ORIGINAL ARTICLE

Decontamination of Artificially Infectious Urinary Catheter using Gamma Irradiation Technique

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ABSTRACT

Background: *Decontaminating urinary catheters prior to disposal or potential reuse is essential for mitigating environmental hazards and reducing the spread of multidrug-resistant (MDR) pathogens.* ***Objective:*** *This study aimed to evaluate the efficacy of gamma irradiation in decontaminating urinary catheters by inactivating MDR uropathogenic bacteria and disrupting their associated biofilms.* ***Methodology:*** *Fifty strains bacteria were isolated from urine samples obtained from catheterized patients and four bacterial isolates were recorded with 100% resistance against all tested antibiotics. Among them, two isolates were selected and identified as strong biofilm producers, Proteus mirabilis and Staphylococcus haemolyticus. The effect of different doses of Gamma radiation on the viability of the drug resistant strains and biofilm formation were studied. The gamma irradiation technique was applied to decontaminate artificially infectious urinary catheters.* ***Results:*** *The study showed that a dose-dependent reduction in bacterial survival and biofilm formation was observed. The lethal gamma doses for P. mirabilis and S. haemolyticus were 0.5 kGy and 1.5 kGy, respectively.* ***Conclusion:*** *Gamma irradiation is an effective strategy for decontaminating urinary catheters and eliminating MDR uropathogenic bacteria, cause biofilm degradation and offering a potential eco-friendly solution for managing infectious medical waste.*

INTRODUCTION

Hazardous medical waste is categorized into infectious waste and non-infectious or dangerous waste. Infectious waste comprises bacteria that can potentially cause diseases in a susceptible individual. As a result, specific treatment is needed to neutralize the associated biohazards.[1] Infectious waste represents between 15% and 25% of total medical waste; it poses significant risks to the environment, staff, and patients. Consequently, the majority of nations on Earth have long categorized hazardous medical waste as extremely dangerous waste.[2]

Among these infectious wastes, catheters are significant sources of hazardous waste because of their inclusion of pathogenic multidrug-resistant bacteria, blood, tissues, and residual antibiotics. Medical catheter demand is rising due to minimally invasive surgeries, diabetes, cardiovascular illness, and urine problems. Over 30 million urine catheters and 150 million intravascular catheters are implanted annually, and 15–20% of hospital admissions involve catheters.[3] An unrestricted release of antibiotic residues associated with infectious waste into the environment poses a unique risk because bacteria get resistant to most antibiotics, and the treatment efficacy falls precipitously. Globally, the issues of antibiotic resistance and infectious waste are very serious nowadays.[4]

Infectious waste of this nature carries elevated levels of pathogens that have the potential to harm food and water sources if disposed to the environment without treatment or decontamination. Water contamination may arise from it coming into touch with solid waste and mixing it with ground waste during the rainy season.[5] Most medical waste is burned to minimize its bulk before being disposed of directly dumped in landfills. If a landfill is not built correctly, it might contaminate drinking water, creating severe hazards. After incineration, toxic gases are released into the air, and ash residue is left behind in areas where waste incineration is performed. Also, Chlorine-containing materials that are incinerated can produce dioxins and furans, which are carcinogenic to humans and have been linked to many harmful health impacts [6].

Therefore, this investigation aims to introduce an alternative, effective decontamination method for the pretreatment of the infectious urinary catheter waste before disposal to prevent the risk that may be generated by the biofilm of multidrug-resistant pathogenic microorganisms associated with these catheters. Gamma irradiation is an alternative, guaranteed, effective technique for treating biofilm-associated bacteria. However, few researchers have examined the effectiveness of low-energy X-rays in inactivating resistant biofilm bacteria on medical waste surfaces.

Biofilm refers to a sessile microbial population that is adhered to biotic or biotic surfaces. Bacterial cells within a biofilm are encased in a self-produced matrix of extracellular polymeric substances that act as a physicochemical barrier, protecting them from various environmental conditions. Forty to eighty per cent of microorganisms can form biofilm, which may consist of a single species or multiple species [7]. Biofilm microorganisms differ from planktonic microorganisms in that they are more resistant to extreme environments such as chemical biocides, bacteriophages, antibiotics and antibodies.[8,9]

Decontamination is the essential procedure that reduces biohazardous substances, such as infectious bacteria, to an acceptable level or below the threshold required to induce infection. According to Sabir, et al. [10], bacterial biofilm growth on catheters can result in continuing infections and heightened antibiotic resistance. Furthermore, germs stuck to catheters may not be entirely eradicated by standard antibiotic treatments alone. As a result, different approaches have been investigated to improve the removal of catheter-associated bacterial biofilms, including gamma radiation and nanoparticles. Gamma irradiation is a form of ionizing radiation that possesses antibacterial characteristics. It can penetrate different substances, such as biofilms, and exhibit its bactericidal properties by directly impairing DNA and disrupting essential bacterial processes. The researchers have found that gamma radiation can effectively eliminate pathogens biofilm attached to catheters [11].

Thus, the main objectives of this investigation are to: a) isolate the pathogenic bacteria from patients receiving urinary catheterization, b) evaluate the antimicrobial activity and MIC of commercial antibiotics on the isolated bacteria, c) examine the potential of most resistant bacterial isolate to biofilm formation, d) Molecular identification of the selected bacterial isolates, e) explore the role of gamma irradiation with different doses in the decontamination of infectious catheters waste by inactivation the associated pathogens biofilm.

METHODOLOGY

Isolation and purification of pathogenic bacterial isolates

Uropathogenic isolates were obtained from urine samples of catheterized patients admitted to Zagazig University Hospitals in the period from March to December 2020. Fresh, not-centrifuged urine samples were collected from the patient who had not received antibiotics in the last 48 hours. The urine samples were put in sterile, wide-mouth containers and transported to the microbiology laboratory at Zagazig University's, Faculty of Science, where the study was conducted.

Urine samples were immediately examined to determine the number of bacterial flora or kept at 4º C. The sterile cotton swab was used to dilute and spread the urine specimen over the surface of the nutrient agar plates. Results can be observed after the incubation of plates at 37 °C for 24 hours. The purified isolates were then maintained on slants of nutrient agar medium. All the slant cultures were stored in the refrigerator, and regular transfers were made every month.

Identification of selected MDR

Antibiotic sensitivity was done by disc diffusion method to select multidrug-resistant bacteria.[12] The antibiotics discs were Amickacin (30µ/disc), Amoxycillin (25µ/disc), Azithromycin (15µ/disc), Cefotaxime (30µ/disc), Chloramphenicol (30µ/disc), Ciprofloxacin (5µ/disc), Clindamycin (2µ/disc), Nitrofurantoin (300µ/disc), Oxacillin (1µ/disc), Streptomycin (10µ/disc), Tetracycline (30µ/disc) and Vancomycin (30µ/disc). From the results, bacterial isolate will be classified as susceptible, intermediate, or resistant. Identification of most resistant bacterial isolates was done by the routine microbiological methods, microscopical and biochemical reactions.

Qualitative assay for the biofilm evaluation

The detection of biofilm production was conducted qualitatively with congo red agar (CRA) assay.[13] The appearance of dry black crystalline colonies indicated that bacteria were strong biofilm producers. Conversely, the presence of pink colonies suggested negative biofilm bacteria, while the absence of crystalline and dry colonies suggested weak biofilm bacteria. Three runs were made at the test.

Quantitative assay for the biofilm evaluation

The microtiter dish test (also known as the crystal violet biofilm staining assay) was used to evaluate the biofilm formation.[14] Microtiter wells filled with BHI broth were inoculated with 200 µL of bacterial culture and incubate 24 h at 37°C. Non-adherent cells were removed, other wells were washed (PBS, pH 7.1), fixed with 99% ethanol (205 µL), stained with 0.1% CV (205 µL, 30 min), then dissolved with 70% ethanol (30 min). Finally, OD_{600} was measured using an ELISA reader (Faculty of Pharmacy, Zagazig University).

Molecular identification

The isolates with the highest level of antibiotic resistance and the most potent ability to form biofilms were selected for molecular identification and further investigations. Following the extraction of genomic DNA, the isolates were identified by 16S rRNA gene sequencing using universal primers, 27F (5'-3') and 1494 R (5′-CGG TTA CCT TGT TAC GAC TT-3′). Macrogen Inc (South Korea) sequenced the purified PCR products. An ABI 377 DNA was used to sequence the purified PCR products. The identical primers as previously mentioned were used by the auto sequencer (PerkinElmer, Applied Biosystems Div., Waltham, USA). The isolates were then sequenced using the Basic Local Alignment Search Tool (BLAST) and submitted to GenBank.

Effect of Gamma radiation on the viability of the resistant bacterial isolate

A cobalt-60 gamma source (Gamma Chamber 4000, India) with rate 0.950 kGy/h was used for the radiation at the National Center for Radiation Research and Technology (NCRRT) in Nasr City, Cairo, Egypt. Five mL of bacterial suspensions were irradiated at varying doses. Gram-positive isolates (more resistant)[15,16] received (0, 1, 2, 3, 4, and 5 kGy), while Gram-negative isolates received (0, 0.2, 0.6, 1, 1.5, and 2 kGy). Post-irradiation, samples were diluted in sterile saline (85% NaCl), plated on nutrient agar (0.1 mL, 37°C, 24 h), and survival curves were plotted. The D_{10} value was determined by taking the negative reciprocal of the survival curve slope according to [17].

Effect of Gamma radiation on the antibiotic's resistance of bacterial isolate

The effect of gamma irradiation on antibiotic resistance of MDR *P. mirabilis* and *S. haemolyticus* was evaluated using the disc diffusion assay. *P. mirabilis* was tested against Nitrofurantoin, Amoxicillin, Ciprofloxacin, Amikacin, and Chloramphenicol; *S. haemolyticus* against Streptomycin, Oxacillin, Azithromycin, Vancomycin, and Clindamycin.

Briefly, 1 mL of irradiated and unirradiated bacterial suspensions was streaked in three directions on sterile nutrient agar plates. Antibiotic discs were placed on the surface, and plates were incubated at 37°C for 24 h. Inhibition zone diameters (including disc) were measured in mm.

Evaluation of bacterial growth and biofilm formation on artificially contaminated urinary catheters

Evaluation of contaminated urinary catheters was achieved according to Maione, et al. [18], with some modifications. Catheter segments (1 cm) were incubated in BHI with *P. mirabilis* or *S. haemolyticus* at 37°C for 48 h; controls lacked bacterial inoculum. Post-incubation, bacterial growth was assessed by OD_{600} using a spectrophotometer (Faculty of Science, Zagazig University). Biofilm formation was quantified via crystal violet staining, as previously described.

Decontamination of artificially infectious urinary catheters

This experiment was designed to detect the lethal irradiation dose that could eliminate the infectious resistant bacteria and inactivate the biofilm formed at the urinary catheter. The artificially contaminated catheter segments that were loaded with selected bacteria separately and collectively were exposed to different doses of gamma irradiation. The microbial growth and biofilm formation were determined for the untreated and treated catheters.

This method was carried according the method described by Jacobsen, et al. [19]. Both *P. mirabilisUA48* and S. *haemolyticusAA1* were cultured in BHI broth for 24hrs. After incubation ,2ml of each culture were added to tested tubes each containing catheter segments (1 cm). All tubes were irradiated at different doses (0, 0.1, 0.2, 0.3, and 0.5 KGy) for *P. mirabilis* and at (0, 0.25, *0.5*, 1.0, and 1.5KGy*)* for *S. haemolyticus.* After irradiation all tubes were incubated at 48hrs and then the growth was detected at 600 nm as (cfu/ml) and the biofilm formation (CV method) at 595 nm as described previously

Statistical analysis

The data were statistically analyzed in a completely randomized design using the PROC ANOVA procedure of Statistical Analysis System (SPSS). The differences among means (at $P \leq 0.05$) were compared by using Duncan's multiple range tests

RESULTS

Characterization and distribution of isolated bacteria

Fifty bacterial isolates were recovered from urine samples. Thirty isolates were found to be Gram-positive (60%) and twenty to be Gram-negative (40%). According to morphological characteristics, the gram +ve bacteria comprised 12 cocci, 24 streptococci, 14 staphylococci, and 10 coccobacilli. Whereas gram –ve isolates have only coccobacilli, bacillus, and streptobacilli with 4, 30 and 6%, respectively. The distribution of isolated bacteria according to their gram stain and morphological characters were illustrated in Fig. 1.

Antibiotic sensitivity test

The disk diffusion method demonstrated that the tested antibiotics exhibited varying susceptibility patterns among the bacterial isolates. The examined isolates revealed a high degree of susceptibility to amikacin (56%), followed by ciprofloxacin, tetracycline, chloramphenicol, and streptomycin (54%, 46%, 42%, and 42% susceptibility, respectively). Conversely, the data indicated that 84% of the bacterial isolates were resistant to amoxicillin, whereas those

resistant to nitrofurantoin, oxacillin, and azithromycin were 76%, 70%, and 66%, respectively. According to the results, four bacterial strains were selected based on its resistance to the wide range of antibiotic according to the Table 1. This is why these bacteria will be the focus of future investigation.

Biofilm formation by most resistant bacteria

All multi-drug resistant bacteria isolates (with 100% resistance) were qualitatively and quantitatively tested for biofilm formation capabilities. Table 2 shows that isolates 7 and 47 produced a moderate biofilm. Based on the findings, isolates coded 36 and 47 were chosen as the most potent biofilm producer isolates for further testing.

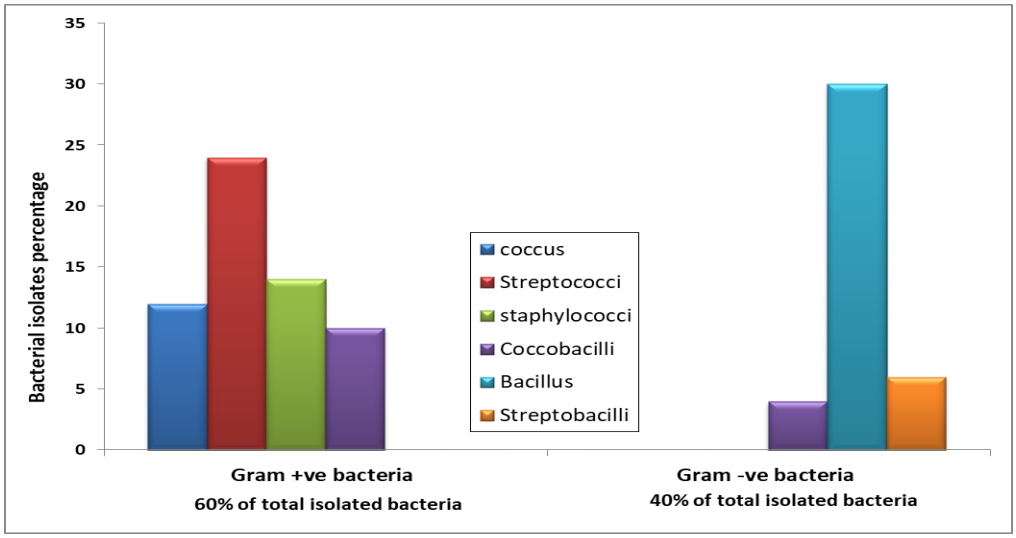

Fig. 1. The distribution of bacterial isolates according to their gram stain and morphological characters

Table 1: Susceptibility of bacterial isolates to different antibiotics.

Antibiotic	Symbol	Conc. µ/disc	Resistant (R)	Intermediate (I)	Susceptible (S)
Amickacin	AK	30	≤14	15-16	≥17
Amoxycillin	AX	25	≤13	14-17	≥18
Azithromycin	AZM	15	≤13	14-17	≥18
Chloramphenicol	C	30	≤12	13-17	≥18
Ciprofloxacin	CIP	5	≤15	16-20	≥21
Clindamycin	DA	2	≤14	15-16	≥17
Nitrofurantoin	F	300	≤14	15-16	≥17
Oxacillin	OX	1	≤13	-	≥15
Streptomycin	S	10	≤11	12-14	≥15
Vancomycin	VA	30	≤9	10-11	≥12

Table 2: Examination of biofilm formation for the selected MDR uropathogenic bacteria (100% resistance).

Bacterial isolates	Qualitative method	Quantitative method	
	CRA method	CV method	
		O.D (600 nm)	Interpretation
Isolate 7	+++	0.22 ± 0.011^{C}	Moderate
Isolate 36	++++	1.34 ± 0.072^{B}	Strong
Isolate 44	++++	1.60 ± 0.144^{A}	Strong
Isolate 47	+++	0.24 ± 0.020^{C}	Moderate

CRA: Congo Red assay, CV method: Crystal Voilet method, O.D: Optical denisty
Calculated mean is for triplicate measurements ± SD; means with different superscripts in the same column are considered statistically different ($p > 0.05$).
(O.D < 0.120): No biofilm formed (-)
(O.D =0.120 to 0.240): Moderate biofilm (+++)
(O.D > 0.240): Strong biofilm (++++)

Molecular identification of selected isolates

By using PCR amplification of the 16S rRNA gene, the two chosen isolates were molecularly identified as *P. mirabilis* UA48 and *S. haemolyticus* AA1, respectively. The amplified genes from both strains had their partial nucleotide sequences uploaded to GenBank under accession numbers OQ607830 and OQ048636, respectively.

Antimicrobial activity of gamma irradiation

In this experiment, the effect of gamma radiation (cobalt-60) at different exposure doses was investigated for the growth of the most powerful biofilm producer isolates with 100% resistance to antibiotics. Then, the dose-response curves were graphically represented, as shown in Fig. 2. The *Proteus merabilis* was subjected to gamma irradiation with different doses as follows: 0, 0.2, 0.5, 1, 1.5, and 2 kGy. In contrast, excessive dosages of gamma irradiation (0, 1, 2, 3, 4, and 5 kGy) were administered to the *Staphylococcus haemolyticus* because the Gram +ve bacterial isolates has more resistance to irradiation. The dose-response curves illustrated that the D_{10} values of *P. merabilis* and *S. haemolyticus* were 0.31 kGy and 0.85 kGy, respectively. The studied bacteria's dose-response curves demonstrated that the decrease in cell number (CFU/ml) was dose-dependent, with linear regression R^2 values ranging from 0.9142 to 0.9922.

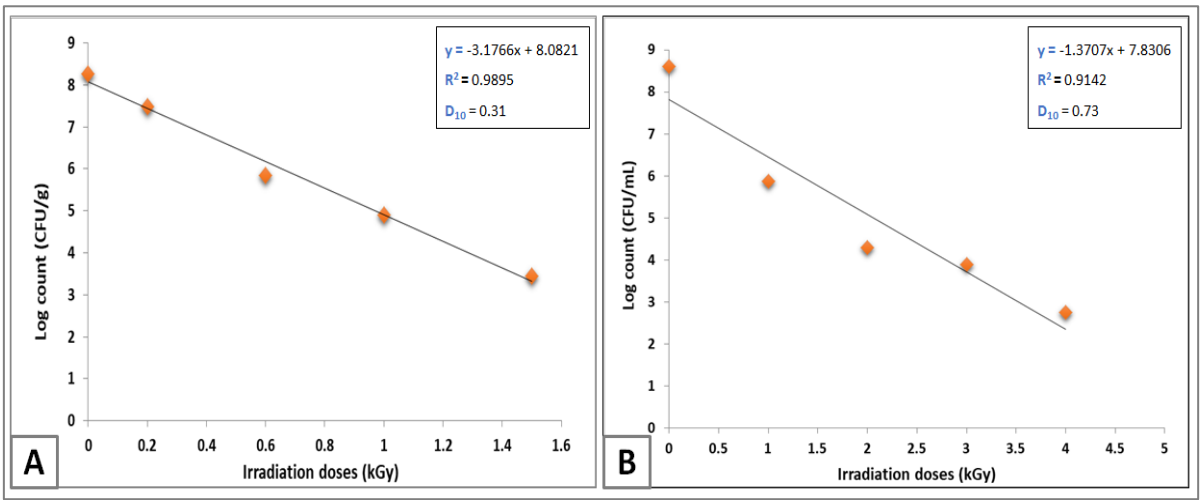

Fig. 2. Dose-response curve comparing bacterial growth across different doses of gamma irradiation A) *Proteus mirabilis* UA48, B) *Staphylococcus haemolyticus* AA1.

The antibiotic resistance assay of irradiated MDR strains

This experiment assessed the impact of gamma radiation on antibiotic resistance in *P. mirabilis* UA48 (D_{10} = 0.31 kGy) and S. haemolyticus AA1 (D_{10} = 0.85 kGy) using the disc diffusion method. As shown in Tables 3, increasing radiation doses led to larger inhibition zones and reduced resistance, with maximum effect at 0.5 kGy for *P. mirabilis* and 1.5 kGy for *S. haemolyticus*. Complete inhibition of *P. mirabilis* was observed at 0.5 kGy for Nitrofurantoin, Amoxicillin, and Chloramphenicol, and at 0.3 kGy for Amikacin and Ciprofloxacin. For *S. haemolyticus*, maximum sensitivity occurred at 1.5 kGy for Oxacillin, Azithromycin, and Vancomycin, and at 1 kGy for Streptomycin and Clindamycin.

Table 3 shows that *P. mirabilis* UA48 moved from resistance (R) to sensitivity (S) at 0.5kGy for Nitrofurantoin and Amoxicillin, 0.3kGy for Chloramphenicol, and 0.1kGy for Amikacin and Ciprofloxacin. Whereas, *S. haemolyticus* AA1 has an (R) to (S) shift at 0.25 kGy for Streptomycin and Clindamycin, 1 kGy for Azithromycin and Vancomycin, and 1.5 kGy for Oxacillin.

Table 3: Influence of gamma irradiation against MDR *P. mirabilis* UA48 and *S. haemolyticus* AA.

Gamma irradiation dose (kGy)	***Proteus mirabilis* UA48**									
	Inhibition zone diameter (mm)									
	Nitrofurantion (300µg/ml)		**Amoxicillin (10 µg/ml)**		**Chloramphenicol (3 µg/ml)**		**Amikacin (25µg/ml)**		**Ciprofloxacin (10 µg/ml)**	
0 (Control)	6.00±0.31^{a}	R	0.00±0.00^{a}	R	0.00±0.00^{a}	R	14.00±0.75^{a}	R	4.00±0.15 ^{a}	R
0.1	7.00±0.26^{a,b}	R	0.00±0.00^{b}	R	4.00±0.56 ^{a,b}	R	26.00±0.61^{a,b}	S	24.00±1.40 ^{a,b}	S
0.2	9.00±0.17^{a,b,c}	R	0.00±0.00^{c}	R	6.00±0.26 ^{a,b,c}	R	34.00±1.53^{a,b,c}	S	47.00±2.19^{a,b,c}	S
0.3	12.00±0.23^{a,b,c,d}	R	6.00±0.10^{a,b,c,d,e}	R	18.00±0.44^{a,b,c,d}	S	No growth	S	No growth	S
0.5	No growth	S	No growth	S	No growth	S	No growth	S	No growth	S
Gamma irradiation dose (kGy)	***Staphylococcus haemolyticus* AA1**									
	Inhibition zone diameter (mm)									
	Streptomycin (10µg/ml)		**Oxacillin (1µg/ml)**		**Azithromycin (15µg/ml)**		**Vancomycin (30µg/ml)**		**Clindamycin (2µg/ml)**	
0 (Control)	11.00±0.90^{a}	R	0.00±0.00^{a}	R	14.00±0.75^{a}	R	9.00±0.60^{a}	R	4.00±0.70^{a}	R
0.25	17.00±0.78^{a,b}	S	0.00±0.00^{b}	R	14.00±1.16^{b}	R	14.00±0.44^{a,b}	R	24.00±0.82^{a,b}	S
0.5	24.00±1.07^{a,b,c}	S	3.00±0.19^{a,b,c}	R	16.00±1.99^{c}	R	16.00±0.56^{a,b,c}	R	30.00±1.32^{a,b,c}	S
1	No growth	S	7.00±0.10^{a,b,c,d}	R	24.00±0.50^{a,b,c,d}	S	23.00±0.78^{a,b,c,d}	S	No growth	S
1.5	No growth	S	No growth	S	No growth	S	No growth	S	No growth	S

R: Resistant, S: sensitive.

Data represented as mean ± standard deviation (SD). Calculated mean is for triplicate measurements from three independent experiments. Different letters (a-d) in the same column for each antibiotic indicate statistically significant differences according to One-way ANOVA at P < 0.05, followed by Tukey post hoc test for pairwise comparisons: *(*Proteus mirabilis* UA48) a: between control radiation dose (0 kGy) and other radiation doses (0.1, 0.2, 0.3, and 0.5), b: between 0.1 radiation dose and other radiation doses (0.2, 0.3, and 0.5), c: between 0.2 radiation dose and other radiation doses (0.3, and 0.5), and d: between 0.3 radiation dose and 0.5 radiation doses. *(*Staphylococcus haemolyticus* AA1) a: between control radiation dose (0 kGy) and other radiation doses (0.25, 0.5, 1, and 1.5), b: between 0.25 radiation dose and other radiation doses (0.5, 1, and 1.5), c: between 0.5 radiation dose and other radiation doses (1 and 1.5), and d: between 1 radiation dose and 1.5 radiation doses.

Effect of gamma irradiation on artificially infectious catheter

In our study , the efficiency of gamma radiation in the process of decontamination of infectious catheters showed that, there was a gradual reduction in the bacterial growth and production of biofilm adhered to the catheter with increasing the dose of gamma irradiation compared with the positive control (the unirradiated bacteria) shown in Table 4 and 5. Generally, it was found that, the doses 0.5KGy and 1.5KGy were used for complete irradiation of bacterial loaded *P. mirabilis* and *S. haemolyticus* and biofilm formation on contaminated catheter segments respectively.

Different letters (a-d) in the same column for each antibiotic indicate statistically significant differences at P < 0.05 according to One-way ANOVA with Tukey's post-hoc test for pairwise comparisons: a: between control radiation dose (0 kGy) and other radiation doses (0.1, 0.2, 0.3, and 0.5), b: between 0.1 radiation dose and other radiation doses (0.2, 0.3, and 0.5), c: between 0.2 radiation dose and other radiation doses (0.3, and 0.5), and d: between 0.3 radiation dose and 0.5 radiation doses.

Different letters (a-d) in the same column for each antibiotic indicate statistically significant differences at P < 0.05 according to One-way ANOVA with Tukey's post-hoc test for pairwise comparisons: a: between control radiation dose (0 kGy) and other radiation doses (0.25, 0.5, 1, and 1.5), b: between 0.25 radiation dose and other radiation doses (0.5, 1, and 1.5 KGy), c: between 0.5 radiation dose and other radiation doses (1, and 1.5 KGy), and d: between 1 radiation dose and 1.5 KGy radiation doses

Table 4: Inhibitory effect of Gamma radiation against bacterial growth and biofilm formation of *P. mirabilis* UA48 associated with catheter.

Gamma Irradiation doses (kGy)	Bacterial growth		Biofilm formation
	Count (CFU/mL)	Log count (CFU/mL)	Optical density at 595nm
0 (Control)	$1.00\pm0.09´10^{8}$	8.00 ± 0.07^{a}	1.15 ± 0.11^{a}
0.1	$1.00\pm0.03´10^{8}$	8.00 ± 0.20^{b}	$0.53\pm0.14^{a,b}$
0.2	$2.00\pm0.19\times10^{6}$	$6.30\pm0.55^{a,b,c}$	$0.37\pm0.03^{a,c}$
0.3	$7.00\pm0.85\times10^{5}$	$5.85\pm0.08^{a,b,d}$	$0.24\pm0.04^{a,b,c,d}$
0.5	0.00±0.00	$0.00\pm0.00^{a,b,c,d}$	$0.00\pm0.00^{a,b,c,d}$

Data are presented as mean ± SD, based on triplicate measurements from three independent experiments.

Table 5: Inhibitory effect of Gamma radiation against bacterial growth and biofilm formation of *S. haemolyticus* AA1 associated with catheter.

Gamma Irradiation doses (kGy)	Bacterial growth		Biofilm formation
	Count (CFU/mL)	Log count (CFU/mL)	Optical density at 595nm
0 (Control)	$4\pm0.09\times10^{8}$	8.602 ± 0.36^{a}	1.37 ± 0.08^{a}
0.25	$4\pm0.01\times10^{8}$	8.602 ± 0.62^{b}	1.21 ± 0.20^{b}
0.5	$1.75\pm0.12\times10^{6}$	$7.24\pm0.09^{a,b,c}$	$0.90\pm0.05^{a,b,c}$
1	$1.33\pm0.10\times10^{5}$	$6.12\pm0.13^{a,b,c,d}$	$0.623\pm0.10^{a,b,d}$
1.5	0.00±0.00	$0.00\pm0.00^{a,b,c,d}$	$0.00\pm0.00^{a,b,c,d}$

Data are presented as mean ± SD, based on triplicate measurements from three independent experiments.

DISCUSSION

Approximately 150 million people worldwide suffer from urinary tract infections (UTI), among the most prevalent bacterial diseases [20]. The patient population and geographic location can impact the dissemination of different types of uropathogenic bacteria [21].

In this work, fifty distinct bacterial isolates were isolated from urine samples of catheterized patients in Egypt. The Gram-positive bacteria represented 60% of the total isolated bacteria. Scientific studies have shown that certain types of uropathogenic bacteria display various virulence factors. One of these features is Biofilm in which microorganisms colonize the mucosal surfaces of the host organs. Bacterial virulence and host susceptibility both have a role in determining the degree of a urinary tract infection. [22]

Our results exhibit that the *Proteus merabilis* and *Staphylococcus haemolyticus* were the strongest biofilm producer isolates with 100% resistance to all tested antibiotics. *Proteus mirabilis*, a highly mobile microbe from the Enterobacteriaceae family, causes clinical infections in many areas, such as the urinary system, abdominal cavity, bloodstream, and indwelling devices. According to Sahal and Bilkay [23], *P. mirabilis* strains are frequently associated with urinary tract infections, especially in individuals with urolithiasis and catheterization. When *P. mirabilis* attaches to live or nonliving surfaces, it can produce a slimy layer called a biofilm, which is a significant virulence factor. This biofilm shields *P. mirabilis* from the host's defence system and antibiotics, resulting in persistent infections [24]. Additionally, *P. mirabilis* produces ammonia and raises the urine's pH to >7.2, encouraging the precipitation and aggregation of apatite or struvite crystals [25]. These crystals cause obstruction and encrustation of catheters because they are immediately deposited onto the surface of the catheter or into microbial biofilms.

Staphylococcus haemolyticus is one of the most frequently coagulase-negative staphylococci (CoNS) isolated from healthcare-associated infections, mainly those related to implanted medical devices. Moreover, among all the CoNS species, *S. haemolyticus* has the highest degree of antimicrobial resistance, and heteroresistance to glycopeptides is widespread. Infection with *S. haemolyticus* is hazardous, and the therapeutic choices are limited [26]. One important *S. haemolyticus* pathogenicity factor is its capacity to colonize medical device surfaces and create biofilms [27].

The contrasting radio-resistance levels between Gram-positive and Gram-negative bacteria in this study highlight the diverse strategies employed by these organisms to counteract radiation-induced damage. Gram-positive bacterial isolates have been known to possess thick peptidoglycan layers in their cell walls, which may provide better protection against radiation.

The catheter is a medical device vital for our life, health, and well-being. However, the waste generated from medical activities can be hazardous and even lethal

because of its high potential for disease transmission. Medical devices are classified as critical, semi-critical, or non-critical according to the level of infection risk associated with their use. This classification played an important role in selecting the most suitable decontamination technique for medical devices [28]. Catheters, an essential medical device, pose the most danger due to their direct contact with blood and open tissues. Therefore, it is necessary to apply a safe, effective, and eco-friendly decontamination method. Single-use catheters are the standard in some nations, but catheter reuse is prevalent in others, depending on factors like personal desire, health insurance, or environmental concerns. However, no recent studies have been conducted on the utilization of gamma irradiation in the decontamination of the catheter for safe disposal or reuse.

Gamma-ray irradiation can affect bacterial DNA, either directly or indirectly. Direct action deposits radiation energy in DNA cells, whereas indirect action includes ionizing radiation interacting with water molecules. Gamma rays interact with water, causing reactive free radicals to destroy DNA. Unrepaired double-strand breaks can lead to cell death. Also, these free radicals can damage cell membranes and protein structures. [29] Biofilms consist of bacterial cells within a protective extracellular polymeric substance (EPS) matrix, which aids adhesion and shields against environmental stress. Gamma irradiation can disrupt glycosidic bonds, degrading the EPS and destabilizing the biofilm.[30,31]

Li, et al. [32] observed **D_{10}** values ranging from 0.3 to 0.8 kGy for bacterial isolates derived from soil samples in the context of comparable results. A strain of *Bacillus* sp. (0.8 kGy) had the highest **D_{10}** value, followed by *Pseudomonas* sp. (0.7 kGy). Similarly, Kim, et al. [33] found that **D_{10}** values for bacterial isolates taken from food processing facilities ranged from 0.2 to 0.7 kGy. A strain of *Listeria monocytogenes* had the highest **D_{10}** value (0.7 kGy), followed by *E. coli* (0.6 kGy), in addition to food-borne pathogenic microorganisms found in meat products, such as *Enterococcus faecalis*, *P. mirabilis*, *S. aureus*, and *Listeria monocytogenes*. According to Kim, et al. [34], a strain of *Enterococcus faecalis* had the highest **D_{10}** value (0.3 kGy), followed by *Pseudomonas aeruginosa* (0.25 kGy).

CONCLUSION

Decontamination of biomedical waste is a social responsibility as well as a legal necessity. This study has shown that ionizing radiation efficiently decreases the populations of both free-floating and bacteria attached to surfaces in a biofilm. Nevertheless, it has been established that biofilms are notably more resistant to elimination and necessitate higher doses for their complete eradication.

Also, we concluded that decontamination of infectious catheters using gamma radiation offers the following benefits: It protects the environment from the harmful effects of extremely hazardous infectious waste. It provides a practical and economical approach to recycling catheters in the face of a severe epidemic and a lack of critical raw materials. It can increase its range of action, which makes it useful against strains that are both vulnerable and resistant to drugs. Finally, it might aid in removing bacterial biofilms, which frequently result in chronic illnesses linked to urinary catheters.

List of abbreviations

BHI: Brain Heart Infusion.
CAUTI: Catheter-Associated Urinary Tract Infection.
CFU: Colony Forming Unit.
CRA: Congo Red agar.
CV: Crystal Violet.
D_{10}: the gamma irradiation dose required to kill the survivors by one log cycle, 90% kill.
MDR: Multi-Drug Resistant.
NaCl: Sodium chloride
OD_{600}: Optical Density at 600 nm
PBS: Phosphate-Buffered Saline.
UTIs: Urinary Tract Infections.

Declarations:

The manuscript has been read and approved by all named authors. The manuscript is not published elsewhere.

Ethics approval and consent to participate:

The study was approved by the Ethics Committee of The Zagazig University-Institutional Review Board under the number ZU-IRB# 192/5-March-2024. As this study only focused on bacterial strains and did not use human material or patient information, also informed consent was obtained from all participants, the Review Board of the Ethics Committee of The Zagazig University Hospital exempted this study from review and waived the need for informed consent. All methods were carried out in accordance with relevant guidelines and regulations.

Experimental research and field studies:

All relevant institutional, national, and international guidelines and legislation were compiled or adhered to in the production of this study.

Availability of data and materials:

The relevant datasets supporting the results of this article are included within the article, and the retrieved ITS sequence was deposited in NCBI GenBank under accession No. 0Q607830 and 0Q048636.
https://www.ncbi.nlm.nih.gov/nuccore/OQ607830
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