Frequency Electromagnetic field effects on Gram-Positive and Gram-Negative Bacteria

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Research note

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Abstract

Abstract Objective: The effects of electromagnetic waves on the growth of living organisms and the determination of the threshold of these radiations have remained elusive. Therefore, in this research, we have investigated the growth rate of gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli) bacteria that had been exposed to the different frequencies of electromagnetic fields. Results: The more frequency increased the slower bacteria grew; however, in gram-positive bacteria such as S. aureus, this effect was seen less. The effect of the 1mT electromagnetic field in the growth of S. aureus was significant between the two groups, nonetheless, in the 2mT electromagnetic field, the effect was not significant between the two groups at different frequencies. Noteworthy, no significant change was observed by increasing the frequency in S. aureus exposed bacteria in comparison to the control group. The study of bacterial growth in terms of frequency in both case and control groups showed an increasing trend. Increasing the frequency from 50 Hz to 150Hz, significantly, enhanced the rate of bacterial growth. On the whole, the magnetic field had an increment effect on the growth of bacteria; in fact, this effect was greater on the gram-negative than on the gram-positive bacteria.

Introduction

Electrical devices, today, have had a great impact on human life. Electromagnetic fields, created by overhead power lines and other electrical devices may lead to any biological changes in living organisms. Being widely exposed to the electromagnetic fields in the last two decades, people have concerned the Low-Frequency Magnetic Fields (ELF-EMF) as the most common magnetic fields in the human environment with the frequencies below 300 Hz, as a great threat to their health quality. (1, 2). Lee HC et al. and Ansari RM et al have investigated the effects of ELF-EMF on the cell functions (1, 2). Other studies, also, have evaluated the effects of ELF-EMF on bacteria (3-5). The results of these studies sometimes contradict each other. For example Inhan-Garip A et al. and Segatore B et al. have shown in their studies that ELF-EMF can negatively affect the living system function (6, 7). On the other hand, some studies reported the positive effects of ELF-EMF on the living organism function, and the bacteria sensitivity to the antibiotics (8, 9). Although the previous studies have shown that the effects of ELF-EMF are depending on some parameters such as frequency and intensity of the field, time of exposure and type of bacteria cells, due to the complexity of biological systems, there is not a final agreement on the effect of ELF on bacteria as a living system (10). Therefore, more investigations are needed to shed light on this controversial subject. In the present study, the effect of ELF-EMF exposure on bacteria growth was investigated in the respect of changing the frequency (50, 75, 100 and 150 Hz), magnetic field intensity (1 and 2 mT) and type of bacteria (gram-positive and gram-negative).

Materials And Methods

Electromagnetic generation system:

Electromagnetic exposures were produced by a homemade electromagnetic generator (previous study) in the Department of Medical Physics at Arak University of Medical Sciences. The system, basically, was consisted of a pair of Helmholtz coils, a power supply and a signal generator (Fig 1).

The developed electromagnetic generator has equipped with buttons that enable the user to select the desired frequency between 0 (as a static electromagnetic field) to 300 Hz (as a pulsating electromagnetic field), and it can produce electromagnetic fields with intensities of 0 to 8 mT. The efficiency and precision of the system were checked using a gauss-meter. The uniformity of the electromagnetic field between the coils provided a simultaneous exposure for the cultures. The yield of the produced electromagnetic field was in agreement with the computation of the field distribution and homogeneity of the electromagnetic field which was calculated by a Laplace equation simulation program. The program takes the finite dimensions of coils into account. In this study, electromagnetic fields with different frequencies of 50, 75, 100 and 150 Hz and intensities of 1 and 2 mT were applied to the two types of gram-positive and gram-negative bacteria.

Table 1: The physical parameters

Physical parameters	properties
Outer diameter	54 cm
Inductance	3 mH
Resistance	4.2 Ω
Number of turns	48
Autotransformer	2 KW and 0-300 V
Transformer	2500W and 12-220 V

Strains

The bacteria used in this study, *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) strains, were provided from the Depatrment of Microbiology, Faculty of Medicine, Arak University of Medical Sciences. Standard biochemical tests such as Gram staining, coagulase, Dnase, Catalase, and Mannitol Fermentation Test were used for the identification of the *S. aureus* strain (11). In order to identify the *E. coli* strain, the samples were cultured on the Simmons citrate agar medium and gram staining procedure was applied to the bacteria. Also, the following tests were performed to determine the *E. coli* strain including Lysine Decarboxylase, Motility, Methyl Red, Indole, Voges–Proskauer, Ornithine Decarboxylase, and Triple Sugar Iron test (12).

Counting the number of colony-forming units

The pour plate method, in a fixed amount of inoculum (generally 1 ml), was used for counting colony-forming units. In the present study, we used a dilution of 0.5 McFarland standard. One milliliter dilution of bacterial strain was poured into an 8 centimeter sterile Petri dish, then Muller Hinton agar (approx. 20mL) was added to the Petri dish and was mixed well. After incubating at 37 °C, and using the following formula: (CFU/mL= CFU * dilution factor * 1/aliquot), the number of colony-forming units in one milliliter of dilution of bacterial strain (CFU/mL) was calculated.

Bacterial culture treatment

A serial dilution of bacteria was provided in the glass tubes, and the tubes were exposed to the electromagnetic fields with different frequencies of 50, 75, 100 and 150 Hz. Then, the exposed bacteria were cultured in the Muller Hinton agar and the number of colony-forming bacteria was counted using the Pour plate method. The dilution was prepared in 1000 µl volume, then 900 µl of each tube was poured into 7 tubes. The media cultures were exposed to the AC electromagnetic fields (0.0 – 1.0 kHz) at a fixed intensity of 450 mT. The properties of the electromagnetic field were determined by a Hall effect probe Gaussmeter. Inside the solenoid, the electromagnetic fields were approximately homogenous in a region ± 3 cm off the center of the coil. The device was kept inside an incubator cabinet at 37°C and the temperature was measured by a thermometer. The clinical strain of *S. aureus* used in this study was obtained from the Tuberculosis and Pediatric Infectious Diseases Research Center, and Department of Microbiology Immunology, Arak University of Medical Sciences. In order to cultivate the bacteria, Nutritive Broth (Merck, Darmstadt) and Plate Count Agar (Difco, Detroit) were used. Salt solution (0.75%) was used to make serial dilutions until 10^{-5}. In this experiment fresh bacterial cultures were applied. Except for the sole exposition to the electromagnetic fields, both control and exposed cultures were kept in the same conditions. In order to quantify the results, the number of colony-forming units (CFU) was counted. To expose the samples with the electromagnetic fields, first, the samples were poured into the glass tubes and were placed on a nonconductive stand electromagnetic generator (homemade) along the axis of the coils, then the tubes were introduced inside the solenoid during exposure times from 0 h to 6 h. So as to reduce uncertainty in our measurements, each test was performed up to 4 times, independently, by keeping the same experimental conditions to obtain reliable results.

Results

Statistical Analysis:

The electromagnetic fields' effect on the bacterium growth was demonstrated by counting colony-forming units on the Muller Hinton agar medium. The CFU/mL for the control group, the bacteria with the control dilution of 10^{-5}-10^{-7}, 0.5 McFarland standards, was more than that of bacterium exposed to the electromagnetic fields. The CFU/mL for the 10^{-5} and 10^{-6} of 0.5 McFarland standards dilution of *E.coli* in the 50 Hz electromagnetic field was 27 and 12, respectively, while this amount for the control group was

720 and 480. To show the CFU/ml difference between the two groups, the logarithm on 10 bases was used to demonstrate the minor changes between the two groups more obvious. Also, the difference between 10^{-5} and 10^{-7} of 0.5 McFarland standard dilution was big and the logarithm has made this difference small. On the other hand, the effect of the electromagnetic field on the bacterium growth was so small and the logarithm has made this difference big. The data analysis was performed and the results are shown in figure 2. The one way ANOVA ststistical test showed this difference (P-value < 0.05) in the electromagnetic field's effect on *E-coli* bacterium growth. In other hand this effect is not significant in *S. aureus* bacterium growth (P-value > 0.05).

Discussion

Electrical devices have a major role in human life and their effects on different biological structures have been discussed in recent years. In the current study, we investigated the effect of electromagnetic field intensity with different frequencies. *E. coli* and *S. aureus* bacteria, gram-negative and gram-positive bacteria, respectively, were selected for this study. These two bacteria can be found in abundance in nature; in addition, they cause various infections including diarrhea, wound infection and septicemia in humans (13, 14). After placing *E. coli* in an electromagnetic field of 1mT, the number of colonies was increased in comparison to the control group that had not been exposed to the electromagnetic field. This result can indicate the significant effects of electromagnetic fields on microorganisms. Furthermore, in both electromagnetic field intensities,1 and 2 milliseconds, the bacterial growth was increased as a result of increasing in electromagnetic field frequency, the effect would be less on the growth of bacteria. In *S. aureus*, ELF-EMF, also, increased bacterial growth in comparison to the control group although the difference was lower than that of *E. coli*. On the other hand, different electromagnetic frequencies had no significant effect on bacterial growth. The present study shows that the electromagnetic fields affect the gram-positive and gram-negative bacteria in different ways. The reason for this phenomenon can lie in the different structures of the cell wall of the gram-positive and gram-negative bacteria. Gram-positive bacteria appear to be less sensitive to electromagnetic waves due to the high thickness of the cell wall caused by the peptidoglycan layers while the cell wall and cytoplasmic membrane structure are completely different in gram-negative bacteria such as *E. coli*. Of course, it should be noted that this is an initial hypothesis, and it is necessary to carry out extensive studies to prove this hypothesis. The effect of electromagnetic waves should be measured on different gram-positive and gram-negative bacteria. The results obtained in the present study are in line with the results obtained by Nawrotek and his colleagues. In their study, *E. coli* and *S. aureus* bacteria which were pretreated with electromagnetic waves showed a significant increase in cell proliferation and survival in comparison to the control group. Therefore, it can be implied that the presence of these bacteria in the vicinity of the electromagnetic field can make it difficult to treat the diseases caused by these pathogens.

Limitations

The Electrical devices that were used in the present study were homemade although they had a major effect on the study. This step can be considered as the main limitation of the study design.

Abbreviations

Exposure to Low-Frequency Electromagnetic Fields (ELF-EMF)

Declarations

Ethics approval and consent to participate

The study was approved in the Ethical Committee of Arak University of Medical Sciences and the ethic approval code is IR.ARAKMU.REC.1397.846. All of the participants in the study gave informed consent prior to enrolment in the study.

Consent for publication

Not Applicable.

Availability of data and material

Please contact the corresponding author (F.S) for data requests.

Competing interests

The authors declare that they have no conflict of interests.

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Authors' contributions

All authors read and approved the final manuscript.

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Figures

Figure 1

Electromagnetic system

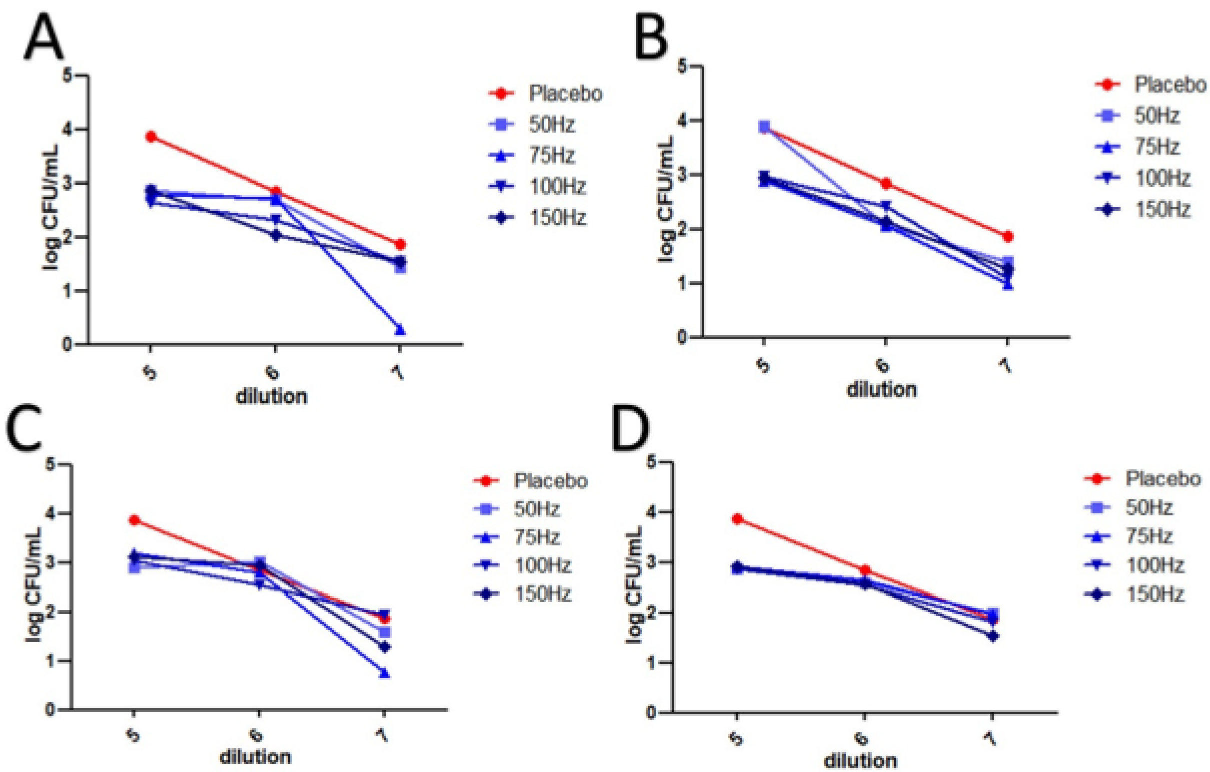

Figure 2

The electromagnetic (1 MT) field’s effect on the bacterium growth on A) E. coli and B) S. aureus The electromagnetic (2 MT) field’s effect on the bacterium growth on C) E. coli and D) S. aureus