Middle-East Journal of Scientific Research 21 (5): 776-781, 2014
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2014.21.05.21602

Radio-Impact of Gamma Radiation on Pathogenic Bacterial Strains Isolated from Rosetta Branch and its Drains of River Nile Water

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Abstract: Determination of the dose response curve of Gram-positive bacterial isolates (*Bacillus cereus, Streptococcus faecalis, Staphylococcus aureus*) and Gram-negative (*E. coli, Pseudomonas aeruginosa* and *Salmonella* sp.) which have been isolated from Rosetta branch and its drains of River Nile water against increasing doses of gamma radiation have been carried out. Four kGy reduced the viability of *Pseudomonas aeruginosa* completely. However 3.0 kGy reduced the viable counts by 4.18 log cycles. The viable count of *Salmonella* sp. was reduced gradually as the doses of gamma radiation increased. Eight kGy reduced *Salmonella* sp. viability completely. In case of *E. coli*, 4.0 kGy reduced its viability completely. However, 6 and 8 kGy reduced the viable count completely of *Staph. aureus* and *Strept. faecalis* respectively. Meanwhile, 10 kGy reduced the viable count of *B. cereus* by 5.24 log cycles. Gram positive bacteria was more resistant to gamma radiation than Gram negative bacteria with the following pattern in descending order *Bacillus cereus*>*Staphylococcus aureus* > *Sterptococcus faecalis* > *Salmonell* sp. > *Pesudomonas aeruginosa* > *E. coli*. Application of gamma radiation in treatment of water contaminated by pathogenic bacteria and protozoa revealed that 10 kGy reduced the viable count of all Gram positive, Gram negative bacteria and protozoa to become safe and clean.

Key words: Gamma radiation · Pathogenic bacteria · River Nile · Rosetta branch · Surface water

INTRODUCTION

Gamma (γ) radiation has a narrow range of length and high energy penetration power resulting from the nuclear disintegration of certain radioactive substances such as the isotopes Cobalt 60 (Co^{60}) and Cesium 137 (Cs^{137}) [1]. Ionizing radiation is defined as radiation that has sufficient energy to remove electrons from atoms and molecules and to convert them to electrically-charged particles called ions. Further reactions of ions and electrons, give rise to the formation of free radicals that are usually highly reactive and which eventually lead to chemical changes in the system that is produced by absorption of ionizing radiation which is known as radiation chemistry [2, 3]. Ionizing radiation can damage the nucleic acids and ultimately kill microbes by direct and indirect hits. Direct hit occur when ionizing radiation directly disrupt nucleic acids, especially DNA. 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 [4]. Some microorganism's exhibit resistance to ionizing radiation and others are sensitive. The relative sensitivity or resistance of different microorganisms to

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ionizing radiation is based on their respective D_{10} value. D_{10} value is defined as the radiation dose required reducing the population by a 10 fold (by one log cycle) or required to kill 90% of total viable number [5, 6]. Extreme ionizing-radiation resistance has been observed in several members of the domains Bacteria and Archaea. Of the genera containing ionizing-radiation-resistant organisms, *Deinococcus*, *Bacillus* and *Rubrobacter* show the highest levels of resistance and all species of these genera have been shown to be either gamma radiation resistant or UV radiation resistant or both [7-9]. Resistance to ionizing radiation can be explained by sulfur-rich cell wall of the bacterial cells which make as scavengers for ionizing radiation or by DNA repair mechanisms [8-10]. So, the aim of the present study is to determine the effect of gamma radiation on pathogenic bacteria isolated from Rosetta branch of River Nile, Egypt to be used as non-conventional method for surface water sterilization and wastewater treatments.

MATERIALS AND METHODS

Bacterial Strains and Specific Media: Six different bacterial strains isolated from Rosetta branch and its drains of River Nile water at Egypt. The isolated strains were three Gram +ve (*B. cereus*, *Staph. aureus* and *Strept. faecalis*) and three Gram –ve (*E. coli*, *Pseudomonas aerugionosa* and *Salmonella* sp.) were isolated on specific agar plate media on Mannitol Egg Yolk Polymixin B-Sulphate (MYP) [11], Baired Parker medium [12] and Kanamycin aesculin azid agar medium (KKA) [13] for *B. cereus, Staph. aureus* and *Strept. Faecalis*, respectively. The three G –ve bacteria were isolated on MacConckey agar [14], Asparagin agar [15] for *E. coli* and *P. aeruginosa*, respectively. However detection for the presence of *Salmonella* have been conducted according to WHO [16] protocol by inoculation equal volume of water sample 50ml into 50 ml of double strength selenite broth at 37°C for 24h, then 1ml from double strength selenite broth transferred to 9 ml of single strength and incubated for another 24h at 37°C. Aloopfull from single strength selenite broth was streaked on the surface of *Salmonella Shigella* agar (SSA) plates [17].

Identification of the Isolated Pathogenic Strains: Biochemical identification of the six isolates have been carried out by the standard methods for Examination of water and wastewater [15] and by API 20E, API 20 NE and AP1 20 Strep [18].

Preparation of Bacterial Suspensions: The well grown separated single colony of each isolated pathogenic bacterium was picked up and inoculated into Lauria broth L.B. broth medium [19] and incubated at 37°C for 48h in shaking incubator (150 rpm). The well grow cultures were centrifuged at 8000 rpm for 10.0 min. The pellets of each isolated strain was washed by sterile saline (0.85% NaCl) twice and then resuspended in sterile saline to form homogenous bacterial suspension.

Determination of Dose Response Curves for Pathogenic Bacterial Strains: Aliquotes (5.0 ml) from each bacterial suspension ($\simeq 5.0 \times 10^8$ CFU/ml) was distributed in 10.0ml sterile screw capped test tubes. The prepared tubes for six pathogenic strains were exposed to different doses of gamma radiation (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 10.0 kGy) from the Indian chamber of Co-60 at National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. The dose rate was 1kGy/12.5 min. for each dose of each strain. The controls (non-irradiated) and the irradiated bacterial suspensions were serially diluted by saline and plated on the surface of L.B. agar plates. The plates were incubated at 37°C for 48h. After that, the counts were determined and the dose response curves have been established. Also examined by light microscope to investigate the presence of protozoa.

Treatment of Water and Wastewater by Gamma Radiation: Three polluted water samples were collected from River Nile water at Rabeea village, Giza Governorate, Kafr El-Zayat City and Edfina city, Beheira Governorate. The three samples were polluted by pathogenic bacteria and Protozoa. The three samples were exposed to 10 kGy gamma radiation. The three samples were plated on L.B. agar medium and specific medium to enumerate the counts of pathogenic bacteria contaminated the water samples before and after irradiation.

RESULTS AND DISCUSSION

Identification of the Isolated Pathogenic Bacterial Strains: Six pathogenic bacterial strains isolated on specific agar plates from polluted water and wastewater of Rosetta branch of River Nile have been identified by a battery of biochemical tests. Also the identification included using of API 20E, API 20NE and API 20 Strep. Results of identification revealed that the Gram +ve strains were *B. cereus, Straphylococcus aureus* and

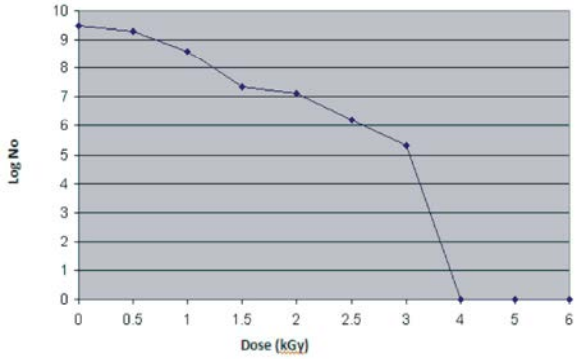

Fig. 1: Effect of gamma radiation on the survival of *Pseudomonas aeruginos*a (Dose response curve).

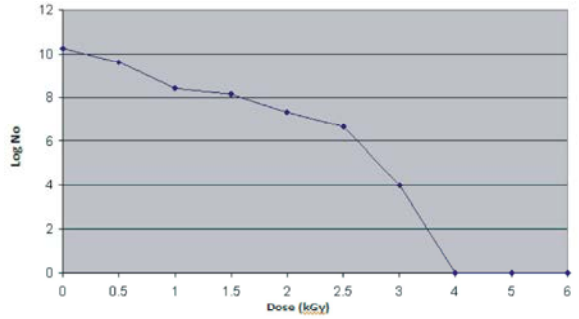

Fig. 2: Effect of gamma radiation on the survival of *Escherichia coli* (Dose response curve).

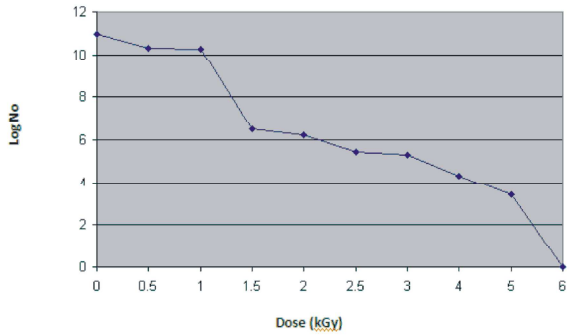

Fig. 3: Effect of gamma radiation on the survival of *Salmonella* sp. (Dose response curve).

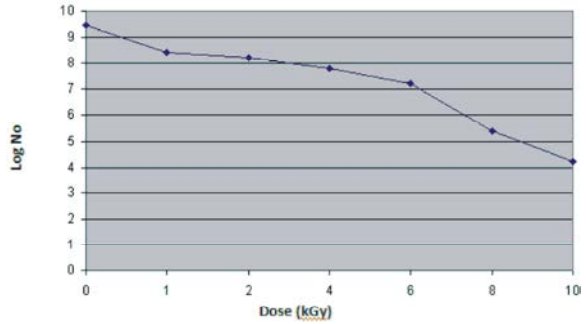

Fig. 4: Effect of gamma irradiation on the survival of *Bacillus cereus* (Dose response curve).

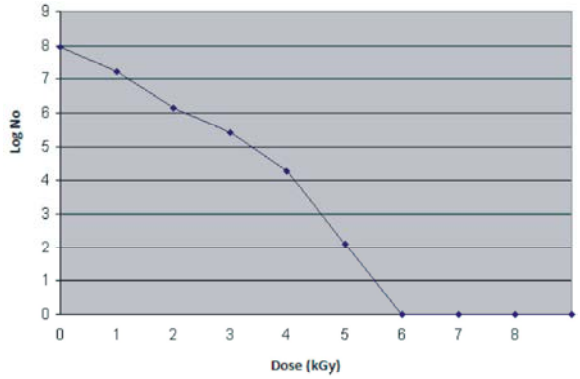

Fig. 5: Effect of gamma radiation on the survival of *Staphylococcus aureus* (Dose response curve).

Streptococcus faecalis. On the other hand, the Gram –ve strains were *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* sp.

Effect of Gamma Radiation on the Pathogenic Bacteria Isolated from Rosetta Branch of River Nile Water: The results revealed that Gram +ve bacterial strains were more resistant to gamma radiation than Gram –ve bacteria. *Pseudomonas aeruginosa* which its viability decreased gradually as the gamma radiation dose increased have been indicated in Fig. 1. Dose 3.0 kGy reduced the viability by 6.18 log cycles, while 4.0 kGy reduced its viability completely. In case of Gram –ve bacteria, 4.0 kGy reduced the viable count of *E. coli* completely. As the dose of gamma radiation increased, the viable count decreased. Three kGy reduced the viability of *E. coli* by 6.25 log cycles as shown in Fig. 2. The response of *Salmonella* sp. to elevated doses of gamma radiation was shown in Fig. 3. The results revealed that, 7.0 kGy reduced *Salmonella* sp. Count by 7.61 log cycles. However, 8.0 kGy reduced the viability of *Salmonella* sp. completely. Exposure of *B. cereus* to increasing doses of gamma radiation was accompanied by decreasing in the viable count i.e. as the dose increased, the viability decreased gradually as shown in Fig. 4. Ten kGy reduced the viable count of *B. cereus* by 5.24 log cycles *Staph. aureus* when exposed to increasing doses of gamma radiation, its count decreased gradually as indicated in Fig.5. Six kGy reduced the viability of *Staph. aureus* completely. However, 5.0 kGy reduced the viable count by 5.84 log cycles.

Increasing the dose of gamma radiation, decreasing the viable count of *Strept. faecalis* gradually as indicated in Fig. 6. Dose of 8.0 kGy reduced the viability of *Strept. faecalis* completely. However, 7.0 kGy reduced its viability by 8.62 log cycles. From the previous results, it was clear

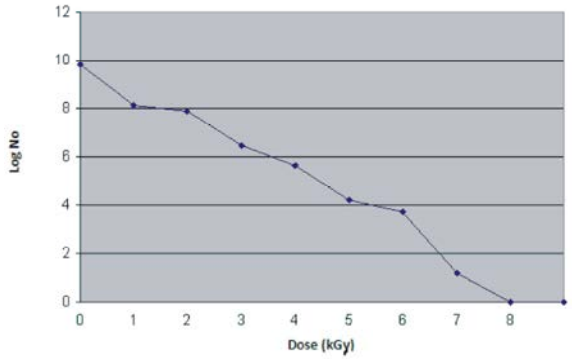

Fig. 6: Effect of gamma radiation on the survival of *Streptococcus faecalis* (Dose response curve).

that Gram +ve bacteria were more resistant than Gram –ve bacteria. Pathogenic bacterial strains followed descending order pattern as the following *B. cereus* > *Staph. aureus* > *Strept. facecalis* > *Salmonella* sp.> *P. aeruginosa* >*E. coli*. The difference between gram +ve and Gram –ve bacterial cells may be explained on the base of difference between them in cell wall structure. Gram-positive bacteria have membrane which surrounded the cell and cell wall primarily made up of peptidoglycan layer. This cell wall rich in sulfur compounds, which protect the cells from harmful gamma radiation and become resistant. Sulfur compound found in cell wall of Gram +ve bacterial cell make as scavenger for free radicals and protect the cells [8, 20, 21]. 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. The base damage is a major component of damage induced by ionizing radiation [22]. The lethal effect of gamma radiation may be explained on the bases that gamma radiation induced DNA-damage, single or double strand breaks and disrupture of protein-DNA complex, so affecting gene expression [23-27]. Our results were in harmony with those observed by Abo-State *et al*. [28] who found that 3 kGy reduced completely the viable count of all Gram-negative short rods bacterium (*Pseudomonas*) isolated from soils and capable of degrading chloroaromatic compounds. Gram-negative bacilli, *Pseudomonas oleovorans*, their viable count was completely reduced by 3.0 kGy of gamma radiation [29]. Abo-State and Khalil [23] indicated that 10 kGy gamma

Table 1: Using gamma radiation for treatment of polluted water sample "Rabeea Village Giza Governorate" of Rosetta branch of River Nile, Egypt.

Bacteria and Protozoa	Before treatment	After treatment
Total bacterial count	4×10^5	Nil
Bacillus cereus	3×10^2	Nil
Escherichia coli	26×10^4	Nil
Enterobacteriaceae	3.3×10^4	Nil
Pseudomonas aeruginosa	1.8×10^2	Nil
Staphylococcus aureus	2.5×10^1	Nil
Streptococcus faecalis	1.6×10^3	Nil
Protozoa	+ve	-ve

Table 2: Using gamma radiation for treatment of polluted water sample "Kafr El Zayat City" of Rosetta branch of River Nile, Egypt

Bacteria and Protozoa	Before treatment	After treatment
Total bacterial count	2×10^4	Nil
Bacillus cereus	1.2×10^1	Nil
Escherichia coli	3×10^2	Nil
Enterobacteriaceae	1×10^3	Nil
Pseudomonas aeruginosa	2×10^1	Nil
Staphylococcus aureus	1.1×10^1	Nil
Streptococcus faecalis	2.4×10^2	Nil
Protozoa +ve	Nil	

Table 3: Using gamma radiation for treatment of polluted water sample Edfina City "Behaira Governorate" of Rosetta branch of River Nile, Egypt

Bacteria and Protozoa	Before treatment	After treatment
Total bacterial count	1×10^2	Nil
Bacillus cereus	Nil	Nil
Escherichia coli	2.3×10^1	Nil
Enterobacteriaceae	4×10^1	Nil
Pseudomonas aeruginosa	3×10^1	Nil
Staphylococcus aureus	Nil	Nil
Streptococcus faecalis	4	Nil
Protozoa	+ve	Nil

radiation reduced the viable count of Bacillus cereus NRRL 569 and ATCC11778 by 5.5 and 2.7 log cycles, respectively. Abo-State *et al*. [10]. Reported that, 10.0 kGy reduced the viable count of *Bacillus* sp. MAM-40 isolated from eye drops and MAM-26 isolated from baby powder and MAM-11 isolated from solution lenses by 4.17, 1.9 and 2.7 log cycles, respectively.

Using Gamma Radiation at 10 kGy for Treatment of Polluted Water Samples of Rosetta Branch of River Nile, Egypt: Three water samples were polluted by pathogenic bacteria and protozoa, total bacterial count, *Bacillus cereus*, *Escherichia coli*, *Enterobacteriaceae*, *Streptococcus faecalis*, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella* sp. The result show that there is no growth or any colony appear on each plates of specific medium after exposure to 10 kGy gamma radiation. Ten kGy reduced the viable count of all Gram positive, negative bacteria and protozoa completely

to become safe and clean water as indicated in Tables 1, 2 and 3. Our results were in harmony with those observed by Kristiansson *et al*. [30], who found that irradiation dose of 1kGy reduced the counts of *Pseudomonas aeruginosa* by 35% of initial count. Meanwhile, the obtained results indicated that the lethal dose was at 4kGy.It seems that, irradiation could be an alternative to traditional chlorination of contaminated water, especially if reuse and or disposal are to consider as an option. The effect of irradiation with accelerated electrons on *Bacillus cereus* and *Bacillus subtilis*, spore counts reduced approximately two log cycles for *B. cereus* and up to five log cycles for *B. subtilis* after radiation dose of 7.6 kGy, with D_{10} values ranging from 1.5 to 3.8 kGy [31]. In accordance with previous opinion, Basfar and Abdel Rehim [32] reported that a dose of 1kGy from ^{60}Co gamma source was effective to cause 99.8% reduction in *Enterobacteriaceae* from unchlorinated effluents; while the same dose resulted in 99.3% reduction in *E. coli* with no regrowth was achieved at a dose of 1.3 kGy. The D_{10} values for both *Enterobacteriaceae* and *E. coli* were 0.3 and 0.4 kGy, respectively. However, with radiation treatment we can avoid the re growth problem as radiation causes complete death of pathogenic organisms. The lethal dose varies according to the pathogen type and initial count. In the opinion of radiation scientists, 6 kGy of ionizing radiation is adequate to complete inactivate pathogens in sewage sludge, while a dose of 1 kGy was sufficient for disinfection of waste water [33, 34]. Radiation doses of 3.5 kGy effectively disinfected effluents with lower concentration of *Ascaris lumbricoides* eggs and *Giardia lambilia*, higher radiation doses of 5kGy were necessary to disinfect effluents with higher eggs concentration of *Ascaris lumbricoides* [35]. Louise *et al* [36] reported that inactivation of microorganisms by gamma and electron beam irradiation comes from the inhibition of DNA repair mechanism by increased energy demand of homeostasis on the cell.

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