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RESEARCH ARTICLE OPEN ACCESS

Antibiotic-resistant *Escherichia coli* Undergoes a Change in *mcr-1* and *qnr-S* Expression after being Exposed to Gamma Irradiation

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Abstract

Human consumption of antibiotics has increased their concentrations in many parts of the environment, including rivers, sediments, soil, and wastewater. Consequently, resistant bacteria originating from these environments are distributed to humans, resulting in illness. The aim of this study was to identify mobilized colistin-resistant (*mcr*) genes and quinolone-resistant (*qnr*) genes in *E. coli* strains obtained from clinical samples. Additionally, the study explored the impact of different radiation dosages on the expression of antibiotic-resistance genes. In this study, conducted in Beni-Suef, Egypt, samples from 430 community-acquired urinary tract infection (UTI) cases resulted in the isolation of 85 different strains of *E. coli*. Conventional microbiological procedures were employed to identify these bacterial isolates. Three bacterial isolates with resistance to both quinolones and colistin underwent examination for their corresponding genetic determinants, which subsequently proved the presence of their respective genes. Furthermore, the expression levels of the *mcr-1* and *qnr-S* genes were assessed using real-time PCR after exposure to gamma irradiation. Remarkably, the use of a sublethal dosage of 3 kGy gamma irradiation treatment on bacterial cells increased their susceptibility to colistin and quinolones post-irradiation. Additionally, there was a notable reduction in the expression levels of both *mcr-1* and *qnr-S* genes, which could be helpful for preventing the storage of antibiotic-resistant *E. coli* in the environment.

Keywords: *mcr-1* Gene, *qnr-S* Gene, Antibiotic-resistant *E. coli,* Gamma Irradiation

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INTRODUCTION

The utilization of antibiotics by humans has resulted in an increase in antibiotic concentrations in waterways, sediments, soil, and sewage.[1] Antibiotics pose a risk of entering the environment through multiple pathways, including the improper disposal of unused or expired antibiotics and the release of animal and human waste. Because of their limited absorption in the digestive systems of humans and animals, a substantial portion of antibiotics is excreted in feces and urine.[2,3] The discharge of antibiotics during industrial antibiotic manufacturing often reaches concentrations of up to 1 mg/l, significantly higher than the amounts observed in human or animal waste.[4]

The persistence of antibiotic-resistant bacteria in the environment is attributed to the genetic variability of antibiotic-resistance genes.[5] Horizontal gene transfer (HGT) plays a pivotal role in disseminating these resistant genes among bacteria. These processes involve the movement or transmission of genes responsible for antibiotic resistance from a pathogenic bacterium to other bacteria. Consequently, the proliferation of these resistance genes is driven by environmental selection.[6] HGT can be facilitated by three distinct processes: conjugation, transduction, and transformation. Among these mechanisms, conjugation stands out as the primary means by which bacteria exchange genes for antibiotic resistance. During conjugation, genetic material, specifically DNA, is transferred among a broad range of bacterial species.[7]

The development of antibiotic resistance in environments is a complicated process because although antibiotic compounds might be present in nature, it is crucial to understand their contribution to antibiotic resistance.[8]

An intriguing aspect is the existence of numerous plasmid-mediated mobile colistin resistance (*mcr*) genes. According to Sun *et al.*,[9] unknown selective pressures were responsible for the continuous evolution of the *mcr* gene, leading to the emergence of various *mcr* variants. Remarkably, within a relatively short timeframe, the *mcr* genes exhibited numerous variations. For instance, *mcr-1* has thirteen variants, each differing from *mcr-1* by one amino acid (*mcr-1.1* to *mcr-1.13*).[10]

Moreover, recent discoveries indicate that mobile genetic components, including plasmids, can serve as mediators for quinolone resistance. Plasmid-encoded genes (*qnr*) mediate quinolone resistance and belong to the pentapeptide repeat proteins. These proteins act as a protective shield for DNA gyrase and topoisomerase IV against quinolone drugs.[11] There are three primary categories of *qnr* elements: *qnr-A*, *qnr-B*, and *qnr-S.*[12,13]

Gamma irradiation is a form of electromagnetic radiation characterized by a low wavelength produced by radioactive isotopes as the unsteady nucleus disintegrates and decays into a stable form.[14] Gamma rays can damage DNA either directly or by inducing ionization of water molecules. Within solutions, ionizing radiation produces three distinct free radicals: hydroxyl radicals (•OH), solvated electrons (e-eq), and hydrogen atoms (•H). This phenomenon occurs through the mechanism of water radiolysis.[15] These free radicals can inflict damage based on reactive oxygen species effects on proteins.[16] Additionally, they contribute to the occurrence of DNA double-strand breaks or single-strand breaks.[15]

To solve the issue of increasing the rise of *E. coli* antibiotic resistance genes, new strategies and approaches must be devised. Our study seeks to tackle this issue by exploring the impact of radiation at various dosages as a strategy to limit the dissemination of antibiotic resistance genes in the environment. This spread typically occurs through horizontal gene transfer (HGT),[6] and our investigation underscores the efficacy of modern applications of gamma rays in contemporary practices, such as the treatment of blood components and the sterilization of medical equipment.

MATERIALS AND METHODS

Samples collection

This study took place between January 2021 to January 2022. A total of 430 urine specimens were collected from specific laboratories, namely Alfa Dar-Elfoad Laboratory and Dar Elkhebra Laboratory, situated in Beni-Suef, Egypt. These specimens represented cases of community-acquired urinary tract infections.

Table 1. Oligonucleotide primers sequences

Gene	Primer sequence (5'-3')	Length of amplified product	Reference
pho-A	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	720 bp	20
mcr-1	CGGT CAGTCCGTTTGTTC CTTGGTCGGTCTGTAGGG	308 bp	21
mcr-2	TGGTACAGCCCCTTTATT GCTTGAGATTGGGTTATGA	1617 bp	22
qnr-A	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516 bp	23
qnr-B	GATCGTGAAAGCCAGAAAGG ACGATGCCTGGTAGTTGTCC	469 bp	23
qnr-S	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	417 bp	23

The urine samples were acquired through either catheterization or mid-stream clean-catch methods.

Isolation and purification of *E. coli*

Approximately 5 milliliters of urine samples underwent centrifugation at a speed of 2000 rpm for 5 minutes. Following centrifugation, a loopful of undiluted urine sediment was aseptically inoculated into suitable culture media, specifically Eosin Methylene Blue and MacConkey agar plates manufactured by Himedia, India. The incubation period lasted for 24 hours at 37°C.

In order to verify the purity of the isolated bacteria, well-isolated individual colonies were subjected to subculturing on the same growth medium. Identification of the isolates was carried out using microscopic and morphological approaches, involving the observation of macroscopic morphology and color on the appropriate agar media. The identification was further confirmed using the Vitek2 computerized system (Vitek 2 GN-card) from bioMerieux SA, France, following the manufacturer's instructions.[17]

Antimicrobial susceptibility testing

The susceptibility of *E. coli* bacterial isolates to seventeen commonly used antibiotics was evaluated using the disc diffusion method. The protocols outlined by the Clinical and Laboratory Standards Institute (CLSI)[18] were followed for this assessment. Antibiotic discs for this research were sourced from Oxoid, United Kingdom. They included colistin (CT) 10µg, piperacillin/tazobactam (TZP) 75/10µg, nitrofurantoin (F) 300µg, ciprofloxacin (CIP) 5µg, cefepime (FEP) 30µg, imipenem (IMP) 10µg, ceftazidime (CAZ) 30µg, levofloxacin (LEV) 5µg, norfloxacin (NOR) 10µg, ampicillin/sulbactam (SAM) 20µg, aztreonam (ATM) 30µg, chloramphenicol (C) 30µg, doxycycline (DOX) 30µg, amikacin (AK) 30µg, cefoperazone/sulbactam (SCF) 105µg, sulfamethoxazole/trimethoprim (SXT) 25µg and amoxicillin-clavulanic acid (AMC) 20/10µg. Fresh bacterial cultures were grown on Mueller-Hinton agar (MHA) plates obtained from Himedia, India. Subsequently, antibiotic discs were applied to the agar plates. After 24 hours of incubation at 37°C, the plates were examined, and the diameter of the inhibitory zone was measured and recorded in millimeters.

DNA extraction and detection of antibiotic resistance genes

Through polymerase chain reaction (PCR), we were able to identify the presence of genes encoding resistance to the antibiotics colistin (*mcr-1* and *mcr-2*) and quinolones (*qnr-A, qnr-B,* and *qnr-S*), following the protocol outlined by Liu *et al.*[19] The DNA extraction process employed the QIAamp DNA micro kit (Qiagen, USA), according to the manufacturer's instructions. The PCR Master Mix was prepared in accordance with the recommendations specified by the manufacturer of the Emerald Amp GT PCR master mix (Takara) (Code No. RR310A kit). The primers used in

Table 2. PCR conditions and annealing temperatures

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
pho-A	94°C	94°C	55°C	72°C	35	72°C
	5 min.	30 sec	40 sec	45 sec		10 min.
mcr-1	94°C	94°C	60°C	72°C	35	72°C
	5 min.	30 sec	30 sec	30 sec		7 min.
mcr-2	94°C	94°C	55°C	72°C	35	72°C
	5 min.	30 sec.	40 sec.	1.2 min.		12 min.
qnr-A	94°C	94°C	55°C	72°C	35	72°C
	5 min.	30 sec.	45 sec	45 sec		10 min.
qn-B	94°C	94°C	55°C	72°C	35	72°C
	5 min.	30 sec.	45 sec	45 sec		10 min.
qnr-S	94°C	94°C	55°C	72°C	35	72°C
	5 min.	30 sec.	45 sec	45 sec		10 min.

this investigation were sourced from Metabion, Germany, and detailed information on primers and conditions is provided in Tables 1 and 2.

Agarose gel electrophoreses

A sterile flask was prepared, containing 1.5 g of high-purity agarose suitable for electrophoresis. This flask was then combined with 100 ml of Tris-acetate-EDTA (TAE) buffer and subjected to microwave irradiation to ensure the complete dissolution of all the agarose granules. Following this, the mixture was allowed to cool to a temperature of 70°C, after which 0.5 mg/ml ethidium bromide was thoroughly blended.

The warmed agarose was promptly transferred into the gel casting device along with the required comb and allowed to cool to room temperature for polymerization. Subsequently, the comb was removed, and TAE buffer was added to the electrophoresis tank. A volume of 20µl was used for each individual PCR item, and both negative and positive controls were placed into the gel. The power source for the tank was set at a rate of 1-5 volts per centimeter of its length. After approximately 30 minutes, the running process was stopped, and the gel was transferred to the UV cabinet. The gel documentation system was used to capture images, and computer software was employed for the analysis and interpretation of the acquired data.[24]

Effect of gamma irradiation on bacterial cells

Ionizing radiation, such as gamma rays, has been employed to eradicate bacterial cells.[25] Gamma irradiation was carried out at the Egyptian Atomic Energy Research Institute in Cairo, Egypt, using a high-level cobalt (^{60}CO) source. The measured radioactivity of the source was estimated to be around 1.47 x 1017 Bq, equivalent to 397,949 Ci. The bacterial samples were cultured in Mueller Hinton broth medium for 24 hours in sterile test tubes. Subsequently, the samples were exposed to various gamma radiation doses: 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 kGy. The dose rate was 0.25 kGy/14.6 minutes during the experiment. Serial dilutions of the irradiated control and non-irradiated control cultures were plated on the surface of Mueller Hinton agar plates (5 µl each) and incubated for 24 hours at 37°C. The viable count was determined and compared.[26]

Determination of *mcr-1* and *qnr-S* genes expression by SYBR Green real-time PCR after gamma irradiation treatment

The evaluation of antibiotic-resistant gene expression in isolates that were synergistically affected by ciprofloxacin or colistin with ZnO-NPs and exposed to varying doses of gamma radiation was conducted. The SYBR Green quantitative PCR (qPCR) test for the identification of mcr-1 and qnr-S was performed using the Quantitate SYBR green PCR kit (Cat. No. 204141) and RevertAid Reverse Transcriptase (Thermo, Fisher) (200 U/L) for master mix preparation for SYBR Green real-time PCR. (No. Cat. EP0441). Detailed information on primers and conditions is provided in Tables 3 and 4. The strata gene MX3005P software was used to calculate Ct values and amplification curves.

Table 3. Oligonucleotide primers in the SYBR Green real-time PCR assay

Gene	Primer sequence (5'-3')	Reference
mcr-1	CGGT CAGTCCGTTTGTTC CTTGGTCGGTCTGTAGGG	21
qnr-S	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	23
16S rRNA	GCTGACGAGTGGCGGACGGG TAGGAGTCTGGACCGTGTCT	28

Table 4. SYBR green real-time PCR cycling conditions.

Target gene	Reverse transcription	Primary denaturation	Amplification (40 cycles)		
			Secondary denaturation	Annealing	Extension (Optics on)
mcr-1	50°C 30 min.	94°C 15 min.	94°C 15 sec.	60°C 30 sec.	
qnr-S				55°C 30 sec.	72°C 40 sec.
16S rRNA				55°C 30 sec.	

Following the computation of gene expression variance in RNA samples using the strata gene MX3005P software, the CT values of each sample were compared with those of the control group using the "Ct" methodology as outlined in the provided reference.[27]

Whereas ΔΔCt = ΔCt reference – ΔCt target

ΔCt target = Ct control – Ct treatment and
ΔCt reference = Ct control- Ct treatment

RESULTS AND DISCUSSION

Bacterial isolates and antibiotic susceptibility

In Beni-Suef, Egypt, 85 different strains of *E. coli* were isolated from 430 urine samples obtained from outpatients experiencing urinary tract symptoms. The identification of bacterial isolates utilized standard microbiological techniques and was further validated using the Vitek2 computerized system (Vitek 2 GN-card) from bioMerieux SA, France, following the manufacturer's instructions.

The investigation aimed to assess the susceptibility of *E. coli* isolates to seventeen commonly used antibiotics spanning different groups. The resistance rates of *E. coli* isolates to the tested drugs are detailed in Table 5.

The results overall indicated a high prevalence of multidrug resistance among the tested isolates. Specifically, the study revealed a significant proportion of *E. coli* isolates with elevated resistance levels to piperacillin/tazobactam (41.2%), sulfamethoxazole/trimethoprim (41.2%), amoxicillin-clavulanic acid (40%) and cefepime (40%). Furthermore, a similar study by Nader *et al.*[29] indicated that *E. coli* isolated from UTIs demonstrated high resistance to the most common antibiotic used in treating UTIs, with the highest resistance rates observed for trimethoprim/sulfamethoxazole (68.75%).

In contrast, the antibiotics with the lowest levels of resistance in the present study were colistin (3.5%), ciprofloxacin (5%), levofloxacin (5%), imipenem (5%), and nitrofurantoin (5%). In previous research, Mashouf *et al.*[30] observed that imipenem, cefepime, and ciprofloxacin

Table 5. Resistance rates of [85] *E. coli* isolate against [17] antimicrobial agents

No.	Antimicrobial agent	No. (%)
1.	Colistin (CT) 10 µg	3 (3.5%)
2.	Nitrofurantoin (F) 300 µg	3 (3.5%)
3.	Ciprofloxacin (CIP) 5 µg	5 (5.8%)
4.	Levofloxacin (LEV) 5 µg	5 (5.8%)
5.	Imipenem (IMP) 10 µg	5 (5.8%)
6.	Norfloxacin (NOR) 10 µg	10 (11.7%)
7.	Amikacin (AK) 30 µg	14 (16.4%)
8.	Ampicillin/Sulbactam (SAM) 20 µg	19 (22.9%)
9.	Ceftazidime (CAZ) 30 µg	21 (24.7%)
10.	Doxycycline (DOX) 30 µg	21 (24.7%)
11.	Chloramphenicol (C) 30 µg	22 (25.9%)
12.	Cefoperazone/Sulbactam (SCF) 105 µg	28 (32.9%)
13.	Aztreonam (ATM) 30 µg	31(36.4%)
14.	Amoxicillin-clavulanic acid (AMC) 20/10µg	34 (40%)
15.	Cefepime (FEP) 30 µg	34 (40%)
16.	Piperacillin/Tazobactam (TZP) 75/10 µg	35 (41.2%)
17.	Sulfamethoxazole/Trimethoprim (SXT) 25µg	35 (41.2%)

demonstrated the most significant antibacterial effects on all tested Gram-negative isolates. Additionally, Randrianirina *et al.*[31] noted a reduced susceptibility to trimethoprim/sulfamethoxazole and ciprofloxacin, and the strains exhibited minimal susceptibility to ceftriaxone, with a documented resistance rate of 53%.

Detection of antibiotic resistance genes

The findings from the polymerase chain reaction (PCR) study revealed that among the investigated *E. coli* isolates, three isolates (3.5%) were found to possess either a *qnr* or *mcr* gene. Notably, a single isolate, *E. coli* A2, was shown to harbor both the *mcr-1* and *qnr-S* genes, while none of the *E. coli* isolates exhibited the presence of *qnr-A*, *qnr-B*, or *mcr-2* genes, as shown in Figure 1.

In another investigation, four isolates of *E. coli* that displayed resistance to colistin based on their phenotype revealed that 3 (75%) contained the chromosomal *mcr-1* genes.[32]

Li *et al.*[33] reported that out of 9 (7.3%) *E. coli* isolates resistant to colistin, PCR screening revealed that 7 (5.7%) harbored the *mcr-1* gene. In another study, Rezazadeh *et al.*[34] reported that *qnr-S1* was present in 4 (2.9%) of the 136 isolates of *E. coli* resistant to quinolones. The clinical isolates in this investigation lacked the *qnr-A* and *qnr-B* genes. Foda *et al.*[35] isolated *E. coli* from clinical samples resistant to colistin with MICs ranging from 6.25 to >200µg/ml, and these strains contained the *mcr-1* gene.

Effect of gamma irradiation on bacterial cells

Radiation is commonly employed in the processing of blood components, sterilization of medical equipment, preservation of food, and preparation of tissue allografts. This use of radiation eliminates the need for elevated temperatures that might pose potential harm to these items.[36] Additionally, it plays a role in sterilizing water

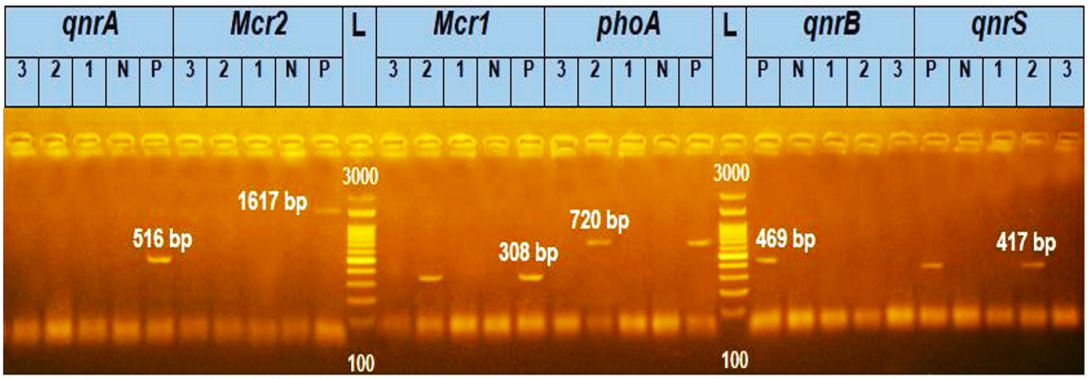


Figure 1. The Agarose gel electrophoresis image showed that *E. coli* strain A2 has *mcr-1* (308 bp) and *qnr-S* (417 bp) genes together. (Notes: (1,2,3) are samples numbers, (N) is a negative control and (P) is a positive control.)

and managing waste in clinical environments. Various factors influence bacterial sensitivity to radiation-induced death, including the rate of cell replication, intracellular water content, DNA content, medium composition, temperature, pH, oxygenation level, and the capacity to repair radiation-induced DNA damage.[25,37-38]

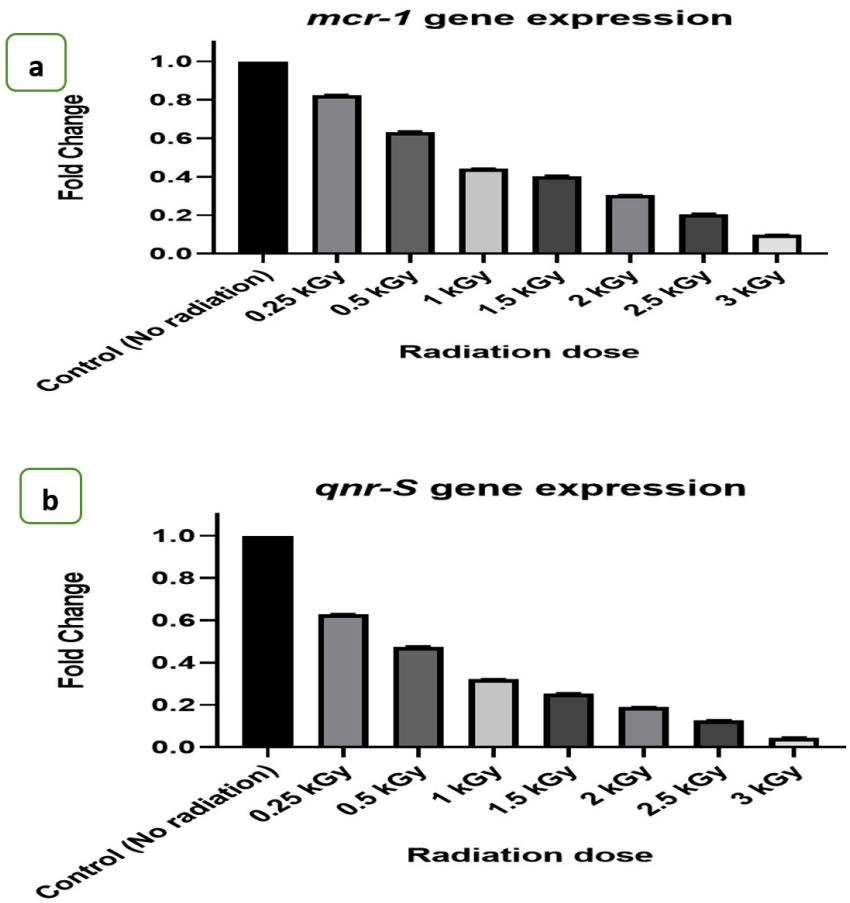
The findings of our investigation indicate that bacterial cells displayed sensitivity to a dose of 3 kGy, which could be classified as either a sensitive or a sublethal dose. Notably, they became susceptible to colistin and quinolones after irradiation.

These findings align with a previous study documenting the impact of gamma radiation at 2.8 kGy and 3.6 kGy on the viability of stationary phase cells of *S. epidermidis* and *E. coli*, respectively.[39] Another study revealed that gamma radiation affected *E. coli* resistance to some antibiotics at doses of 2, 3, and 4 Gy, without inhibiting it. However, following exposure to 5 Gy, a small zone of inhibition was observed.[40]

Furthermore, our results are consistent with those of Aziz *et al.*,[41] where a dose of 3 KGy ultimately reduced the viable count of Gram-positive short-rod bacteria isolates.

Determination of *mcr-1* and *qnr-S* genes expression after gamma irradiation treatment by SYBR Green real-time PCR

The gene expression levels of *mcr-1* and *qnr-S* genes were quantitatively measured using the sensitive technique of quantitative RT-PCR following gamma irradiation treatment.



Figures 2. a) The effect of radiation doses on fold changes of *mcr-1* genes expression, b) The effect of radiation doses on fold changes of *qnr-S* genes expression

Transcriptional alterations in these genes were determined from the fold change, as illustrated in Figures 2a and b.

Our study's results concerning the clinical isolate *E. coli* A2 indicate that exposure to gamma irradiation led to a decrease in the expression levels of the *mcr-1* and *qnr-S* genes. These genes are known contributors to the antibiotic-resistant properties of bacteria, particularly against colistin and quinolones antibiotics. Consequently, the susceptibility of the bacteria to these antibiotics would be enhanced.

Another study that examined the effects of radiation on *E. coli* reported a radioprotective system against the initial instance of DNA harm or damage and to promote long-term survivability, the *dna-K* gene was strongly activated in response to gamma radiation.[42] and in contrast to the findings of research, this is in agreement with Yamaguchi *et al.*[43] who showed that mutations in the *dna-K* genes increased the susceptibility of the bacteria to levofloxacin and that the *dna-K* genes affect the antibacterial action of the fluoroquinolone in *E. coli*. Another study found that a mutation in the *dna-K* gene improved the susceptibility of methicillin-resistant *Staphylococcus aureus* to oxacillin and methicillin.[44]

An additional study investigating the impacts of gamma radiation on uropathogenic *E. coli* expressing *colibactin* and *Cnf-1*, aimed at investigating their impact on cytotoxicity, revealed a significant rise in the expression of *clbA* and *clbQ* genes in cultures subjected to irradiation at doses resembling these received during pelvic radiotherapy.[45]

An extra study was conducted to examine the impact of radiation on the treatment of abdominal tumors through radiotherapy. The findings of this study indicated alterations in the microbiota composition, resulting in a reduction in diversity. Specifically, there was a decrease in the presence of *Lactobacillus spp.* and *Bifidobacterium spp.*, while *Staphylococcus spp.* and *E. coli* exhibited an increase. The increased number of Pathogenic *E. coli* results in the repositioning of *claudin-1*, *occludin*, and *ZO-1* within tight junctions. This condition exacerbates radiation enteritis and enhances the expression of inflammatory factors.[46]

CONCLUSION

According to the results obtained from our investigation, it was observed that gamma irradiation had a beneficial effect on the antibiotic resistance of *E. coli*. This was evidenced by a reduction in the expression levels of both *mcr-1* and *qnr-S* genes, leading to an increased susceptibility to colistin and quinolones antibiotics. Consequently, gamma irradiation shows promise in limiting the spread of antibiotic resistance genes within the environment, which occurs through horizontal gene transfer (HGT). This proves the effectiveness of modern applications of gamma rays in contemporary practices, such as the treatment of blood components and sterilization of medical equipment.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

GMES conceptualized and supervised the study. AGM collected resources and analyzed the data. AOEG, MSA and HMEK applied methodology. AFA and AGM performed the experiments. GMES performed formal analysis. AOEG performed investigation. HMEK performed visualization. MSA and HMEK data curation. AGM wrote the manuscript. AGM, AOEG and GMES reviewed the manuscript. AGM and GMES edited the manuscript. All authors read and approved the final manuscript for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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