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Research Article

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Evaluation of the Effects of Alternative Magnetic Fields on Growth of Escherichia Coli and Staphylococcus Aureus

Mohamad Reza Bayatiani*

Associate professor, Department of Medical Physics and Radiotherapy, Arak university of Medical Sciences and Khansari hospital, Arak, Iran

***Corresponding author:** Mohamad Reza Bayatiani, Associate professor, Department of Medical Physics and Radiotherapy, Arak university of Medical Sciences and Khansari hospital, Arak, Iran.

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Abstract

Purpose: *This research aimed to evaluate the effect of different intensities and frequencies of alternative electromagnetic fields on the growth situations of Escherichia coli and Staphylococcus aureus strains.*

Materials and Methods: *The effect of 1 and 2 mT alternative magnetic fields with 50, 75, 100, and 150 Hz frequencies were evaluated on Escherichia coli and Staphylococcus aureus strains by standard plate count technique as viable colony counts on nutrients agar culture medium. Incubation was at 37 °C for 24 hours and then colonies were counted as colony-forming units per millilitre (CFU/ml).*

Results: *The results of colony-forming units showed that the number of Escherichia coli colonies treated with 1 and 2 mT magnetic fields with 50, 75, 100 and 150 Hz frequencies were more than the colonies of the control groups but CFU/ml of Staphylococcus aureus treated with the magnetic fields were less than the colonies of control groups.*

Conclusion: *The highest decrease of the CFU and the negative magnetic field effect on bacterial growth was observed with Gram-positive Staphylococcus aureus. The smallest magnetic field effect (increase of CFU) was achieved for Escherichia coli.*

Keywords: Alternative Magnetic Field, Growth of Bacteria, Escherichia Coli, Staphylococcus Aureus

Introduction

Nowadays due to advances in science and technology, most people are exposed to electromagnetic fields from electricity transfer lines and high number of electrical devices [1, 2]. Especially in the last two decades in scientific and social communities, there were worries about possible damages from exposure to very low-frequency magnetic fields, which have frequencies less than 300 Hz. These concerns also exist in the on text of the effect of magnetic field on subcellular, cellular and tissue functions [3-6].

Various studies have focused on the effect of electromagnetic fields on biological systems, however, due to the complexity of such systems and processes there is not a final agreement regarding these effects. For example, while several epidemiological studies approve of the relationship between exposure to magnetic fields and induction of cancer there are other in-vivo and in-vitro studies which do not agree with these results [7-10].

As a sample, it was approved by some studies that electromagnetic fields have roles in the production of stress and free radicals and disturbing the equilibrium of antioxidants [11]. Suzuki et al [12]. reported an increased frequency of micronucleus in the balb C mouse due to exposure to static magnetic field. Udroiu et al reported an increased frequency of micronucleus in the blood of newborns of mice whose parents were exposed to low-frequency electromagnetic fields during their pregnancies [13].

One of the subjects that recently followed is to evaluate the effect of electromagnetic fields and nonionizing radiations on the growth of different positive and negative gram bacteria and their biological changes. With this regard, some results approve the controlling effect of such fields on the growth of bacteria and some others reported inverse effects [14-19]. The contradictory results which are reported by different studies are likely due to the fact that the growth, function, survival and morphological changes of bacteria in a magnetic field can be a function of type of bacteria, field intensity, frequency, type of magnetic field (being constant or alternative), duration of field exposure and other physical factors [20].

Nawrotek et al evaluated the effect of a 30 mT rotating magnetic field with frequency of 50 Hz on the growth and cell metabolic activity of Escherichia coli and Staphylococcus aureus bacteria [21]. Their results showed that the rotating magnetic field had an increasing effect on the growth and cell metabolic activity of the bacterial strains. This effect was dominant in the time interval of 30-150 min and also for the Staphylococcus aureus cultures. A rapid and significant decrease in the growth was observed in the case of Escherichia coli samples. The results showed that the 30 mT rotating magnetic field with 50 Hz frequency has a stimulatory effect on the growth and metabolic activity of both bacterium types.

Nafisi et al evaluated the effect of high and low-frequency electromagnetic fields (700 milli gauss) on the Escherichia coli bacterium. The case sample was treated with the electromagnetic field for a time of six hours and the control sample was put aside. The obtained results showed a significant increase in the number of Escherichia coli treated with high-frequency electromagnetic field, while a significant decrease was observed in the number of Escherichia coli in the low-frequency electromagnetic field group. There were also negative effects of electromagnetic fields on the biochemical properties of Escherichia coli, Fojt et al [22, 23].

Evaluated the morphology changes of bacteria exposed to 50 Hz and 10 mT magnetic fields. The evaluated bacterial strains were Escherichia coli and Paracoccus denitrificans. There was a decrease in viability of different bacterial strains due to exposure to an alternating magnetic field. On the other hand, no change was observed in the bacterial morphology for both bacterial types after 1 h exposure to 50 Hz and 10 mT magnetic field.

Esmekaya et al have performed a study and evaluated the effects of extremely low-frequency magnetic field in the presence of FeCl3 on growth and morphology of bacteria [24]. The magnetic field was a 50 Hz sinusoidal magnetic field. The field was approximately uniform throughout the axis of the coil pair. Escherichia coli bacterial samples were treated with different concentrations of FeCl3 and exposed for 24 hours to 50 Hz and 2 mT magnetic fields. Viable colony numbers were counted, and scanning electron microscopy was used to investigate the morphology of bacteria. The extremely low-frequency magnetic field and FeCl3 treatment did not affect cell viability. On the other hand, some morphological changes were observed in the Escherichia coli samples.

Segatore et al investigated the effects of extremely low-frequency electromagnetic fields (with intensity of 2 mT and frequency of 50 Hz) on the growth and antibiotic sensitivity of Escherichia coli Pseudomonas aeruginosa bacteria [20]. The results showed that the electromagnetic field treatment significantly changed the growth rate of the two bacterial strains when incubation was performed in the presence of 1 μg/mL kanamycin and 0.5 μg/mL amikacin. In other words, at 4, 6, and 8 h of incubation, the number of cells was significantly decreased but at 24 h of incubation, the percentage of cells increased. The antibiotic susceptibility was not remarkably changed in both bacteria due to exposure to the electromagnetic fields.

Bayir et al investigated the effect of 2 and 4 mT in magnetic intensities with 20, 40 and 50 Hz frequencies and 1, 2, 4 and 6 h exposure times on the growth of bacteria including Staphylococcus aureus and Escherichia coli. Cell suspensions were inoculated to Mueller-Hinton Agar plates after exposure to the fields [25]. For both bacterial samples, the characteristic responses depended on the magnetic intensity, frequency and exposure time of the fields. A statistically significant decrease was observed in colony forming for samples exposed to the electromagnetic field, compared to the control groups, especially at longer exposure times.

Fojt et al evaluated the effect of 5 Hz and 10 mT frequency electromagnetic fields with exposure time of less than 30 min on Escherichia coli, Leclercia adecarboxylata and Staphylococcus aureus [16]. The results showed that for all bacterial types, viability decreased with longer exposure time and/or higher magnetic field intensity. For Escherichia coli, the highest decrease of viability and the biggest magnetic field effect was observed. On the other hand, the smallest magnetic field effect was for the Staphylococcus aureus strain.

There are also other studies on the effect of magnetic fields on bacteria [26-31].This research aims to evaluate the effect of different intensities and frequencies of alternative electromagnetic fields on the growth of Escherichia coli and Staphylococcus aureus.

This area of study is important because many studies revealed that the effect of magnetic fields on bacteria is not fully understood and is variable depending on the type of microorganism. Therefore, the studies have controversial results [29]. Different bacterium genus showed variable responses when exposed to different magnetic fields depending on the exposure time and magnetic field intensity levels, Suggesting sustained effect of magnetic fields upon the growth rate of bacteria. The present study aimed to examine and analyze the growth situations of E. coli in the different intensities of magnetic fields.

Materials and Methods

Bacteria

In this study, the effect of 1 mT and 2 mT alternative electromagnetic fields with 50, 75, 100 and 150 Hz frequencies on the growth of gram-negative Escherichia coli and gram-positive Staphylococcus aureus was evaluated. For each bacterial strain, two groups including the case group as exposed to alternative magnetic field and control group (un-exposed) were considered.

Exposure Treatments

The effects of different magnetic field exposure effect on growth rate were assessed on tested strains in terms of viable colony counts. For Escherichia coli and Staphylococcus aureus two groups of tubes as the control (without magnetic field exposure) and case groups separately were subjected at room temperature to 1 and 2 mT magnetic fields with 50, 75, 100 and 150 Hz frequencies respectively (10 hours). After the exposure, viable cells were determined in case and control groups by serial dilutions and plate counting on Nutrient agar as explained below [24-32].

Viability Tests

Minimal Inhibitory Concentrations (MIC) were determined by conventional method. An inoculum of 5×105 colony-forming units in millilitre (CFU/mL) as suggested by CLSI was used [33]. For each treatment, 3 dilutions in 3 petri dishes were provided. Dilutions of control and exposed groups were poured on Nutrient Agar (Merck, Germany) culture medium by pour plating and incubated overnight at 37°C. Cell growth was examined for each group by plating serial tenfold dilution of bacterial suspensions and CFU was used to quantify our results. Results were reported as dilution values at which not no visible growth occurred (MICs values). The effect of the alternative magnetic field was evaluated based on the change in CFU/ml as a criterion for live bacteria for different field intensities, and frequencies. The tests were repeated in three parallels and the magnetic field exposure effect was determined by comparing the viable bacterial counts [24-32].

Alternative Magnetic Field Exposure

The applied alternative magnetic fields were 1 mT and 2 mT fields with 50, 75, 100 and 150 Hz frequencies. The magnetic fields were produced using a specially designed device which was constructed for this study. The device included electronic circuits and Helmholts coils (Fig. 1). By use of calibration tables, adequate voltages were applied to the Helmholtz coils so that the considered magnetic fields and frequencies be produced between the two Helmholtz coils space. The design of the Helmholts coil was so that a relatively uniform field be produced in the experiment space which was between the two Helmholts coils. To ensure that the magnetic field is uniform, the magnetic field intensity was measured by a GM2 gaussmeter (Alphalab Inc., USA) before each exposure to the magnetic field.

The main circuit and Helmholtz coil used for the production of alternating magnetic fields in this study are presented in Fig 1.

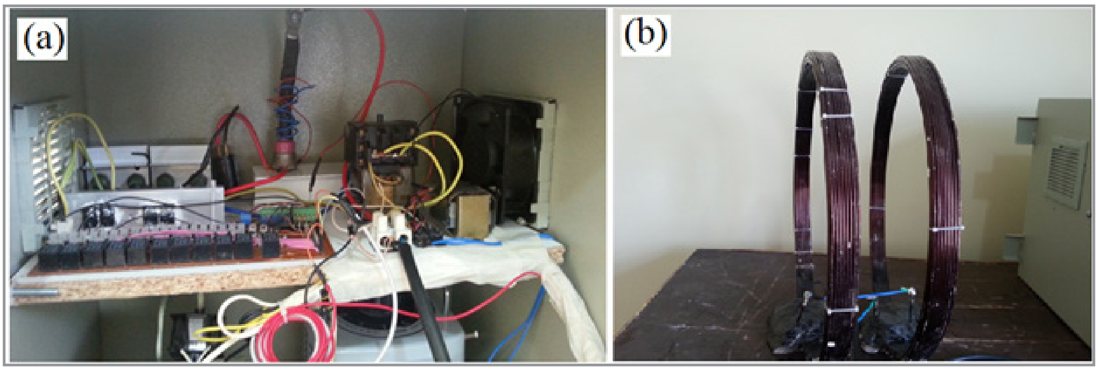

Figure 1: Main circuit (a) and Helmholtz coil (b) were used for production of alternating magnetic fields in this study.

Results

Escherichia Coli Viability

The results of colony-forming units for Escherichia coli as number of bacterial colonies (CFU/mL) in the control and case groups for 1 and 2 mT magnetic fields with 50, 75, 100 and 150 Hz frequencies were summarized in Table 1. Each experiment was repeated three times and the average ±SD value was written in the tables. The results showed that the number of Escherichia coli colonies treated with magnetic fields was higher than that of the control.

Table 1: Number of bacterial colonies (CFU/mL) for Escherichia coli in the control and case groups for 1 and 2 mT magnetic fields with 50, 75, 100 and 150 Hz frequencies.

	Frequency	Dilution 5	Dilution 6	Dilution 7		Frequency	Dilution 5	Dilution 6	Dilution 7
Control group (0 mT)	-	72	12	20	Case group (1 mT)	50 Hz	720	480	27
	-	310	42	18		75 Hz	605	520	2
	-	103	19	18		100 Hz	450	210	35
	-	122	37	47		150 Hz	720	107	35
Control group (0 mT)	Frequency	Dilution 5	Dilution 6	Dilution 7	Case group (2 mT)	Frequency	Dilution 5	Dilution 6	Dilution 7
	-	640	104	18		50 Hz	800	560	385
	-	920	141	19		75 Hz	1552	616	90
	-	880	184	14		100 Hz	1072	340	85
	-	800	207	18		150 Hz	1304	872	200

Staphylococcus Aureus Viability

Some bacterial colonies (CFU/mL) for Staphylococcus aureus for the control and case groups for 1 and 2 mT magnetic fields with 50, 75, 100 and 150 Hz frequencies are presented in Table 2. As a result, CFU/ml of Staphylococcus aureus treated with magnetic fields was smaller than that of the control.

Table 2: Number of bacterial colonies (CFU/mL) for Staphylococcus aureus for the control and case groups for 1 and 2 mT magnetic fields with 50, 75, 100 and 150 Hz frequencies.

	Frequency	Dilution 5	Dilution 6	Dilution 7		Frequency	Dilution 5	Dilution 6	Dilution 7
Control group (0 mT)	-	82	10	2	Case group (1 mT)	50 Hz	1340	118	24
	-	210	36	10		75 Hz	800	114	10
	-	170	84	34		100 Hz	920	254	12
	-	40	32	8		150 Hz	856	137	18
	Frequency	Dilution 5	Dilution 6	Dilution 7		Frequency	Dilution 5	Dilution 6	Dilution 7
Control group (0 mT)	-	598	71	13	Case group (2 mT)	50 Hz	668	102	16
	-	480	50	10		75 Hz	592	43	8
	-	868	115	4		100 Hz	832	82	5
	-	744	128	14		150 Hz	720	105	13

Discussion

The effect of 1 and 2 mT alternative magnetic fields with 50, 75, 100, and 150 Hz frequencies was evaluated on Escherichia coli (positive grams) and Staphylococcus aureus (negative grams) bacteria. As our obtained results, the highest decrease of the colony-forming units (the negative magnetic field effect) on bacterial growth was observed with Gram-positive Staphylococcus aureus. On the other hand, the smallest magnetic field effect (an increase of colony-forming units) was achieved for Gram-negative Escherichia coli.

Based on the results presented in Table 1 for Escherichia coli bacteria with application of 1 mT magnetic field, in the dilutions 5 and 6 in the control group (the group without magnetic field) the numbers of colonies are less than the case group (the group with magnetic field). However, it can be concluded from the data that the application of 1 mT or 2 mT alternative magnetic field would increase the growth of Escherichia coli. The decrease of CFU was observed for S. aureus. This bacterium was susceptible to the magnetic field.

The main theories that try to explain the effects of magnetic fields on the biological system are the effects on the formation of free radicals and permeability of the ionic channels in the cytoplasmic membrane and subsequently disruption of ion transport into the cells [33, 34].

The results of the present study indicate that the trend for growth of Escherichia coli Staphylococcus aureus bacteria is different. This effect does not depend on the applied frequency. This may be due to differences in the cellular membranes of these two bacterial strains. Positive gram bacteria have a thicker membrane and, therefore, they are more resistant to mechanical pressures and other factors. On the other hand, negative gram bacteria have thinner cellular membranes but with two cytoplasmic layers and this results in more control of entrance and exiting of different materials into the bacteria.

There are assumptions that electromagnetic fields have effects on the permeability of ionic channels on the cytoplasmic membranes of cells. These channels control the entrance and exiting of materials in cells and bacteria and have important role in their vital activities. Other probable effects can be formation of free radicals due to application of magnetic fields and their effects on the cell membrane. In previous studies like Strašák et al, Fojt et al, and Inhan-Garip et al, it was reported that the growth of bacteria decreased by application of extremely low-frequency and low-frequency magnetic fields [14-16].

In other studies, by Justo et al and Gaafar et al [18, 19]. it was reported that application of extremely low frequency on Escherichia coli increased its growth. The results of the present study were in agreement with the results reported by Justo et al. and Gaafar et al., since in this study the growth of Escherichia coli and Staphylococcus aureus was increased in different intensities and frequencies of alternative magnetic fields.

There has been a large amount of conflicting evidence regarding the effect of magnetic field on bacterial proliferation. This implies that such effect is related to the magnetic field intensity and frequency, exposure time, bacterial type, morphology, physiology, metabolism and so on [35]. It was demonstrated that the effect of magnetic field on the cell was related to an increase in the formation of free radicals, disintegration of the cell wall, permeability of membrane ion channels, efflux of the cytoplasmic contents and blebbing [14-16].

Conclusion

In this paper, we focused on the effect of magnetic fields on the growth rate of gram-positive and gram-negative bacteria. Based on the obtained results, an alternative magnetic field has an increasing effect on the growth of Gram Negative and a decreasing effect on the growth of Gram-Positive bacteria suggesting a progressive adaptive response.

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