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Research article

Escherichia coli's response to low-dose ionizing radiation stress

Jaouhra Cherif[1,*], Anis Raddaoui[2], Ghofrane Ben Fraj[3], Asma Laabidi[3] and Nada Souissi[4]

[1] Laboratory of Biophysics and Medical Technologies, LR13ES07, Higher Institute of Medical Technologies of Tunis, University of Tunis El Manar, Tunis, Tunisia
[2] Laboratory Ward, National Bone Marrow Transplant Center, LR18ES39, Faculty of Medicine of Tunis, University of Tunis El Manar, Tunis, Tunisia
[3] Higher Institute of Medical Technologies of Tunis, University of Tunis El Manar, Tunis, Tunisia
[4] Bacteriology Laboratory, Tunisian Institute of Veterinary Research, University of Tunis El Manar, Tunis, Tunisia

* **Correspondence:** Email: jaouhra.cherif@istmt.utm.tn.

Abstract: Low-dose ionizing radiation can trigger a phenomenon known as hormesis in microorganisms, in which exposure to mild stressors like radiation results in beneficial adaptive responses. This study investigated the impact of low-dose X-rays on *Escherichia coli*'s viability and their potential influence on antibiotic susceptibility. The irradiated samples displayed increased bacterial viability compared to non-irradiated controls, with a significant increase observed at 5 and 10 mGy of X-ray radiation exposure. This suggests a stimulating effect of low-dose ionizing radiation on *E. coli*'s viability. To explore the correlation between viability and antibiotic susceptibility, we assessed the inhibition zone diameters for various antibiotics in non-irradiated and irradiated samples. The obtained results showed that the exposure of bacteria to low-dose ionizing radiation resulted in a significant reduction in the inhibition zone diameters for marbofloxacin, amoxicillin/clavulanic acid, ceftiofur, and cefoxitin. These findings suggest that low-dose X-ray radiation exposure can enhance *E. coli*'s viability and its ability to withstand antibiotics, raising potential concerns.

Keywords: antibiotic susceptibility; *Escherichia coli*; low-dose ionizing radiation; radiation hormesis; stress response

1. Introduction

The discovery of X-rays and radioactivity in the late 19th century initiated extensive research into the effects of ionizing radiation on biological cells and organisms. Today, ionizing radiation is increasingly utilized in research and the medical field for diagnostic and therapeutic purposes. This type of radiation interacts with biological cells by depositing energy within their structures, being capable of rupturing chemical bonds and generating unpaired electrons. Consequently, this interaction induces alterations in molecular structures [1,2], leading to the generation of reactive oxygen species (ROS) such as hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2) [3]. OH^- is the most powerful among the ROS produced in biological systems, being able to react with cellular components. In the DNA molecule, OH^- attacks purine and pyrimidine bases, inducing genetic mutations [4]. High doses of ionizing radiation are well-known for their detrimental effects, causing extensive damage to DNA and inactivating or disrupting proteins and lipids, ultimately leading to cell death or impairing cellular function [5,6]. In contrast, the effects of low doses of ionizing radiation are still under debate and the subject of scientific investigation. Some studies suggest that low doses may have beneficial effects, as they can trigger an adaptive response that protects cells against subsequent higher doses of radiation. This adaptive response is known as radiation hormesis, which has been observed in various organisms including bacteria [7–10]. Hormesis refers to a phenomenon in which a stressor, typically harmful at higher doses, elicits a beneficial or stimulatory response in living organisms when administered in a lower dose. Min et al. [11] conducted a study on *Escherichia coli* demonstrating that exposure to low doses of gamma radiation can stimulate repair mechanisms, potentially minimizing DNA damage and mutation. This observation aligns with the concept of radiation hormesis, which challenges the assumptions of the linear no-threshold (LNT) model. The LNT model suggests a linear relationship between radiation dose and harm, proposing that even low doses of ionizing radiation can cause damage [12]. At the cellular level, hormesis can activate DNA repair pathways, induce change in genes' expression, increase the production of antioxidants, and improve cellular defense systems [13,14]. Moreover, radiation hormesis stimulates the secretion of specific growth factors and cytokines and activates the cell-membrane receptors [15]. Tubiana et al. [16] reported that adaptive responses have been observed at doses ranging from 1 to 500 mGy. Especially, doses below 10 mGy have been found to activate effective DNA damage repair, reduce the risk of mutations, and enhance defense pathways [17]. According to Khan et al. [18], low-dose radiation significantly influences the proliferation, activation, and function of immune cells. These responses contribute to enhancing the cell's ability to cope with subsequent stressors. However, research has also shown that even low doses of ionizing radiation can be harmful in certain contexts, contributing to DNA mutations and posing potential long-term health risks [19]. As such, the impact of low-dose ionizing radiation on cells remains an essential area of ongoing research to fully understand its potential benefits and risks.

Bacteria, like other living organisms, can be exposed to low doses of ionizing radiation from various sources, including cosmic rays and natural background radiation in the environment [20–22]. Additionally, in medical settings, bacteria can encounter low doses of radiation during diagnostic imaging studies, such as X-rays used for medical imaging. The study of bacterial responses to low-dose radiation is of great interest as it can shed light on microbial reactions to environmental stressors and their implications for health and research. Recent studies have revealed intriguing findings on the adaptive responses of bacteria to low-dose radiation exposure, similar to those observed in higher organisms [10,22]. This adaptive response may enable bacterial cells to cope with stress, enhancing their survival and ability to respond to subsequent challenges, including antibiotic exposure.

In light of these observations, our study aims to investigate the effects of low-dose ionizing radiation on bacterial viability, with a particular focus on *E. coli*. As a common bacterium found in various environmental settings, *E. coli* has been extensively studied concerning stress responses. We will also explore the potential impact of low-dose ionizing radiation on antibiotic susceptibility in *E. coli*. This research may contribute to the advancement of our understanding of radiation hormesis and its potential applications in medicine and public health, as well as provide insights for future therapeutic and antimicrobial strategies.

2. Materials and methods

2.1. Bacterial strain preparation

In this study, the bacterial strain *E. coli* 31521, isolated in the bacteriology laboratory of the Veterinary Research Institute of Tunisia, was selected as the subject of investigation. The bacterial strain preserved at −80 °C was later subcultured from the frozen stocks onto Bromo Cresol Purple agar (BCP) (Biokar, France) and then incubated aerobically at 37 °C for 24 h for recovery. This preparation ensured the viability of the bacterial strain, enabling it to be ready for irradiation.

2.2. Radiation instrument and irradiation

The X-ray irradiation process was performed using the Stephanix Movix 4.0 Mobile X-ray system, which operates at a low-energy range from 40 to 115 kV. For the experiments involving X-ray exposure, bacterial suspensions were prepared by transferring isolated colonies from a fresh subculture of *E. coli* into sterile plastic tubes containing 5 mL of liquid culture medium. The bacterial concentrations were adjusted to correspond to 0.5 McFarland standards using a McFarland densitometer (Grant Bio). Bacterial suspensions were then exposed to low doses of X-ray radiation at 5 and 10 mGy. The dose received by the sample was measured using the TNT 12000 X-ray test tools system (Fluke Biomedical). Throughout the entire experiment, it is important to note that both the irradiated samples (test group) and non-irradiated samples (control group) were carefully maintained under identical environmental conditions.

2.3. Determination of viable cell counts

The viable cell count of bacterial cultures was determined using a standardized protocol. Irradiated bacteria were first diluted with sterile saline solution and thoroughly stirred to ensure a homogenous cell suspension. Serial dilutions were prepared, and 100 µL of each diluted bacterial suspension was spread onto plate count agar (PCA) medium (Bio-Rad, France). Subsequently, the agar plates were incubated at 37 °C for 24 h to allow bacterial growth, and the resulting bacterial colonies were carefully counted. The viable cell count was determined by considering the number of colonies counted, the plated volume, and the dilution factor (10^3 for untreated samples, used as a control group, and 10^4 for treated samples). The viability of *E. coli* was assessed by quantifying the surviving fraction as the $\log_{10}$ of the colony-forming units (CFU/mL).

2.4. Determination of antimicrobial susceptibility

Antimicrobial susceptibility testing was determined by the disk diffusion method [23] on Mueller Hinton (MH) agar (Bio-Rad, Marnes-la-Coquette, France) according to Antibiogram Committee of the French Society of Microbiology (CA-SFM) [24]. Eight antibiotics were tested using 6.5 mm discs (Bio-Rad, Marnes-la-Coquette, France): amoxicillin (AMX) (25 μg), amoxicillin/clavulanic acid (AMC) (20/10 μg), cefalexin (CN) (30 μg), cefoxitin (FOX) (30 μg), ceftiofur (XNL) (30 μg), gentamicin (GM) (15 μg), neomycin (N) (30 UI), and marbofloxacin (MAR) (5 μg). Both irradiated and non-irradiated samples were subcultured on BCP agar and incubated at 37 °C for 24 h. This step allowed for bacterial repair and regrowth, activating responsive and adaptive mechanisms prior to exposure to antibiotics, following the methodology of Oskouee et al. [25].

Bacterial suspensions were prepared from each sample, obtaining a turbidity equivalent to 0.5 McFarland. A bacterial inoculum with approximately 1.5×10^8 CFU/mL was then applied to the surface of MH agar plates. The antibiotic disks were placed on the inoculated agar surface, and the plates were incubated at 37 °C for 24 h prior to reading. The inhibition zone around each antibiotic disk was measured to the nearest millimeter, and the zone diameter of each drug was interpreted according to CA-SFM recommendations [24] as either susceptible, intermediate, or resistant. As a control group, non-irradiated samples were used for comparison. Three replicate agar plates were used in each experiment group. To ensure the accuracy of susceptibility testing results, reference strain *E. coli* ATCC 25922 was employed as quality control.

2.5. Statistical analysis

The experimental data were presented as means ± standard deviation (SD) and analyzed by one-way analysis of variance (ANOVA). Statistical significance was considered at $P < 0.05$, indicating differences between the irradiated and non-irradiated samples.

3. Results

In this study, we investigated the effects of low doses of ionizing radiation on the viability of *E. coli*. As shown in Figure 1, irradiated samples exhibited increased bacterial viability compared to the non-irradiated control group (denoted as dose 0 in Figure 1). When exposed to 5 mGy of X-ray radiation, the number of viable colonies in the irradiated samples showed a significant increase of 0.86 $\log_{10}$ CFU/mL ($p < 0.001$). Furthermore, with a dose of 10 mGy, the stimulatory effect on bacterial viability was even more pronounced, resulting in an increase of 0.98 $\log_{10}$ CFU/mL ($p < 0.001$). These findings indicate that low doses of ionizing radiation have a stimulating effect on *E. coli* viability.

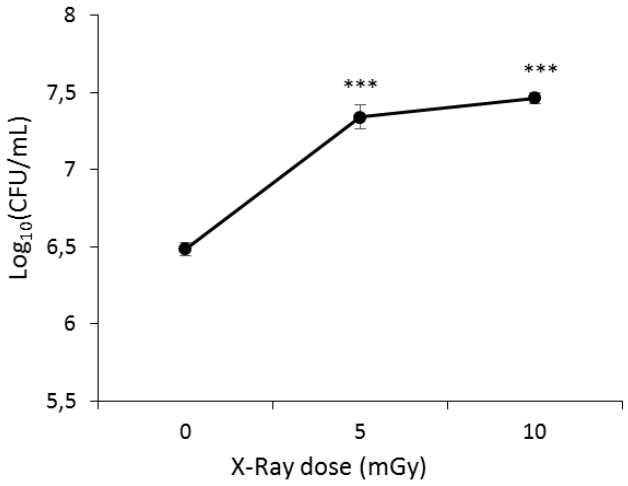

Figure 1. Viability of *E. coli* following irradiation with 5 and 10 mGy low-dose X-rays. Viability data is presented as the $\log_{10}$ of surviving colony-forming units per milliliter (CFU/mL). Data in this figure are expressed as mean ± SD, n = 6, *** $p < 0.001$ compared to control**.**

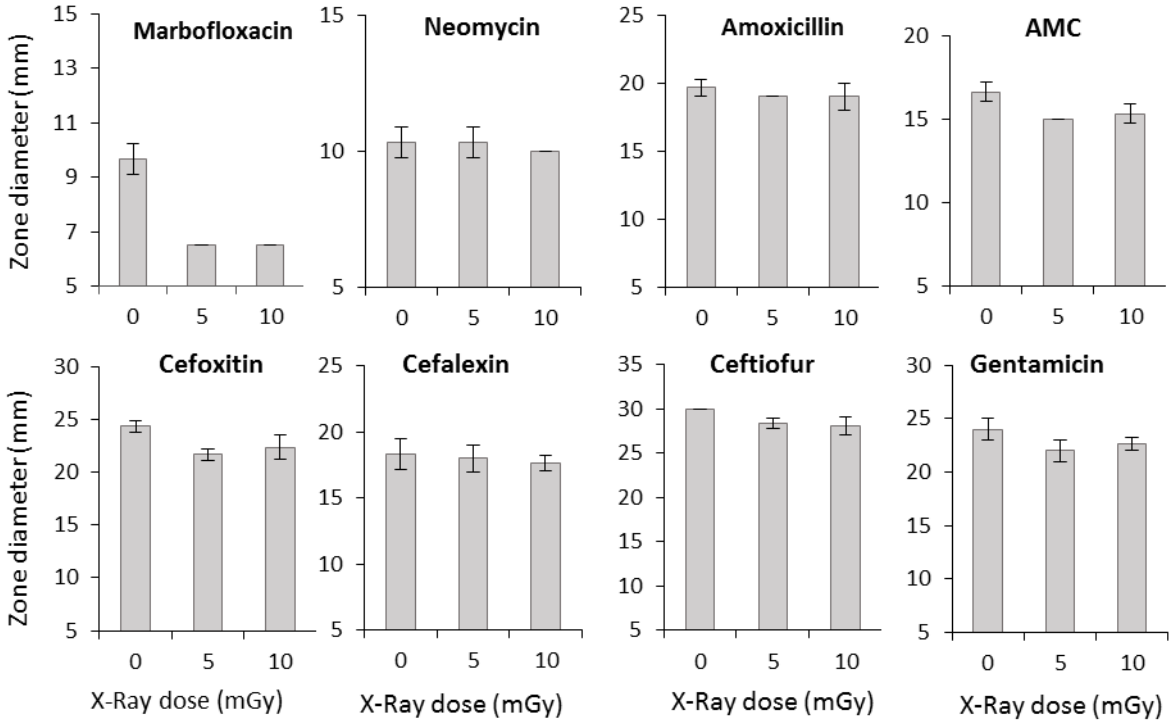

Figure 2. Mean diameter of the inhibition zone of *E. coli* in non-irradiated and irradiated bacteria exposed to low-dose X-rays radiation. Each value represents a mean ± SD obtained from three independent experiments. AMC: Amoxicillin/clavulanic acid.

To further explore the correlation between bacterial viability and antibiotic susceptibility of *E. coli* when exposed to low-dose ionizing radiation, we evaluated the mean diameters of inhibition zones for various tested antibiotics in non-irradiated control samples and samples exposed to 5 and 10 mGy of X-ray radiation (Figure 2). The results revealed a reduction in the diameter of inhibition zones for the majority of antibiotics after irradiation. Interestingly, we observed a notable effect of marbofloxacin, where the mean diameters of the growth inhibition disks were significantly reduced by 3.16 mm after exposure to radiation doses of 5 mGy and 10 mGy ($p < 0.001$) compared with non-irradiated bacteria (Table 1). This suggests that the bacteria became more resistant to marbofloxacin after irradiation (Table 2).

Table 1. Comparison of inhibition zone diameters of tested antibiotics between non-irradiated and irradiated bacteria exposed to low-dose X-ray radiation.

Comparison of inhibition zone diameters				
Antibiotics	5 mGy		10 mGy	
	p-value*	Difference	*p*-value*	Difference
Marbofloxacin	<0.001	HS	<0.001	HS
Neomycin	0.630	NS	0.630	NS
Amoxicillin	0.116	NS	0.374	NS
AMC	0.015	S	0.027	S
Cefoxitin	0.021	S	0.047	S
Cefalexin	0.725	NS	0.422	NS
Ceftiofur	0.044	S	0.031	S
Gentamicin	0.070	NS	0.116	NS

***** *p*-value of the one-way ANOVA comparison between non-irradiated and irradiated bacteria. NS: non-significant from non-irradiated bacteria. S: significant from non-irradiated bacteria at $p < 0.05$. HS: highly significant from non-irradiated bacteria at $p < 0.001$.

Table 2. Antibiotic susceptibility of non-irradiated and irradiated *E. coli* exposed to low-dose X-ray radiation.

Antibiotics	Non-irradiated bacteria	Irradiated bacteria	
		5 mGy	10 mGy
Marbofloxacin	R	R+++	R+++
Neomycin	R	R	R
Amoxicillin	I	I	I
AMC	I	I	I
Cefoxitin	S	I	S
Cefalexin	S	S	I
Ceftiofur	S	S	S
Gentamicin	S	S	S

R: resistant, R+++: more resistant, I: intermediate, S: susceptible.

Table 1 provides a clear comparison between the inhibition zone diameters of irradiated and non-

irradiated bacteria, revealing a statistically significant reduction for AMC, ceftiofur, and cefoxitin after exposure to both 5 and 10 mGy of radiation ($p < 0.05$). Notably, the susceptibility of *E. coli* to cefoxitin was altered to an intermediate level ($p = 0.021$) after exposure to a low dose of 5 mGy of X-ray radiation (Table 2). In contrast, low-dose ionizing radiation did not significantly alter the main diameter of growth inhibition zones for amoxicillin, cefalexin, gentamicin, and neomycin.

These findings indicate that low-dose ionizing radiation may enhance the viability of *E. coli* and could potentially influence their response to certain antibiotics.

4. Discussion

This study explores the effect of low-dose X-ray radiation exposure, as a physical environmental stress, on the adaptive responses of *E. coli*. The choice of the *E. coli* bacterium, known for its high inherent antibiotic resistance rates, establishes a relevant model for investigating the impact of low-dose radiation on antibiotic resistance and understanding how environmental factors influence bacterial resistance mechanisms. The findings from this study reveal that exposure to these low doses significantly activates bacterial viability and induces notable changes in bacterial susceptibility to specific antibiotics. To the best of our knowledge, this is the first study to suggest that low-dose X-ray radiation exposure can enhance *E. coli* viability and its ability to withstand antibiotics.

The increased bacterial viability observed in *E. coli* following exposure to low-dose X-ray radiation aligns with findings from various studies indicating similar stimulatory effects on bacterial viability. For instance, Kolesnik et al. [26] demonstrated that low doses of alpha- and beta-emitting radionuclides activated bioluminescence in *Photobacterium phosphoreum*, suggesting a hormetic response. Additionally, research on *Synechococcus lividus* showed that exposure to low-dose gamma radiation could potentially enhance the proliferation rate of this cyanobacteria [27]. Furthermore, previous research on the impact of non-ionizing frequency electromagnetic fields on bacterial growth rates revealed that exposure to extremely low-frequency electromagnetic fields, specifically 2 mT at 50 Hz, enhanced the growth rate of both *E. coli* and *Pseudomonas aeruginosa* [28]. These collective outcomes underscore the potential of low-dose radiation to positively influence *E. coli* viability, thereby promoting increased colony formation. When exposed to stressors, Gram-negative bacteria trigger the expression of stress-related genes, which is governed by the RNA polymerase sigma factor (σ^s). This σ^s factor plays a key role in transcribing genes essential for bacterial replication and growth [29]. In addition, the σ^s factor provides protection to *E. coli* against diverse stress conditions and facilitates the activation of numerous genes essential for ensuring cell survival during the stationary phase [30].

Extensive research over the past two decades has increasingly supported the concept that low-dose ionizing radiation can induce hormetic effects on living organisms. These effects prominently include the activation of distinct metabolic pathways and physiological functions [31]. Notably, studies by Rozhko et al. [10] revealed that low-dose ionizing radiation can induce the production of ROS in bacterial cells. These ROS molecules function as important secondary messengers within diverse signal transduction pathways that are vital for cellular growth and proliferation [32,33], potentially influencing the development of an adaptive response. This influence stems from their involvement in the damage-sensing process, particularly following exposure to conditioning doses. In their study, Kim et al. [34] reported that low-dose ionizing radiation has the capacity to activate repair mechanisms. These mechanisms effectively mend the initial damage, providing protection to the organism against

subsequent stressors, whether they are radiation-related or arise from other forms of exposure. The observed increase in bacterial viability in *E. coli* following exposure to low doses of ionizing radiation suggests a potential enhancement in the cells' ability to cope with various forms of stress, including antibiotics.

Our study revealed a significant reduction in bacterial susceptibility to antibiotics, including marbofloxacin, AMC, ceftiofur, and cefoxitin, after exposure to low-dose ionizing radiation. This finding aligns with our earlier report, where changes in antibiotic susceptibility were noted in *Staphylococcus aureus* and *Salmonella enteritidis* after exposure to low-dose X-ray radiation [35]. Furthermore, recent research by Li et al. [36] demonstrated that insufficient doses of ultraviolet radiation lead to reduced antibiotic susceptibility in *Pseudomonas aeruginosa*. These results are attributed to the activation of an adaptive response. This concept could be behind the reduced susceptibility of *E. coli* to specific antibiotics, as observed in our study. The exposure to low-dose ionizing radiation can be viewed as an environmental stressor that has the potential to influence the expression of virulence genes in bacteria [37] and initiate complex molecular rearrangements at the cellular metabolic level, intricately influencing the modulation of bacterial reactions to antibiotics [38]. When exposed to an environmental stress, bacteria are equipped to engage a range of reparative mechanisms and induce the activity of DNA polymerases. This phenomenon has been associated with an expedited emergence of antibiotic resistance, as discussed by Lukačišinová et al. [39]. Moreover, Tahmasebi et al. [40] reported that the expression of the σ^s factor under stress conditions is linked to reduced susceptibility of bacteria to different antibiotics.

The specific effect of low-dose radiation on marbofloxacin resistance observed in *E. coli* is intriguing and merits further investigation. Interestingly, a similar effect was previously noted where low-dose X-ray radiation induced resistance to marbofloxacin in strains of *Staphylococcus aureus* and *Salmonella enteritidis* [35]. While the underlying mechanisms are not yet fully understood, it is reasonable to hypothesize that radiation-induced changes in cellular processes, such as modifications in membrane permeability or drug efflux pumps, could contribute to the increased resistance to this particular antibiotic [41,42]. Gram-negative bacteria, including *E. coli*, feature an outer membrane that contains numerous protein channels known as porins. These channels primarily facilitate the entry of antibiotics [43]. The changes observed in antibiotic susceptibility of *E. coli* after exposure to low-dose ionizing radiation could potentially stem from a reduction in the influx through these outer membrane porins. Moreover, low-dose radiation has the potential to induce mutations [44]. If these mutations affect genes associated with antibiotic uptake or efflux pumps, they could consequently influence susceptibility [45]. Additional investigations are required to clarify the possible role of membrane porins and efflux pumps in the modifications to bacterial susceptibility to antibiotics induced by low-dose ionizing radiation.

Resistance to marbofloxacin can occur via other mechanisms. This antibiotic belongs to the fluoroquinolone family, where resistance primarily arises through mutations in genes gyrA and parC. These genes encode the primary and secondary targets DNA gyrase and topoisomerase IV, respectively. Mutations in gyrA and parC lead to alterations in the target protein structure, resulting in changes to the drug-binding affinity of the enzyme [46]. In *E. coli*, DNA gyrase is the primary target for fluoroquinolones, although topoisomerase IV also becomes a target once gyrA is mutated [47]. Our findings suggest that X-rays induce genetic mutations in gyrA and parC, potentially contributing to the observed enhancement of resistance to marbofloxacin in *E. coli*. Further DNA sequencing analysis is warranted to confirm these findings.

5. Conclusions

The findings of this study corroborate the notion that low-dose ionizing radiation can elicit adaptive responses that could potentially enhance the viability of *E. coli* and influence its response to specific antibiotics. While this phenomenon opens up possibilities for beneficial effects, it also raises concerns about the potential risk of altered bacterial behavior, which could impact antibiotic effectiveness and contribute to the development of antibiotic resistance. Further research is needed to fully understand these implications and assess any associated risks.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Conflict of interest

The authors declare no conflicts of interest.

References

1. Freneau A, Dos Santos M, Voisin P, et al. (2018) Relation between DNA double-strand breaks and energy spectra of secondary electrons produced by different X-ray energies. *Inter J Radiat Biol* 94: 1075–1084. https://doi.org/10.1080/09553002.2018.1518612
2. Rappole CA, Mitra K, Wen H (2012) Dynamic fluorescence imaging of the free radical products of X-ray absorption in live cells. *Opt Nano* 1: 5. https://doi.org/10.1186/2192-2853-1-5
3. Chang RL, Stanley JA, Robinson MC, et al. (2020) Protein structure, amino acid composition and sequence determine proteome vulnerability to oxidation-induced damage. *EMBO J* 39: e104523. https://doi.org/10.15252/embj.2020104523
4. Courtade-Saïdi M (2007) The biological effects of very low-doses of ionizing radiation at the occupational exposure level. *Morphologie* 91: 166–172. https://doi.org/10.1016/j.morpho.2007.10.008
5. Liu F, Li N, Zhang Y (2023) The radioresistant and survival mechanisms of *deinococcus* radiodurans. *Radiat Med Prot* 4: 70–79. https://doi.org/10.1016/j.radmp.2023.03.001
6. Islam MT (2017) Radiation interactions with biological systems. *Int J Radiat Biol* 93: 487–493. https://doi.org/10.1080/09553002.2017.1286050
7. Calabrese EJ (2018) Hormesis: path and progression to significance. *Int J Mol Sci* 19: 2871. https://doi.org/10.3390/ijms19102871
8. Shibamoto Y, Nakamura H (2018) Overview of biological, epidemiological, and clinical evidence of radiation hormesis. *Int J Mol Sci* 19: 2387. https://doi.org/10.3390/ijms19082387
9. Lau YS, Chew MT, Alqahtani A, et al. (2021) Low dose ionising radiation-induced hormesis: therapeutic implications to human health. *Appl Sci* 11: 8909. https://doi.org/10.3390/app11198909
10. Rozhko TV, Nogovitsyna EI, Badun GA, et al. (2019) Reactive oxygen species and low-dose effects of tritium on bacterial cells. *J Environ Radioact* 208–209: 106035. https://doi.org/10.1016/j.jenvrad.2019.106035

11. Min J, Lee CW, Gu MB (2003) Gamma-radiation dose-rate effects on DNA damage and toxicity in bacterial cells. *Radiat Environ Biophys* 42: 189–192. https://doi.org/10.1007/s00411-003-0205-8
12. Doss M (2013) Linear no-threshold model vs. radiation hormesis. *Dose Response* 24: 480–497. https://doi.org/10.2203/dose-response.13-005.Doss
13. Broome EJ, Brown DL, Mitchel REJ (1999) Adaptation of human fibroblasts to radiation alters biases in DNA repair at the chromosomal level. *Int J Radiat Biol* 75: 681–690. https://doi.org/10.1080/095530099140014
14. Eken A, Aydin A, Erdem O, et al. (2012) Induced antioxidant activity in hospital staff occupationally exposed to ionizing radiation. *Int J Radiat Biol* 89: 648–653. https://doi.org/10.3109/09553002.2012.702295
15. Szumiel I (2012) Radiation hormesis: autophagy and other cellular mechanisms. *Int J Radiat Biol* 88: 619–628. https://doi.org/10.3109/09553002.2012.699698
16. Tubiana M, Feinendegen LE, Yang C, et al. (2009) The linear-non-threshold relationship is inconsistent with radiation biologic and experimental data. *Radiology* 251: 13–22. https://doi.org/10.1148/radiol.2511080671
17. Mullenders L, Atkinson M, Paretzke H, et al. (2009) Assessing cancer risks at low-dose radiation. *Nat Rev Cancer* 9: 596–604. https://doi.org/10.1038/nrc2677
18. Khan AUH, Blimkie M, Yang DS, et al. (2021) Effects of chronic low-dose internal radiation on immune-stimulatory responses in mice. *Int J Mol Sci* 7: 7303. https://doi.org/10.3390/ijms22147303
19. Rashid AM, Ramalingam L, Al-Ja wadi A, et al. (2018) Low dose radiation, inflammation, cancer and chemoprevention. *Int J Radiat Biol* 95: 506–515. https://10.1080/09553002.2018.1484194
20. Castillo H, Smith, GB (2017) Below-background ionizing radiation as an environmental cue for bacteria. *Front Microbiol* 8: 117. https://doi.org/10.3389/fmicb.2017.00177
21. Lampe N, Marin P, Coulon M, et al. (2019) Reducing the ionizing radiation background does not significantly affect the evolution of Escherichia coli populations over 500 generations. *Sci Rep* 17: 14891. https://doi.org/10.1038/s41598-019-51519-9
22. Lu YY, Fu Y, Chen LT, et al. (2023) Combined effect of simulated microgravity and low-dose ionizing radiation on structure and antibiotic resistance of a synthetic community model of bacteria isolated from spacecraft assembly room. *Life Sci Space Res* 38: 29–38. https://doi.org/10.1016/j.lssr.2023.04.005
23. Bauer AW, Kirby WMM, Sherris JC, et al. (1966) Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 45: 493–496. https://doi.org/10.1093/ajcp/45.4_ts.493
24. CA-SFM, Comité de l'Antibiogramme de la Société Française de Microbiologie, Recommandations vétérinaires, Paris, France, 2021. Available from: https://www.sfm-microbiologie.org/2021/12/10/casfm-veterinaire-2021/
25. Oskouee S, Feghhi SAH, Soleimani N (2020) Antibiotic susceptibility variations of Methicillin-resistant Staphylococcus aureus after gamma irradiation. *Intern J Radiat Biol* 96: 390–393. https://doi.org/10.1080/09553002.2020.1704301
26. Kolesnik OV, Rozhko TV, Kudryasheva NS (2023) Marine bacteria under low-intensity radioactive exposure: model experiments. *Int J Mol Sci* 24: 410. https://doi.org/10.3390/ijms24010410

27. Planel H, Soleilhavoup JP, Tixador R, et al. (1987) Influence on cell proliferation of background radiation or exposure to very low, chronic gamma radiation. *Health Phys* 52: 571–578. https://doi.org/10.1097/00004032-198705000-00007
28. Segatore B, Setacci D, Bennato F, et al. (2012) Evaluations of the effects of extremely low-frequency electromagnetic fields on growth and antibiotic susceptibility of *Escherichia coli* and *Pseudomonas* aeruginosa. *Int J Microbiol* 2012: 587293. https://doi.org/10.1155/2012/587293
29. Gottesman S (2019) Trouble is coming: signaling pathways that regulate general stress responses in bacteria. *J Biol Chem* 294: 11685–11700. https://doi.org/10.1074/jbc.REV119.005593
30. Kazmierczak MJ, Wiedmann M, Boor KJ (2005) Alternative sigma factors and their roles in bacterial virulence. *Microbiol Mol Biol Rev* 69: 527–543. https://doi.org/10.1128/mmbr.69.4.527-543.2005
31. Vaiserman A, Cuttler JM, Socol Y (2021) Low-dose ionizing radiation as a hormetin: experimental observations and therapeutic perspective for age-related disorders. *Biogerontology* 22: 145–164. https://doi.org/10.1007/s10522-020-09908-5
32. Milkovic L, Cipak Gasparovic A, Cindric M, et al. (2019) Short overview of ROS as cell function regulators and their implications in therapy concepts. *Cells* 8: 793. https://doi.org/10.3390/cells8080793
33. Huang MZ, Li JY (2020) Physiological regulation of reactive oxygen species in organisms based on their physicochemical properties. *Acta Physiol* 228: e13351. https://doi.org/10.1111/apha.13351
34. Kim BM, Hong Y, Lee S (2015) Therapeutic implications for overcoming radiation resistance in cancer therapy. *Int J Mol Sci* 16: 26880–26913. https://doi.org/10.3390/ijms161125991
35. Cherif J, Raddaoui A, Trabelsi M, et al. (2023) Diagnostic low-dose X-ray radiation induces fluoroquinolone resistance in pathogenic bacteria. *Int J Radiat Biol* 99: 1971–1977. https://doi.org/10.1080/09553002.2023.2232016
36. Li HB, Hou AM, Chen TJ, et al. (2021) Decreased antibiotic susceptibility in *Pseudomonas aeruginosa* surviving UV irradition. *Front Microbiol* 12: 604245. https://doi.org/10.3389/fmicb.2021.604245
37. Fang FC, Frawley ER, Tapscott T, et al. (2016) Bacterial stress responses during host infection. *Cell Host Microbe* 20: 133–143. https://doi.org/10.1016/j.chom.2016.07.009
38. Sheikh SW, Ali A, Ahsan A, et al. (2021) Insights into emergence of antibiotic resistance in acid-adapted enterohaemorrhagic *Escherichia coli*. *Antibiotics* 10: 522. https://doi.org/10.3390/antibiotics10050522
39. Lukačišinová M, Novak S, Paixão T (2017) Stress-induced mutagenesis: Stress diversity facilitates the persistence of mutator genes. *PLoS Comput Biol* 13: e1005609. https://doi.org/10.1371/journal.pcbi.1005609
40. Tahmasebi H, Dehbashi S, Arabestani MR (2021) Antibiotic resistance alters through iron-regulating sigma factors during the interaction of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Sci Rep* 11: 18509. https://doi.org/10.1038/s41598-021-98017-5
41. Maurya N, Jangra M, Tambat R, et al. (2019) Alliance of efflux pumps with *beta-Lactamases* in multidrug-resistant *Klebsiella pneumoniae* isolates. *Microb Drug Resist* 25: 1155–1163. https://doi.org/10.1089/mdr.2018.0414

42. Zhao L, Wang S, Li X, et al. (2020) Development of in vitro resistance to fluoroquinolones in Pseudomonas aeruginosa. *Antimicrob. Resist Infect Control* 9: 124. https://doi.org/10.1186/s13756-020-00793-8
43. Ghai I (2023) Barrier to entry: examining the bacterial outer membrane and antibiotic resistance. *Appl Sci* 13: 4238. https:// doi.org/10.3390/app13074238
44. Nagashima H, Shiraishi K, Ohkawa S, et al. (2018) Induction of somatic mutations by low-dose X-rays: the challenge in recognizing radiation-induced events. *J Radiat Res* 59: 11–17. https://doi.org/10.1093/jrr/rrx053
45. Davin-Regli A, Pages JM, Ferrand A (2021) Clinical status of efflux resistance mechanisms in gram-negative bacteria. *Antibiotics (Basel)* 10: 1117. https://doi.org/10.3390/antibiotics10091117
46. Li S, Li Z, Wei W, et al. (2015) Association of mutation patterns in GyrA and ParC genes with quinolone resistance levels in lactic acid bacteria. *J Antibiot* 68: 81–87. https://doi.org/10.1038/ja.2014.113
47. Redgrave LS, Sutton SB, Webber MA, et al. (2014) Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends Microbiol* 22: 438–445. https://doi.org/10.1016/j.tim.2014.04.007

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