X-ray resistance in multidrug-resistant Gram-negative nosocomial pathogens: An *in vitro* assessment of bacterial survival across radiation dose thresholds

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ABSTRACT

Background: X-rays used for diagnostic and therapeutic purposes have various effects on nosocomial pathogens. Thanks to advanced repair mechanisms, bacteria, among the earliest life forms on Earth, can survive exposure to ionizing radiation. It is important to determine the radiation threshold for nosocomial pathogens with drug resistance. In this study, we investigated the survival responses of Gram-negative bacteria, which exhibit antimicrobial resistance in the clinical setting, to different doses of ionizing radiation. **Materials and Methods:** Bacterial isolates obtained from pure culture and adjusted to 0.5 McFarland turbidity were exposed to radiation doses of 0 (Control), 12.5, 25, 50, and 100 Gy in an X-ray machine used in clinical treatment. The results obtained were evaluated by comparing the number of microorganisms that remained alive after exposure to X-ray radiation with the control group. **Results:** No significant reduction in bacterial growth was observed following exposure to 12.5 Gy and 25 Gy. At 50 Gy, 3 isolates exhibited reduced viability. Exposure to a dose of 100 Gy induced a substantial reduction in Colony-Forming Unit (CFU) counts in 14 isolates and caused complete lethality in P. mendocina. These effects followed a dose-dependent trend. **Conclusions:** MDR Gram-negative pathogens are resilient against radiation doses typically applied in clinical practice. Bactericidal effects were only observed at doses ≥50 Gy, levels far exceeding what is biologically tolerable for human tissues. These findings underscore the need for precise dose classification when discussing bacterial radiation responses and emphasize that such high doses are suitable only for external sterilization, not patient-based applications.

INTRODUCTION

The increasing prevalence of antibiotic resistance among pathogenic microorganisms represents a significant and escalating global public health challenge. Multidrug-resistant (MDR) bacterial infections, particularly those originating in nosocomial environments, complicate clinical management and therapeutic outcomes. Gram-negative pathogens, including *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*, are of particular concern due to their inherent ability to resist multiple antibiotic classes [1, 2].

Beyond antimicrobial resistance, bacterial resistance to physical stresses such as ionizing radiation poses additional complications for infection control strategies. Exposure of bacterial pathogens to X-ray radiation may act as an environmental stressor, prompting the bacterium to increase mutational events, potentially leading to enhanced antibiotic resistance and alterations in its genotypic profile. Notably, exposure to ionizing radiation has been suggested to influence bacterial adaptation processes [3].

While the overuse and misuse of antibiotics remain primary drivers of antimicrobial resistance, non-lethal radiation exposure has also been implicated in promoting bacterial adaptation and resistance development. Sub-lethal doses of ionizing radiation can induce mutagenic events, facilitating bacterial survival under adverse conditions [4, 5].

Extensively used for diagnostic and therapeutic purposes, X-rays exert dose-dependent lethal or mutagenic effects on microbial populations. Radiation-induced DNA damage can be fatal or generate genotypic variations that enhance bacterial adaptability [3]. Therefore, understanding the impact of low-dose radiation exposures on clinically relevant bacterial isolates is crucial.

Although the survival strategies of radiation-resistant extremophiles have been well studied, there remains limited information regarding the radiation tolerance of clinically isolated MDR bacteria [6]. The ability of nosocomial pathogens to withstand ionizing radiation exposure may facilitate the persistence and transmission of drug-resistant infections, particularly

in oncology patients, for whom infections remain a significant cause of mortality.

This study aimed to assess the survival responses of MDR Gram-negative bacteria isolated from clinical specimens to varying doses of X-ray radiation. Specifically, the goal was to determine the threshold radiation doses capable of inactivating these pathogens and to evaluate the implications for clinical sterilization practices.

MATERIALS AND METHODS

Clinical ısolation and characterization of multidrug-resistant gram-negative bacteria

Twenty-three MDR Gram-negative bacterial isolates, purified and identified from clinical specimens at the Medical Microbiology Laboratory of Atatürk University, were included in this study. Among these isolates, 21 were obtained from blood samples, one from a wound specimen, and one from a tracheal aspirate. Species identification and antimicrobial susceptibility testing were performed using the VITEK 2 automated system (BioMérieux, France). The isolates comprised both enteric and non-fermentative Gram-negative bacilli.

The bacterial species tested are listed in table 1, and their antimicrobial resistance profiles are presented in figure 1. Isolates were stored at −80 °C in Tryptic Soy Broth (TSB) medium supplemented with 20% glycerol until further use. Before the experiments, the isolates were subcultured onto Sheep Blood Agar (Oxoid, UK) and incubated under aerobic conditions at 37 °C for 18–24 hours.

Following incubation, colonies were suspended in 10 mL of phosphate-buffered saline (PBS) and adjusted to a turbidity equivalent to a 0.5 McFarland standard using a densitometer (DensiCHEK Plus; BioMérieux, France). A volume of 1.5 mL from each bacterial suspension was transferred into sterile plastic tubes (five tubes per isolate) for subsequent X-ray resistance testing.
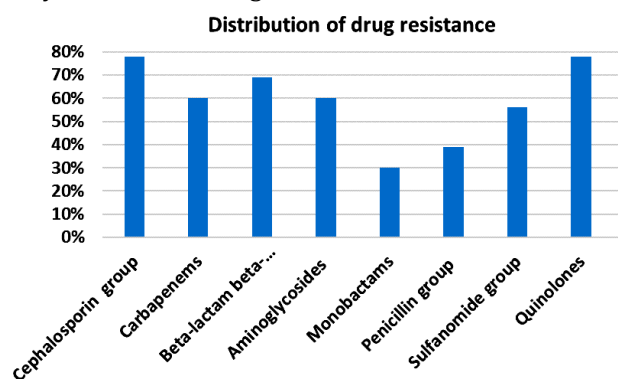

Figure 1. Distribution of antimicrobial resistance among multidrug-resistant Gram-negative clinical isolates.

Radiation exposure

Irradiation was performed at the Department of Radiation Oncology using an Elekta Synergy linear accelerator (Elekta AB, Stockholm, Sweden). The bacterial suspensions were exposed to single-dose fraction doses of 12.5, 25, 50, and 100 Gy. The linear accelerator was calibrated to deliver 1 Gy per 1 Monitor Unit (MU). A control group (0 Gy) was handled identically, with no irradiation applied.

Before irradiation, all samples were placed in sterile plastic tubes, and their positioning within the irradiation field was standardized to ensure uniform exposure. The tubes were positioned at the maximum dose point, with laser alignment and field setup carefully adjusted to achieve homogeneous dose distribution across all samples. The source-to-surface distance (SSD) was maintained at 100 cm for all groups. Calibration was conducted to deliver 1 Gy per 1 MU, and the laser was adjusted accordingly.

During the entire experiment, both irradiated (test) and non-irradiated (control) samples were maintained under identical environmental conditions to ensure that any observed differences in bacterial survival could be attributed solely to X-ray exposure. Following irradiation, all samples were transported to the microbiology laboratory for viable cell count under sterile conditions [7-9].

Determination of viable cell counts

The viable cell count of bacterial cultures was determined using a standardized protocol. The quantification of viable bacteria after irradiation was performed through the culturing method. To perform viable cell counts of irradiated and control group bacteria, the samples were brought to the microbiology laboratory without waiting and diluted in a sterile saline solution. After 24 hours of incubation at 37 °C, 0.1 mL of the appropriate dilution (10^{-4}) was then spread evenly over the surface of the TSA medium. The viable cell count was determined by considering the number of colonies counted, the plated volume, and the dilution factor. The results were compared with the control groups, and the percentage of viable cells was calculated [9, 10].

Statistical analysis

Data were analysed using SPSS software (version 19) and nonparametric statistical Kruskal-Wallis test. Statistical significance was considered at $p<0.05$.

RESULTS

The viable bacterial cells after the applied doses were compared with those in the control group. Presented in Table 1 which shows the dose-dependent viable cell counts (CFU/ml) for each bacterial species.

Bacterial growth response to radiation
12.5 Gy and 25 Gy (Subclinical Range)

All isolates showed full colony growth on the

culture medium, similar to the control group. No measurable reduction in CFU was observed compared to the controls ($\sim 1.5 \times 10^8$ CFU/mL).

50 Gy (Threshold Level)

Slight reductions in viability were observed in three isolates, while the remaining 20 strains exhibited growth patterns indistinguishable from those in the lower-dose groups.

100 Gy (High Lethal Range)

A significant decline in CFU counts was recorded in 14 isolates. Pseudomonas mendocina showed complete lethality, with no detectable viable cells.

Table 1. Bacterial species and dose-dependent viable cell count (CFU/ml).

Bacteria Type	1st Dose (12.5/Gy)	2nd Dose (25/Gy)	3rd Dose (50/Gy)	4th Dose (100/Gy)	Control (0/Gy)
Acinetobacter baumannii	Growth(+)	Growth(+)	Growth(+)	5.2×10^4	Growth(+)
Klebsiella pneumoniae	Growth(+)	Growth(+)	Growth(+)	Growth(+)	Growth(+)
Klebsiella pneumoniae	Growth(+)	Growth(+)	Growth(+)	6.4×10^4	Growth(+)
Escherichia coli	Growth(+)	Growth(+)	Growth(+)	Growth(+)	Growth(+)
Klebsiella pneumoniae	Growth(+)	Growth(+)	Growth(+)	Growth(+)	Growth(+)
Proteus vulgaris	Growth(+)	Growth(+)	Growth(+)	Growth(+)	Growth(+)
Pseudomonas aeruginosa	Growth(+)	Growth(+)	Growth(+)	5.8×10^4	Growth(+)
Enterobacter aerogenes	Growth(+)	Growth(+)	Growth(+)	Growth(+)	Growth(+)
Escherichia coli	Growth(+)	Growth(+)	Growth(+)	5.3×10^4	Growth(+)
Escherichia coli	Growth(+)	Growth(+)	Growth(+)	1.1×10^3	Growth(+)
Acinetobacter baumannii	Growth(+)	Growth(+)	Growth(+)	5.6×10^4	Growth(+)
Klebsiella pneumoniae	Growth(+)	Growth(+)	Growth(+)	5.2×10^4	Growth(+)
Escherichia coli	Growth(+)	Growth(+)	Growth(+)	5.1×10^4	Growth(+)
Klebsiella pneumoniae	Growth(+)	Growth(+)	Growth(+)	6.2×10^4	Growth(+)
Sphingomonas paucimobilis	Growth(+)	Growth(+)	Growth(+)	5.5×10^4	Growth(+)
Proteus mirabilis	Growth(+)	Growth(+)	Growth(+)	Growth(+)	Growth(+)
Acinetobacter baumannii	Growth(+)	Growth(+)	Growth(+)	Growth(+)	Growth(+)
Klebsiella pneumoniae	Growth(+)	Growth(+)	8.5×10^4	5.4×10^4	Growth(+)
Acinetobacter baumannii	Growth(+)	Growth(+)	8.7×10^4	4.8×10^4	Growth(+)
Klebsiella pneumoniae	Growth(+)	Growth(+)	Growth(+)	Growth(+)	Growth(+)
Klebsiella pneumoniae	Growth(+)	Growth(+)	Growth(+)	7.2×10^4	Growth(+)
Klebsiella pneumoniae	Growth(+)	Growth(+)	Growth(+)	8.8×10^4	5.3×10^4	Growth(+)
Pseudomonas mendocina	Growth(+)	Growth(+)	Growth(+)*	No growth**	Growth(+)

*After irradiation, there was the same growth as the control group ($\sim$1,5x108). **No growth after irradiation.

These data demonstrate a clear dose-dependent response. While sub-therapeutic and moderate doses had a negligible effect, high-dose exposure resulted in partial to complete bacterial inactivation in several strains. The data revealed a clear pattern of resistance and sensitivity among bacterial populations as the radiation dose increased. This demonstrates the proportional effect of each dose on the total viable bacterial population (figure 2). A graph showing the number of viable cells (CFU/mL) by radiation dose, including the control group, was created (figure 3). This graph indicates the radiation threshold below which the tested bacteria cannot survive. The obtained values highlight the critical dose-dependent relationship between X-ray radiation and bacterial viability, as well as the potential mechanisms for bacterial DNA damage repair and the limits of bacterial resistance to ionizing radiation.
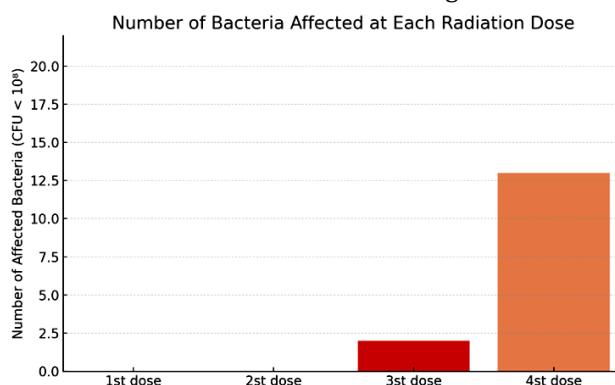

Figure 2. Dose-dependent total live cell count (CFU/ml). First to 4th dose 12.5, 25, 50, and 100 Gy respectively.
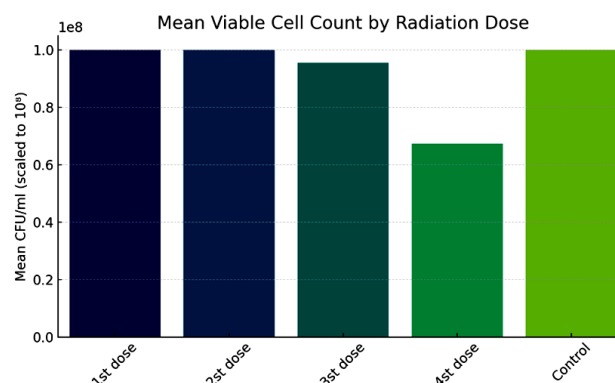

Figure 3. Relationship between increasing radiation dose and number of viable cells. First to 4th dose 12.5, 25, 50, and 100 Gy respectively.

Comparing the effect of the 4th dose (100 Gy) on the total number of viable cells (CFU/mL) with the control group, as shown in Figure 2, a significant decrease in bacterial viability is clearly observed. This indicates a marked reduction in the number of viable cells compared to the control group, which did not receive any dose. This graph effectively emphasizes the bactericidal efficacy of the 100 Gy dose of radiation exposure.

As shown in figure 3, bacterial viability changes with different radiation doses. There is a noticeable decrease in the number of viable cells as the dose increases, especially at 100 Gy. This emphasizes the significant impact of higher radiation doses on bacterial survival.

Statistical significance was considered at $p < 0.05$, indicating significant differences between the irradiated (50 Gy, 100 Gy) and non-irradiated (control) samples. This p-value is well below the commonly accepted significance threshold of 0.05, suggesting that radiation doses significantly affect bacterial viability. The results confirm the bactericidal effect of ionizing radiation, particularly at doses ≥50 Gy, as illustrated in figure 2.

DISCUSSION

As a result of clinical X-ray exposure, bacteria can survive radiation without sustaining damage, survive with DNA damage and genomic instability due to radiation-induced, or die with the applied radiation dose [3].

The mechanism of microbial inactivation by ionizing radiation results from direct or indirect damage to nucleic acids. Direct damage involves a direct collision between radiation energy and DNA, while indirect damage is observed when radiation ionizes water molecules and creates free transient radicals that react with the genetic material. A single strand of DNA can break, or energy and electrons can break a double strand of DNA. Single-strand breaks may not be fatal, but if their number exceeds the bacterium's ability to repair them, they cause the death of the cell. Double-strand breaks are lethal because they are beyond the ability of biological systems to repair. DNA lesions accumulating above the threshold level also lead to cell death [7, 11].

Research reports that radiation tolerance in biodiversity has increased over the last 25 years [12]. Ionizing radiation, if not at lethal doses, causes mutations in bacterial DNA. Studies showed that X-rays, which are considered an environmental stress for bacteria, trigger mutagenesis and accelerate the development of microbial pathogenesis and antibiotic resistance. The development of antimicrobial resistance increased in pathogens that survived exposure to non-lethal doses of X-rays [4, 13].

While extensively studied in extremophiles such as *D. radiodurans*, the radiation tolerance of clinical bacterial isolates remains insufficiently characterized [8, 14]. Given the widespread use of ionizing radiation in medical diagnostics and therapy, understanding whether nosocomial pathogens can survive incidental or direct exposure is essential.

Literature data show that prokaryotic cells have more radiation resistance than eukaryotic cells [15]. In humans, exposure to ionizing radiation above certain levels (e.g., 1 Gy) has been shown to cause adverse biological effects by damaging critical biomolecules such as DNA and proteins [16, 17]. The X-ray dose given to kill bacteria is much higher than the dose given to the patients for diagnosis and treatment, and the limit dose that reduces the number of bacteria in our study has been identified to be 50 Gy. Our findings indicate that clinically relevant doses typically under 10 Gy for diagnostic procedures and up to ~70 Gy total in fractionated radiotherapy are insufficient to exert a bactericidal effect on all tested pathogens. The observed resilience of these isolates suggests that radiation-based microbial control, if pursued, must exceed 100 Gy to ensure consistent inactivation. Such doses, however, are not applicable *in vivo*, as they far surpass the biological limits of human tissue [17, 18].

When the radiation resistance of the pathogens tested in our study was evaluated, of the bacteria tested, a total of 8 isolates, 3 of which were *K. pneumoniae*, grew at the same intensity as the control group at radiation doses of 50 and 100 Gy. Researchers have reported that K. pneumoniae shows moderate resistance to ionizing radiation, and doses of 1.5 kGy are required for undetectable [19].

Another aspect of low-dose radiation exposure in bacteria is the so-called radiation hormesis, in which sublethal radiation levels can trigger cellular stress responses and increase the bacteria's potential to survive and develop resistance to antibiotics. in medical settings, bacteria can encounter low doses of radiation during diagnostic imaging studies, such as X-rays used for medical imaging. For example, Cherif et al. reported in their study with S. aureus and S. enteritidis bacteria that exposure to low doses of X-ray radiation had the opposite effect on the bacteria, increasing the number of viable bacterial colonies and altering the antimicrobial resistance profile of the bacteria. The researchers interpreted this as radiation hormesis [10]. It has also been reported that short-term exposure to non-ionizing diagnostic ultrasonic waves alters antibiotic action profiles, making them resistant [20, 21]. Revealing the mechanisms by which non-lethal doses of radiation for bacteria can trigger the development of resistance is important for reevaluating hospital infection control policies and diagnostic imaging protocols.

Members of the genus *Pseudomonas* are frequently isolated in environments contaminated with radionuclides [22]. While one *P. mendocina* species in our study maintained its viability steadily at a dose of 50 Gy, a lethal effect was suddenly observed in all cells at a dose of 100 Gy, and no viable bacterial cells survived. In the study by Ezzat *et al*., the lethal dose of gamma irradiation for drug-resistant Pseudomonas aeruginosa isolates was reported to be 3 kGy. [23]. In contrast, our findings demonstrated a significant decline in viable cell count in P. aeruginosa following exposure to a considerably lower dose of 100 Gy. Moreover, the same dose resulted in complete cell death in P. mendocina isolates. This pronounced difference in radiation response suggests that P. mendocina may possess a greater intrinsic sensitivity to ionizing radiation compared to *P. aeruginosa*. Notably, this effect was observed exclusively in P.

mendocina, indicating potential interspecies variations in biological processes such as DNA repair pathways or oxidative stress response mechanisms.

The dose-response relationship obtained in the findings of our study has important consequences for the fields of microbiology and radiobiology. It provides insights into the potential of radiation to be used as a bactericidal tool in sterilization processes and the need to optimize radiation doses to achieve the desired results without developing resistance. In our study, a radiation dose of 100 Gy was determined to be the highest lethal dose only for *P. mendocina* among the bacteria tested, and no vital cells remained in this dose range.

Studies in the literature mostly focus on killing microorganisms, inactivation, or sterilization [24]. A WHO report found that irradiating foodstuffs with ionizing radiation up to 10 kGy increased microbiological safety without increasing toxicity [25]. In our study, we planned to investigate bacterial activity against X-ray dose values (Gy) commonly used in medical applications and therefore tested lower limits.

Similar to our study, Fırat *et al.* [24] tested the effect of routine diagnostic doses released from X-ray and computed tomography *in vitro* against common bacteria in the human microbiota. They reported that the X-ray doses released from computed tomography reduced the growth efficiency of living microbiota members, while the dose released from X-rays did not have such an effect, except on *E. coli* strains. The resistance observed in bacterial populations at low doses suggests the existence of effective DNA repair mechanisms or other adaptive responses that enable survival under sublethal stress conditions. Further studies on the radiation resistance of MDR isolates may encourage more in-depth research into the molecular mechanisms underlying bacterial resistance to ionizing radiation, thereby contributing to the development of strategies aimed at more effectively combating bacterial pathogens.

Clinical relevance and limitations

It is essential to emphasize that the effective bactericidal dose observed in this study (≥100 Gy) is not compatible with human application. Doses beyond 10 Gy are already associated with serious clinical risks, including radiation sickness and organ damage. Therefore, the application of such doses must be limited to *in vitro* sterilization methods (e.g., medical equipment, pharmaceuticals, or food irradiation). Additionally, the study is limited by its *in vitro* design and lack of molecular analysis of resistance pathways. Further studies should explore microbial responses at the molecular level and assess possible synergistic effects with antibiotics or chemical sterilants.

CONCLUSION

In conclusion, this study highlights the complex relationship between bacterial survival and the bactericidal effects of X-ray radiation. It provides a clear dose-dependent model, which is of significant practical and theoretical importance for microbiology and radiobiology.

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