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EFFECT OF GAMMA AND BETA RADIATION ON MULTI –DRUG RESISTANT(MDR) *PSEUDOMONAS AERUGINOSA* RESISTANCE COLISTIN ISOLATED FROM SKIN, BURNS, WOUND INFECTIONS AND INHIBITION OF MEXXY, MEXAB-OPRM EFFLUX PUMPS

Nebras R. Mohammed[1*], Hanna S. Sabaa[2], Mohammed F. Al-Marjani[3] and Khalid H. Mahdi[4]

[3,1]Department of Biology – College of Science – Al- Mustansiriya University.
[2]Department of Physics – College of Science – Al- Mustansiriya University.
[4]Department of Physics – College of Education-Ibn Al Haitham– Baghdad University.

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***Correspondence for Author**
Dr. Nebras R. Mohammed
Department of Biology – College of Science – Al-Mustansiriya University.

ABSTRACT

It was obtained *P.aeruginosa*, it has been activated then held conducted her dilution according to the method used below, it has been exposed to Beta and Gamma irradiation that emitted from ^{137}CS source with activity 1.712 **µ**ci, 5 **µ**ci, 9 **µ**ci and ^{60}Co source with activity 1 **µ**ci and ^{22}Na source with activity 1 **µ**ci, in different dose calculated of KGy which emitte Beta and Gamma irradiation. for a time of (2,3,) hours for a contenouse exposure, it has been calculated number of colony and percentage of killing of *P.aeruginosa* resistance Colistin. It has been obtained to increase the times increased the percentage of killing of *P.aeruginosa*, the morphology of colony were different from origin appear her clear change Detection of Gene expression of *P.aerginosa* resistance Colistin before exposure to Beta and Gamma irradiation and after exposure to Beta and Gamma irradiation was performed done by using q RT-PCR technique after RNA extraction of *P.aerginosa* and cDNA synthesis, calculated gene expression according to Livak equation to detection of gene expression of MexXY and MexAB-OprM efflux pumps by studied *mexB* and *mexX* gene, The results showed that gene expression were high to MexXY and MexAB-OprM efflux pumps of *P.aerginosa* resistance Colistin before exposure to Beta and Gamma irradiation. but The results showe gene expression were very fewer to MexXY and MexAB-OprM efflux pumps of *P.aerginosa* after exposure to Beta and Gamma irradiation, the gene expression of MexXY, MexAB-OprM efflux pumps reach zero. The Beta and Gamma

irradiation was efficiency inhibitors to MexXY and MexAB-OprM efflux pumps. and efficiency to killing *P.aeruginosa* resistance Colistin. This result give indicat to DNA was effected by Beta and Gamma irradiation and not occur gene expression of efflux pumps.

KEYWORDS: *P.aeruginosa*, Gamma and Beta irradiation.

INTRODUCTION

P.aeruginosa is a Gram-negative bacillus that is most frequently associated with opportunistic infection an opportunistic pathogen that causes life-threatening infections in individuals with compromised immune systems, such as cancer patients undergoing chemotherapy or patients with cystic fibrosis. Immunocompromised individuals are at high risk of becoming infected in a hospital setting where *P.aeruginosa* causes a variety of nosocomial infections, including pneumonia, urinary tract infections, surgical wound infections and blood stream infections. Biofilms are structured communities of bacteria embedded in a polysaccharide matrix and growth in this form renders *P.aeruginosa* innately resistant to antimicrobial treatment (3). The fact that *P.aeruginosa* is resistant to treatment with antibiotics demands alternative strategies to treat infections with this pathogen. (Pukatzki *et al*., 2001). The ubiquitous bacterium *Pseudomonas aeruginosa* is the quintessential opportunistic pathogen. Certain isolates infect a broad range of host organisms, from plants to humans (He *et al*., 2003).

The range of *P.aeruginosa* infections varies from localized infections of the skin to life-threatening systemic disease. Many *P.aeruginosa* infections are marked by characteristic cutaneous manifestations, The ability of *P.aeruginosa* to rapidly acquire antibacterial resistance is an increasingly well recognized phenomenon, and the correct application of antipseudomonal therapy is therefore of the utmost importance. Rapid clinical recognition of *P.aeruginosa* infection aided by the identification of characteristic cutaneous manifestations can play a critical role in the successful management of potentially life-threatening disease (Wu *et al*., 2011).

Beta rays is a electrons or neutrons (positively charged electron), it possesses high speed produced from the nucleus as a result of the disintegration of the proton or neutron and accompanying emitted particle known as the neutrino or anti neutrino in respectively, Gamma rays is a electronmagnetic radiation is issued as a result of moving the nucleus from the excited state to the ground state directly or in stages to move to a state of less then signal

down to the ground state as a result of any other nuclear process kanavat alpha, beta or another nuclear reaction to get rid of excitation energy (Ahmed and Mohammed, 1988).

q RT-PCR is one of the molecular biological techniques are used to amplify the gene and determine its existence, as well as identifying a gene expression even for a new mount of genes, allow to amplify the product Complementary DNA (c DNA) copied inversely from mRNA, this so-called quantitative reverse transcription Real Time PCR (q RT-PCR) (Gudnason *et al*., 2007).

The aim of this study was to detect the effect of gamma and Beta irradiation on *P. aeruginosa* resistance Colistin isolated from skin, burns. wound infection and study of gene expression of efflux pumps before and after exposure to Beta, Gamma irradiation.

MATERIALS AND METHODS

Bacterial isolates

A total of 50 *P.aeruginosa* isolates were collected from several samples (abscess, wound and burns 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 a nutrient agar plate. The results were read after 24 h of incubation at 37°C. And antibiotic sensitivity was done to different antibiotic them Polymyxins B, Colistin E, The isolates of *P.aeruginosa* were inoculated on Muller Hinton Agar plate. The results were read after 24 of incubation at 37°C.

Effect of Beta and Gamma irradiation on MDR *P. aeruginosa* resistance Colistin

The irradiation facility used was gamma (γ) and Beta ( ) irradiation unit of (^{22}**Na,** ^{137} **Cs**, ^{60}Co) isotope. The *P.aeruginosa* resistance Colistin was grown on Nutrient broth for 24 h. The well grown bacterial culture was centrifuged at 8000 rpm for 15 minutes. 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 and Beta irradiation, using triplicates for each dose. 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 (Trampus *et al*., 2006).

RESULTS AND DISSCUSION

The lethal effect of ionizing radiation on microorganisms, as measured by the loss by cells of colony-forming ability in TSA, has been the subject of detailed study. Much progress has been made towards identification of the mechanism of inactivation, but there still considerable doubt as to the nature of the critical lesions involved, although it seems certain that lethality is primarily the consequence of genetic damage. 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 (Greez *et al*, 1983).

The percentage of killing calculated from equation below

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

This study aims to prove the effect of Gamma and Beta irradiation directly on the *P.aeruginosa* resistance Colistin. After the exposure of *P.aeruginosa* resistance Colistin to different doses of irradiation (^{60}**Co,** ^{22}**Na,** ^{137}**Cs**) isotope, the viability of these cells determined using count of *P.aeruginosa* colony in table (1).

Table (1): Dose, Energy, Activity of different isotopes and Killing ration of *P.aeruginosa* resistance Colistin after exposure to Gamma and Beta irradiation.

Isotope	Type of decaye	Activity	Dose (KGy)	Killing ration %	Dose (KGy)	Killing ration %
			2 hr		3 hr	
^{60}Co	β	1µci	$0.8262*10^{-10}$	89.5%	$1.239*10^{-10}$	95.07%
^{137} Cs	ɣ, β	5µci	$0.257*10^{-10}$	96%	$0.3863*10^{-10}$	84.9%
	ɣ, β	9µci	$1.3158*10^{-10}$	85%	$1.973*10^{-10}$	99.6%
^{22} Na	ɣ, β	1µci	$1.0225*10^{-10}$	97.5%	$1.533*10^{-10}$	99.3%

In table (1) show killing ration of *P.aeruginosa* resistance Colistin after exposure to Gamma and Beta irradiation in different time (2, 3) hr. of (^{60} **Co,** ^{22}**Na,** ^{137}**Cs**) isotope, this result give highly precentage of killing of MDR *P.aeruginosa* resistance Colistin when exposed to Gamma and Beta irradiation, because the irradiation effected on cell membrane, DNA, cytoplasmic membrane by absorbance irradiation and therby cause damage to this bacteria.

Gamma rays cause damage at a cellular level and are penetrating, causing diffuse damage throughout the body. However, they are less ionising than alpha or beta particles, which are less penetrating. (Rothkamm *et al*., 2003). therefore by mechanisms of Gamma and Beta irradiation can be elimination of *S.aureus* that causes many infection to skin of human, particularly *S. aureus* infections can spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing hyaluronidase that destroys tissues, and contact with objects such as towels, sheets, clothing, or athletic equipment used by an infected person. Deeply penetrating *S. aureus* infections can be severe. (Zhu *et al.*, 2008).

A study by Shokier *et al*., (2010) show Seventy one samples were randomly collected from patients suffering from different bacterial skin infections as *S.aureus* and *P.aeruginosa*. the isolates were resistant to Ciprofloxacin. the antibiotic sensitivity was retested for the most resistant bacterial isolates after irradiated by different doses of gamma radiation (0.5 ,1 ,2) Gy. when exposure to irradiation that lost resistance but become sensitive to antibiotics. very low doses of gamma irradiation increase the release rate of capsule content in both types of bacteria.

Study By Ezzat *et al*., (2014) was conducted to determine the effect of different doses of gamma radiation on Multi-drug resistant *P.aeruginosa* isolated from River Nile at Rosetta branch and associated drains in Egypt. Water samples were processed using membrane filtration, Irradiation of bacterial isolates was processed using gamma irradiation unit of cobalt (^{60}Co). The viable counts of MDRPA decreased with increasing radiation doses of gamma rays up to the lethal dose (3 kGy). Contaminated fresh water may act as reservoirs for antibiotic resistant pathogens. Gamma radiation demonstrates a potential value for wastewater treatment and pollution control.

Table (2): Gene expression of MexAB-OprM and Mex XY efflux pumps of *P.aeruginosa* resistance Colistin by using qRT-PCR Before exposure to irradiation.

sample	Calibrator			Treated			ΔΔCt	Ration (Fold 1)
	mexX	***rps***	**ΔCt**	***mexX***	***rps***	**ΔCt**		
1	26.11	16.11	10	20.25	12.94	7.31	-2.69	6.4
2	23.26	16.33	6.93	23.94	18.85	5.09	-1.84	3.6
3	32.74	33.95	-1.21	29.8	35.82	-6.02	-4.81	28.1
4	27.54	17.27	10.27	33.49	34.47	-0.98	-11.25	2435.5
sample	**Calibrator**			**Treated**			**ΔΔCt**	**Ration (Fold 1)**
	mexB	***rps***	**ΔCt**	***mexB***	***rps***	**ΔCt**		

1	20.78	16.33	4.45	7.13	5.34	1.79	-2.66	6.3
2	21.11	16.11	5	22.2	18.9	3.3	-1.7	3.2
3	25.54	16.11	9.43	26.71	22.3	4.41	-5.02	32.4
4	25.54	16.11	9.43	26.71	22.3	4.41	-5.02	32.4

In table (2): gene expression of MexAB-OprM and MexXY efflux pumps of *P.aeruginosa* resistance Colistin before exposure to Beta and Gamma irradiation, show all isolates possess gene expression of MexXY and MexAB-OprM efflux pumps of *P.aeruginosa* resistance Colistin when added 200µL/L Gentamycine to detection of MexAB-OprM and added 2 mg/ L Colistin powder to detection of MexXY Of *P.aeruginosa* resistance Colistin by using q RT-PCR.

Aprevious study by Dumas *et al*., (2006) Use *mexX* gene to known gene expression by using Real Time PCR, which found gene expression increase from 8-12 once the existence Tetracycline. also study by Vettoretiti *et al*., (2009) on *mexB* gene to known gene expression by using Real Time PCR, found gene expression increase from 4 to 64 once with added Gentamycine, this result agree with our study.

Study by Poonsuk and Chuanchuen, (2014) show the antibiotic substrates specific for each Mex systems were used as phenotypic markers including carbenicillin, MexAB-OprM, erythromycin, MexCD-OprJ, norfloxacin and imipenem, MexEF-OprN and gentamicin, MexXY-OprM. The methods were validated with reference strains with known genotypes of the Mex systems and the potential applicability in clinical practice was tested with clinical isolates. The results for the reference strains support that the combination of resistance phenotype and mRT-PCR is a potential-attractive method for diagnosis of efflux-mediated resistance in *P.aeruginosa*. Further development to make it more practical for clinical use and study in a larger number of clinical isolates is required.

Diagnosis of efflux-mediated resistance generates data that is helpful for both routine clinical analysis (rationalizing the antibiotic selection and dose) and epidemiological studies (monitoring the existing and prevalent resistance mechanisms), As yet, no EPIs are approved for clinical use. (Mesaros *et al*., 2007).

Efflux pump inhibitors (EPIs) have been under investigation as an alternative to the development of new antibiotics for treatment of *P. aeruginosa* infection (Lomovskaya *et al.*, 2001).

Table (3): Gene expression of MexAB-OprM and MexXY efflux pumps of *P.aeruginosa* resistance Colistin by using q RT-PCR after exposure to irradiation.

Isotope	Activity	sample	Calibrator			Treated			ΔΔCt	Ration 1) (Fold	Killing ration (3hr.)
			mexX	*rps*	ΔCt	*mexX*	*rps*	ΔCt			
Co	1µci	1	0	18 .0	-18	22.98	24.94	-1.96	16.04	0.0	95.07%
CS	5µci	2	26.50	7.12	9.38	24.8	14.0	10.8	1.42	0.3	84.9%
CS	9µci	3	0	18.0	-18.0	22.0	23.94	-1.94	16.06	0.0	99.6%
Na	1µci	4	0	15.0	-15.0	23.98	25.94	-1.96	13.04	0.0	99.3%
Isotope	**Activity**	**sample**	**Calibrator**			**Treated**			**ΔΔCt**	**Ration (Fold 1)**	**Killing ration (3hr.)**
			mexB	*rps*	ΔCt	*mexB*	*rps*	ΔCt			
CO	1µci	1	0	18.0	-18 .0	22.0	23.94	-1.94	16.06	0.0	95.07%
CS	5µci	2	25.54	16.11	19.43	24.8	14.8	10	0.57	0.6	84.9%
CS	9µci	3	0	17.27	-17.27	23.98	25.94	-1.96	15.31	0.0	99.6%
Na	1µci	4	34.0	40.0	6.0	30.0	0.0	30.0	24.0	0.0	99.3%

Trampuz *et al*., (2006) show Gamma irradiation is widely used for sterilizationr, its effect on elimination of amplifiable DNA. The effect of gamma irradiation on the viability of *Staphylococcus epidermidis* and *Escherichia coli* (using quantitative cultures) and on their DNA (using quantitative 16S rRNA gene PCR) was evaluated. Viability was abrogated at 2.8 and 3.6 kGy for *S. epidermidis* and *E. coli*, respectively.

With few new antibiotics and the rising incidence of MDRPA worldwide as well as growing water scarcity in parallel to increased levels of water pollution, it becomes increasingly important to utilize strategies that will minimize antibiotic resistant strains spread and mediate wastewater treatment and reuse. In this study we suggested gamma irradiation as a new, non-conventional and effective method for wastewater treatment. Lethal dose of gamma rays to MDRPA was recorded at 3 kGy. It is well known that, exposure of bacterial 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. Gamma radiation induced three types of damage in DNA, single strand breaks, double strand breaks and nucleotide damage which include base damage and damage in the sugar moiety (Farrag *et al*., 1996). The base damage is a major component of damage induced by ionizing radiation (Pouget *et al*., 1999). Gamma irradiation also affects protein fingerprinting and enzymes as indicated by (Abo-State *et al*., 2001; Abo-State *et al*., 2004).

CONCLUSIONS

This study support use Beta and Gamma irradiation of ^{137}CS source with activity 1.712 µci, 5 µci, 9 µci and ^{60}Co source with activity 1 µci and ^{22}Na source with activity 1 µci to, in different doses KGy to killing *P.aeruginosa* resistance Colistin isolated from skin infection, Burns and Wounds. also use Beta, Gamma irradiation as inhibitors to gene expression of MexAB-OprM and MexXY efflux pumps of *P.aruginosa* by using Real Time PCR (q RT-PCR).

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