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Survival Rate Evaluation of Escherichia coli Exposed to Low-Frequency Electromagnetic Field

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ABSTRACT

This study aimed to investigate the effects of low-frequency (LF) electromagnetic field (EMF) radiation on the survival rate of *E. coli*. The viability of these bacteria was assessed before and post-exposure to determine growth rates through viable count techniques, which measure the reduction in count forming units (CFU) numbers, indicating the number of bacteria surviving the treatments. The exposure apparatus produced consistent, time-varying magnetic fields between 5 and 500 Hz and 1 and 3 mT, which exposed the bacteria to radiation for 90 minutes.

All irradiated *E. coli* bacterial samples exhibited decreased growth rates compared to the control sample. The exposure to 100 Hz or above was more effective in reducing bacterial viability than those less than 100 Hz, with the lowest CFU value observed at 300 Hz and 2 mT with a maximum percentage change of (71.3%). The lowest percentage change was observed after exposure to 5 Hz at 2 mT equal to 15.2%. Moreover, non-linear fluctuating behavior was observed demonstrating that bacterial samples were highly responsive to radiation when exposed to both magnetic flux density and low electromagnetic field.

Our study demonstrated that certain LF-EMF parameters profoundly impact the growth rate of *E. coli*. These findings suggest that optimized LF EMF parameters could potentially affect microbial infections, reduce inflammation, and accelerate wound healing.

Keywords: Colony-forming unit, *Escherichia coli*, Low-frequency electromagnetic field.

1. INTRODUCTION

The exploration of electromagnetic fields (EMFs) and their impact on biological systems has shown significant scientific interest over recent decades. These fields are widely present in both natural and man-made environments, from the Earth's geomagnetic activity to electrical appliances and power lines. Recently, using very low-frequency electromagnetic (VLF-EM) radiation as an alternative treatment for many diseases is of great interest [1].

Low-frequency electromagnetic (LF-EM) radiation represents a particular subset of EMFs, characterized by extremely- Low frequencies (ELF) typically ranging from 3 Hz to 30 Hz, super-low frequencies ranging from 30 Hz to 300 Hz, and ultra-low frequencies (ULF) from 300 Hz to 3 KHz. ELF-EMF radiation has been shown to speed up the healing process. EMF influences various cellular -LF epithelialization. They also -processes involved in tissue repair, including stem cell proliferation and re chemical mediators that play roles in tissue injury repair across affect the expression of specific bio different types of tissues, including skin and bone. Applied biological targets experience a non-thermal influence from LF-EMF radiation [2].

Unfortunately, various factors might affect the tissue repair rate and impact wound healing, like bacterial wound infection, where the wounds become colonized by bacteria or other microorganisms that either slow down the healing process or worsen the wound itself [3]. Gram-negative *Escherichia coli* is a perfect model organism that has been researched extensively in molecular biology and microbiology due to its widely distributed presence in the environment and human gut flora, as well as its relatively simple and well-characterized genome [4].

Many studies have examined the relationship between LF-EMF and biological systems, focusing on its potential therapeutic applications, as well as its possible adverse effects. The exact methods by which LF-EMF affects cellular and molecular processes, however, are still not fully understood [5], [6]. So, in this study, we tried to understand how *E. coli* responds to LF-EMF which can shed light on broader biological implications, that may have an impact on environmental science, medicine, and microbiology.

Previous studies have demonstrated varied impacts of LF-EMF on bacterial cells, ranging from alterations in growth rates and morphological changes to impacts on genetic expression and metabolic activity [7]. For instance, some research has suggested that exposure to LF-EMF can enhance bacterial growth and biofilm formation, potentially due to alterations in cell membrane permeability and increased nutrient uptake [8], [9]. In contrast, other studies have reported inhibitory effects on bacterial proliferation, hypothesizing that LF-EMF might induce stress responses or disrupt critical cellular processes [10]. These contradictory findings figure out the complexity of LF-EMF interactions with microbial systems and highlight the necessity for more targeted and systematic investigations.

LF-EMF therapy has been investigated for its potential to target microbial infections, reduce inflammation, and accelerate wound healing. Understanding how these fields affect bacteria can help in enhancing therapy regimens and guaranteeing safety [11]. Moreover, understanding how LF-EMF affects

bacterial populations can help with bioengineering, food preservation, and wastewater treatment procedures in both industrial and environmental settings [12].

When the magnetic fields interact with biological tissues, they induce electric fields which stimulate cellular processes by affecting ion channels, membrane potentials, and other cellular structures. There are two ways that LF EMF interacts with biological tissues: direct and indirect. Changes in ion channel activity and cell membrane permeability are among the direct impacts on cellular activities. Signal transduction mechanisms that affect gene expression and protein synthesis may be involved in indirect effects. Because LF-EMF is non-thermal, its effects come mostly from induced electric fields, not from heating [13-15].

For therapeutic applications where the objective is to promote repair processes without inflicting thermal damage, this distinction is critical. LF-EMF has been studied for its potential in treating chronic pain, reducing inflammation, and improving wound healing in medicinal applications. Clinical trials supporting its usage in treating osteoarthritis and speeding bone regeneration indicate better results compared to conventional therapies [13].

This study aimed to evaluate the impacts of low-frequency electromagnetic radiation on the growth of *Escherichia coli*. The viability of bacterial *E. coli* isolates and the number of colony-forming units (CFUs) were examined pre and post-radiation exposure. The variation of the exposure irradiation parameters, such as frequency (5-500 Hz) and magnetic induction (1-3 mT). We aimed to elucidate whether these conditions can affect bacterial growth and thereby the public health improvements.

2. MATERIALS AND METHODS

2.1. Bacterial Strain: In this study, we used *E. coli K*-12 from the American Type Culture Collection (ATCC 10798). Bacterial culture was first cultivated onto a MacConkey Agar plate (Oxoid, Hampshire, UK), and incubated aerobically at 37°C for 24 hours. Pure *E. coli* colonies were suspended in Mueller Hinton Broth (Oxoid, Hampshire, UK) to a specific density equal to 0.1A at 600 nm, which ensured controlled standard experimental conditions **[14], [15]**.

2.2. Electromagnetic Field Exposure System: Bacterial isolates samples were exposed to a static magnetic field (0 Hz) ranging from 1 mT to 3 mT and simultaneously subjected to electromagnetic radiation within the radio frequency (RF) range of 5 Hz to 500 Hz. A custom-built Helmholtz coil system (Model: XYZ-123, Serial No. 456789) was employed to generate the static magnetic field, which was adjustable and calibrated using a Gaussmeter (Model: ABC-456) to ensure field uniformity. The coil system comprised two coils, each with an average radius of 13.0 ± 0.5 cm, and 800 turns made of 2 mm² wire. The resistance of each coil was 2.4 Ω, and the inductance was 39 ± 1 mH, with an average vertical distance between the coils of 13.5 ± 0.5 cm. This configuration provided a magnetic field uniformity of better than 1% within a cylindrical exposure area that accommodated either a stack of four 96-well Falcon multi-well plates or twelve 20 mL glass tubes containing bacterial samples.

The exposure system was further equipped with a secondary coil system integrated into the primary Helmholtz setup to generate the RF electromagnetic field. The RF field, ranging from 5 Hz to 500 Hz, was applied by passing alternating current (AC) through the secondary coils, and the field amplitude was set at 1 mT at each frequency step. An oscilloscope (Model: DEF-789) monitored the waveform to ensure precise amplitude across all frequency ranges.

A magnetic field strength meter (Tenmars, model TM-191 portable) was used to measure the static magnetic field as a function of distance along the axis of symmetry of the Helmholtz coils, with variations in current controlling the intensity of the magnetic field. The static field intensity was adjusted incrementally from 1 mT to 3 mT across different experimental groups. A sinusoidal waveform with a frequency of 50 Hz and an amplitude of 2.0 mT was generated by a waveform generator and amplified using a current amplifier. This magnetic flux density (B) was measured at the center of the coils using an FW gaussmeter (Model 912, RFL Industries, Boonton, NJ), while a digital multimeter (Agilent 34401A) monitored the current through the system.

The system was housed within an incubator, maintaining a constant temperature of 37.0 ± 0.5°C, verified by a thermometric sensor (Fluke 51-II, Fluke, WAQ3). This temperature was chosen to simulate physiological conditions for the bacteria. In sham field experiments, the current was reversed, creating a null magnetic field, while ensuring that environmental parameters, such as temperature and gas tension, remained unaffected.

2.3. Experimental Conditions and Exposure: Bacterial samples were divided into two groups: an LF-EMF exposed group and a control group. The LF-EMF exposed group was placed at the center of the solenoid, where a homogeneous sinusoidal magnetic field was generated. The RF field frequency was varied incrementally between 5 Hz, 25 Hz, 50 Hz, 100 Hz, 200 Hz, 300 Hz, 400 Hz, and 500 Hz, while the static magnetic field was varied across 1.0, 1.5, 2.0, 2.5, and 3.0 mT in separate experiments. The RF field amplitude was maintained at 1 mT throughout all experiments to ensure uniform exposure. Each bacterial sample, resuspended in the appropriate medium, was exposed to the electromagnetic field for 90 minutes under controlled conditions.

The control group was placed in a separate incubator under identical environmental conditions but without exposure to the electromagnetic fields. Both groups were maintained at a stable temperature of 37.0 ± 0.3°C to eliminate temperature as a variable in the experimental outcomes.

2.4. Bacterial Growth: The optical density (OD) of the control and treated samples was measured at 600 nm every hour for the first three hours before irradiation and then again at 4.5, 5, and 5.5 hours after irradiation. The CFU value per milliliter (mm) was calculated using serial dilution. A volume of 100 μL from the final tube was inoculated using the spread plate technique on agar plates (dilution factor 1×10^{6}) following five successive dilutions. The colonies that developed on the plates were visually counted following incubation. Except for being subjected to magnetic fields, control cultures were maintained in identical circumstances for all tests.

Statistical Analysis: All experiments were replicated at least three times, and the statistical significance of each difference observed among the mean values was determined by standard error analysis. A paired t-test was used for the statistical analysis using GraphPad Prism software (version 7.0, GraphPad Software Inc., La Jolla, CA, USA); $P < 0.05$ was considered to be statistically significant. All data were expressed as mean ± standard deviation (mean ± SD).

3. RESULTS

In this research, we studied the effect of low-frequency electromagnetic radiation on the growth of *E. coli* in a CFU. Bacterial cultures were irradiated with a wide range of radiofrequencies from 5 Hz to 500 Hz and magnetic densities (1.0, 1.5, 2.0, 2.5, and 3.0 mT). According to previous results, irradiation lasting for less than one hour had no significant effects on bacterial cultures and didn't decrease the number of living cells **[16]**. Therefore, we extended the irradiation duration for 1.5 hours.

CFU values of the irradiated and control samples were calculated at the chosen physical conditions. Large CFU numbers indicated a lower relative change in bacterial growth. Similarly, lower CFU numbers showed that LF EMF had the greatest impact on bacterial growth, accounting for a greater relative change (%) value. As shown in **Table 1**, our results revealed that the maximum percentage change (71.3%) was observed at 300 Hz and 2 mT, followed by (62%) at 200 Hz, 2.5 mT, and 3 mT. The minimum percentage change was observed after irradiation at 5 Hz at 2 mT and 3 mT equal to 15.2%

Table 1: Minimal CFU values and maximal percentage for the studied frequencies and magnetic flux densities on *Escherichia coli*

Frequency (Hz)	**Minimum CFU Count (n)**	**Maximum Change (%)**	**Magnetic Field (mT)**
5	67	15.2%	2 and 3
25	65	16.7%	3
50	52	30.7%	1.5
100	50	34.2%	1
200	30	62%	2.5 and 3
300	23	71.3%	2
400	31	60.8%	2
500	39	46.8%	1

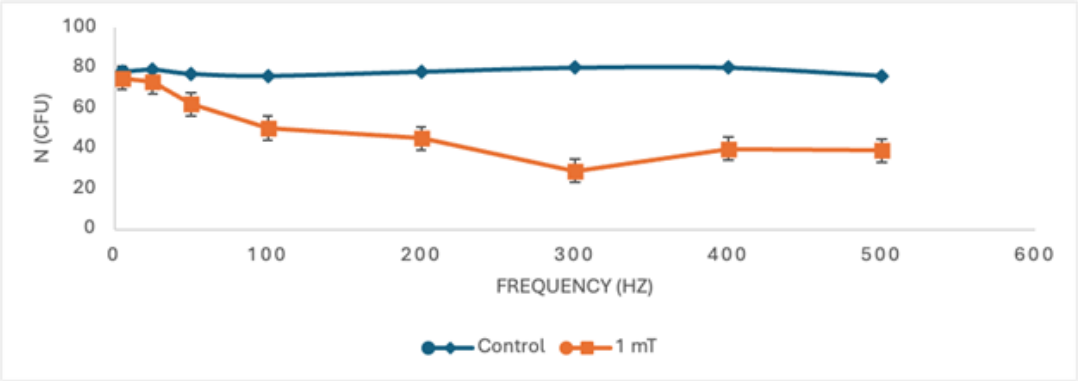

Figure 1: CFU count of E. coli after irradiation at 1 mT. Data is represented as means ± SD from 3 different experiments.

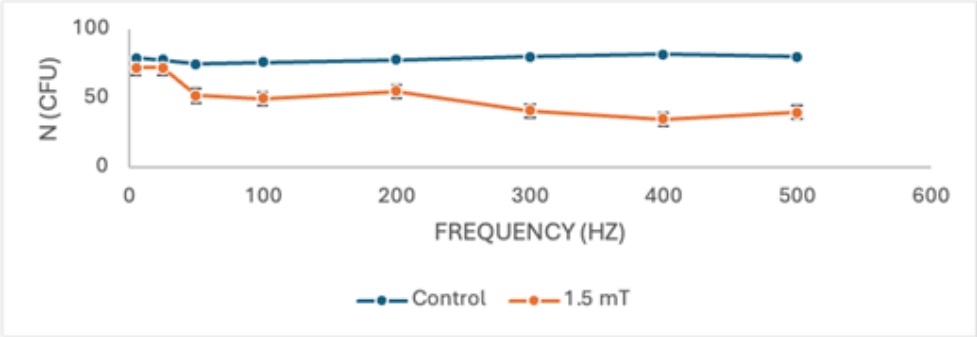

Figure 2: CFU count of E. coli after irradiation at 1.5 mT. Data is represented as means ± SD from 3 different experiments.

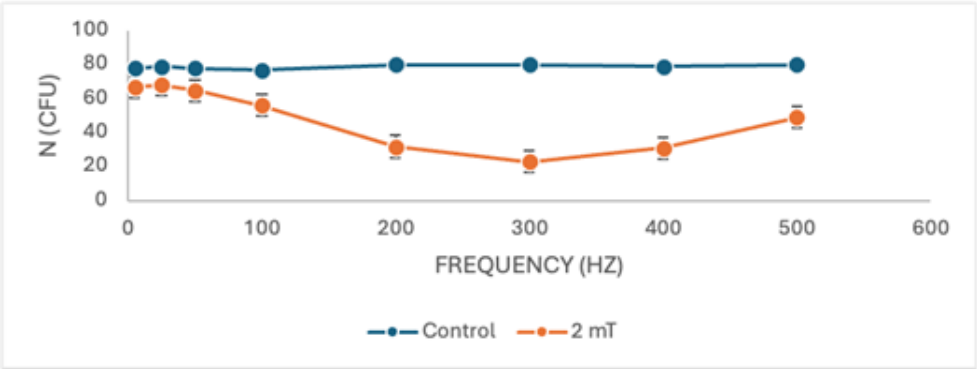

Figure 3: CFU count of E. coli after irradiation at 2 mT. Data is represented as means ± SD from 3 different experiments

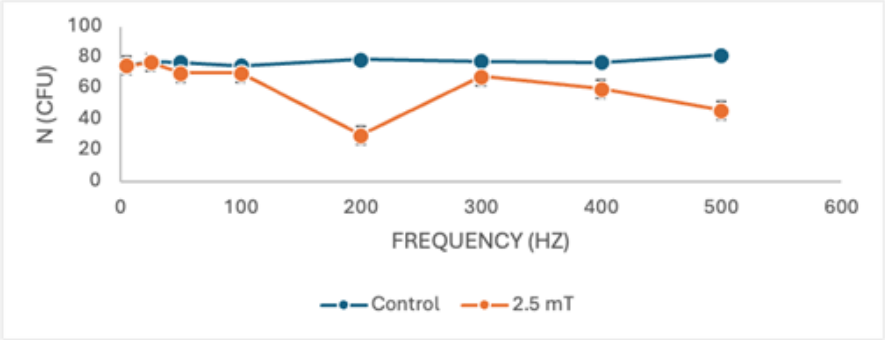

Figure 4: CFU count of E. coli after irradiation at 2.5 mT. Data is represented as means ± SD from 3 different experiments.

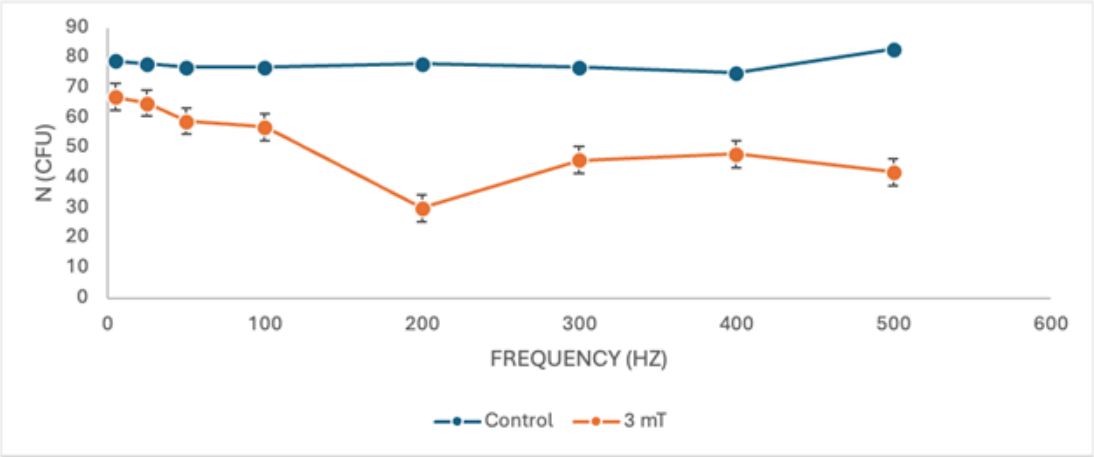

Figure 5: CFU count of E. coli after irradiation at 3 mT. Data is represented as means ± SD from 3 different experiments.

Figure 1-5 showed the great difference between the CFU numbers of irradiated and control *Escherichia coli* culture, where (N) represented the bacterial count in 100 µL of suspension. The percentage change in CFU between the irradiated and non-irradiated samples was measured for each experiment. Our results showed a general decline in the bacterial count of the exposed samples to radiation more than 100 Hz compared to the control samples. The effect of irradiation was statistically significant with the lowest P value (0.001) recorded at 3 mT. It is noteworthy that across the whole spectrum of various LF-EMF exposures, the bacterial count for the non-irradiated samples stayed nearly stable, at roughly 76.

Our results revealed that bacterial count at 5 Hz and 25 Hz examined at the 5 different magnetic fields were consistently higher (minimal change) in contrast to the counts observed at the higher frequencies. After exposures to 5 Hz, bacterial count was recorded as 75 CFU at 1 mT, and 67 for both 2 mT and 3 mT. This corresponded to a relative percentage decline of 3.8% at 1 mT **Figure 1**, 14.1% at 2 mT **Figure 3**, and 15.1 % at 3.0 mT **Figure 5.** Moreover, there was a slight decrease in CFU values after exposures at 25 Hz with a relative percentage decrease of 7.5%, 13.9%, and 16.6% at 1 mT, 2 mT, and 3 mT, respectively.

On the other hand, results represented in **Figure 1 and Figure 3** revealed that maximal declines of CFU counts (N = 23 and N= 29) were recorded at 300 Hz and magnetic fields of 2 mT and 1 mT. This corresponded to relative reductions of 64.6% and 68.6%, respectively.

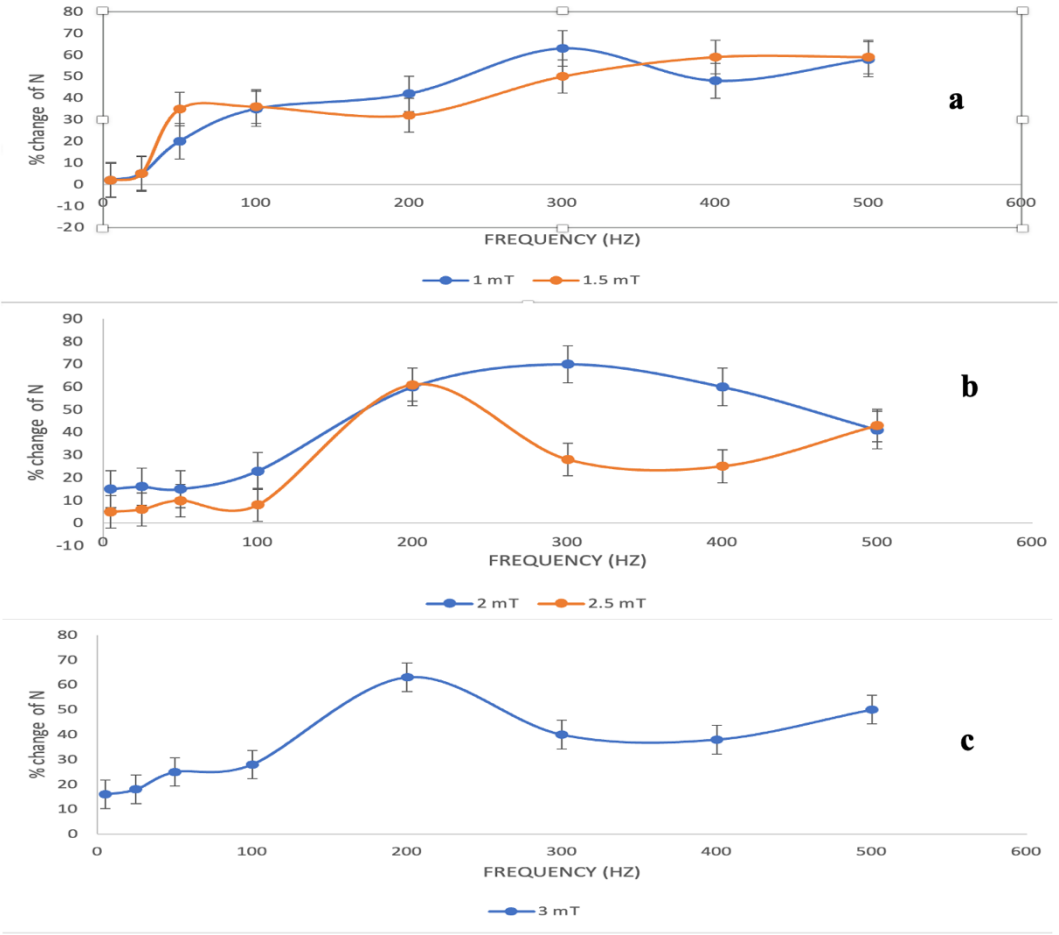

Figure 6: Percentage change (%) of CFU number after exposure at 1 mT /1.5 mT (a), at 2 / 2.5 mT (b) and at 3 mT (c). Data is represented as means ± SD from 3 different experiments.

Non-linear fluctuating behavior was observed in **Fig. 6** in bacterial counts following electric and magnetic exposure. The peak values are dispersed throughout a broad range of frequencies rather than concentrated at a single frequency, which demonstrated that bacterial cultures are highly affected by radiation when exposed to the low electromagnetic field. The percentage change of CFU number was not statistically significant when comparing 1mT with 1.5 mT (P value equal to 0.855) while the P value recorded with 2 mT and 2.5 mT was 0.041.

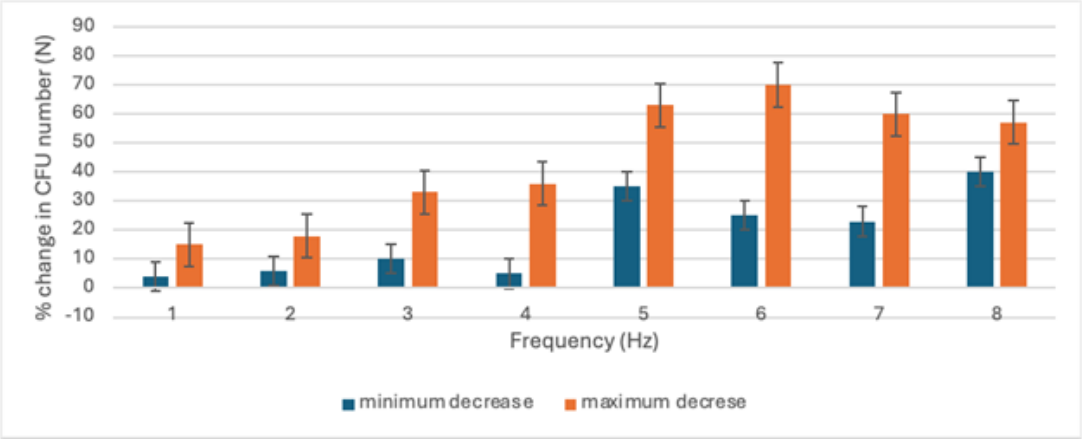

Figure 7: Relative minimal and maximal change (%) in bacterial count after LF-EMF exposure.

Fig. 7 represented the relative percentage changes (%) in CFU numbers for the shown frequencies. The minimum decline corresponding to the magnetic flux density range of 1-3mT was detected within 5 Hz to 100 Hz and changed from 4% to 10%. A maximal percentage decline was noticed at 300 Hz, where a percentage of 70% was noted followed by 63% at 200 Hz. In general, the maximum changes in CFU numbers noticed after exposure to 200 Hz and more were considerably higher in contrast to the exposures in the lower end of the LF spectrum.

4. DISCUSSION

Our study concluded that exposing *E. coli* to LF-EMF significantly affected their viability, which is measured by CFUs. The CFU method links the reduction in CFU numbers post-exposure to bacterial death, indicating the number of bacteria surviving the treatments [12], [17], [18].

By studying the effectiveness of different frequencies and magnetic flux densities, we found that control samples, which were not exposed to LF-EMF, maintained stable CFU values around 76. After the statistical analysis, results showed that the difference between exposed and control samples was always significant ($P < 0.05$).

Frequencies above 100 Hz were more effective in reducing bacterial viability compared to lower frequencies. The most significant reduction in CFU was observed at 300 Hz and 2 mT, showing a 71.3% decrease. This finding suggests potential applications of optimized LF-EMF parameters in managing microbial infections, reducing inflammation, and accelerating wound healing [14].

According to Ahmed *et al*, 2013, exposures lasting less than one hour had no significant effect on bacterial cultures. So, subsequent exposures were standardized to 90 minutes [16].

On the other hand, this study demonstrated some variations with magnetic flux densities; at 5 Hz, minimal decreases in CFU values were observed, indicating low effectiveness at this frequency. According to EbrahimPour, 2012, the following factors could be the reason for the decline in *E. coli* colonies in the field within the frequency range of 10 Hz; first, modifications in the permeability of bacterial membranes, which could lead to biological changes in the organism [12].

Higher frequencies resulted in more significant decreases in CFU values, particularly at 300 Hz with magnetic flux densities of 2 mT and 1 mT, showing decreases of 64.6% and 68.6% respectively. According to Inhan-Garip *et al* 2012, for all strains, very low-frequency electromagnetic radiation was seen to cause a statistically significant drop ($p < 0.05$) in bacterial viability, which remained until the strains reached the stationary phase. For the majority of the strains, the gap in growth rates between the irradiated bacteria and the controls narrowed during the 5^{th} and 6^{th} hours. Gram-positive and Gram-

negative bacteria similarly responded to LF-EMF; that is, no discernible differences in the reductions in growth rates were seen [19].

We also found that relative changes in CFU values following LF-EMF radiation exhibited non-linear oscillatory patterns. Peak values were dispersed across a broad range of frequencies rather than concentrated at a single frequency, demonstrating that bacteria are highly affected by specific combinations of magnetic fields and radiofrequency. These patterns are similar to those seen in our previous study on the effect of LF-EMF on Collagenase enzyme kinetics [12].

The exact mechanism by which magnetic fields inhibit bacterial growth is still unclear. According to Fojt *et al,* 2004, the research did not find the reason behind how the magnetic field can inhibit bacteria growth. Primary ideas that attempt to explain how electromagnetic fields affect biology are predicated on potential impacts on the selective permeability of the membrane's ionic channels. This may have an impact on ion transport into the cells, which may alter the microorganisms' biology. Another possible impact is the production of free radicals due to magnetic field exposures [15].

LF-EMFs affect biological systems, often showing "windows" of effective parameters [20]. Weaker magnetic fields can be more effective, causing interference in ion quantum states and altering ion-protein dissociation probabilities, suggesting a nonlinear physical mechanism. Frequency and magnetic field "windows" were particularly noted at frequencies above 250 Hz at 0.5 mT, 200 Hz at 1.0 mT, and 150 Hz at 2.0 and 2.5 mT [21].

Other studies have also reported reduced growth rates in bacterial cultures subjected to LF-EMF, with observed morphological changes in *E. coli*, such as cytoplasmic alterations, without cell wall disruption. LF magnetic fields affect biological systems, showing specific "windows" of effective parameters [22].

LF-EMFs may interact with biological systems through mechanisms such as electron displacement in DNA, local charging leading to biopolymer disaggregation, changes in ion channel permeability, and free radical formation. These interactions suggest that LF-EMFs can significantly alter bacterial cell structure and function, impacting their viability [14], [23], [24].

EMF) -frequency electromagnetic fields (LF-to low *E. coli* Our study demonstrated that exposing significantly reduced bacterial viability, with frequencies above 100 Hz showing the most pronounced effects. These findings suggest potential applications in various fields, including biomedicine, and food -invasive antibacterial treatment. Studies indicated that ELF-EMF has shown promise as a non-safety. LF offering ،*Staphylococcus aureus* and *E. coli* resistant-EMF exposure can reduce the viability of multidrug found that 2003 *.et al* Additionally, Pickering .[8] ic therapya potential alternative to traditional antibiot associated infections on orthopedic -EMF exposure enhances antibiotic efficacy in treating biofilm .[25] implantsLF-EMF has been reported to accelerate wound healing and tissue regeneration. Tofani *et al*. 2001 demonstrated that LF-EMF exposure inhibits tumor growth and induces apoptosis, which may have implications for tissue repair [6]. Similarly, Ehnert *et al.* (2019) showed that LF-EMF enhances bone healing and osteogenesis [13].

.et al safety. Segatore thermal method for bacterial inactivation in food-EMF has potential as a non-LF ،*Pseudomonas aeruginosa* and *E. coli* EMF exposure on-observed significant effects of LF 2012 suggesting possible applications in food sterilization[20]reported 2008 *.et al* Additionally, Cellini . MF exposure, which could be leveraged for changes in bacterial growth dynamics under 50 Hz E microbial control in food industries[10].

5. CONCLUSION

In our study, we examined the effects of a broad range of low-frequency electromagnetic radiation (5-500 Hz) with magnetic field densities between 1 mT and 3 mT on the bacteria *E. coli*. We employed the CFU method, which has been used to assess bacterial viability after LF-EMF exposure. Clinical studies

suggested that EMF therapy could be beneficial and should be incorporated into standard treatments to inhibit bacterial infections, thereby enhancing antibiotic therapy.

Our investigation showed that LF-EMF exposure led to a decline in CFU in all treated samples in comparison to controls, with the most notable effects at frequencies higher than 100 Hz. The optimal effects were observed at 300 Hz and 2 mT, with CFU reductions of nearly 20% for frequencies over 200 Hz across all tested magnetic field densities.

Non-linear physical responses, categorized as "window" effects, were observed in bacterial suspensions upon exposure to very low-frequency electromagnetic radiation. Identifying the optimal LF-EMF parameters for bacterial elimination is crucial for developing effective and non-invasive treatments for infected tissues, thereby promoting wound healing. These parameters can also guide further exploration of LF-EMF's potential applications.

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7. CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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9. DATA AVAILABILITY

Data will be available upon request through Dr/ May El-Antrawy email: mayantrawy92@gmail.com.

10. AUTHOR CONTRIBUTIONS

EAH contributed to the study conception, methodology, resources, data analysis, and supervision. MAE-A contributed to methodology, data analysis, writing draft, review, and editing. All authors read and approved the final version of the manuscript.

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