International Journal of Biomedicine 12(2) (2022) 293-298
http://dx.doi.org/10.21103/Article12(2)_OA17

ORIGINAL ARTICLE

Experimental Biology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Antibacterial Control of an Extremely Low Frequency Electric Field on *Escherichia coli*

Amr A. Abd-Elghany[1,2]; Ebtesam A. Mohamad[2]; Fadel M. Ali[2]; Ahmed M. M. Yousef[1]; Marwa Samy[2]; Nawal Ayman[2]; Seham AbdelFatah[2]; Eslam Atef[2]; Mohamed A. El-Sakhawy[3,4]

[1]*Radiology and Medical Imaging Department, College of Applied Medical Sciences, Prince Sattam Bin Abdul-Aziz University, Alkharj, Saudi Arabia*
[2]*Biophysics Department, Faculty of Science, Cairo University,Cairo, Egypt*
[3]*Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Prince Sattam Bin Abdul-Aziz University, Al-Kharj, Saudi Arabia*
[4]*Department of Medicinal and Aromatic Plants, Desert Research Center, Cairo, Egypt*

Abstract

The aim of this study was to investigate the electric field frequency and the time of exposure that causes maximum inhibition of *Escherichia coli* (*E. coli*) growth.

Methods and Results: Bacterial suspensions were subjected to an extremely low frequency electric field (ELFEF) with a 0.1 Hz interval between 0.2 Hz and 0.4 Hz. The bacterial growth was observed through optical density (OD) readings. OD values were taken every hour for four hours to monitor bacterial growth in both exposed and unexposed samples. The antibiotic susceptibility test was done to determine the difference between the susceptibility of both exposed and unexposed bacterial samples. Structural changes in the exposed bacterial samples were monitored by transmission electron microscope (TEM). The bacterial growth curve revealed a highly significant growth inhibition after being exposed to 0.2 Hz at 2 hours' exposure time. *E. coli* suspension exposed to ELFEF at inhibition frequency 0.2 Hz showed a significant increase in susceptibility to antibiotics Keflex, meropenem, and piperacillin-tazobactam.

Conclusion:The current data suggest that treating E. coli with 0.2 Hz for 2 hours is an effective, prospective, and novel technique for reducing cellular growth and dramatic alteration in the cell membrane. TEM clarified the great destruction of the bacteria cell wall. (**International Journal of Biomedicine. 2022;12(2):293-298.**)

Key Words: extremely low frequency electric field • *E. coli* • optical density • transmission electron microscope

For citation: Abd-Elghany AA, Mohamad EA, Ali FM, Yousef AMM, Samy M, Ayman N, AbdelFatah S, Atef E, El-Sakhawy MA. Antibacterial Control of an Extremely Low Frequency Electric Field on *Escherichia coli*. International Journal of Biomedicine. 2022;12(2):293-298. doi:10.21103/Article12(2)_OA17

Abbreviations

ELFEF, extremely low frequency electric field; **OD**, optical density; **TEM**, transmission electron microscope.

Introduction

There is an interest in applications of a pulsed electromagnetic field with different frequencies in the medical field, such as pain control, enhancing the efficacy of anticancer drugs, cancer cell proliferation and apoptosis, and transdermal delivery of low permeant drugs.[1-4] Previous studies on extremely low frequency electromagnetic waves succeeded in controlling the growth of Ehrlich tumors.[5,6] It was shown that extremely low frequencies from 6 Hz up to 500 Hz radiation can process wound repair,[7,8] skin, and bones.[7-10] Cellular activity of microorganisms has recently been controlled using

extremely low electromagnetic waves with a very low field strength that resonate with bioelectric signals created during a specific metabolic activity.[11,12] The exposure of Agrobacterium tumefaciens (the common cause of crown gall disease in plants) to 1 Hz square amplitude modulating waves for 90 minutes changed its cellular activity and DNA structure, inhibiting growth and affecting virulence.[13]

Extremely low frequency electric field (ELFEF) effects on bacteria have been investigated. Depending on the bacterial strain and the physical parameters such as frequency and field strength, it has been demonstrated that ELFEF can negatively or positively affect cell growth, viability, and bacterial antibiotic sensitivity.[14,15]

The category of microorganisms includes a massive range of organisms, including bacteria, fungi, viruses, algae, archaea, and protozoa.[16] Some microorganisms that are seen to be beneficial to health are termed probiotics and are available as dietary supplements, or food additives.[17] But some microorganisms, particularly bacteria, are harmful to plants, animals, or humans by attacking their cells and causing disease, for example, Salmonella, E. coli, and MRSA.[18] There is a type of bacteria that can be harmful or useful for the human body, such as E. coli, which normally dwell in the intestines of humans. It can also be found in the intestines of some animals.[19]

The majority of E. coli strains are safe and even beneficial to the digestive system. Some strains like enterotoxigenic E. coli (ETEC), enteropathogenic E. coli (EPEC), and Shiga toxin-producing E. coli (STEC), can cause breathing issues, pneumonia, diarrhea, and urinary tract infections. E. coli is responsible for 75 to 95% of urinary tract infections.[20] Healthy persons who have been infected with E. coli typically feel better within a week. However, some people have a significant kidney issue known as hemolytic uremic syndrome.[21] The elderly and children are more likely to be affected.[22] The traditional and effective method to destroy bacteria and other harmful microorganisms in the body is antibiotics, such as Amoxicillin, Doxycycline, Cephalexin, Ciprofloxacin, Clindamycin, Gentamycin, and Azithromycin.[23]

Bacteria often develop resistance to antibiotics; therefore, there is a constant search for alternative ways to combat harmful bacteria. The application of a pulsed electric field will help in preventing bacterial resistance. It has been reported that stimulation or inhibition of bacterial growth is dependent on the field strength of the electric field radiation and types of bacteria.[24-26]

Therefore, this research studied the effects of electric field frequencies on bacterial growth at different time exposures, as well as the effects of the chosen range of electric field frequency (0.2-0.4H z) on bacteria. The optical density (OD), which is a measure of the quantity of light absorbed by a bacterial suspension, was used to determine the growth in the organism's cell mass. The TEM examined the ultrastructure of the bacteria.

Materials and Methods

Throughout the study, test organism E. coli (ATCC 27853) was obtained from the plant and microbiology department, Faculty of Science, Cairo University, Giza, Egypt. The bacterial strain was subcultured on nutrient agar plates.

Preparation of bacterial suspension

To prepare broth subculture, two cultured colonies of bacteria grown on a nutrient agar plate were inoculated into 5ml sterile nutrient broth of pH 7.4±0.2 (Lab M Limited, UK) in a test tube and incubated at 37°C for 24 hours (incubator of plue pard, PH050A, Italy). After adjusting the bacterial cell concentration to the 0.5 McFarland standard,(27) a standardized bacterial suspension of 20µl (1.5×108 CFU/ml) was distributed into 1.5ml broth media tubes: 1.5ml of broth medium (organism-free) was used as a blank tube (negative control), 1.5ml of broth media seeded with the microorganism was used as a positive control, and a corresponding number of test tubes were produced to be subjected to various frequencies; each experiment was performed in triplicate, with the average taken into account. The tubes were cultured for 24 hours at 37°C in a shaking (20 rpm) incubator (Orbital/ Reciprocal Shaking Water Bath, Boekel). Spectrophotometer measurements of OD at 560nm per hour were used to create growth curves for bacterial cell cultures (Humalyzer Primus manufactured for Human GmbH. Germany).

Experimental setup

The exposure system was constructed at the Biophysics Department, the Cairo University, to develop an electric field generator with a frequency range of 0.1-20 Hz and frequency intervals of 0.1 Hz. For each frequency, the duty cycle was increased by 10% each time until there was a 100% variation. When 12V was utilized as an input source, the microcontroller was automated to manage a power supply circuit (Venus Scientific INC, NY) that can generate high output voltage up to 1500V of direct current signals. During the exposure duration, two parallel copper electrodes were utilized to conduct the signal, and the sample was positioned between them as shown in Figure 1.

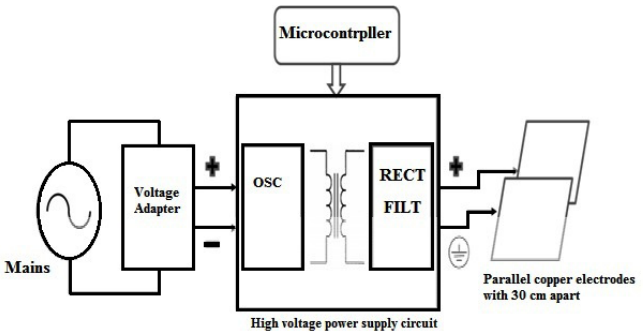

Fig. 1. *Schematic diagram for the exposure device.*

Investigation of the inhibition frequency of the E. coli bacteria

The standardized bacterial suspension (Luria Broth) was used to make the cultured broth medium, which was then incubated at 37°C for 24 hours. Subsequently, the bacteria were divided into two groups: a control group that has not been exposed and a group that has been exposed to ELFEF in the frequency range (0.2-0.4Hz) with an interval frequency of 0.1 Hz. OD values were taken every hour for four hours

to monitor bacterial growth in both exposed and unexposed samples.

Determination of the optimal exposure time

The standardized bacterial suspension was exposed to an electric field at 0.2 Hz for different exposure time ranges from 0.5 h to 2.5 h with an interval of 0.5 h. The samples were incubated at 37°C at the end of the exposure duration. The samples were shaken every hour and the absorbance was recorded.

Antibiotic susceptibility test

Antimicrobial Drugs

Susceptibility testing was determined by three distinct antibiotics: Keflex (K), piperacillin-tazobactam (PTZ), and meropenem (MEM) against *E. coli* ATCC 27853 bacterial strain.

Antibacterial Tests

The disc diffusion method was used to determine *E. coli* susceptibility to these antibiotics. The fresh incubated *E. coli* was suspended in sterilized saline (0.9% NaCl) until it was adjusted to an 0.5 McFarland turbidity standard, resulting in a suspension of 1.5×10^8 CFU/ml. *E. coli* suspension was separated into two groups, control (unexposed) and exposed, to ELFEF for 2 hours at inhibition frequency 0.2 Hz. After the exposure period (2h), bacterial *E. coli* suspensions of two groups were streaked on Mueller Hinton agar (MHA) plates. With sterile forceps, the appropriate antibiotic discs were placed on the agar surface; mild pressure was applied over the surface of each disc to ensure contact. The plates were incubated for 24 hours at 37°C. After that, each inhibition zone's diameter was measured by millimeters of the growth inhibition zones. All tests were performed in triplicate.

Dielectric relaxation measurements for the bacterial cells

The dielectric measurements were carried out for the samples in the frequency range 10Hz-MHz using a Loss Factor Meter (type: HIOKI 3532 LCR Hi TESTER, version 1.02,1999, Japan), with a sample cell (PW 9510/60, Philips). The sample cell had two squared platinum black electrodes of 0.64 cm^2 area (A), which were separated 1 cm apart (d). The capacitance of the samples was measured at each frequency and the resistance was recorded at room temperature. The absolute dielectric increment (ε_o), the relative permittivity (έ), loss tangent (tan δ), dielectric loss (ε″), conductivity (C) and relaxation time (τ) of the samples were calculated for each frequency using the following relations;

$\varepsilon' = C\,d / \varepsilon_o A$ Eq. (1)

$(\delta) = 1/2\pi f C$ Eq. (2)

$\varepsilon'' = \varepsilon'(\delta)$ Eq. (3)

$\tau = 12\pi f_c$ Eq. (4)

$\sigma = 2\pi f\, \varepsilon''\varepsilon_o$ Eq. (5)

where f_c is the critical frequency corresponding to the mid-point of dispersion curves.

Morphological examination by TEM

TEM (JEM-1400; JEOL Ltd., Akishima, Tokyo, Japan) was used to determine the morphological changes in *E. coli* caused by exposure to an electric field at the most effective frequency (0.2 Hz) for the most effective time (2 h). The bacteria sample was centrifuged and the pellet was passed through some processing according to Demicheli et al.[28] CCD camera model AMT, an optronics camera with 1632×1632 pixels, was used to capture the images for the processed bacterial samples.

Statistical analysis was performed using the statistical software package SPSS version 16.0 (SPSS Inc, Chicago, IL). Variables were presented as the mean (M) and standard deviation (SD). Multiple comparisons were performed with one-way ANOVA. A probability value of $P<0.05$ was considered statistically significant.

Results

Inhibition frequency of the *E. coli* bacteria

The bacterial growth curve of groups subjected to electric pulses of frequencies in the range 0.2-0.4 Hz was compared to that of the control group (Figure 2). After the groups were exposed to 0.2 Hz, the findings revealed a highly significant ($P<0.05$) growth inhibition. The changes in absorbance as a function of the applied frequency between bacterial groups exposed to the range 0.2-0.4 Hz and the control group are shown in Figure 3. When compared to the control, the highest growth inhibition ($P<0.05$) occurred after exposure to 0.2 Hz.

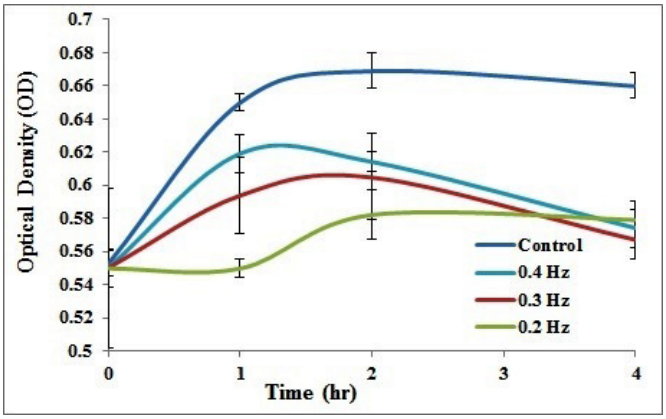

Fig. 2. *The growth curve for control (unexposed group) and exposed bacterial samples for 0.2, 0.3, and 0.4 Hz.*

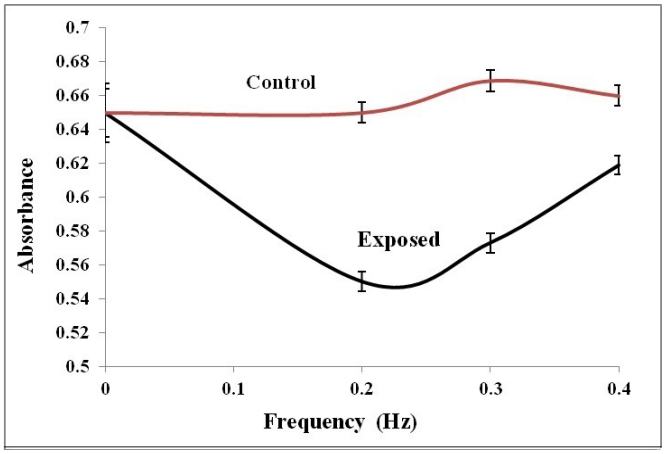

Fig. 3. *The variation in absorbance as a function of frequency after 12 hours of incubation compared to the control.*

Determination of exposure time

The growth inhibition of *E. coli* following exposure to 0.2Hz for various exposure times was studied (Figure 4). Two-hour treatment resulted in the greatest growth inhibition (P<0.05) at the specified frequency (0.2 Hz).

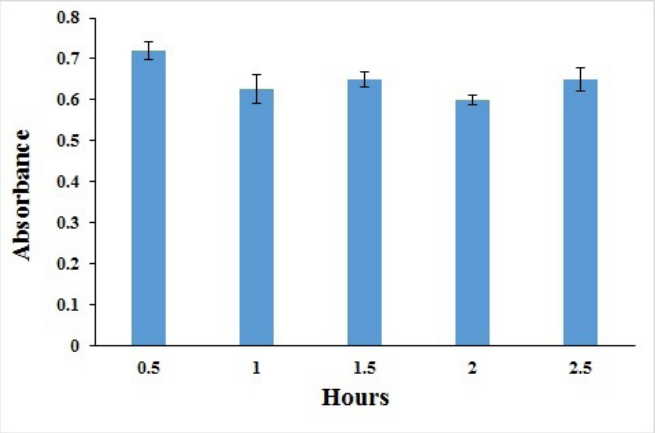

Fig. 4. *The exposure time for inhibition against OD. The samples were exposed for different exposure times ranging from 0.5 h. to 2.5 h with an interval of 0.5 h.*

The antibiotic sensitivity test

Table 1 demonstrates the antibiotic sensitivity for unexposed and exposed *E. coli* to 0.2 Hz for 2 hours, revealing a substantial difference between the unexposed (control) and exposed samples. Samples exposed to 0.2 Hz exhibited high, significant sensitivity (P<0.01) to antibiotics Keflex, Meropenem, and Piperacillin-tazobactam (cell wall synthesis inhibitors) (Table 1). All antibiotics (K, MEM, and PTZ) used in this study showed antibacterial activity against exposed and unexposed test organism *E. coli* (Table 1). On the other hand, the activity of the antibiotics was remarkably increased against *E. coli* exposed to ELFEF, compared to unexposed *E. coli*. Antibacterial activity of Keflex showed activity (21.5 mm and 17.5 mm) against exposure to ELFEF and unexposed *E. coli,* respectively. Meropenem showed activity (20 mm and 10 mm) against exposure to ELFEF and unexposed *E. col;*, finally, Piperacillin-tazobactam showed activity (19.5 mm and 16.0 mm) against exposed and unexposed *E. coli,* respectively.

Table 1.
The average diameter in mm of the inhibition zones of antibiotics against unexposed and exposed E. coli to ELFEF (0.2 Hz for 2 h)

Test organism	Mean of inhibition zone diameter (mm)		
	Antibiotics		
	K	MEM	PTZ
E. coli	17.50±0.27	10.00±0.10	16.00±0.24
Exposed *E. coli* to 0.2 Hz ELFEF	21.50±0.22	20.00±0.19	19.50±0.16

K, Keflex; MEM, Meropenem; PTZ, Piperacillin-tazobactam

The dependence of dielectric properties on ELFEF

Table 2, represented the values of the relaxation time (τ), the dielectric increment (Δέ), and the electric conductivity (σ) for the control and exposed sample. The obtained data indicated a high increase (P<0.05) in the relaxation time, dielectric increment, and conductivity for the exposed sample, compared to the control.

Table 2.
The relaxation time (τ), dielectric increment (Δέ) and conductivity (σ) of control and exposed samples to ELFEF (0.2 Hz for 2 h)

Test organism	Dielectric parameter		
	Relaxation time (τ) ($\times 10^{-6}$sec)	$\Delta\grave{\varepsilon}=(\grave{\varepsilon}0 - \grave{\varepsilon}_{\infty})$	Conductivity (σ) At 1 MHz ($\times 10^{-3}$ S/M)
E. coli control	2.2±0.26	1900.05 ±0.96	47.83 ±0.63
Exposed *E. coli* to 0.2 Hz PEF	4.9±0.67	5159.43 ±0.53	167.44 ±0.33

Investigation of morphological changes by TEM

TEM pictures of control *E. coli* cells are shown in Figure 5 (a,b), demonstrating that the cells' contents are un-damaged and their shape is preserved. The cells have a smooth outer membrane and a well-preserved cell envelope. Furthermore, the cells displayed binary fission, indicating active metabolic processes. Figure 5 (c,d) shows TEM images of cells subjected to 0.2 Hz, which demonstrate breakdown and disintegration of the cell wall as well as aberrant septation.

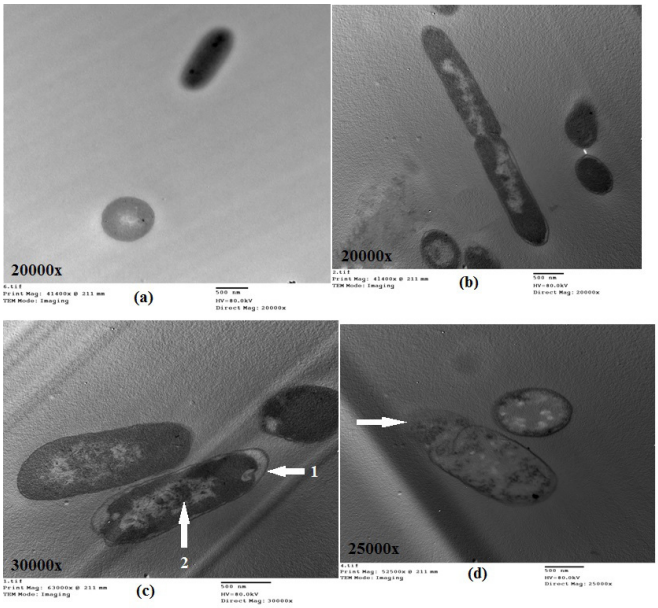

Fig. 5. (a-d) *TEM images of exposed and unexposed E. coli ATCC 27853 to ELFEF (0.2 Hz for 2 h). Transmission electron micrographs of (a) Unexposed (normal) E. coli a normal morphology and had a smooth and compact cell membrane with homogeneous electron density in the cytoplasm, (b) Unexposed E. coli cells undergoing binary fission, (c) Exposed E. coli demonstrating deformation of bacterial cell as the cytoplasm shrinks (arrow 1). The nucleus is completely damaged (arrow 2), (d) Exposed E. coli exhibit cell-wall disruption, damage to the cell membrane, and leakage of intracellular cytoplasmic constituents was observed (arrow).*

Discussion

Plotting cell growth (absorbance) versus incubation time can be used to investigate bacterial growth. It was possible to obtain a sigmoid standard growth curve. Thus, increasing the turbidity of the broth medium indicated an increase in the *E. coli* cell mass. When *E. coli* was placed into a freshly prepared medium, it took time to adapt to the new surroundings. This phase was called the Lag phase, in which cellular metabolism was accelerated, cells grew larger, but the bacteria were unable to multiply, and therefore cell mass was maintained. During the log phase, *E. coli* was growing rapidly. All their metabolic activity was increased, and the bacterial cells duplicated at a consistent pace by binary fission. The maximum amount of growth medium was used, the culture attained its maximum rate of growth, and the number of bacteria increased logarithmically (exponentially). It is known that *E. coli* split every 20 minutes, resulting in a 20-minute generation time. *E. coli* consumes all of the nutrients in the growth medium as the bacterial population grows, resulting in fast multiplication. As a result, waste products, harmful metabolites, and inhibitory chemicals like antibiotics accumulate in the medium. This changes the medium's parameters, such as pH and temperature, making the environment unsuitable for bacterial growth. The bacterium's reproduction rate will drop down until it eventually stops dividing entirely. The cell enters the stationary phase of its life cycle.

The current study presented a novel strategy for inhibiting the growth of hazardous *E. coli* strains by utilizing a low-frequency electric field at an inhibitory frequency. The current findings revealed that exposing *E. coli* to 0.2 Hz for 2 hours resulted in highly significant growth inhibition. The low-frequency, inhibitory impact of the electric field was caused by interference with bioelectric signals created by the bacterial cells' physiological processes, which disrupted the physiological process in progress.[29] It could also be linked to pH changes, antimicrobial agent synthesis, electrophoretic transfer of antimicrobial compounds into the biofilm,[30] hyperoxygenation, or the formation of extra biocide ions. Changes in cellular membrane permeability or structure may result in the loss of cell inter-constituents and/or the penetration of extracellular elements into the cell. The main source of the extremely substantial increase in electric charges is assumed to be changed in the charge distribution on the protein molecules of the cellular membrane, which can be markers of structural changes in the cellular membrane.

Antibiotic sensitivity tests involving inhibition of cell wall synthesis revealed a variation in diameter of the inhibition zone significantly, approving the influence of the inhibitory frequencies on the cell wall. Keflex, Meropenem, and Piperacillin-tazobactam act as cell wall synthesis inhibitors. All antibiotics (K, MEM, and PTZ) used in this study showed antibacterial activity against exposed and unexposed test organism *E. coli* (Table 1). On the other hand, the activity of the antibiotics was remarkably increased against *E. coli* exposed to ELFEF in comparison to unexposed *E. coli*. In previous studies, Meropenem displayed antibacterial activity as cell wall inhibitors against Enterobacteriaceae (*E. coli* and *Pseudomonas aeruginosa*); moreover, Piperacillin/tazobactam was less active against Enterobacteriaceae but not *P. aeruginosa*. Keflex (cephalexin) showed moderate activities against *E. coli* isolated from children with urinary tract infections. According to the results of A. Abduzaimovic et al.,[31] the sensitivity of *E. coli* to cefuroxime was 89.87%, ciprofloxacin - 89.24%, gentamicin - 89.24%, cefalexin - 87.97%. *E. coli* resistance to ciprofloxacin was 9.49%, gentamicin - 8.86%, cephalexin - 8.23%.

TEM images showed the rupture of the cell wall at 0.2Hz, allowing the observed loss of intercellular contents. The results of dielectric properties indicated a pronounced increase in the average values of the dielectric increment, the relaxation time, and the conductivity for exposed samples. The relaxation time and electrical conductivity are directly related to the macromolecular electric dipole moment which in turn is dependent on the size and charge of the macromolecule and thus, the significant changes in dielectric relaxation may be due to the redistribution of cellular and molecular charges. It has been verified that ELF-EMF can affect membrane functions. The frequency range used in the present study was extremely low, compared to the previous studies that used 50Hz with a moderate effect on *E. coli*.[32,33]

Conclusion

The current data suggest that treating *E. coli* with 0.2 Hz for 2 hours is an effective, prospective, and novel technique for reducing cellular growth and dramatic alteration in the cell membrane. It has the feature of being non-invasive, rapid, safe, and inexpensive when compared to conventional therapies, and it may be used to treat human illnesses and to pasteurize and sterilize food products. Future in vivo investigations on the implementation of this approach to assess its suitability as a biophysical therapy for *E. coli* infection will be possible based on the results of this research.

Acknowledgments

This publication was supported by the Deanship of Scientific Research at Prince Sattam Bin Abdulaziz University.

Competing Interests

The authors declare that they have no competing interests.

References

1. Shupak NM, Prato FS, Thomas AW. Human exposure to a specific pulsed magnetic field: effects on thermal sensory and pain thresholds. Neurosci Lett. 2004 Jun 10;363(2):157-62. doi: 10.1016/j.neulet.2004.03.069.
2. Rageh MM, El-Garhy MR, Mohamad EA. Magnetic

***Corresponding author:** Dr. Amr A. Abd-Elghany. Radiology and Medical Imaging Department, College of Applied Medical Sciences, Prince Sattam Bin Abdulaziz University, Alkharj, Saudi Arabia. Biophysics Department, Faculty of Science, Cairo University, Egypt. E-mail: amrabdelghany25@gmail.com*

fields enhance the anti-tumor efficacy of low dose cisplatin and reduce the nephrotoxicity. Naunyn Schmiedebergs Arch Pharmacol. 2020 Aug;393(8):1475-1485. doi: 10.1007/s00210-020-01855-9.
3. Akbarnejad Z, Eskandary H, Vergallo C, Nematollahi-Mahani SN, Dini L, Darvishzadeh-Mahani F, Ahmadi M. Effects of extremely low-frequency pulsed electromagnetic fields (ELF-PEMFs) on glioblastoma cells (U87). Electromagn Biol Med. 2017;36(3):238-247. doi: 10.1080/15368378.2016.1251452.
4. Amr A. Abd-Elghany, Ebtesam A. Mohamad. Ex-vivo Transdermal delivery of Annona Squamosa Entrapped in Niosomes by Electroporation. Journal of Radiation Research and Applied Sciences 2020; 13 (1): 164–173.
5. Ali FM, El-Gebaly RH, Hamad AM. Combination of bacteriolytic therapy with magnetic field for Ehrlich tumour treatment. Gen Physiol Biophys. 2017 Jul;36(3):259-271. doi: 10.4149/gpb_2016051.
6. Fadel MA, El-Gebaly RH, Aly AA, Ibrahim FF. Control of Ehrlich Tumor Growth by Electromagnetic Waves at Resonance Frequency (In Vivo Studies). Electromagnetic Biology and Medicine 2005;24(1):9-21. doi: 10.1081/JBC-200054263.
7. Milgram J, Shahar R, Levin-Harrus T, Kass P. The effect of short, high intensity magnetic field pulses on the healing of skin wounds in rats. Bioelectromagnetics. 2004 May;25(4):271-7. doi: 10.1002/bem.10194.
8. Athanasiou A, Karkambounas S, Batistatou A, Lykoudis E, Katsaraki A, Kartsiouni T, Papalois A, Evangelou A. The effect of pulsed electromagnetic fields on secondary skin wound healing: an experimental study. Bioelectromagnetics. 2007 Jul;28(5):362-8. doi: 10.1002/bem.20303.
9. Ahmadian S, Zarchi SR, Bolouri B. Effects of extremely-low-frequency pulsed electromagnetic fields on collagen synthesis in rat skin. Biotechnol Appl Biochem. 2006 Feb;43(Pt 2):71-5. doi: 10.1042/BA20050086.
10. Akpolat V, Celik MS, Celik Y, Akdeniz N, Ozerdem MS. Treatment of osteoporosis by long-term magnetic field with extremely low frequency in rats. Gynecol Endocrinol. 2009 Aug;25(8):524-9. doi: 10.1080/09513590902972075.
11. Ali FM, Elgebaly RH, Elneklawi MS, Othman AS. Role of duty cycle on Pseudomonas aeruginosa growth inhibition mechanisms by positive electric pulses. Biomed Mater Eng. 2016 Aug 12;27(2-3):211-25. doi: 10.3233/BME-161577.
12. Fadel MA, Mohamed SA, Abdelbacki AM, El-Sharkawy AH. Inhibition of Salmonella typhi growth using extremely low frequency electromagnetic (ELF-EM) waves at resonance frequency. J Appl Microbiol. 2014 Aug;117(2):358-65.
13. Fadel MA, El-Gebaly RH, Mohamed SA, Abdelbacki AMM. Biophysical control of the growth of Agrobacterium tumefaciens using extremely low frequency electromagnetic waves at resonance frequency. Biochem Biophys Res Commun. 2017 Dec 9;494(1-2):365-371. doi: 10.1016/j.bbrc.2017.10.008.
14. Cellini L, Grande R, Di Campli E, Di Bartolomeo S, Di Giulio M, Robuffo I, Trubiani O, Mariggiò MA. Bacterial response to the exposure of 50 Hz electromagnetic fields. Bioelectromagnetics. 2008 May;29(4):302-11.
15. Belyaev I. Toxicity and SOS-response to ELF magnetic fields and nalidixic acid in E. coli cells. Mutat Res. 2011 May 18;722(1):56-61. doi: 10.1016/j.mrgentox.2011.03.012.
16. Schopf JW. Fossil evidence of Archaean life. Philos Trans R Soc Lond B Biol Sci. 2006 Jun 29;361(1470):869-85.
17. Dixit Y, Wagle A, Vakil B. Patents in the Field of Probiotics, Prebiotics, Synbiotics: A Review. Journal of Food: Microbiology, Safety & Hygiene 2016; 01(02).
18. Fuchs JG. Chapter 11. Interactions between Beneficial and Harmful Microorganisms: From the Composting Process to Compost Application. In: Insam H. et al., editors. Microbes at Work. Springer –Verlag Berlin Heidelberg. 2010;213-229.
19. Köhler CD, Dobrindt U. What defines extraintestinal pathogenic Escherichia coli? Int J Med Microbiol. 2011 Dec;301(8):642-7. doi: 10.1016/j.ijmm.2011.09.006.
20. Subashchandrabose S, Mobley HLT. Virulence and Fitness Determinants of Uropathogenic Escherichia coli. Microbiol Spectr. 2015 Aug;3(4):10.1128/microbiolspec.UTI-0015-2012. doi: 10.1128/microbiolspec.UTI-0015-2012.
21. Fakhouri F, Zuber J, Frémeaux-Bacchi V, Loirat C. Haemolytic uraemic syndrome. Lancet. 2017 Aug 12;390(10095):681-696. doi: 10.1016/S0140-6736(17)30062-4. Epub 2017 Feb 25. Erratum in: Lancet. 2017 Aug 12;390(10095):648.
22. Ruggenenti P, Noris M, Remuzzi G. Thrombotic microangiopathy, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. Kidney Int. 2001 Sep;60(3):831-46. doi: 10.1046/j.1523-1755.2001.060003831.x.
23. Goldwater PN, Bettelheim KA. Treatment of enterohemorrhagic Escherichia coli (EHEC) infection and hemolytic uremic syndrome (HUS). BMC Med. 2012 Feb 2;10:12. doi: 10.1186/1741-7015-10-12.
24. Hunt RW, Zavalin A, Bhatnagar A, Chinnasamy S, Das KC. Electromagnetic biostimulation of living cultures for biotechnology, biofuel and bioenergy applications. Int J Mol Sci. 2009 Nov 20;10(10):4515-58. doi: 10.3390/ijms10104515.
25. Cellini L, Grande R, Di Campli E, Di Bartolomeo S, Di Giulio M, Robuffo I, et al. Bacterial response to the exposure of 50 Hz electromagnetic fields. Bioelectromagnetics. 2008 May;29(4):302-11. doi: 10.1002/bem.20391.
26. Kohno M, Yamazaki M, Kimura I I, Wada M. Effect of static magnetic fields on bacteria: Streptococcus mutans, Staphylococcus aureus, and Escherichia coli. Pathophysiology. 2000 Jul;7(2):143-148. doi: 10.1016/s0928-4680(00)00042-0.
27. JORDEN J. Textbook of Diagnostic Microbiology: Edited by C.R. MAHON and G. MANUSELIA. 1995. ISBN 0-7216-4028-1. Philadelphia, Pa: WB Saunders; 1995. Journal of Medical Microbiology 1996;45(5):391-391.
28. Demicheli MC, Goes AM, de Andrade AS. Ultrastructural changes in Paracoccidioides brasiliensis yeast cells attenuated by gamma irradiation. Mycoses. 2007 Sep;50(5):397-402.
29. Leonard DA, Bonomo RA, Powers RA. Class D β-lactamases: a reappraisal after five decades. Acc Chem Res. 2013 Nov 19;46(11):2407-15. doi: 10.1021/ar300327a.
30. Mohammad EA, Elshemey WM, Elsayed AA, Abd-Elghany AA. Electroporation Parameters for Successful Transdermal Delivery of Insulin. Am J Ther. 2016 Nov/Dec;23(6):e1560-e1567.
31. Abduzaimovic A, Aljicevic M, Rebic V, Vranic SM, Abduzaimovic K, Sestic S. Antibiotic Resistance in Urinary Isolates of *Escherichia coli*. Mater Sociomed. 2016 Dec;28(6):416-419. doi: 10.5455/msm.2016.28.416-419.
32. Segatore B, Setacci D, Bennato F, Cardigno R, Amicosante G, Iorio R. Evaluations of the Effects of Extremely Low-Frequency Electromagnetic Fields on Growth and Antibiotic Susceptibility of Escherichia coli and Pseudomonas aeruginosa. Int J Microbiol. 2012;2012:587293. doi: 10.1155/2012/587293.
33. Oncul S, Cuce EM, Aksu B, Inhan Garip A. Effect of extremely low frequency electromagnetic fields on bacterial membrane. Int J Radiat Biol. 2016;92(1):42-9. doi: 10.3109/09553002.2015.1101500.