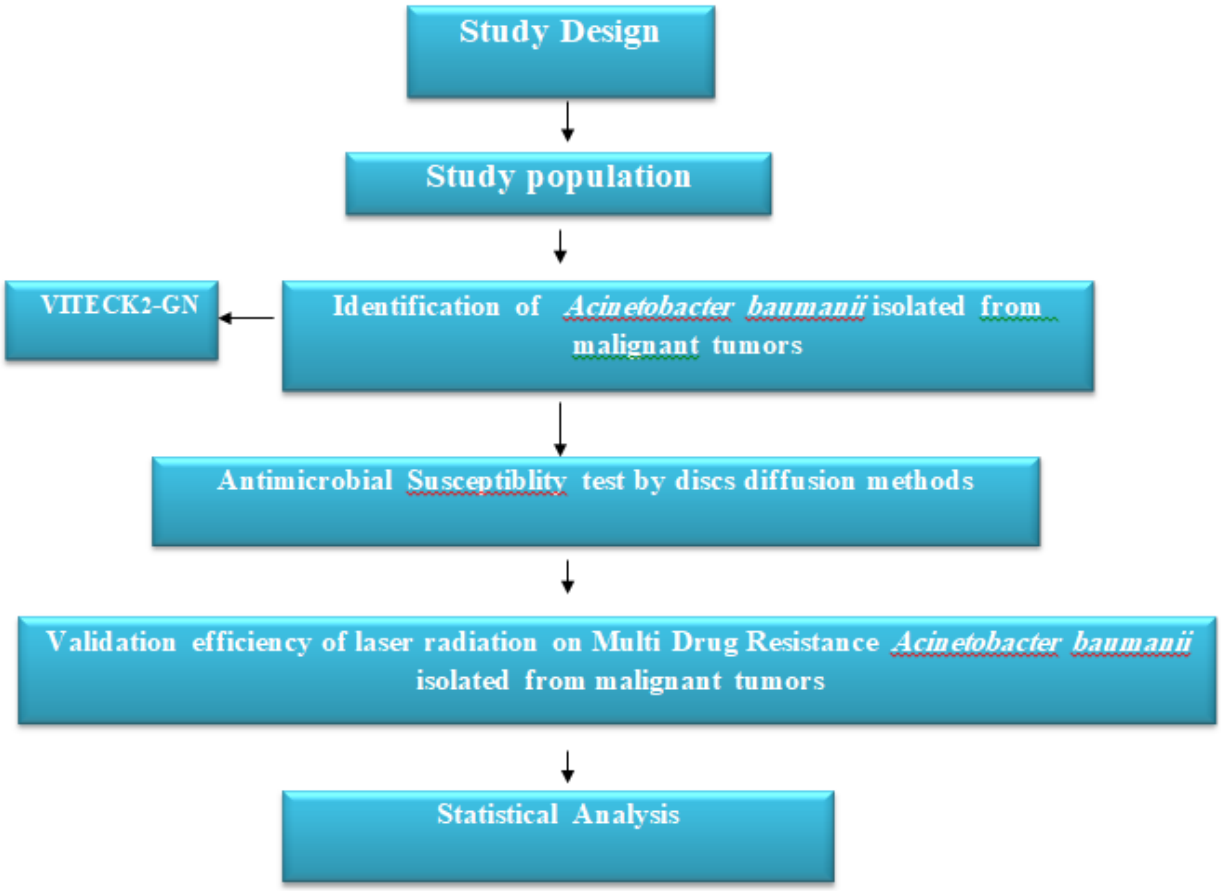
Semiconductor laser is capable of decontaminating implant surfaces. Surface characteristics determine the necessary power density to achieve a sufficient bactericidal effect. The rapid heat generation during laser irradiation requires special consideration of thermal damage to adjacent tissues .(Kreisler *et al*., 2003) .Due to the advantages of semiconductor laser such as small body, light weight, long life span, high efficiency, it has been used widely in the medical fields[8].

Methodology

Clinical examination include collection of sample from different malignant tumors,identification by VITECK2-GN, antimicrobial susceptiblity tests for antibiotics, Validation efficiency of laser radiation on MDR *Acinetobacter baumanii* in this study**.**

Study design

Study desin is the formulation of trials and experiments, as well as observational studies in medical,clinical or other types of research (e.g:epidemiological) involving human beings



Scheme (1): Methodology of research project

Study populations

A total of 25 *Acinetobacter baumanii* isolates were collected from tumors from Baghdad hospitals in 2019.These isolates were identified by conventional biochemical reactions according to the criteria established by [9].The isolates were inoculated a Nutrient agar, the results were remain for 24 h of incubation at 37°C.

Identification of MDR *A.baumanii* isolated from malignant tumors by VITECK2-GN

The Gram Negative (GN) card is used for the automated identification of most significant Gram-Negative bacteria.The GP identification card is based on established biochemical methods and newly developed substrates.There are 64 biochemical tests measuring carbon source utilization, enzymatic activities and antibiotics resistance (Collins and Lawson, 2000; Barros *et*

al., 2001).Final identification results are available in approximately eight hours or less (Bio Merieux, 2010).

Procedure:

Prepared the inoculums from a pure culture, In case of mixed cultures, a re-isolation step is required. It is recommended that a purity check plate was done to ensure that a pure culture was used for testing .

1. Aseptically transferred 3.0 ml of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) into a clear plastic (polystyrene) test tube (12 mm x 75 mm).
2. A sufficient number of morphologically similar colonies were transferred to the saline tube prepared in step 1 to Prepared the homogenous suspension of the organism with a density equivalent to a McFarland No. 0.70 to 0.110.

Note: Age of suspension must not exceed 30 minutes before inoculating card. While Suspension Turbidities used for Card Inoculation (McFarland turbidity) must be range from 0.50-0.63.

Antimicrobial Susceptiblity test by discs diffusion methods

Antimicrobial susceptibility test was done by discs diffusion method of antibiotic discs to 25 isolates for 12 antibiotics that shown in table 1 and compared with the recommendation of (CLSI,2020).

Table 1 :antibiotics Code and Concentrations of discs

No.	Discs concentrations (µg/disc)	Symbol	المضاد المايكروبي	Company
1	5	OFX	Ofloxacin	Bioanalyse (Turkey)
2	300	PB	Polymyxin	
3	10	CN	Gentamicin	
4	10	TOB	Tobramycin	
5	30	ATM	Aztreonam	
6	25	AX	Amoxicillin	
7	25	PY	Carbenicillin	
8	30	NA	Nalidixic Acid	
9	10	LOM	Lomefloxacin	
10	5	LEV	Levofloxacin	

	11	10	CIP	Ciprofloxacin	
	12	10	NOR	Norfloxacin	

Validation efficiency of laser radiation on Multi Drug Resistance *Acinetobacter baumanii* isolated from malignant tumors

A.baumanii cultivation was done according to [10] with some modifications as follows: *A.baumanii* were cultivated in Nutrient broth at 37° C for 24 h to reach the stationary-phase culture, then culture was centrifuged (5000 rpm for 10 minutes). The pellet was suspended in 150 ml of sterile normal saline ,then 1 ml of this solution was exposed to Semiconductor laser in different time (10, 20, 30) min., in comparison with control (without exposure).

The equation for calculating the killing of MDR *A.baumanii*

$$\text{Killing of MDR } A.baumanii \ \% = \frac{\text{Control – patronize}}{\text{Control}} * 100$$

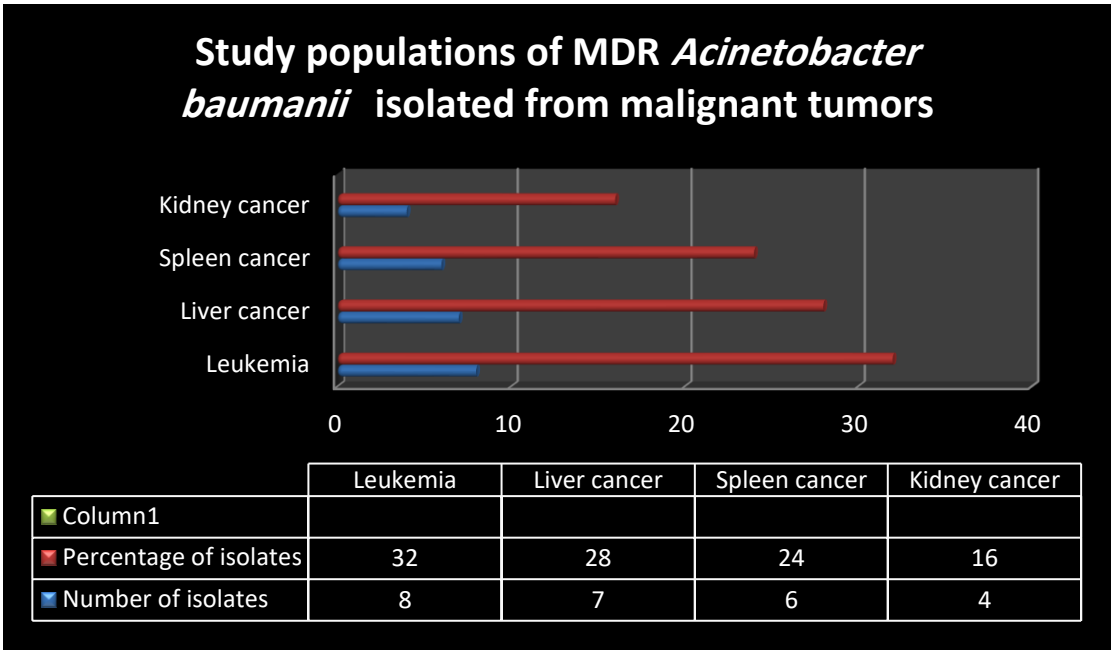
Results and Discussions

Study design

In this descriptive study design of 25 MDR *A.baumanii* by cross-sectional study design collected from malignant tumors.

Study populations

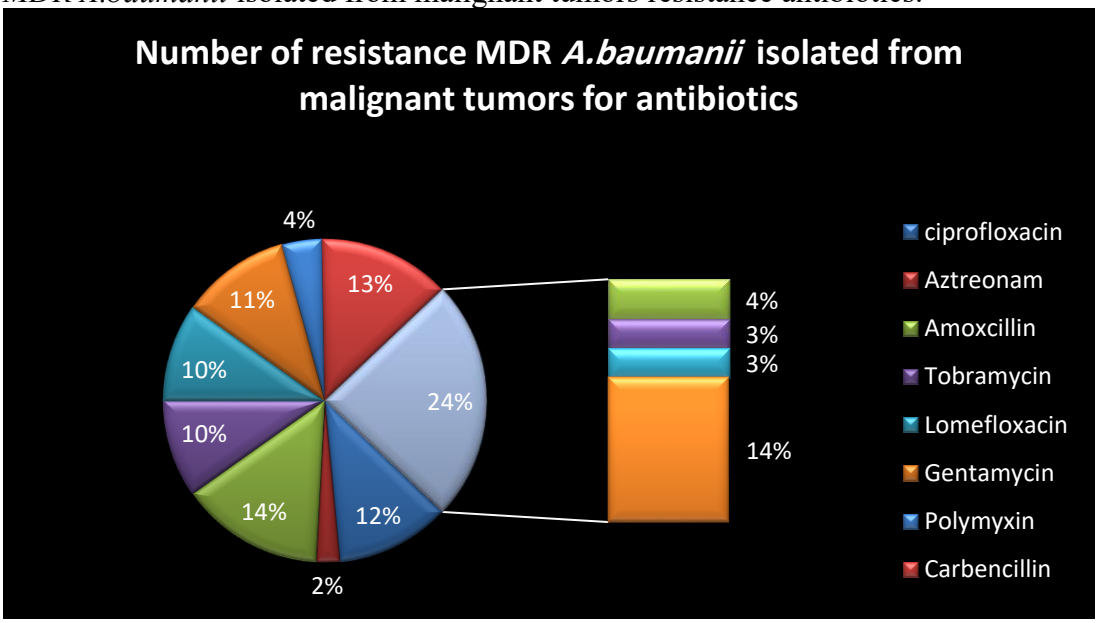
MDR *A.baumanii* (25) isolated from malignant tumors from leukemia, liver cancer, spleen cancer and kidney cancer from Baghdad hospitals through 2019. Identification by using vitek2-GN system. The results shown in figure(1) of isolation from human inclusive: 8(32%) leukemia, 7(28%) liver cancer, 6 (24%) spleen cancer and 4(16%) kidney cancer.



Figure(1): Study populations of MDR *Acinetobacter baumanii* isolated from malignant tumors

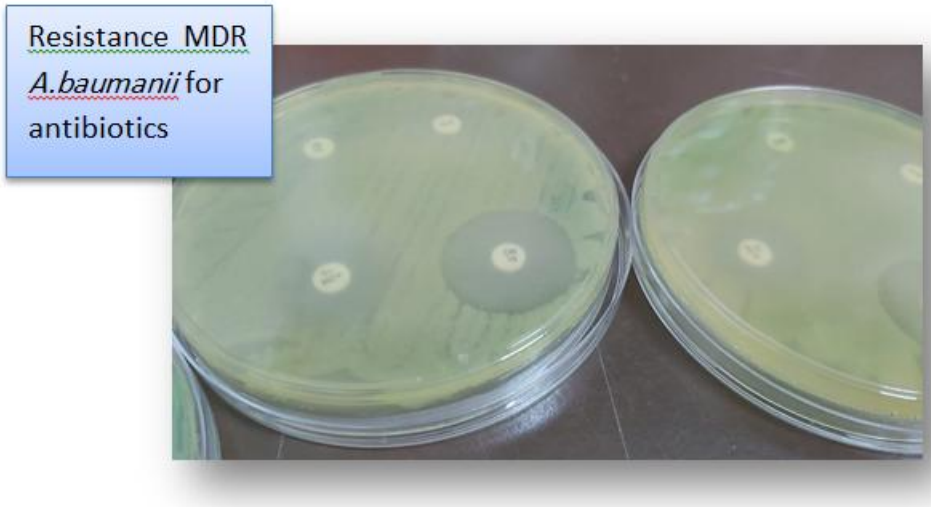
Antimicrobial Susceptiblity test by discs diffusion methods

The antibiotic resistance for Ciprofloxacin 500(100%), Norfloxacin 475(95%), Carbenicillin 475(95%), Ofloxacin 175(35%),Polymyxin 225(45%), Gentamycin 425(85%), Tobramycin 125(25%), Aztreonam 75(15%), Amoxcillin 75(15%), Nalidixic acid 50(10%), Lomefloxacin 25(5%) and Levofloxacin 25(5%) showed in figure(2) respectively that represent the number of *MDR A.baumanii* isolated from malignant tumors resistance antibiotics.



Figure(2):Number of resistance *MDR A.baumanii* isolated from malignant tumors for antibiotics.

Results in figure (3) explain inhibition zone around antibiotics by discs diffusion methods for MDR *A.baumanii* .



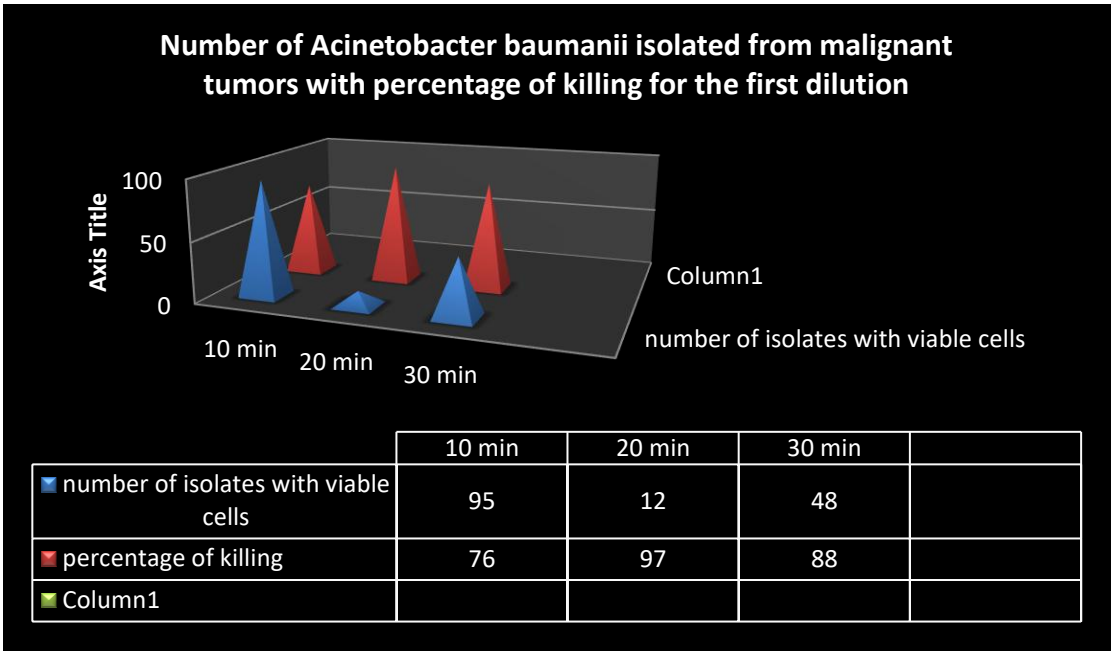
Figure(3): Inhibition zone around antibiotics by discs diffusion methods for MDR *A.baumanii*.

Antimicrobial resistance greatly limits the therapeutic options for patients who are infected with this organism, especially if isolates are resistant to the carbapenem class of antimicrobial agents. Because therapeutic options are limited for multidrug-resistant*Acinetobacter* infection, the development or discovery of new therapies, well-controlled clinical trials of existing antimicrobial regimens and combinations, and greater emphasis on the prevention of health care–associated transmission of multidrug-resistant *Acinetobacter* infection are essential [7].

Validation efficiency of laser radiation on MDR *A.baumanii* isolated from malignant tumors

The results as shown in figure(4) and figure(5)explain Number of *Acinetobacter baumanii* isolated from malignant tumors with percentage of killing for the first dilution after exhibition to radiation emit from Semiconductor laser at (10, 20, 30) min, in power 5mW, with Wavelenghth 650+-10 nm, in power 5 Mw, Max out put<100W with control.Validation efficiency of laser radiation on MDR *A.baumanii* isolated from malignant tumors with increase percentage of killing because its effect directly or indirectly on cell membrane, DNA, cytoplasmic membrane via heat generated from semiconductor laser .

Results in figure(4) indicate influence or validation of semiconductor laser radiation on MDR *A. baumanii* with fertile 95 cells in 10 min,12 cells in 20 min and 48 cell in 30 min, compared control=400 cell, the proportion of killing was highly and the fertile cells was fewer than control .

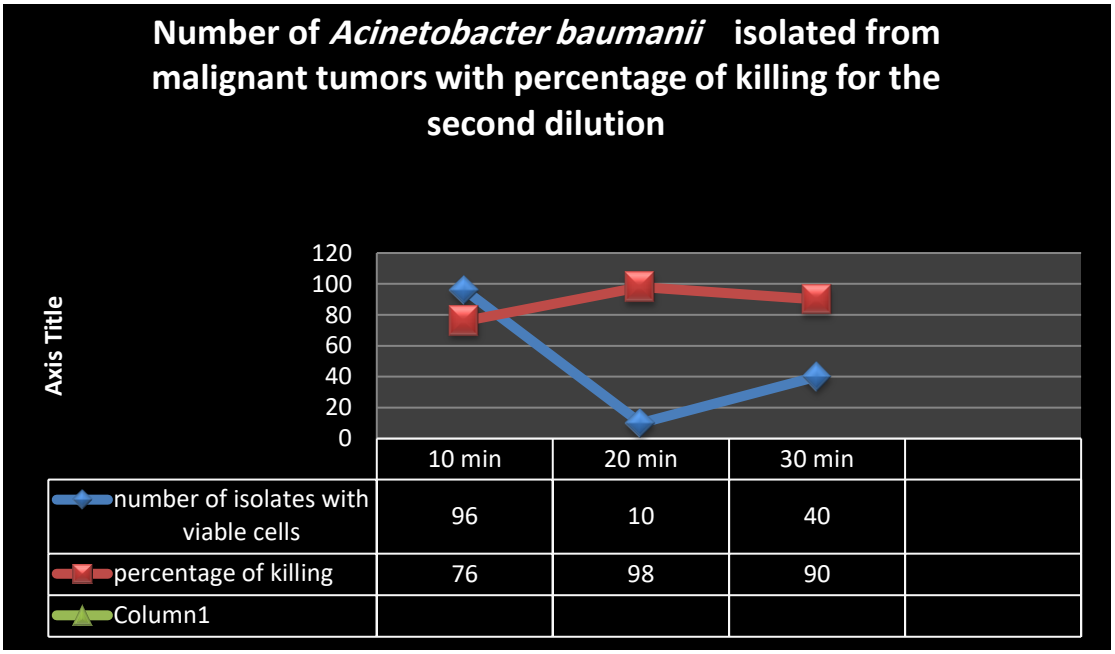


	10 min	20 min	30 min	
number of isolates with viable cells	95	12	48	
percentage of killing	76	97	88	
Column1				

Figure(4): Number of *Acinetobacter baumanii* isolated from malignant tumors with percentage of killing for the first dilution exhibition for Semiconductor laser with (P< 5 m W), Wavelenghth = 650 +-10 nm.

Results in figure(5) show validation of semiconductor laser radiation on MDR *A. baumanii* from second dilution with fertile 96 cells in 10 min,10 cells in 20 min and 40 cell in 30 min, compared control=400 cell, the proportion of killing was highly and the fertile cells was fewer than control .

Validation efficiency of laser radiation on Multidrug resistance *Acinetobacter baumanii* isolated from malignant tumors high efficiency to killing bacteria resistance antibiotics in a short time.



Figure(5): Number of *Acinetobacter baumanii* isolated from malignant tumors with percentage of killing for the second dilution exhibition for Semiconductor laser with (P< 5 m W), Wavelenghth = 650 +-10 nm.

Many hypotheses have been proposed and tested regarding the mechanism of cell damage by radiation. Some scientists proposed the mechanism thought 'radiotoxins' that are the toxic substances produced in the irradiated cells responsible for lethal effect. Others proposed that radiation was directly damaging the cellular membranes. In addition, radiation effects on enzymes or on energy metabolism were postulated. The effect on the cytoplasmic membrane appears to play an additional role in some circumstances [11].

There are two main mechanisms of semiconductor lasers in medical applications:The first is bio-stimulation mechanism.Stimulus is a concept on the biological functions. According to the biological function, autonomic nerve reflex, Pavlov's molecular biology and the principle of theory, can think that the weak interaction between laser and organism, laser is a source of stimulation, and the organism has special feel for various stimuli[12].

The second mechanism is thermal effect. The thermal effect is the main factor of biological effect.It plays a role in all of the laser irradiation. High power semiconductor laser is mainly thermal effects of its application.

Results in figure(6) exhibition number of *Acinetobacter baumanii* isolated from malignant tumors in Iraqi laboratory with percentage of killing on Nutrient agar medium for exhibition for Semiconductor laser rays.

Figure(6):
Number of *Acinetobacter baumanii* isolated from malignant tumors in Iraqi laboratory with percentage of killing on Nutrient agar medium for exhibition for Semiconductor laser rays.

Semiconductor laser is capable of decontaminating implant surfaces. Surface characteristics determine the necessary power density to achieve a sufficient bactericidal effect. The rapid heat generation during laser irradiation requires special consideration of thermal damage to adjacent tissues[13].Due to the advantages of semiconductor laser such as small body, light weight, long life span, high efficiency, it has been used widely in the medical fields [8].

Conclusions

1-There are high number of *Acinetobacter baumanii* resistance for antibiotics isolated from malignant tumors.

2- Validation of radiation emitted from semiconductor laser have high efficiency for killing Multidrug resistance *Acinetobacter baumanii* isolated from malignant tumors through a short time.

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