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The Bactericidal Activity of Gamm ,Beta and of Semiconductor Laser Irradiation Effect on *Klebsiella pneumoniae* (*in vitro*)

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Abstract

This work evaluated the effect of gamma , beta and Semiconductor laser irradiation on *Klebsiella pneumoniae* isolated from sputum . The experiment included a control and 4 doses of each gamma and beta irradiation ,which ranged(1.317×10^{-4} -0.320µSv) and (10.573-96.950µSv) respectively.

The total effect of gamma and beta irradiation on *K. pneumoniae* viability was abrogated at (96.915177 & 63.100 µSv) by ^{137}Cs and ^{90}Sr respectively, the percentage of killing was highly (81%) and (85%) respectively and the viable cells was fewer than control . This work evaluated also the effect of Semiconductor laser on *K pneumoniae*. The experiment included a control and triplicate of *K. pneumoniae* exposed to Semiconductor laser in power 5 mW , in Wavelength 650 nm. The effect of Semiconductor laser on the viability of *K. pneumoniae* was counted and percentage of killing was counted , the number of viable cells of *K. pneumoniae* was fewer than control (without exposure to this laser) , but percentage of killing was higher than control . Semiconductor laser was efficient to killing *K. pneumoniae* , and the Gamma and Beta irradiation is efficient to killing *K. pneumoniae* that cause many infection to human and may be cause death .

Keywords: *Klebsiella pneumonia* ,Semiconductor laser , Gamma and Beta irradiation

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Introduction

Klebsiella pneumoniae was Gram-negative bacteremia, encapsulated bacterium, initially named bacillus, was renamed *Klebsiella* in 1886 , not only colonizing the human gastrointestinal tract, skin and nasopharynx, but also able to cause urinary and biliary tract infections, osteomyelitis and bacteremia ,and cause pneumonia , The virulence factors playing an important role in the severity of *K. pneumoniae* infections are capsular polysaccharides, , are essential to the virulence of *Klebsiella* , The capsular material, forming fibrillous structures that cover the bacterial surface , protects the bacterium from phagocytosis and prevents killing of the bacteria by bactericidal serum factors, are non-flagellar (Vuotto *et al.*, 2014) .

Due to its high pathogenicity *K. pneumonia* in the pre-antibiotic era, was considered as an important causative agent of community-acquired (CA) infections, including a severe form of pneumonia, especially in alcoholics and in diabetic patients (Lederman *et al*., 2005 ; Laupland *et al*, 2007) . The greater adhesiveness and presumably also the invasiveness of strains may play an important role in the recurrent infections, *K. pneumoniae* strains being able to persist despite appropriate antibiotic treatment (Lin *et al*., 2014 ; Vuotto *et al.*, 2014 ; Ko *et al*., 2002),

, as a physical cold process, has been Gamma irradiation is a physical means of decontamination, because it kills bacteria by breaking down bacterial DNA, inhibiting bacterial division. Energy of gamma rays passes through hive equipment, disrupting the pathogens that cause contamination (Kátia ,2012) Radiation sterilizationwidely used in many developed and developing countries for the sterilization of health care products. A historical review shows clearly that ionizing radiation was used extensively for the treatment of many types of infections before the advent of antibiotics(Calabrese and Baldwin ,2000) It is well known that, exposure of microbial cells to ionizing radiation presents an additional stress to the cells which tends to disturb their organization. Nucleic acids, especially DNA, are the primary target for cell damage from ionizing radiation. Breaks in the DNA chain disrupt function of the molecule in several ways (Scala, 1990).

Because of these shortages of antimicrobials, the use of laser irradiation has become a topic of much interest and is a promising field in periodontal therapy (Sennhenn *et al*., 2007). Antimicrobial effect of laser is considered as a safe coadjutant in nonsurgical treatment, The most common laser wavelengths used in periodontics include those of diode, (Aoki *et al*., 2004).

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 (Jialiang , 2015) .

The aim of this primary study was to detect the effect of gamma and Beta irradiation and the effect of Semiconductor laser on the viability of *Klebsiella pneumonia* isolated from sputum .

Material and method
Bacterial isolates

A total of 25 *Klebsiella pneumonia* isolates were collected from sputum samples from patients who were admitted to Baghdad hospitals in 2015.These isolates were identified by conventional biochemical reactions according to the criteria established by (Forbes *et al* ., 2007). The isolates were inoculated on Nutrient agar for 24 h of incubation at 37°C.

Effect of Gamma, Beta Irradiation and Semiconductor laser on *K. pneumoniae* isolates.

K. pneumoniae cultivation was done according to Tramps *et al.,* (2006) with some modifications as follows:

The irradiation facility used was gamma (γ) and Beta (β)irradiation of different dose and different energy using many sources for constant exposure time(3 h.) . The *K. pneumoniae* isolates was grown in Nutrient broth for 24 h. on shaker (150 rpm) at 30ºC. The well grown bacterial culture was centrifuged at 8000 rpm for 15minutes. The supernatant was decanted and the pellets were suspended in sterile saline.
The suspended cells were collected in a clean sterile flask to form pool. The bacterial suspension of the pool (5ml) was distributed in clean sterile screw cap test tubes and exposed to different doses of gamma radiation using three replicates for each dose, so as for beta. The non-irradiated control and the irradiated cultures were serially diluted and plated on the surface of Trypton soy agar plates and the viable count was determined .

K. pneumoniae also cultivation was done according to Tramps *et al.*, (2006) with some modifications ,also exposed to Semiconductor laser 1 ml of this solution was exposed to Semiconductor laser in different time (5 , 10 , 20 , 30) min , in comparison to control group (without exposure) . Each run was done in triplicate and inoculated in Trypton soy agar .

The percentage of killing calculated from following equation :

$$\text{percentage of killing } \% = \frac{\text{Control} - \text{treated}}{\text{Control}} * 100$$

Disscusion and Results

The lethal effect of ionizing radiation on microorganisms, as measured by the loss cells of colony-forming ability in Trypton soy agar, has been the subject of detailed study.
From Table (1) find that the increase in radiation dose leads to increase the percentage of killing (ie, in response by more bacteria) , as well as when drawing the radiation energy with percentage of killing relationship for beta and gamma fiugers (1 & 2) , find that an increase of energy less response and the reason for that is due the fact that the increase energy means the higher penetration of radiation and interaction is less, than when the energy is less. As well , we find that the percentage of killing by beta higher than the gamma and this is due to the nature of each radiation and by the fact that the beta is acharge partical and its specfic ionizaition higher than the gamma which is EMF radiation .So we can choose low energy with high activity to get high dose which is efficient to killing *K.pneumoniae*, because irradiation effect directly or indirectly on cell membrane , DNA , cytoplasmic membrane by absorbance irradiation from this bacteria and therby cause damage to this bacteria .

Table (1): The percentage of killing *K. pneumoniae* colony after exposure to Gamma & Beta irradiation, doses & energies.

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Isotope	Type of decaye	E (MeV)	Do (µSv)	Killing ration %
^{90}Sr	β	0.198	63.100	85%
^{60}Co	$β^-$	0.318	10.573	51.81%
	γ	1173.1332	1.3178*10^{-4}	4.96%
				56.77%
^{22}Na	β +	0.513	32.444	31.67%
	γ	1.275	1.416*10^{-4}	32.33%
				64.00%
^{137}Cs	β	0.514	96.950	29.47%
	γ	0.662	1.776*10^{-4}	51.02%
				80.49%
^{241}Am	α	5485.6 5442.8	non	55%
	γ	0.060	0.320	

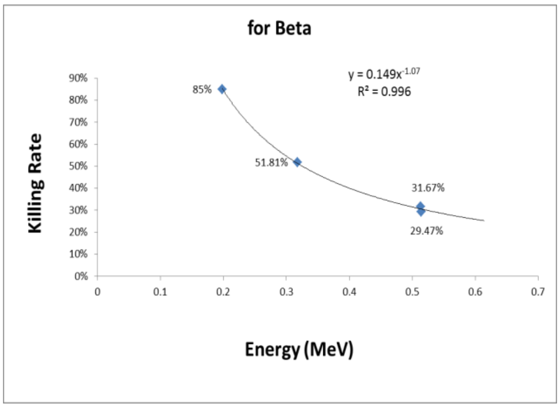

Fig (1): Energy and killing Rate for Beta irradiation

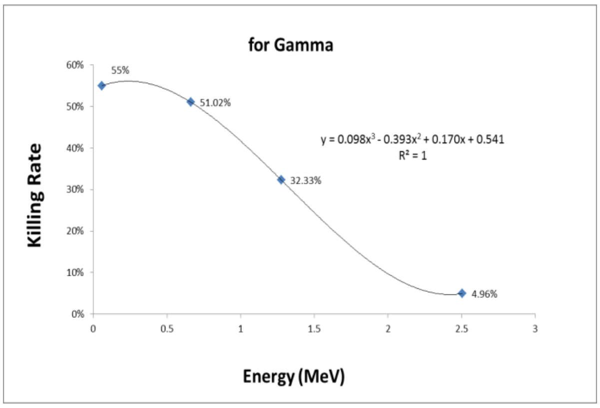

Fig (2): Energy and killing Rate for Gamma irradiation .

Table (2): The percentage of killing *Klebsiella pneumonia* colony (1). after exposure to Semiconductor laser .

	percentage of cell killing exposed to semiconductor laser							
	5min		10 min		20 min (killing)		30 min	
	Viable cells	Precentage of killing	Viable cells	Precentage of killing	Viable cells	Precentage of killing	Viable cells	Precentage of killing
K1	23	92.3 %	20	93.3 %	10	96.6 %	8	98 %
K2	22	92.6 %	20	93.3 %	9	97 %	8	98 %
(P< 5 m W) , Wavelenghth = 650 nm . ; control = 300 colony .								

From Table (2) Number of viable cells and percentage of killing after exposure to Semiconductor laser in (5, 10, 20, 30) minute , in power 5mW , in Wavelenghth 650 nm , the results showed when increase exposure *K. pneumoniae* to semiconductor laser increased percentage of killing of this bacteria , because semiconductor laser effect directly or indirectly on cell membrane , DNA , cytoplasmic membrane , by heat generated of semiconductor laser .

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