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Efficacy of radiation technologies in reducing microbial contamination in hospital wastewater

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Abstract

This study evaluates the effectiveness of ionizing radiation in reducing the microbial loads of hospital wastewater, comparing to on-site hospital treatment plant and membrane bioreactor system. The gamma irradiation above 2 kGy significantly outperformed other processes. Gamma treatment at 4.5 kGy provided more efficient treatment with a 4 log reduction in total aerobic mesophilic bacteria, compared to 1.4 and 2.9 log reductions for the on-site and combined on-site/membrane treatment systems. *Enterococcus faecium* was identified as the most radio-resistant strain. The investigation of dose rate effect on *E. faecium* inactivation revealed that the D_{10} value decreased (2.17, 1.49, and 0.76 kGy) with an increase in dose rate (257 Gy/h for gamma-ray, 1087 Gy/h for gamma-ray, and 2500 Gy/s for e-beam). These findings demonstrate that radiation processing offers a robust solution for reducing global health risks associated with hospital wastewater.

Keywords Hospital wastewater · Gamma ray · Electron beam · Bacteria · D_{10} value · Dose rate

Introduction

The prevalence of antimicrobial-resistance bacteria (ARB) has escalated recently, posing substantial health risks [1]. The World Health Organization (WHO) identifies antimicrobial-resistance as one of the top ten global health threats and jeopardizes over a century of medical progress. Currently, the United Nations estimates that approximately 700,000 deaths annually from ARB infections [2]. The misuse and overuse of antibiotics in both humans and animals is considered the primary driver behind the emergence and dissemination of ARB, which now affects not only clinical settings but also natural ecosystems [3].

Humans serve as one of the main sources of ARB, contributing resistant bacteria to the environment through household sewage or, more critically, through hospital wastewater (HWW) discharges [4]. In many developing countries, HWW is often discharged into municipal sewage without adequate treatment, contributing to the spread of ARB and other pathogens. Recent studies indicate that HWW can be 5–15 times more harmful than regular municipal wastewater and may cause operational challenges in wastewater treatment plants (WWTPs) [5].

Discharging hospital wastewater into sewers without adequate treatment at the on-site hospital treatment plant may contribute to the spread of ARB in the environment. Studies have shown that plasmids from laboratory strains present in hospital wastewater can transfer to indigenous bacteria through the wastewater disposal system [6–8]. Antibiotic resistance genes may then spread across diverse bacterial populations and environmental ecosystems, potentially resulting in human infections [8, 9]. Moreover, ARB can contaminate food via surface water, leading to foodborne and waterborne diseases, such as salmonellosis and campylobacteriosis [6].

One notable case occurred in 2021, when *Salmonella* Typhimurium was detected in chocolate products. Although the strain remained susceptible to certain antibiotics, it showed resistance to six antibiotic groups, illustrating the complexity and unpredictability of resistance patterns [10].

Among resistant pathogens, particular concern centers around the ESKAPE —*Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Escherichia coli*—which are responsible for most drug-resistant

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hospital-acquired infections [11–14]. Among them, *E. coli* serves as a significant reservoir of antibiotic resistance genes in the community and contributes to the spread of resistant pathogens [15]. For instance, resistance rates as high as 92.9% to ciprofloxacin and 36.0% to third-generation cephalosporins have been reported for *E. coli* [2]. Consequently, reducing *E. coli* levels in wastewater may play a critical role in mitigating antimicrobial-resistance.

Given the high microbial load and the presence of resistant organisms, drug residues, and hazardous pollutants in HWW, effective on-site treatment is essential. Without appropriate treatment, HWW can contaminate groundwater and surface water, disrupt ecological balance, and significantly accelerate the dissemination of ARB. There are various treatment processes, such as chlorine exposure, ozonation, gamma radiation, and ultraviolet (UV) processes, to destroy microorganisms in wastewater [6, 9, 16, 17]. However, each method has limitations, and no single chemical or physical treatment consistently inactivates all microbial species. However, radiation-based disinfection offers a promising alternative [18, 19]. Irradiation is widely regarded as an efficient, environmentally friendly technology that leaves no residual by-products. Furthermore, the bacterial DNA is damaged by the direct and indirect effect of ionizing radiation, which results in the inactivation of ARB [20, 21]. Recent irradiation technology recent studies have demonstrated that the irradiation process can effectively eliminate microorganisms in foods, animal and poultry feeds, medical devices, wastewater, and sludge [22]. The potential of radiation processing to reduce microbial loads in HWW below regulatory limits highlights its value as a pre-treatment or an on-site treatment method.

Microbial inactivation by radiation processing follows a predictable kinetic model similar to that of thermal treatment. The inactivation rate depends on various factors, including radiation dose, microbial type, and environmental conditions. The D_{10} value, defined as the radiation dose required to achieve a 90% reduction in viable cells, is commonly used to evaluate the sensitivity of specific organisms to irradiation. This parameter guides the determination of appropriate exposure levels for wastewater disinfection [16, 23–25]. However, some microorganisms possess the ability to repair DNA strand damage induced by irradiation, particularly single-strand breaks and base alterations, which are classified as radiation-resistant microorganisms. Therefore, it is essential to employ the appropriate radiation technology for treatment.

This study aims to investigate the application of irradiation technology in the treatment of hospital wastewater to reduce the spread of hospital-acquired microorganisms and thereby protect human health and mitigate environmental pollution. In this regard, the effect of gamma and electron beam irradiation on the inactivation of pathogenic microorganisms present in real HWW samples was investigated, and the results were compared with the on-site treatment facility efficiency.

Material and method

Wastewater sampling

HWW samples were taken from a medical institution in the Marmara Region of Türkiye. Samples were collected using automatic samplers from the entrance of the on-site medical waste treatment facility and the discharge point of the hospital effluent into the municipal sewage system. Firstly, the samples were subjected to physicochemical and microbiological characterization to assess the wastewater quality. The membrane bioreactor system was also employed as an additional process to treat the effluent from the on-site hospital treatment plant to evaluate its effect on microbial load reduction [26]. Then, the performance of gamma irradiation in reducing the bacteria load was evaluated and compared with the on-site and membrane treatment efficiencies. To determine the dose rate effect, the samples were also subjected to electron beam and gamma irradiation at different dose rates.

Determination of physicochemical parameters and initial microbial load

The analysis of physicochemical parameters was conducted using Standard Methods for the Examination of Water and Wastewater (APHA, 2005). COD (Chemical Oxygen Demand), BOD (Biochemical Oxygen Demand), TSS (Total Suspended Solids), TKN (Total Kjeldahl Nitrogen), and ammonia were determined by APHA (2005) 5220–D, APHA (2005)–5210 B, APHA (2005) 2540–D, APHA (2005) 4500–N_{org}–B, and APHA (2005) 4500-NH_3–B, respectively [26].

The samples were then analyzed to identify and quantify the microorganisms present, specifically total aerobic mesophilic bacteria, coliforms, *E. coli*, Pseudomonads, and Enterococci.

The samples underwent serial dilution, and each dilution was plated using the spread plate technique on Tryptic Soy Agar for total aerobic mesophilic bacteria (TAMB), Slanetz Bartley Agar for Enterococci, and Cetrimide Agar for Pseudomonads. Petri dishes for total bacteria were incubated at 28 °C for 72 h post-inoculation, while plates for Enterococci and Pseudomonads were incubated at 37 °C for 48 h. After incubation, the plates were assessed, and results were recorded as colony-forming units per milliliter (CFU/mL).

To determine coliforms and *E. coli* counts, the samples were diluted, and each dilution was inoculated into

tubes containing Lauryl Sulfate Tryptose Broth with 4-methylumbelliferyl-β-D-glucuronide (MUG). The tubes were incubated at 37 °C for 48 h. After incubation, gas production was evaluated to quantify coliforms. The presence of *E. coli* was confirmed by detecting blue fluorescence under a portable UV lamp emitting light at a wavelength of 366 nm in gas-positive tubes. Additionally, an indole test was performed by adding 0.2 mL of dimethylaminobenzaldehyde to the culture medium and observing the formation of a red ring, indicating a positive indole reaction. Results for coliforms and *E. coli* were expressed as the most probable number per milliliter (MPN/mL).

Irradiation and radiosensitivity of microorganisms

HWW samples were exposed to gamma radiation at varying doses (up to 4.5 kGy) using a Co-60 irradiator (Ob-Servo-Sanguis Co-60 irradiator, 12 kCi maximum activity, Hungary) at dose rates of 1087 Gy/h and 257 Gy/h. Absorbed doses were quantified using calibrated standard dosimeters (Harwell Perspex dosimeters, UK). Microbial counts were determined before and after treatment to evaluate the effectiveness of the irradiation. For electron beam treatment, mid-energy electron accelerator (Vivirad, 500 keV-20 mA ICT type, France) was used at 500 keV–1 mA irradiation conditions (2500 Gy/s), and doses were validated using radiochromic film dosimeter (FWT-60, Far West Technology, USA).

The untreated sample was divided into sealed glass bottles, each with a volume of 100 mL, to assess the radiosensitivity of the total bacterial population and to identify the bacteria exhibiting the highest resistance to radiation. The samples were exposed to gamma rays up to 4.5 kGy, with a dose rate of 1087 Gy/h.

Following the irradiation, the samples underwent serial dilution and were inoculated onto Tryptic Soy Agar plates. The plates were incubated at 28 °C for 72 h before the assessment. After the incubation period, the plates were assessed for microbial growth and the results were expressed as colony-forming units per milliliter (CFU/mL).

Colony isolation was also performed on samples irradiated at 3.8 and 4.5 kGy to determine the radio-resistant bacteria. Colonies were selected based on distinct morphological characteristics. Isolates were preserved in tryptic soy broth supplemented with 50% glycerol at − 80 °C for further analysis. Preliminary identification included Gram staining, spore formation assessment, catalase and oxidase testing, and biochemical assays according to Bergey's Manual of Determinative Bacteriology.

For precise identification, the MALDI Biotyper® Sirius One RUO/GP rapid identification system (Bruker GmbH, Germany) containing the MALDI Biotyper® Library was used. A spot of the microorganism culture was deposited onto a disposable MALDI Biotarget plate, followed by the addition of an MBT Galaxy® Matrix and formic acid solution were then added to each spot. The plate was then analyzed using the MALDI Biotyper® Sirius One RUO/GP system for microbial identification. *Escherichia coli* ATCC 25922 was used as the reference strain during rapid identification. In addition, antibiotic susceptibility testing was performed using the disk diffusion method to determine the multidrug resistance profile of *Enterococcus faecium*. Briefly, bacterial suspensions were adjusted to a 0.5 McFarland standard (approximately 8 log CFU/mL) and inoculated onto Mueller–Hinton agar plates. Antibiotic disks were placed on the agar surface, and the plates were incubated at 35 ± 1 °C for 18–24 h.

Comparison of different dose rates

The effect of dose rate on the reduction of radio-resistant bacteria was evaluated using gamma irradiation at dose rates of 1087 Gy/h and 257 Gy/h. Additionally, control samples and samples artificially inoculated with *E. faecium*, which was isolated from irradiated influent and identified as the most radio-resistant bacterium, were subjected to electron beam irradiation at a dose rate of 2500 Gy/s. The electron beam irradiation of the samples was performed in a rectangular glass container (4.5 cm wide and 40 cm long), ensuring a sample thickness of 1 mm to allow penetration of the 500 keV electron beam. Post-treatment microbial survival was assessed to determine the D_{10} values.

Statistical analysis

Analyses were conducted in duplicate, and the results were presented as means ± standard deviations. A one-way analysis of variance (ANOVA) was performed on the mean values of the groups, with a significance level set at $\alpha = 0.05$. A *p*-value less than 0.05 was considered statistically significant.

Results and discussion

Determination of physicochemical parameters and initial microbial load

HWW samples may exhibit high levels of ammonia, biological oxygen demand (BOD_5), chemical oxygen demand (COD), total nitrogen and suspended solids [17]. In this context, the physicochemical parameters (pH, COD, BOD_5, NH_3-N, TKN and TSS) of influent and effluent from the on-site treatment process were determined in this study as the initial step to assess the quality of wastewater. The results were given in Table S1. It was found that the physicochemical parameters of the samples were within acceptable

discharge limits of the regulatory bodies [27]. Therefore, the subsequent phase of the study focused on the microbiological parameters of the HWW samples.

Comprehensive analysis of the microbiological composition of HWW samples is essential for evaluating pollution levels and determining appropriate radiation doses for bacterial load reduction. In this study, microbiological investigations were conducted to determine the initial microbial levels and radiation treatment efficiency. These included analyses of TAMB, coliforms, *E. coli*, Enterococci, and Pseudomonads. The outcomes of microbiological analyses are provided in Table 1.

The main objective of TAMB analysis is to quantify the bacterial load, which is essential for evaluating potential environmental risks [28, 29]. It was found that on-site treatment at the hospital significantly decreased TAMB count by 1.37 log units (log CFU/mL$_{\text{non-treated HWW}}$ − log CFU/mL$_{\text{treated HWW}}$). Following membrane treatment of the effluent, the TAMB count was reduced by an additional 1.55 log units.

Fecal contamination was monitored through coliform and *E. coli* tests (Fig. 1). HWW contained a high level of coliform bacteria (Table 1), exceeding the permitted limit of 1000 cells/100 mL, as specified in hospital wastewater quality guidelines [6]. The analyses revealed that the coliform and *E. coli* counts decreased by 3.38 and 2.51 log units, respectively, after the on-site treatment. The membrane treatment further reduced the coliform count by 1.3 log unit, while the *E. coli* count decreased to an undetectable level (< 1 CFU/ml).

Among the bacteria responsible for hospital-acquired infections, *P. aeruginosa* is a prevalent Gram-negative opportunistic pathogen that spreads within healthcare environments. These bacteria are naturally resistant to multiple antibiotics and have an ability to rapidly acquire additional resistance genes [7]. Figure 2 shows the analysis of Pseudomonads on cetrimide agar under UV light in this study. In HWW samples, Pseudomonads were detected at a level of 4.0 log CFU/mL and decreased by 0.52 log unit following the on-site treatment. An additional 1.28 log unit reduction

Table 1 Microbial load of the HWW samples (influent), the samples after the on-site treatment (effluent), and membrane treatment

Microbial analysis	Influent (log)	Effluent (log)	Membrane treated (log)
TAMB (CFU/mL)	6.10	4.73	3.18
Coliform (MPN/mL)	5.04	1.66	0.36
E. coli (MPN/mL)	2.87	0.36	< 1
Pseudomonads (CFU/mL)	4.00	3.48	2.20
Enterococci (CFU/mL)	3.57	< 1	< 1

Fig. 1 Gas production, blue fluorescence, and indole ring formation tests for coliform and *E. coli*, obtained from LST broth medium supplemented with MUG

was found in the effluent from the membrane treatment process.

Another member of ESKAPE, *Enterococcus* spp., is a gram-positive, fermentative, and aerotolerant bacteria that can survive in the presence of oxygen. While Enterococci are part of the normal gastrointestinal flora in humans and animals, typically serving as harmless commensals, they can become opportunistic pathogens when the commensal relationship with the host is disrupted [30–32]. In this study, the count of Enterococci was found to be 3.57 log CFU/mL in untreated wastewater. However, Enterococci counts remained below the detectable limits in on-site-treated and membrane-treated wastewater.

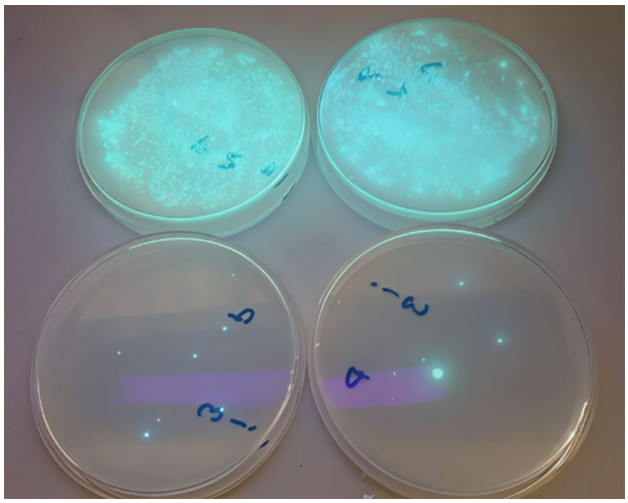

Fig. 2 Blue-green fluorescence under ultraviolet light of Pseudomonads colonies on cetrimide agar

Bacteria isolated from the HWW samples were also analyzed using MALDI Biotyper®, achieving a score of > 2.0. As could be seen from Table S2, *Enterococcus* spp. strains were identified as the predominant bacteria in the sample.

The microbial counts in this study revealed that untreated HWW exhibited elevated bacterial levels, which can pose significant environmental and public health risks. A recent study highlighted that antibiotic resistance pollution primarily results from the essential use of antibiotics in medical treatments [33]. On-site treatment and membrane filtration reduced bacterial loads marginally; however, residual ARB remained. Radiation technology can be a more effective and a promising option for the treatment of microbiological contaminants.

Effect of irradiation and isolation of radio-resistant bacteria

In this section of the study, firstly, the inactivation rate of microorganisms in untreated HWW samples subjected to gamma irradiation up to 4.5 kGy (dose rate of 1087 Gy/h) was investigated. Figure 3 and Table S3 illustrate the reduction in TAMB counts and Table S4 shows the one-way ANOVA results. After treatment with a 2 kGy irradiation dose, the bacterial count diminished significantly ($p < 0.05$) from 6.10 ± 0.02 log CFU/mL to 2.71 ± 0.16 log CFU/mL. The gamma irradiation at dose of 4.5 kGy reduced ($p < 0.05$) the counts of TAMB count by approximately 4 log unit (6.10 ± 0.02 log CFU/mL to 2.12 ± 0.11 log CFU/mL).

The treatment efficiencies of radiation processing, the on-site treatment plant, and the combined on-site/membrane treatment system were compared by evaluating the log reduction (log $CFU/mL_{non\text{-}treated\ HWW}$ − log $CFU/mL_{treated\ HWW}$) of the TAMB counts (Fig. 4). As can be clearly seen in Fig. 4, gamma irradiation at doses of ≥ 2 kGy has reduced the total bacterial load in HWW more than other processes. Similar

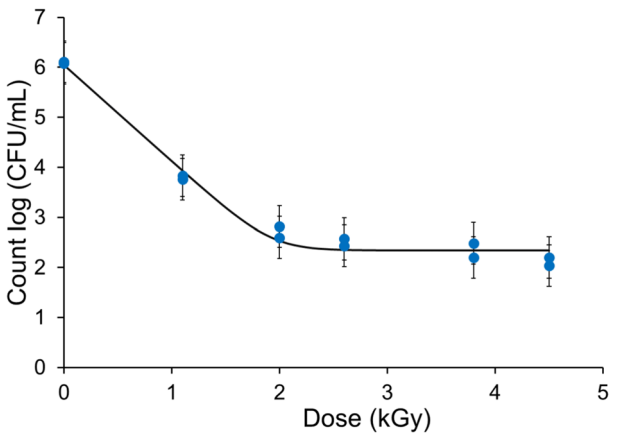

Fig. 3 Reduction of TAMB after irradiation treatment

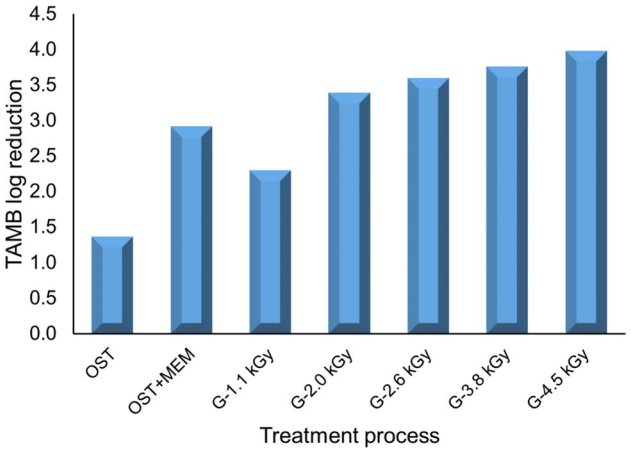

Fig. 4 Comparison of TAMB log reduction (log $CFU/mL_{non\text{-}treated\ HWW}$ − log $CFU/mL_{treated\ HWW}$) by different treatment processes (OST: on-site treatment, OST + MEM: On-site/membrane treatment combination, G-1.1 kGy: Gamma treatment at 1.1 kGy, G-2.0 kGy: Gamma treatment at 2.0 kGy, G-2.6 kGy: Gamma treatment at 2.6 kGy, G-3.8 kGy: Gamma treatment at 3.8 kGy, and G-4.5 kGy: Gamma treatment at 4.5 kGy

findings were reported that ionizing radiation provided high and stable disinfection efficiency (95%) for microbiological load at radiation doses > 0.25 kGy, inhibiting bacterial regrowth [28, 34–37]. Based on these findings, it can be suggested that gamma radiation has provided a more efficient treatment for TAMB than the on-site treatment and even the membrane treatment that was employed as an additional process.

As mentioned above, the TAMB reduction by gamma irradiation was more pronounced up to 2 kGy; however, TAMB inactivation reached nearly a plateau at doses beyond 2 kGy (Fig. 3). According to one-way ANOVA results (Table S4), there was no significant difference between the colony counts (log CFU/mL) of TAMB obtained from the samples irradiated at 2.0, 2.6, 3.8, and 4.5 ($p = 0.06$), indicating the increase in gamma dose within the range of 2.0–3.8 kGy did not lead to any further inactivation. This finding can be attributed to the presence of radio-resistant bacteria in HWW samples.

To identify the radio-resistant bacteria, isolates obtained from the samples treated with 3.8 and 4.5 kGy were grouped based on colony morphology. For preliminary identification, Gram reaction, spore-forming ability, and biochemical characteristics were examined. After 3.8 kGy irradiation, the dominant flora consisted of *Bacillus* spp. and *Enterococcus* spp. On the other hand, the only microorganism detected after 4.5 kGy irradiation was *Enterococcus* spp., indicating gamma irradiation at dose of 4.5 kGy was effective in inactivating coliforms, *E. coli*, and Pseudomonads.

To achieve accurate species-level identification of radio-resistant strains, MALDI Biotyper® analysis was performed.

Bacillus strains were identified as *Bacillus cereus*, with identification scores exceeding 2.0 (Table S5). Furthermore, the most radio-resistant isolates after 4.5 kGy irradiation treatment were accurately identified as *E. faecium* using the MALDI Biotyper®, with scores exceeding 2.0 (Table 2). In this study, it was found that *E. faecium* strains demonstrated the greatest resistance to radiation among the bacterial populations present in the HWW samples.

In addition, antibiotic resistance assays were conducted on radiation-resistant *E. faecium* strains to evaluate their resistance to various antibiotics, including vancomycin, gentamicin, and ciprofloxacin, which are frequently used in hospitals. In this study, commercial antibiotic discs were used, and the results were presented in Table S6. As could be seen from Table S6, *E. faecium* shows resistance against the tested second and third generations of antibiotics as well.

Enterococci are considered as more reliable indicators of fecal pollution compared to fecal coliforms [25]. The widespread distribution of antibiotic-resistant *Enterococcus* spp. (particularly *E. faecalis* and *E. faecium* pathogens) in various environments, such as soil, aquatic systems, vegetation, and food, poses a significant threat to human and animal health [25, 38–40]. In this study, gamma irradiation at 4.5 kGy with a dose rate of 1087 Gy/h was found to be efficient treatment process for the reduction of TAMB and fecal contamination. However, the isolation of *E. faecium* strains from irradiated wastewater samples has revealed the necessity of implementing a more efficient radiation process due to the potential health risks mentioned above. In this context, the effect of electron beam irradiation on reducing the populations of *E. faecium* (due to being both antibiotic and radiation resistant strain) was investigated in the following section. As electron beam processing delivers radiation energy much faster than gamma irradiation due to higher dose rate, this technology can provide greater treatment efficiency along with a shorter treatment time.

Comparison of dose rates

In order to better understand the radio-resistance of *E. faecium* and to examine the effect of the dose rate on the reduction of these strains, sterile wastewater samples (irradiated at 25 kGy) were artificially contaminated with *E. faecium* strains isolated from the HWW samples. These samples were exposed to gamma radiation up to 1.5 kGy at different dose rates (1087 Gy/h and 257 Gy/h) and electron beam irradiation at a dose rate of 2500 Gy/s. Figure 5 and Table 3, and Table S7 shows the dose response of *E. faecium* at different dose rates. D_{10} values, representing the dose required for a 90% reduction in microbial population (one-decimal logarithm decline), were determined from linear regression models of surviving fractions plotted against absorbed irradiation dose (kGy) at each dose rate. As shown in Table 3 and Table S8, the D_{10} value significantly ($p < 0.05$) decreased as the dose rate increased, indicating *E. faecium* exhibited greater resistance to irradiation at lower dose rates.

The difference in radiation resistance of *E. faecium* can be attributed to DNA repair and oxidative defense mechanisms. Verde et al. [24] stated that microorganisms had more time to repair radiation-induced damage at a lower dose rate, which was reflected in higher surviving fractions after irradiation. However, Taghipour [41] reported that variations in gamma radiation dose rates did not significantly impact the extent of inactivation at a given total dose. In our study, the reduced inactivation rate at the low dose rate (Table 3) suggested that *E. faecium* had more opportunities to repair or adapt to irradiation-induced damage, underscoring the significance of dose rate in enhancing the efficacy of irradiation for bacterial inactivation. Thus, it is assumed that low dose rates facilitate the functioning of bacterial repair systems.

The findings indicate that e-beam irradiation due to the high dose rate is a more effective treatment method for HWW than gamma rays. Selambakkannu et al. [42] investigated the effects of electron beam irradiation on sewage sludge, reporting a decrease in *Enterococci* counts to 2.30, 2.01, 1.74, and 0.60 log CFU/mL at dose rates of 0.560, 1.120, 1.587, and 1.867 kGy/s, respectively. Jebri et al. [43] demonstrated that a 7 kGy dose effectively inactivated pathogenic bacteria and viruses by electron beam irradiation.

As a result, both gamma irradiation and electron beam irradiation are effective treatment methods for inactivating bacteria populations in HWW compared to on-site treatment and combined processes with membrane treatment. However, electron beam radiation demonstrated superior efficacy than gamma rays.

Table 2 Bacteria isolated from wastewater sample irradiated at 4.5 kGy

Sample name	Sample ID	Organism (best match)	Score value	Organism (second best match)	Score value
A1(+++)(A)	*E.coli**	*E.coli*	2.13	*E.coli*	2.05
A2(+++)(A)	*E.coli**	*E.coli*	2.34	*E.coli*	2.33
A3(+++)(A)	N501	*E. faecium*	2.44	*E. faecium*	2.44
A4(+++)(A)	N501	*E. faecium*	2.46	*E. faecium*	2.40

*ATCC25922

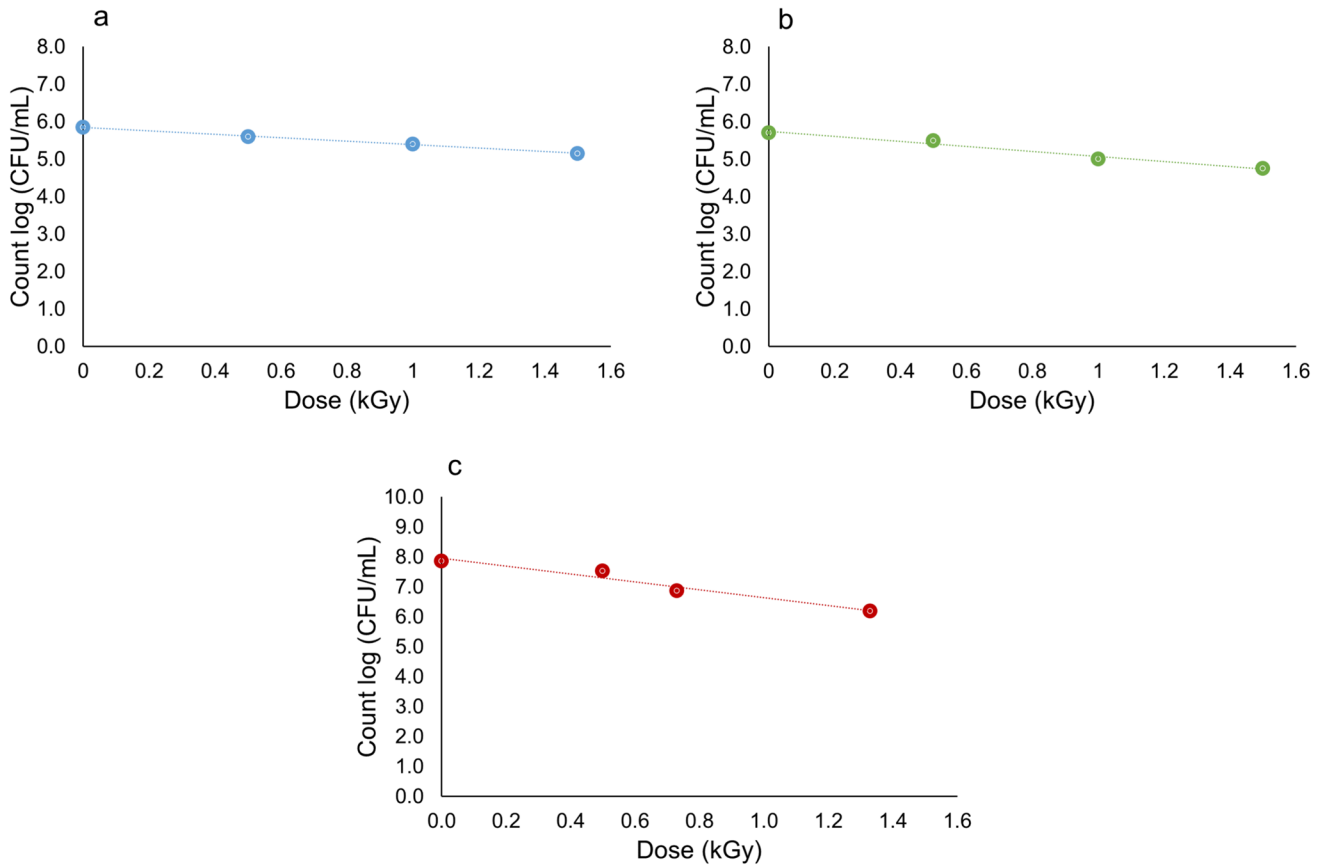

Fig. 5 Treatment of *E. faecium* by **a** gamma-ray at dose rate of 257 Gy/h, **b** gamma-ray at dose rate of 1087 Gy/h, and **c** e-beam at dose rate of 2500 Gy/s

Table 3 Comparison of dose rate effect for *Enterococcus faecium*

	Gamma-ray (257 Gy/h)	Gamma-ray (1087 Gy/h)	E-beam (2500 Gy/s)
Regression equation	$y = -0.46x + 5.845$	$y = -0.67x + 5.74$	$y = -1.3193x + 7.9526$
Inactivation rate	0.46	0.67	1.32
R^2	0.9981	0.9727	0.9514
D_{10} (kGy)	2.17	1.49	0.76

Conclusion

Untreated hospital wastewater is a significant source of environmental contamination and contributes to the spread of ARB. The conventional on-site hospital treatment plants have significant limitations in reducing the microbial load in the wastewater, particularly in eliminating the multidrug-resistant pathogens. This research highlights ionizing radiation as a highly effective treatment process for real hospital wastewater.

Key findings reveal that:

- Gamma irradiation at doses ≥ 2 kGy provides a more efficient treatment TAMB reduction compared to traditional hospital treatment plants as well as combined on-site/membrane treatment systems.
- Gamma irradiation at 4.5 kGy significantly reduced total bacterial populations (a 4 log reduction). Following the irradiation, coliforms, *E. coli*, and Pseudomonads could not be detected.
- *Enterococcus faecium* was identified as the most radioresistant pathogen in HWW irradiated at 4.5 kGy, dis-

playing persistance to second and third-generation antibiotics as well.

- The dose rate of ionizing radiation was a critical parameter in the treatment of *E. faecium*. The higher dose rates (particularly e-beam processing) significantly enhanced the rate of the microbial inactivation and reduced the D_{10} value.
- The electron beam treatment, having the highest dose rate of 2500 Gy/s, yielded the lowest D_{10} value (0.76 kGy) for *E faecium*. This finding supports the hypothesis that rapid energy deposition overwhelms cellular repair mechanisms.

Consequently, the utilization of radiation technologies, particularly electron beam accelerators due to their speed and efficiency, offers a promising process for the treatment of hospital wastewater. Moreover, most studies on the use of ionizing radiation to inactive ARB have been conducted in laboratory conditions on small samples of synthetic wastewater. This study, which used real hospital wastewater, highlights the importance of industrial applicability.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10967-026-10755-w.

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Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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