Inhibition gene expression MexAB-OprM and MexXY efflux pumps of *Pseudomonas aeruginosa*(XDR) by Novel inhibitors Levofloxacin, Silver nanoparticles and Beta rays.

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Abstract

This study was achieved in order to determine the inhibition of gene expression of MexAB-OprM and MexXY efflux pumps by using different Novel inhibitors including: Levofloxacin, AgNPs and Beta rays emitted by Tl^{208} and Sr^{90} on *P.aeruginosa* (XDR). Efflux pumps are responsible for pumps various kinds of antibiotics including quinolones(fluoroquinolones),Aminoglycosides, B-Lactam and Polymyxin in *Pseudomonas aeruginosa* (XDR).

Levofloxacin utilized as Novel inhibitor to MexXY and MexAB-OprM effiux pumps to *P.aeruginosa*(XDR),the percentage of killing determined.Detection of gene expression by using qRT-PCR,improved no gene expression of MexXY and MexAB-OprM when adding Levofloxacin 50 mg/ml when study gene expression of *mexX* and *mexB* encoded to MexXY and MexAB-OprM efflux pumps.

Silver nanoparticles(AgNPs) used as New inhibitor of MexXY and MexAB-OprM effiux pumps of *P.aeruginosa*(XDR), the percentage of killing calculated , the survey of gene expression by utilizing qRT-PCR was determined. The results exhibit no gene expression of the MexXY and MexAB-OprM when adding Silver nanoparticles 100 mg/ml with study of *mexX* , *mexB* and compared with control (without treating).

Beta rays utilized as new inhibitors emitted of Tl^{208} radiosource with activity 1 **µ**ci, dose $12.93*10^{-10}$ KGy for 3 hr.; also exposure to Sr^{90} radiosource with activity 1 **µ**ci, dose $1.937*10^{-10}$ KGy for 3 hr. and exposure to Sr^{90} radiosource with activity 9 **µ**ci, dose $6.3*10^{-10}$ KGy for 3 hr. The results exposition Beta radiation *P.aeruginosa*(XDR) showed the number of colonies less than the control with rise the percentage of killing of *P.aeruginosa*(XDR), also it found the morphology of the colonies changed compared with the original(control).

Detection gene expression of *P.aerginosa*(XDR) prior exposition to Levofloxacin, AgNPs, Beta rays and after exposition to Levofloxacin, AgNPs, Beta rays was achieved by qRT-PCR technique, RNA extraction of *P.aerginosa*(XDR),synthesis of cDNA ,calculate gene expression according to Livak equation to detect gene expression of MexXY and MexAB-OprM efflux pumps with study of *mexB* and *mexX* gene. The results exhibit gene expression prior exposition to Levofloxacin, AgNPs and Beta rays were highly in MexXY and MexAB-OprM efflux pumps of *P.aerginosa*(XDR) but fewer after exposition to Levofloxacin, AgNPs and Beta rays.The Levofloxacin, AgNPs, Beta rradiation were ecompetence to killing *P.aeruginosa* and qualification

as novel inhibitors to inhibit gene expression of MexXY and MexAB-OprM efflux pumps *P.aerginosa*(XDR).

Keywords: Antibiotics, Nanoparticles, types of radiosources, types of radiation, *P.aeruginosa*.

Introduction

Pseudomonas aeruginosa is gram negative bacteria, causing many diseases in the human body, including wounds infection ,burns infection,eye infection, skin infections ,urinary tract infection ,middle ear infections, bacteremia and Nosocomial infection also causing disease who suffer from HIV disease and[1].

Efflux pumps are membrane- bound transporter proteins having a wide spectrum of antibiotics specificity and expel drug [2], protein channel that located in cytoplasmic membrane have an important role in different of excluded agents. Chromosomal genome of *Pseudomonas aeruginosa* encodes for many types of efflux pumps, especially family Resistance Nodulation cell-Division (RND),so–called multidrug efflux pumps (MDR-Efflux pumps) like MexAB-OprM and MexXY[3,4]. The antibiotics exclusion from the bacterial cell and release out including: Novobiocin, Quinolones ,Chloramphenicol, Tetracycline, B-Lactams,Trimethoprim and Macrolides[5].The found of mutation in genes cause rapid development of resistance of *P.aeruginosa* to antibiotics during a short period in the treatment, this a danger because the development during treatment cause a failure in treatment from bacterial infection [6,7].

MDR(Multi Drug Resistance) is acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR(Extensively Drug Resistance) is as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (bacterial isolates remain susceptible to only one or two categories) and PDR(Pan Drug Resistance)is non-susceptibility to all agents in all antimicrobial categories[8].

A quinolone antibiotic is a member of broad-spectrum bactericides,they are used in human medicine to treat from bacterial infections [9].Nearly all quinolone antibiotics in use are fluoroquinolones which contain a fluorine atom in chemical structure and effective against both Gram-negative and Gram-positive bacteria. One example is ciprofloxacin, one of the most widely used antibiotics to treat from many types of bacteria [10].

Fluoroquinolones are a first-line therapy for genitourinary infections and treatment of hospital-acquired

infections such as urinary catheters, in community-acquired infections for multidrug resistance, for acute cases of pyelonephritis andbacterial prostatitis where hospitalised, [11].Fluoroquinolones are used prominently in guidelines for the treatment of hospital-acquired pneumonia [12].

Levofloxacin, Levaquin is a quinolone antibiotic, antimicrobial spectrum for Gram positive bacteria and Gram negative bacteria used to treat different bacterial infections including acute bacterial sinusitis, pneumonia, urinary tract infections, chronic prostatitis and gastroenteritis. It used along with other antibiotics to treat tuberculosis, meningitis, pelvic and inflammatory disease.The action of Levofloxacin by inhibition topoisomerase (DNA gyrase), which inhibits relaxation of supercoiled DNA and allow to breakage of double stranded DNA [13,14]

The reason for the difficulty of the treating because resistance to many of the antimicrobials including : B-lactam, Macrolides , Flouroquinolones work it one of the most dangerous pathogen[15],that resistance to Polymyxin , Colistin (polymyxin E) a group of polypeptide antibiotics that used extensively in topical and ophthalmic solutions, two forms of Colistin are commercially available , Colistinsulfate and Colistimethate sodium, that binds with the anionic lipopolysaccharide molecules by displacing calcium and magnesium from outer cell membrane of gram negative bacteria, leading to permeability changes in the cell envelope, leakage of cell contents and cell death[16,17].

qRT-PCR is one of the molecular biological techniques are used to amplify the gene and determine its existence gene ,as well as identifying a gene expression even for few mount of genes , allow to amplify the product Complementary DNA (c DNA) copied from mRNA ,so-called quantitative reverse transcription Real Time PCR(qRT-PCR) ,which depends on the use of Reverse Transcriptase responsiple for converting RNA to cDNA , in qRT-PCR pigments used them SYBR Green dye is linked to a specialist in Minor groove of double strand DNA , it is fast and sensitive for the detection of a small amount of mRNA, based on the data mathematically values of cycle threshold (ct) that reflect the values of the amount of change gene expression[18,19,20].

Nanoparticles are particles between 1 and 100 nanometres (nm) in size with a circumference interfacial layer. The interfacial layer is an integral part of nanoscale matter,essentially affecting all of its properties. The interfacial layer consists of ions, inorganic and organic molecules[21].

Silver nanoparticles (AgNPs) are antimicrobial agents,widely used in

medical applications [22].Silver types are Ag+ and $AgNO_3$ used as a source of silver ion, AgNPs or Silver sulfadiazine, which is an effective inhibitor [23,24].AgNPs have lower cellular toxicity than $AgNO_3$ used as a source of silver ion. Silver ions are widely used as disinfectants to control burns and eye injuries. Silver ions can block the bacterial cell wall that penetrate the cell,….. interfere with physiological functions of respiration and cell metabolism by binding to the thiol group in the protein thus causing the membrane to break down [25].

Colloidal AgNPs prepare by Chemical reduction,radioactive chemical reduction methods, acoustic chemistry and chemical chemical reduction method. The size of AgNPs is 5nm to 40nm.The most important in the preparation of metal nanoparticles (AgNPs) is the biological method. Many microorganisms use to preparation nanoparticles include bacteria, yeasts and fungus to produce different sizes of silver particles are: *Lactobacillus spp., Bacillus megaterium, Klebsiella pneumonia, Bacillus licheniformis, Corynibacterium spp., Proteus mirabilis, Bacillus spp., Staphylococcus aureus, Pseudomonas aeruginosa*,(AgNPs) are effective against Gram positive and negative bacteria [26].

Metallic nanoparticles not only enhance the antimicrobial activity but bactericidal activity that extensively used to treat infections in human cell because their low toxicity [27,28].

Beta rays is an 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 emitte particle known as the neutrino or anti neutrino,beta or another nuclear reaction to get rid of excitation energy[29].

Materials and methods

Bacterial isolates

A total of 200 *P .aeruginosa* isolates were collected from several samples (abscess, wound , burns , Sputum , blood , urinary tract catheter , urine and tumors) from patients who were admitted Baghdad hospitals to 2017-2018.These isolates were identified by conventional biochemical reactions according to the criteria established by [30].The isolates were inoculated a nutrient agar plate. The results were read after 24 and 48 hr. of incubation at 37°C and antibiotic sensitivity was done to different antibiotic Colistin(Polymyxin E),PolymyxinB, Gentamicin, Nalidixic acid, Aztreonam, Carbenicillin, Lomefloxacin, Levofloxacin, Ciprofloxacin, Norfloxacin,Tobramycin, Amoxicillin and Ofloxacin, 100 *P .aeruginosa* isolates were XDR (Extensively Drug

Resistance) inoculated on Muller Hinton Agar plate.

Effect of Antimicrobial agents (Antibiotics) as inhibitors on *P.aeruginosa* (XDR):

Antimicrobial Susceptibility

This test was indicated that all isolates by using disc diffusion test to thirteen type of antibiotics included: Colistin ,PolymyxinB, Gentamicin, Nalidixic acid, Aztreonam,Carbenicillin,Lomefloxacin,Levofloxacin,Ciprofloxacin,Norfloxacin,Tobramycin,Amoxicillin and Ofloxacin. The results were compared according to the recommendation of CLSI [31].

Table(1):Antibiotics test used by disc diffusion method:

Origin/ Company	Concentration of disc (µg/disc)	Symbol	Types of Antimicrobial	No.
Bioanalyse (Turkey)	10	CT	**Colistin**	1
	300	PB	**Polymyxin**	2
	10	CN	**Gentamicin**	3
	10	TOB	**Tobramycin**	4
	30	ATM	**Aztreonam**	5
	25	AX	**Amoxicillin**	6
	25	PY	**Carbenicillin**	7
	30	NA	**Nalidixic Acid**	8
	10	LOM	**Lomefloxacin**	9
	5	LEV	**Levofloxacin**	10
	10	CIP	**Ciprofloxacin**	11
	10	NOR	**Norfloxacin**	12
	5	OFX	**Ofloxacin**	13

Effect of Levofloxacin as Novel inhibitors on *P.aeruginosa* (XDR):

The method used to inhibit gene expression of MexXY and MexAB-OprM efflux pumps of *P.aeruginosa* (XDR) by using qRT-PCR technique when adding Levofloxacin with different concentrations 50, 60, 70 mg/ml with study of the *mexX* and *mexB* that encode to MexXY and MexAB-OprM efflux pumps of *P.aeruginosa*(XDR) and compared with control (without treating to Levofloxacin).

Effect of Silver nanoparticles (AgNPs) as new inhibitors on *P.aeruginosa* (XDR):

Determination of the minimum inhibitory concentration (MIC) of Silver nanoparticles(AgNPs):-

1- Determine the Minimum Inhibitory Concentration (MIC) of silver nanoparticles.

2-Added different concentrations of Silver nanoparticles(AgNPs) (50,60,70, 100 and 120 mg / ml).

3-The lowest inhibitory concentration was identified as the least concentration of the antibiotics that preventing the emergence of bacterial growth.

4-The gene expression of the *mexX*, *mexB* gene were studied after extraction of RNA from *P.aeruginosa*(XDR) of sub-MIC for Silver nanoparticle as inhibitor to efflux pumps.

5-cct was calculated for the isolation, then compared with gene expression of the before adding Levofloxacin as Novel inhibitory(control).

Effect of Beta rays (Tl^{208} and Sr^{90} radiosources) as inhibitors on *P.aeruginosa* (XDR):

P.aeruginosa (XDR) cultivation was done according to [32]with some modifications as follows:

P.aeruginosa (XDR) were cultivated in Pseudomonas agar at 37° C for 24 hr. to reach the stationary-phase culture, centrifuged (5000 rpm for 10 minutes), the supernatant was removed and the precipitate was re-suspended using sterile normal saline (physiological saline), the suspension was mixed using vortex to get homogenous suspension, which compared with the MacCfarland solution ($1.5*10^{8}$ CFU/ml), then 5 ml of this solution was exposed to Beta ray of Tl^{208} radiosource with activity 1 **µ**ci, dose $12.93*10^{-10}$ KGy for 3 hr., also exposure to Sr^{90} radiosource with activity 1 **µ**ci, dose $1.937*10^{-10}$ KGy for 3 hr. and exposure to Sr^{90} radiosource with activity 9 **µ**ci, dose $6.3*10^{-10}$ KGy for 3 hr. inoculated in Trypton soya agar at 37° C for 24 hr. , the colonies were counted, the effect of inhibition was evaluated and

calculate the percentage of killing (reduction of bacterial growth) counted by using the equation below:

The equation used to calculate percentage of Killing:

$$\text{Percentage of Killing } \% = \frac{\text{Control} - \text{treated}}{\text{Control}} * 100$$

Detection gene expression of MexXY and MexAB-OprM efflux pumps by using Quantitative Real Time PCR (qRT-PCR) :

Detection of *mexX* gene , *mexB* gene that encode to MexXY and MexAB-oprM efflux pumps by using qRT-PCR with use (Go Taq®1-Step RT-qPCR kit) and extraction of RNA with use (SV Total RNA Isolation System Kit),kept in (-20°c) , and placed in qRT-PCR **Smart Cycler:**

Table (2): The components used for the detection of gene expression :

Component	Final concentratiom	Size of interaction Mixture of one Tube (Microliter)
Go Taq®1-Step RT-qPCR	**1x**	12.5
Forword Primer (10 Picomole)	**1 Picomole**	2.5
Reverse Primer (10 Picomole)	**1 Picomole**	2.5
RT mix	**1x**	0.5
Template RNA		5
Distill Water (deionized)		2
Final size		25

Programmed device Smart Cycler (Quantitative Real Time PCR) as follows :

Steps		Process
1		One cycle for 15 minute in 95°c to primary denaturation of cDNA template .
2		40 cycle included:
	A	(20)sec. in tempreture 49°c denaturation of cDNA
	B	(20)sec. in tempreture 60°c to annealing cDNA template with Primer.
	C	(30)sec. in tempreture 72°c to Elongation Primers linked with cDNA
3		One cycle for (5)minute in 72°c to final Elongation to cDNA .

The calculated Ct (Cycle threshold) that in form Dissassociation Curve represent value of gene expression of *mexB* and *mexX* gene , calculated the gene expression according to Livake method[33]:

ΔCt(Treated sample**) = Ct GOI – Ct norm.**
ΔCt(Calibrator**) = Ct GOI – Ct norm.**

ΔΔCt = ΔCt(treat.) - ΔCt(cal.).

Fold change $= 2^{-\Delta\Delta Ct}$ **.**

Treated : refers to *P.aeruginosa* (XDR) treated with Antibiotics, AgNPs and Beta rays.

Calibrator : refers to *P.aeruginosa* (XDR) not treated with Antibiotics, AgNPs and Beta rays.
GoI : refers to value of target gene (*mexB* ,*mexX* gene).
Ct norm .: refers to value of Ct gene *rps* (house keeping gene) .

Results and Discussion

Detection gene expression of MexXY and MexAB-OprM efflux pumps by using Quantitative Real Time PCR (qRT-PCR) :

qRT-PCR is a technique of molecular biology based on the polymerase chain reaction (PCR) that is used to amplify the gene and

determine its presence, as well as determine its gene expression even if a small number of genes, it is a sensitive method, allows to amplify the product of cDNA (Complemantry DNA) replicated inversely from mRNA, Quantitative reverse transcription Real Time PCR (qRT-PPR) which is based on the use of reverse transcriptase, responsible for the conversion of RNA to cDNA, compared target gene with house keeping gene(It is essential for cell life and the mRNA construction of it is stable and safe in different tissues) [34].

Table (2):Primer sequences used in study gene expression of MexAB-OprM and MexXY efflux pumps of *P.aeruginosa* (XDR) by using q RT-PCR:

No.of Nucleotide	Sequences of gene	Gene		No.
19	'5-ATCCGCCAGACCATCGCCA-3'	mexB1	*mexB*	1
22	'5-CATCACCAGGAACACGAGGAGG-3'	mexB2		
23	'5-TGAAGGCGGCCCTGGACATCAGC -3'	mexX1	*mexX*	2
23	' 5-GATCTGCTCGACGCGGGTCAGCG-3'	mexX2		

Table (4):gene expression of MexAB-OprM and MexXY efflux pumps of *P.aeruginosa* (XDR) by using qRT-PCR Before exposure to inhibitors:

sample	Calibrator			Treated			ΔΔCt	Ration (Fold 1)
	mexX	*rps*	ΔCt	*mexX*	*rps*	ΔCt		
1	25.54	16.11	9.43	26.71	22.3	4.41	-5.02	**32.4**
2	23.26	16.33	6.93	23.94	18.85	5.09	-1.84	**3.6**

3	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	25.54	16.11	9.43	26.71	22.3	4.41	-5.02	**32.4**
2	25.22	15.76	9.46	27.41	22.12	5.29	-4.17	**18.0**
3	26.11	16.11	10	20.25	12.94	7.31	-2.69	**6.4**

The antibiotic specificity for each Mex efflux pumps were used as including Carbenicillin,MexABOprM; Erythromycin,MexCD-OprJ; Norfloxacin, Imipenem, MexEF-OprN and Gentamicin, MexXY. The qRT-PCR is a potential-attractive method for diagnosis of efflux-mediated resistance in *P. aeruginosa*. Diagnosis of efflux-mediated resistance is helpful for clinical analysis (rationalizing the antibiotic selection and dose) and epidemiological studies (monitoring the existing and prevalent resistance mechanisms) [35,36].

Effect of Antimicrobial agents and effect of Levofloxacin as Novel inhibitors on *P.aeruginosa* (XDR) :

Antibiotic resistance is not a recent original sin, it has observed when the first use of penicillin, resistance development in some bacterial isolates rapidly [37].

Antimicrobial resistance to antibiotics is the emergence of bacterial isolates do not inhibit their growth by MIC inhibitor. Antimicrobial resistance that cause problems are Multi-Drug Resistant (MDR) that observed in Gram-negative bacteria resistance to a wide range of antibiotics and increase morbidity and mortality[38,39].

The most important antibiotic resistance in *P.aeruginosa* by permeability barrier [40], alteration in Target Site [41], production of antimicrobial enzymes [42] and efflux Pumps[43].

Table (5):Antimicrobial agents test of *P.aeruginosa* by Disc diffusion method:

No.	CIP	NOR	LEV	OFX	LOM	NA	TOB	CN	ATM	PY	AX	PB	CT
1	R	R	S	R	R	R	R	R	R	R	R	R	R
2	R	R	S	R	R	R	R	R	R	R	R	R	R
3	R	R	S	R	R	R	R	R	R	R	R	R	R
4	R	R	S	R	R	R	R	R	R	R	R	R	R
5	R	R	S	R	R	R	R	R	R	R	R	R	R
6	R	R	S	R	R	R	R	R	R	R	R	R	R
7	R	R	S	R	R	R	R	R	R	R	R	R	R
8	R	R	S	R	R	R	R	R	R	R	R	R	R
9	R	R	S	R	R	R	R	R	R	R	R	R	R

Bacterial resistance to antibiotics is affected by several factors such as of bacteria, location of infection, concentration of antibiotics and the immune system [44].*P.aeruginosa* has little sensitivity to most of the antibiotics in medical treatment and making it bacteria a dangerous that threat human healthy[45].The resistance of *P.aeruginosa* to antibiotics is because having resistance genes either on chromosome or on plasmids that cause the development bacteria to antibiotic resistance [46].

The resistance of Polymyxins is either intrinsic or acquired. The resistance of colstin of bacteria based on phoP-phoQ systems done in a low magnesium environment when the concentration of magnesium is inhibited the isolates become sensitive to polymyxin; phoQ sensor kinase activation of phoP activating PmrA-PmrB activates the expression of genes responsible for encoded enzyme that necessary for Lipid-A[47]. The resistance of colstin also caused by gene mutation of *mexZ* that requlation MexXY[48].

Quinolone antibiotics have a lethal effect on microorganisms that inhibit DNA replication by inhibiting DNA-gyrase by break the helical binding of a DNA strand[49]. Resistance genes to antibiotics may carry on plasmid or on chromosome [50].Resistance occurs by lack of permeability of the outer membrane or production of OprM proteins cause a reduce in permeability of the outer membrane of bacteria and reduce the accumulation of antibiotics inside the cell of bacteria[51,52].

The resistance of bacteria to aminoglycoside antibiotics is because several mechanisms including the production of aminoglycoside modifying enzyme (AMEs) [53]or resistance by change membrane permeability[54]. The efflux pump inhibitors as new therapeutic agents bdue to development of multidrug resistance(MDR) of pathogens that result difficulties to elimination of many infectious diseases because overproduces express of efflux pumps that are responsible for the expeling the antibiotics from the cells, chemotherapeutics is a new direction used efflux pump inhibitors. The compounds used for inhibition efflux pump: either analoges for antibiotic or use chemical compounds as inhibitor (EPI) for efflux pumps [55].

Table (6):gene expression of MexAB-OprM and MexXY efflux pumps of *P.aeruginosa* (XDR) by using q RT-PCR after adding Levofloxacin as Novel inhibitor:

sample	Calibrator			Treated			ΔΔCt	Ration (Fold 1)
	mexX	*rps*	ΔCt	*mexX*	*rps*	ΔCt		
1	0	18.0	-18.0	22.98	24.94	-1.96	16.04	**0.0**
2	34.0	40.0	6.0	30.0	0.0	30.0	24.0	**0.0**
3	0	18.0	-18.0	22.0	23.94	-1.94	16.06	**0.0**
sample	Calibrator			Treated			ΔΔCt	Ration (Fold 1)
	mexB	*rps*	ΔCt	*mexB*	*rps*	ΔCt		
1	0	15.0	-15.0	23.98	25.94	-1.96	13.04	**0.0**

3	0	17.27	-17.27	23.98	25.94	-1.96	15.31	**0.0**
2	27.0	18.0	9.0	25.0	15.0	10.0	0.3	**0.0**

The results showed in table (6) by using Levofloxacin as Novel inhibitor to MexXY and MexAB-OprM effiux pumps of *P.aeruginosa*(XDR), calculate the percentage of killing, study gene expression by using qRT-PCR , fold calculate by Livake equation that mean gene expression. The results of MexXY and MexAB-OprM not expressed by adding Levofloxacin when studied *mexX* and *mexB* with adding 50 mg/ml and compared with control (without treating to Levofloxacin) have very high gene expression 2435.5. Levofloxacin is an effective inhibitor(Novel inhibitor) to efflux pump MexXY and MexAB-OprM of *P.aeruginosa*(XDR).

Effect of Silver nanoparticles(AgNPs) as new inhibitor on *P.aeruginosa* (XDR):

Nanoparticle action is the direct bound of metal nanoparticles to the active site of efflux pumps and expelling antibiotics outside the cells. Metal nanoparticles act as a competitive inhibitor of antibiotic for the binding site of efflux pumps [56].

Another action of nanoparticles are distruction of efflux kinetics of MDR efflux pump, MexAM-OPrM in *P. aeruginosa*, cause close of proton gradient and disruption of membrane possible or loss of proton motive force (PMF), resulting in damage of driving power necessary for efflux pump activity [57,58].

Also the combination metallic nanoparticles with an antibiotic that diminish their concentration drug dosage and reduce the toxicity to human cell lines [59].

Zinc oxide nanoparticles a novel efflux pump inhibitory on NorA efflux pumps of *S. aureus*, 22% raise zone inhibition for ciprofloxacin in the existence of zinc oxide nanoparticles in *S. aureus* and *E. coli* [60].

Table (7): Gene expression of MexAB-OprM and MexXY efflux pumps of *P.aeruginosa* (XDR)by using qRT-PCR after exposure to AgNPs as new inhibitors:

sample	Calibrator			Treated			ΔΔCt	Ration (Fold 1)
	mexX	*rps*	ΔCt	*mexX*	*rps*	ΔCt		
1	0	18.0	-18.0	22.0	23.94	-1.94	16.06	**0.0**
2	0	15.0	-15.0	23.98	25.94	-1.96	13.04	**0.0**
3	27.7	33.5	-5.8	25.5	25.2	0.3	6.1	**0.01**
sample	Calibrator			Treated			ΔΔCt	Ration (Fold 1)
	mexB	*rps*	ΔCt	*mexB*	*rps*	ΔCt		
1	0	18.0	-18	22.98	24.94	-1.96	16.04	**0.0**
3	0	17.27	-17.27	23.98	25.94	-1.96	15.31	**0.0**
2	27.7	18.0	9.7	25.0	15.0	10.0	0.3	**0.8**

The results in table(7) showed use Silver nanoparticles(AgNPs) as New inhibitor of MexXY and MexAB-OprM effiux pumps of *P.aeruginosa*(XDR), calculate the percentage of killing ,fold that mean gene expression that calculate by Livake equation when study *mexX* and *mexB* that encode to MexXY and MexAB-OprM efflux pump, the results were zero gene expression (no gene expression of efflux pumps)when adding AgNPs 100mg/ml) and compared with control (have high gene expression befor added silver nanoparticles). Silver nanoparticles (AgNPs) is an effective new inhibitor of MexXY and MexAB-OprM efflux pumps of *P.aeruginosa*(XDR).

Effect Beta rays on *P.aeruginosa* (XDR):

The lethal effect of ionizing radiation on microorganisms, determined by the absence of colony-forming unit(CFU) in medium. Some scientists suggest the mechanics of killing microorganisms by 'radiotoxins' that are the toxic matter created in the irradiated cells responsible for lethal effect. Others suggest the radiation was directly deleterious to the cellular membranes, enzymes energy, metabolism and cytoplasmic membrane[61].

Table (8):Colonies of *P.aeruginosa* (XDR) after exposure to Beta rays, Time, Dose and Percentage of killing :

Radiosource (Isotope)	Activity (Mci)	Time (hr.)	Dose (KGy)	Number of colonies of *P.aeruginosa* (XDR)	Percentage of Killing %
Tl^{208}	**1**	**3**	**$I\beta=12.39*10^{-10}$**	10	**93.3 %**
Sr^{90}	**1**	**3**	**$I\beta=1.973*10^{-10}$**	15	**90 %**
Sr^{90}	**9**	**3**	**$I\beta=6.3*10^{-10}$**	6	**96 %**
Control = 150 colonies					

(Control : Means *P.aeruginosa* without exposure to radiation**).**

From table (8) Beta rays as new inhibitors that emitte by Tl^{208} radiosource with activity 1 **µ**ci, dose $12.93*10^{-10}$ KGy for 3 hr., also exposure to Sr^{90} radiosource with activity 1 **µ**ci,dose $1.937*10^{-10}$ KGy for 3 hr. and exposure to Sr^{90} radiosource with activity 9 **µ**ci, dose $6.3*10^{-10}$ KGy for 3 hr. It was obtained after exposure to were colonies of

P.aeruginosa(XDR) less than the control with increase the percentage of killing of *P.aeruginosa*(XDR), also the morphology of the colonies changed compared with the original(control).

Table (9): Gene expression of MexAB-OprM and MexXY efflux pumps of *P.aeruginosa* (XDR)by using qRT-PCR after exposure to Beta ray:

sample	Calibrator			Treated			ΔΔCt	Ration (Fold 1)
	mexX	*rps*	ΔCt	*mexX*	*rps*	ΔCt		
1	0	18 .0	-18	22.98	24.94	-1.96	16.04	**0.0**
2	34 .0	40 .0	6.0	30 .0	0	30.0	24.0	**0.0**
3	26.50	17.12	9.38	24.8	14.0	10.8	1.42	**0.3**
sample	Calibrator			Treated			ΔΔCt	Ration (Fold 1)
	mexB	*rps*	ΔCt	*mexB*	*rps*	ΔCt		
1	0	18.0	-18 .0	22.0	23.94	-1.94	16.06	**0.0**
2	0	15.0	-15.0	23.98	25.94	-1.96	13.04	**0.0**
3	23.0	14.0	9	24.50	15.12	9.38	0.38	**0.7**

The results in table(9) showed Beta rays also used as new inhibitors of MexXY and MexAB-OprM effiux pumps of *P.aeruginosa*(XDR), emitted from Tl^{208} radiosource with activity 1 **µ**ci, dose $12.93*10^{-10}$ KGy for 3 hr.; exposure to Sr^{90} radiosource with activity 1 **µ**ci, dose $1.937*10^{-10}$ KGy for 3 hr. and exposure to Sr^{90} radiosource with activity 9 **µ**ci, dose $6.3*10^{-10}$ KGy for 3 hr. calculate fold(mean gene expression) calculated by Livake equation when study *mexX* and *mexB* encoded to MexXY and MexAB-OprM efflux pump, the results were zero gene expression (no gene expression of efflux

pumps)when exposure to radiation and compared with control (have high gene expression befor exposure to radiation). Beta radiation is an effective new inhibitor of MexXY and MexAB-OprM efflux pumps of *P.aeruginosa*(XDR).

Radiation-induced ionizations labor directly on the cellular content molecules or indirectly on water molecules causing water-derived radicals; Radicals interact with close molecules resulting in fraction of chemical bonds and cause oxidation (addition of oxygen atoms) of the influenced molecules, the great impact in cells is DNA fracture[62,63].

The radiation is a physical process to decontamination because it put to death bacteria by destroy bacterial DNA, inhibiting bacterial division [64]. Radiation a physical cold sterilization method, the ionizing radiation was used widely for the treatment of different types of infections [65].The insinuation of microbial cells to ionizing radiation push to the cells which damage their organization, nucleic acids(especially DNA) are the prime target for cell deterioration from ionizing radiation [66].

With modern antibiotics and the hight happening of MDRPA worldwide raised levels of water pollution, use lethal dose of gamma rays to MDRPA at 3 kGy insinuation of bacterial cells to ionizing radiation which effected on cell organization, Nucleic acids(DNA), there are three types of deterioration in DNA, single strand breaks, double strand breaks and nucleotide break(base damage) and destroy in the sugar moiety[67,68].

A preceding study by Brown,2008 and Fischer,2014, used radiation (ionizing radiation)for cancer treatment because it organize ions in the cells of the tissues. ions element electrons from atoms and molecules, this put to death cells, change genes that pause growing. The radiation is significant in therapy[69].The *radiation oncologist* (a doctor treat cancer with radiation) chose the type of radiation that's most convenient for each patient's cancer type and location. Beta particles are mainly generated by particular radioactive material that injected or put into the body.It is called "Radiopharmaceuticals", radiation cause deleterious to the genes of DNA in cells when radiation damages the genes of cancer cells, they can't develope and divide any further, the cells passaway [70,71].

Beta particles are speedy dynamic electrons released from the nucleus during radioactive degeneration. Humans are exposed to beta particles from man-made and natural radiation provenance, such as Tritium, Carbon-14 and Strontium-90. Beta particles are more penetrative than Alpha particles but are less deleterious, they travel

space in air but can be minimize or paused by a layer of clothing or by Aluminum, Beta-emitters are most dangerous when they are snaffed or guzzled [72].

Beta particles are high-energy, high-speed electrons released by particular types of radioactive nuclei such as Potassium-40, the beta particles emitted are a form of ionizing radiation also known as beta rays ,when radiation react with substance,beta has the medium penetrative power and the medium ionizing force [73].

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