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The Bactericidal Activity of Gamma ,Beta on *Vibrio cholerae* resistance to Many of antibiotics (*in vitro*)

الفعالية القاتلة لاشعة كاما وبيتا على بكتريا ضمات الكوليرا المقاومة لعدد من المضادات الحيوية خارج الجسم الحي

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Abstract

This study evaluated the effect of gamma , beta irradiation on *Vibrio colerae* isolated. The experiment included control (without exposure to irradiation) and 5 replicated doses of each gamma and beta irradiation ,which ranged (1.218×10^{-4} -0.320µSv) and (63.100 -96.950µSv) respectively. The total effect of gamma and beta irradiation on *Vibrio colera*e viability was abrogated at (96.915177 & 63.100 µSv) by ^{137}Cs(1µci) and ^{137}Cs(9µci) respectively, the percentage of killing was highly (84%) and (85%) respectively and the viable cells was fewer than control. Gamma and Beta irradiation is efficient to killing *Vibrio cholerae* that cause many infection to human and may be cause death. The sensitivity of antibiotics of *Vibrio cholerae* to ten of antibiotic was tested, the results showed that percent of resistant against *V. cholerae* to Erythromycin , Methicillin, Ampicillin and Tetracycline was 70%, the percent of resistant to Nalidixic acid was 50%, percent of resistant to Amikacin was 10%, while percent of resistant to Chloramphenicol, Streptomycin, Ciprofloxacin and Norfloxacin was 0%.

Keywords: *Vibrio cholerae* , Irradiation , Type of decay

الخلاصة

الدراسة قيمت تأثير اشعة كاما وبيتا على عصيات الكوليرا المعزولة . التجربة تضمنت نماذج السيطرة بدون التعرض للاشعاع و (5) جرع من الاشعاع لكل من اشعة كاما واشعة بيتا بمعدل (1.218×10^{-4} -0.320µSv) و (63.100 -96.950µSv) على التوالي. التاثير القاتل لاشعة كاما واشعة بيتا على عصيات الكوليرا تم ابقاءه على (96.915177) و (63.100 µSv) بواسطة ^{137}Cs(1µci) و^{137}Cs(9µci) وعلى التوالي .

نسبة القتل كانت عالية (84%) و (85%) على التوالي والخلايا القادرة على الحياة كانت اقل من السيطرة .

اشعة كاما واشعة بيتا كانت فعالة في قتل عصيات الكوليرا والتي تسبب العديد من الاصابات الى الانسان وربما تسبب الوفاة .اختبرت حساسية عصيات الكوليرا العشرة من المضادات الحيوية والنتائج اظهرت بان نسبة مقاومة عصيات الكوليرا لمضاد الارثرومايسين والمثيسلين والامبسلين كانت (70%) ونسبة المقاومة لمضاد النالدكسك اسد كانت (50%) ونسبة المقاومة لمضاد الاميكاسين كانت (10%) بينما كانت المقاومة لمضاد الكلورامفينيكول والستربتومايسين والسبروفلوكساسين والنورفلوكساسين (0%) .

Introduction

Vibrio species are halophilic, Gram-negative bacilli ,facultative anaerobic, genus *Vibrio* belongs to the *Vibrionaceae* family **[1].** Approximately(190- 200) serogroups compose the *V. cholerae* species according to the surface O antigen of the lipopolysaccharide **[2]**. The cholera toxinis also called(Choleragen)of the most important virulence by *V. cholerae*, which is Exotoxins factors**[3]**. *V. cholerae* O1 strains are classified into two biotypes depending on phenotype and genotype properties:'classical' and 'El Tor'**[4]**.hemolysin product by V. cholerae (Eltor) strains but (classical) strains non-producing hemolysin**[5] .** hemolysin plays a clear role in the events of diseases, caused by events holes in the target membranes of cells, where the bacteria analysis of red

blood cellsin order to get the iron is essential for making proteins needed by bacteria in the metabolic processes**[6]**.

V. cholerae exist predominantly as matrix enclosed, surface associated communities known as biofilms which confer a marked survival advantage in hostile environments by providing safety against harmful sources such as antibiotics , pH variations including those within the human digestive tract, and host defense mechanisms **[7]** ,as a physical cold process, has been Gamma irradiation it killed bacteria by inhibiting bacterial division , breaking down DNA of bacteria **[8]**.

Many antibiotics used to treatment *V. cholera* such as Chloramphenicol, Doxycycline, Furazolidone Cotrimoxazole, Erythromycin, Tetracycline, Azithromycin are used as first-drug for treatment pregnant women and children ,while Doxycycline used in treatment this bacteria for adult, some antibiotics inhibit the protein synthesis in bacteria such as Chloramphenicol and Tetracycline **[9].** The aim of this study was to detect Comparative Activity of Gamma and Beta Irradiation and Some of Antibiotics Against *Vibrio cholerae* bacteria**.**

Material and Methods

Bacterial isolates and Irradiation

25 isolates of *cholera bacterial* were sampled from patients who were admitted to Baghdad hospitals in 2015.These isolates were identified depending on the criteria established by conventional biochemical reactions by Forbes **[10].** The *Vibrio cholerae* isolates was grown in (Nutrient broth) for(1 day). 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 deferred cells were collected in a sterile flask to form pool. The bacterial suspension of the pool (4-5ml) was distributed in sterile screw cap test tubes and exposed to different quantities of Gamma radiation using five replicates for each dose, as well as for Beta. used dilution and plated on the surface of (Trypton soy agar)plates and determined the viable count .

The ratio of killing calculated from following equation :

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

Antibacterial Susceptibility Test

All isolates of *V. cholera* were tested of sensitivity to 10 of antibiotic are Ceftriaxone (CRO 30µg) , Ceftazidime (CAZ 30µg) , Imipenem (IPM10 µg) ,Cefepime (FEP 30µg) , Amikacin (AK 30µg) , Gentamicin (GM 10 µg) ,Ciprofloxacin (CIP 5µg), Piperacillin (PIP100µg) , Aztreonem (AT 30µg) and Ticarcillin (Tcc 75µg) by disk method on Mueller-Hinton agar .All isolates were tested for antibacterial susceptibility dependent on the inhibition zone **[11].**

Determination of the minimum inhibitory concentrations of ciprofloxacin

Two fold dilution method was used on Mueller-Hinton agar for determinate MIC_s of Ciprofloxacin**[11].**

Effect of Gamma, Beta Irradiation on *Vibrio cholerae* isolates:

The isolates be there inoculated on Nutrient agar for 24 hr. of incubation at 37°C. The irradiation used was gamma (γ) and Beta (β) irradiation of different energy and different dose using different sources for different time of exposure .The *Vibrio cholerae* isolates was grown in (Nutrient broth) for(1 day). 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 sterile flask to form pool. The bacterial suspension of the pool (4-5ml) was distributed in sterile screw cap test tubes and exposed to different doses of Gamma radiation using five replicates for each dose, as well as for Beta. used

dilution and plated on the surface of (Trypton soy agar)plates and determined the viable count **[12].**

Results and Discussion

The isolates were cultured on trypton soy agar decrease in number after exposure to radiation ,these effect can be measured by loss and died cells on this media, 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) 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. we find that the effect of Beta higher than the Gamma in killing of bacteria and this is ay be because the nature of Beta and Gamma radiation and by specfic ionizaition higher than the gamma and it is the fact that the beta is acharge partical. As well as , we can choose low energy with high activity to get high dose which is efficient to killing *Vibrio cholerae* because irradiation effect directly or indirectly on cell membrane , DNA , cytoplasmic membrane by absorbance irradiation from this bacteria and thereby cause damage to this bacteria **.**

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

Isotope				
	Type of decaye	E (MeV)	Do (µSv)	Killing ration %
^{137}Cs(1µci)	β	0.198	63.100	85%
	γ	0.662	$1.28*10^{-4}$	10%
^{60} Co(1µci)	β^-	0.318	10.573	51.81%
	γ	1173.1332	$1.3178*10^{-4}$	4.96%
				55%
^{137}Cs (5µci)	β +	0.514	32.444	31.67%
	γ	0.662	$1.416*10^{-4}$	32.33%
				84%
^{137} Cs (9µci)	β	0.514	96.950	29.47%
	γ	0.662	$1.776*10^{-4}$	51.02%
				87 %
^{241}Am(10µci)	α	5485.6 5442.8	non	72%
	γ	0.060	0.320	

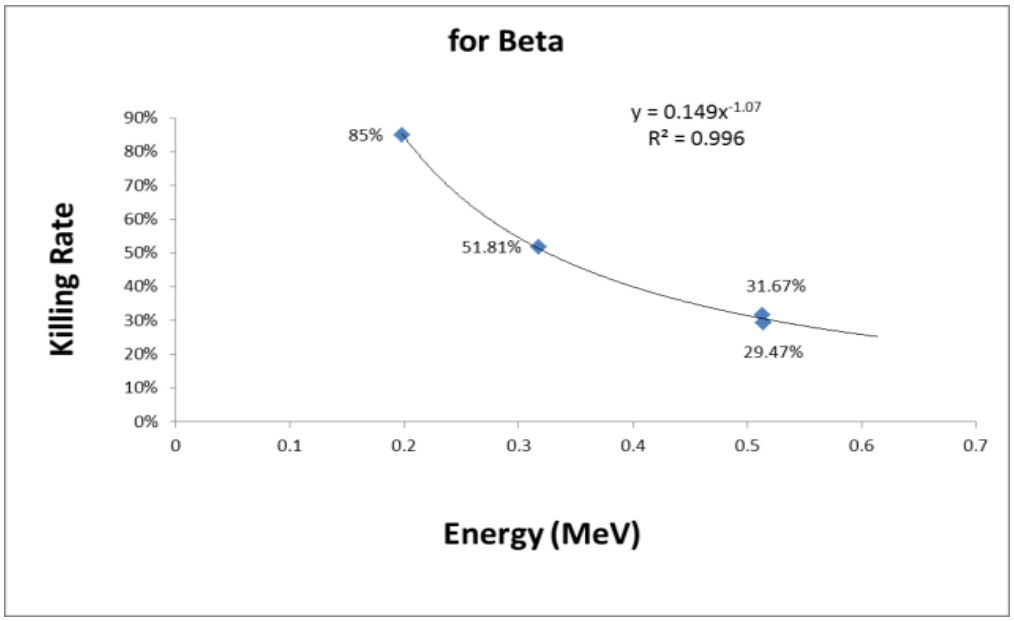

Fig (1): Energy and killing Rate for Beta irradiation

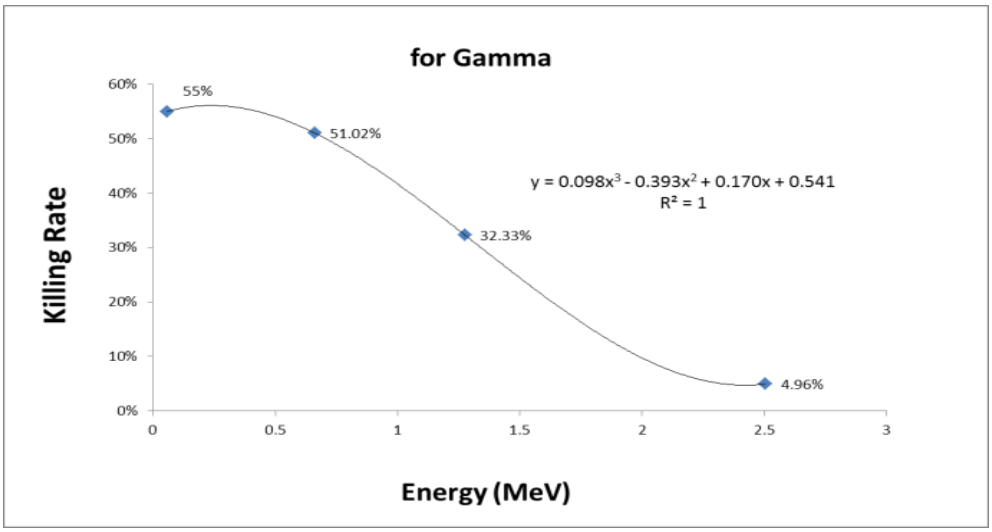

Fig (2): Energy and killing Rate for Gamma irradiation .

The percentage of resistance against *V. Cholerae* to Erythromycin , Methicillin, Ampicillin and Tetracycline was 70%,the percent of resistant to Nalidixic acid was50%, percent of resistant to Amikacin was 10%, while percent of resistant to Chloramphenicol, Streptomycin, Ciprofloxacin and Norfloxacin was 0% shown in fig.(3).

1-Erythromycin
2-Methicillin
3-Ampicillin
4-Tetracycline
5-Nalidixic acid
6-Amikacin
7-Chloramphenkol
8-Streptomycin
9-Ciprofloxcin
10-Norfloxacin

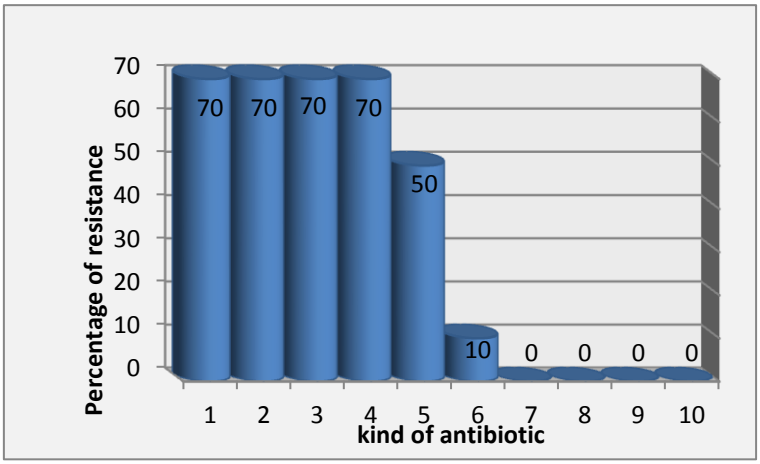

Figure (3): The percent of resistant to antibiotic for *Vibrio cholerae*

many of antibiotic inhibit the protein synthesis in bacteria such as chloramphenicol and Tetracycline **[14]**.with widespread antibiotic usage cause multiply resistant bacteria ,induce several changes in gene expression due to sub-MICs of antibiotics **[15]**.

from antibiotics resistance mechanism are conjugative plasmids , integrons ,spontaneous chromosomal mutation, and SXT elements **[16]**.

In local study was done by Alnaddawi *et al* **.[17]** they found the clinical isolates of *V. cholerae* were showed variable conduct against these antibiotics, these showed (90%) resistance to ciprofloxacin, as a maximum value and (50%) for

amoxicillin as a lowest value.

The activity of radiation can be divided into indirect effect and direct effect on living organisms, the biological effectively of radiation duo to discrete changes in the molecular structure and nucleus of the cells**.** The genetic damage affecting succeeding generations happen because of permanent hereditary changes in surviving cells **,** subsequent tissue damage reproductive death of cells **[18,19].**

Gamma radiation caused three kinds of damage in nucleic acid. Thus irradiation cause damage which can produces mutations which cause change in the biological activities of the bacterial cell, discrete changes in the nucleus from these biological activities of radiation, the absorption of ionizing radiations duo to chromosomal alterations **[20,21]**.

Misrepair could occur either because of base sequence information or repair occurring on a template error-prone repair system **[22,23]**. Gamma and Beta radiation effect on *V. Cholerae* especially DNA and Nucleic acid include three kinds of damage in the cell, double strand breaks ,single strand breaks, and nucleotide damage which include damage in the sugar moiety and a major component of damage is base damage,some bacterial cells after exposure to irradiation, contain exhibit heritable changes, while in the other cells abnormal sets of chromosomes **[24]**.

The recommendation in this study :Gamma and Beta irradiation is active against *V. cholerae* bacteria and can be used as alternative of antibiotic because the side effect for these antibiotic and the cost.

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