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ORIGINAL ARTICLE

THE EFFECT OF 2.4 GHZ ELECTROMAGNETIC RADIATION ON THE GROWTH AND VIABILITY OF *ESCHERICHIA COLI*

Richard Galajda[1]*, Dagmar Mudroňová[2], Lucia Selnekovičová[1], Marián Maďar[2], Ján Molnár[3]

[1]Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia; [2]Department of Microbiology and Imunology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia; [3]Department of Theoretical and Industrial Electrical Engineering, FEI TUKE, Technical University in Košice, Košice, Slovakia

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***Correspondence:** richard.galajda@uvlf.sk

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

The impact of radiofrequency electromagnetic field (RF-EMF) on living organisms is an important and current topic of scientific research, especially in the context of the growing use of wireless technologies. The aim of this study was to investigate the effects of non-ionizing electromagnetic radiation with a frequency of 2.4 GHz on the growth and viability of a selected model organism – *Escherichia coli*. The study was aimed at analyzing how exposure to this frequency affects the growth and viability of bacteria. The samples were exposed to RF-EMF for 2, 4, and 24 hours, then stained and analyzed by flow cytometry. The results showed a statistically significant inhibition of bacterial growth during the first 2 hours of exposure compared to the control sample. However, no significant differences were observed at the 4- and 24-hour time points. This effect is likely due to the selection of resistant bacterial subpopulations, reflecting the rapid life cycle and high adaptability of *Escherichia coli* to environmental stress. These findings suggest that short-term exposure to 2.4 GHz RF-EMF can temporarily suppress bacterial growth but also demonstrate the role of adaptive responses during long-term exposure.

Key words: *Escherichia coli*; exposure; growth; RF-EMF; viability

INTRODUCTION

Electromagnetic radiation

Electromagnetic radiation can be divided into ionizing and non-ionizing based on wavelength, frequency, source, and also photon energy. The limit for ionization of an atom or molecule is generally accepted as a photon energy of 10 electron volts (eV), which after conversion corresponds to 124 nm of wavelength. Electromagnetic radiation (RF-EMF) includes the electromagnetic spectrum: gamma rays, X-rays, ultraviolet radiation, visible radiation, infrared radiation, microwave radiation, and radio waves [1] (Fig.1).

Living organisms have adapted exclusively to natural electromagnetic radiation from natural sources during

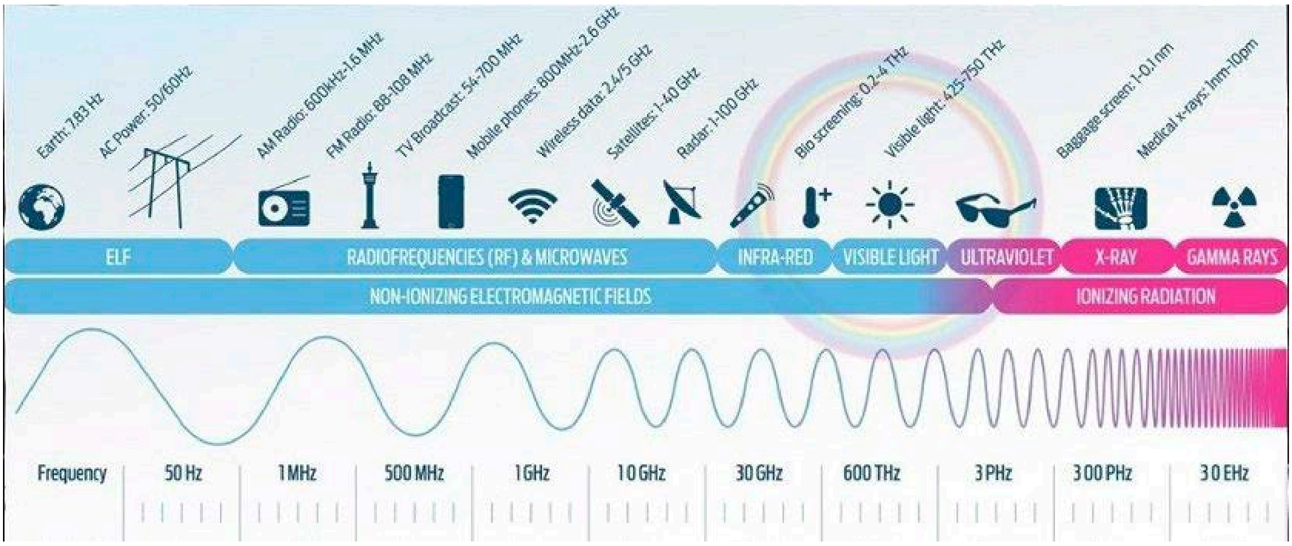

Fig. 1. Distribution of electromagnetic radiation from several aspects

evolution, which suggests that artificial RF-EMF are foreign to biological systems and may have a beneficial effect in some cases, but more often an adverse effect [2].

Thermal and non-thermal effects of electromagnetic radiation

The effects of electromagnetic radiation on living organisms can be divided into two categories: thermal and non-thermal. Thermal effect is caused by an increase in temperature upon absorption of energy, while non-thermal effect occurs without this heating and is more complex, including the induction of electrical currents, cell stimulation, and various interactions at the molecular level. Although the division is somewhat artificial, thermal effects are better understood and documented, while non-thermal effects are the subject of ongoing research and debate, as their mechanisms and health consequences are not fully understood [3, 4, 5].

Wi-Fi and routers

The term "Wi-Fi" refers to a wireless local area network that allows devices to be connected wirelessly using non-ionizing radiation at various frequencies, mainly 2.4 GHz and 5 GHz. Wi-Fi is used to exchange data and access the Internet in various environments from homes to public spaces. A Wi-Fi router is an electronic device that functions as a router and a wireless access point. Depending on the manufacturer and model, it can work in a wired local area network, in a wireless network, or in a mixed network. The most important parameter of a router is the frequency band and the number of channels. The difference between the 2.4 GHz and 5 GHz frequencies lies in the number of channels that a Wi-Fi router uses in its operation, which affects the stability and speed of the network connection [6, 7]. The 2.4 GHz frequency band is 70 MHz wide, and devices are usually limited to three 20 MHz channels. The advantage is a longer range and better overcoming obstacles (walls); the disadvantages include slower data throughput under heavy load and fewer available channels. The 5 GHz Wi-Fi spectrum is approximately 500 MHz wide, and devices can use up to six larger 80 MHz channels. The advantage is the high speed and reliability of the connection, suitable for e.g. streaming; the disadvantages include shorter range and worse overcoming obstacles (walls) [7]. Exposure limits for Wi-Fi radiation are set by international organizations such as ICNIRP in order to protect people from potentially harmful effects [8].

Effects of Wi-Fi on living organisms

Wi-Fi uses electromagnetic radiation at frequencies of 2.4 GHz and 5 GHz to transmit data. Although it is non-ionizing radiation, which does not have enough energy to directly damage DNA like ionizing radiation (e.g., X-rays), its potential effects on living organisms are the subject of scientific research. The potential health effects of wireless technologies such as WiFi and 3G/4G signals are the subject of various research studies [9, 10, 11].

Current findings indicate negative effects of acute and long-term exposure in animal models, including changes in the cardiovascular system, behavioral changes, the occurrence of oxidative stress, and cell damage [12]. Regarding the effects in humans, systematic reviews of studies do not confirm harmful effects of WiFi at levels below the regulatory limits applicable to protect human health. The current state of knowledge on this issue presents diverse and not always consistent research results [13,14].

Several studies have shown that Wi-Fi radiation can affect different types of bacteria. It has been found that shorter exposures can stimulate the growth and production of lactic acid in probiotic bacteria, while longer and continuous exposure can increase antibiotic resistance, as well as motility and biofilm formation in some pathogenic bacteria. The overall effects depend on the type of bacteria and the duration or frequency of radiation [15, 16, 17].

In our study, we set out to find out how 2.4 GHz Wi-Fi affects the growth and viability of the bacterium *Escherichia coli*.

MATERIALS AND METHODS

The experiment used the Network Flood software in a setup consisting of a Mikrotik hAP ac3 router and two HP ProBook laptops. Due to the precisely set transmission speed between the two laptops, a constant data transfer was established between these devices. (Fig. 2A). This setting resulted in the router being forced to operate in constant transmission conditions. This achieved a precisely defined electromagnetic radiation between the two antennas of the router, which operated at a frequency of 2.4 GHz and a radiated power density of 0.062 W. In the experiment, we installed the router in a closed space of the thermostat and placed bacterial cultures between the antennas of the router (Fig. 2B).

To determine the lifespan, number of bacteria, and oxidative stress after exposure to RF-EMF, a suspension of *Escherichia coli* (CCM3954) was used, which was cultured for 24 hours at 37 °C on Endoagar (Himedia, India) (Fig. 2C). After collecting the colonies into tubes with sterile physiological solution and filtered PBS (phosphate buffered saline) (MP Biomedicals, France) through a 0.2 µm filter, the optical density was adjusted to 0.5 McFarland (DEN-1 McFarland Densitometer, Biosan). Subsequently, a control sample (KVz) and a sample exposed to RF-EMF (EVz) were created by adding 100 µl of the sample to 900 µl of BHI broth (Brain-Heart Infusion broth; Himedia, India). The control and exposed samples were created in triplicate to determine the number of bacteria. The numbers were determined before the experiment (0 h) and then after 2 h, 4 h, and 24 h of RF-EMF exposure by the standard 10-fold dilution plating method on Endoagar. The agar plates were cultured at 37 °C for 24 h, and *E. coli* counts are expressed as colony-forming units per milliliter (CFU/ml). Subsequently, we determined the viability of the bacteria and oxidative damage, which we analyzed before exposure to 0 h and then after 2 h and 4 h of exposure.

Bacterial viability was determined by staining with carboxyfluorescein diacetate (cFDA; Sigma Aldrich, USA) [18, 19]. Staining procedure: 2.5 µl of 1 mM cFDA and 222.5 µl of PBS (phosphate-buffered saline; MP Biomedicals, France) were added to 25 µl of bacterial samples diluted 1:100 in PBS. The sample was incubated for 30 minutes at 37 °C in a water bath. Oxidative damage to bacterial membranes was assessed using the fluorescent dye BODIPY C11 [20]. BODIPY C11 is an analogue of fatty acids, which can be incorporated into the cell membranes. In its unoxidized form, it produces red fluorescence, which shifts to bright green upon undergoing peroxidation. Ten µl of a 20 µM BODIPY 581/591 C11 solution of 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (Molecular Probes, ThermoFisher Scientific, Scientific) was added to 25 µl of bacterial samples (diluted 1:100 in PBS). After 15 min incubation at 37 °C, 1.F5 µl of PI (1 mg/ml) was added and incubated for another 15 min. After incubation, 200 µl of PBS was added, and the samples were analyzed. Analysis was performed on a BD FACSCantoTM flow cytometer using BD FACS DivaTM software (Fig. 3). The location of the bacteria was delineated on a dot plot of forward scatter (FSC-A) versus side scatter (SSC-A) (Fig. 4), and the bacterial viability as well as oxidative damage of the membranes were determined on the histograms for green fluorescence using fluorescein isothiocyanate (FITC-A) versus counts (Fig. 5). Statistical analysis of cell viability was performed using an unpaired T-test within individual samples in the statistical program GraphPad Prism version 3.00.

Fig. 2. A- Set of equipment used in the experiment, B- Placement of the exposed sample in the thermostat between the router antennas, C- *E. coli* cultured on Endoagar

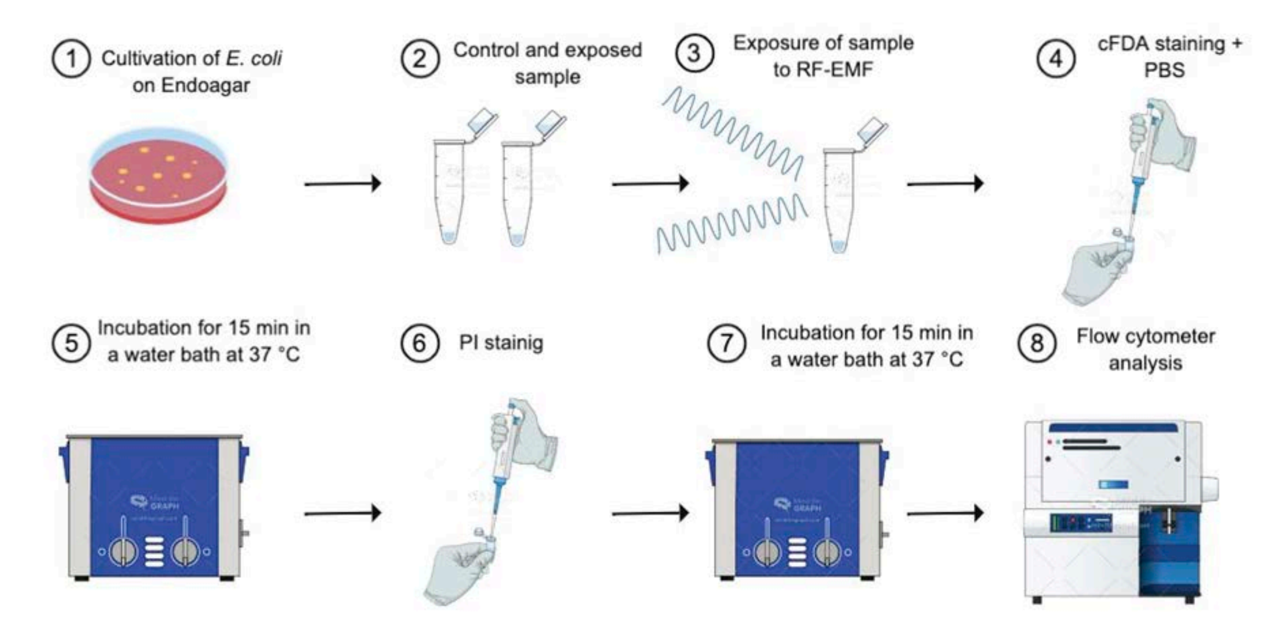

Fig. 3. Scheme of the experimental methodology

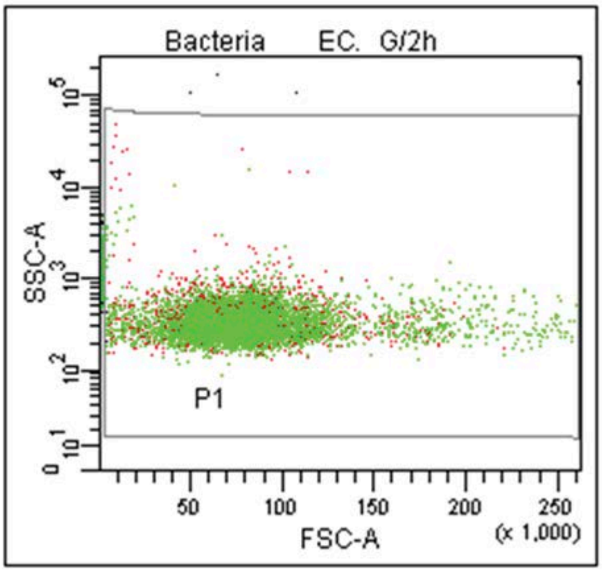

Fig. 4. Delineation of the position of bacteria (P1) on a basic dot plot showing size (FSC-A) versus granularity (SSC-A)

RESULTS

Based on the above-described experiment, the samples were analyzed on a flow cytometer. Before RF-EMF exposure, we measured the bacterial counts: control sample (KVz) 1.97 x 10^6 /ml and exposed sample (EVz) 1.97 x 10^6 /ml. After two hours of RF-EMF exposure, there was a significant decrease in the number of *E. coli*: control sample 1.1 x 10^7 /ml and exposed sample 4.73 x 10^6 /ml. After four hours of exposure, the values of the number of *E. coli* ranged from 1.0 x 10^7 /ml in the control sample to 1.85 x 10^7 /ml in the exposed sample. Then after twenty-four hours of exposure, we measured the value of 2.0 x 10^9 /ml in the control sample and 6.15 x 10^8 /ml in the exposed sample (Tab. 1, Fig. 6). So after two hours of exposure, we

Table 1. ***E. coli*** **counts expressed as cfu/ml before and after exposure to 2.4 GHz radiation**

E. coli	0 h	2 h	4 h	24 h
KVz	1.97×10^6 /ml	1.1×10^7 /ml	1.0×10^7 /ml	2.0×10^9 /ml
EVz	1.97×10^6 /ml	4.73×10^6 /ml	1.85×10^7 /ml	6.15×10^8 /ml

KVz – control sample, EVz – exposed sample

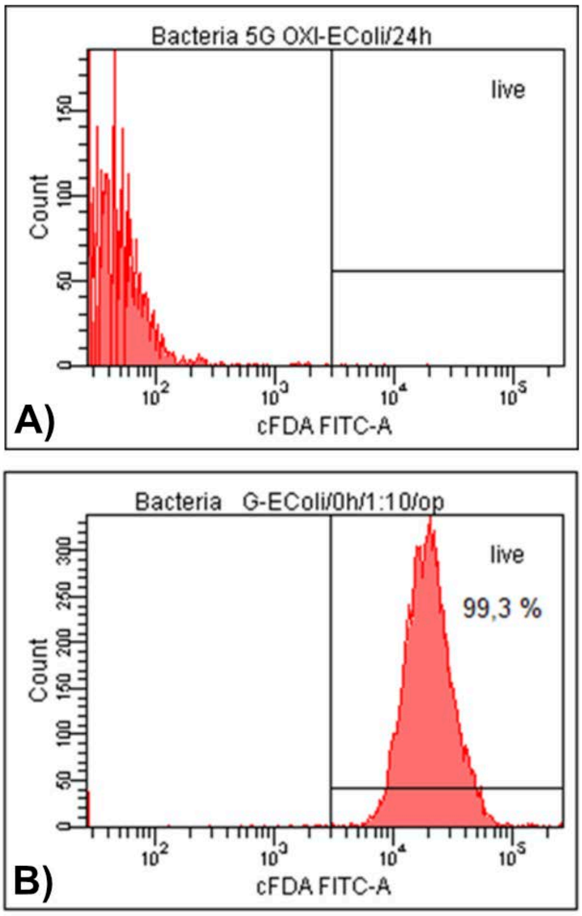

Fig. 5. Bacterial viability determined on the histograms for green fluorescence (FITC-A) versus count, A) negative sample – unstained; B) live bacteria – 0 h

observed a significant negative effect on *E. coli*, but then after four hours of exposure, we did not see a significant difference in the number. The result showed that resistant *E. coli* were selected, and in the following multiplication cycles (the rate of cell division of *E. coli* is very high – on average once every 30 min), their number began to increase again. After twenty-four hours of RF-EMF exposure, the exposed sample began to overtake the control sample in number.

Analysis of oxidative damage in *E. coli* recorded the largest increase after 2 h of RF-EMF exposure, up to 7.5%, but after 4 h of exposure it decreased again to 1.1% (Tab. 2, Fig. 7).

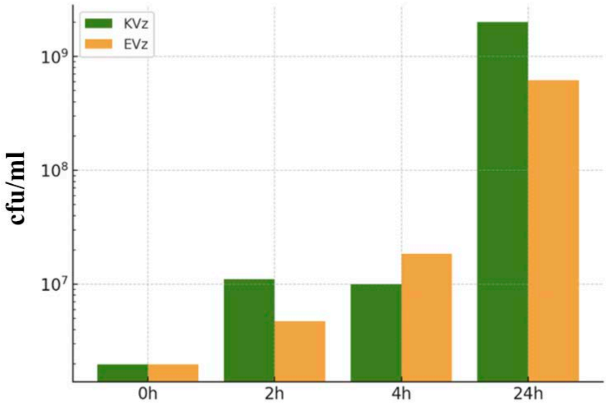

Fig. 6. ***E. coli*** **counts expressed as cfu/ml before and after exposure to 2.4 GHz radiation, KVz – control sample, EVz – exposed sample**

Table 2. Percentage expression of oxidative stress in ***E. coli*** **before and after exposure to 2.4 GHz radiation**

E. coli	0 h	2 h	4 h
KVz	0.2%	2%	1.6%
EVz	0.2%	7.5%.	1.1%

KVz – control sample, EVz – exposed sample

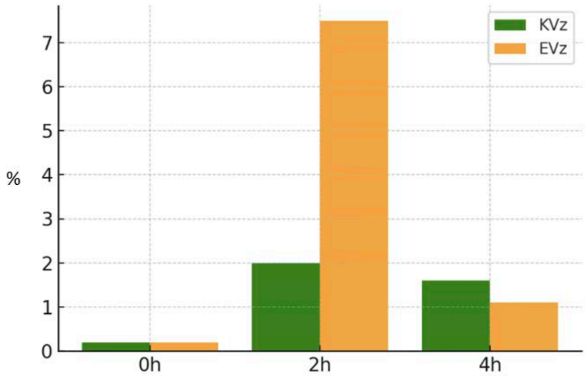

Fig. 7. Percentage expression of oxidative stress in ***E.coli*** **before and after exposure to 2.4 GHz radiation, KVz – control sample, EVz – exposed sample**

Table 3. Percentage expression of ***E. coli*** **viability before and after 2.4 GHz exposure**

E. coli	0 h	2 h	4 h
KVz	99.3 %	72.0 ± 1.35	51.9 ± 2.16
EVz	99.3 %	59.4 ± 2.16	27.5 ± 0.33

KVz – control sample, EVz – exposed sample

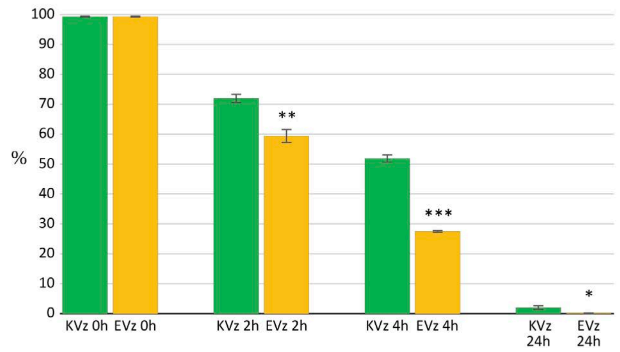

Fig. 8. Percentage of viable *E. coli* before and after exposure to 2.4 GHz radiation
* significantly different from control (** $p<0.01$, * $p<0.05$, *** $p<0.001$)

When analyzing the viability of E. coli, we observed a decrease after 2 h of RF-EMF exposure of approximately 12.6% and after 4 h of exposure of approximately 24.4% (Tab. 3, Fig. 8, 9, 10, 11, 12, 13).

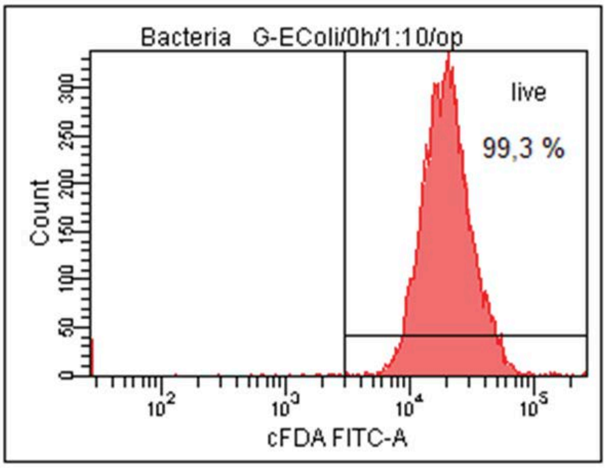

Fig. 9. Histogram of the proportion of live bacteria emitting green fluorescence after staining with cFDA (FITC-A), at initial values (0 h)

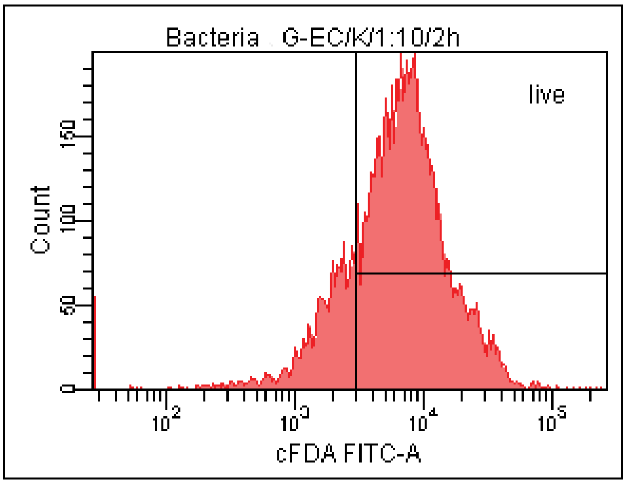

Fig. 10. Histogram of the proportion of live bacteria emitting green fluorescence after staining with cFDA (FITC-A), in the control sample after 2 h

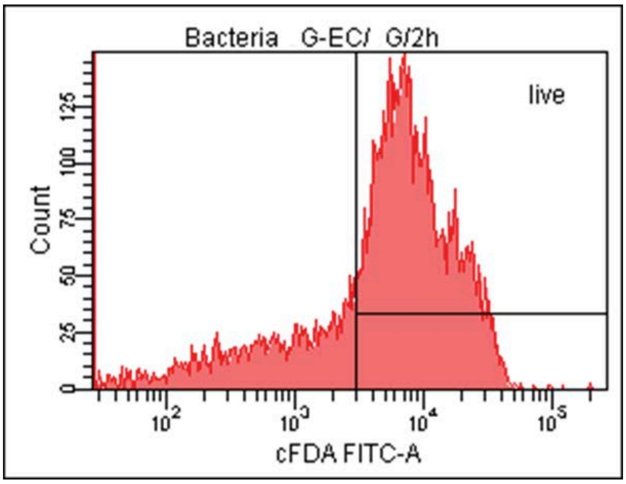

Fig. 11. Histogram of the proportion of live bacteria emitting green fluorescence after staining with cFDA (FITC-A), in the exposed sample after 2 h

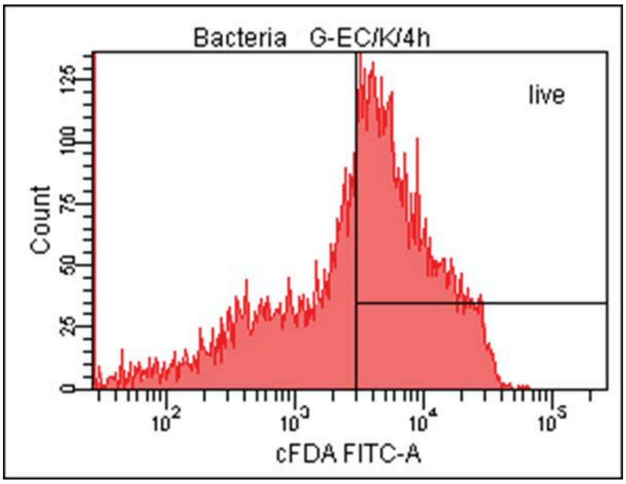

Fig. 12. Histogram of the proportion of live bacteria emitting green fluorescence after staining with cFDA (FITC-A), in the control sample after 4 h

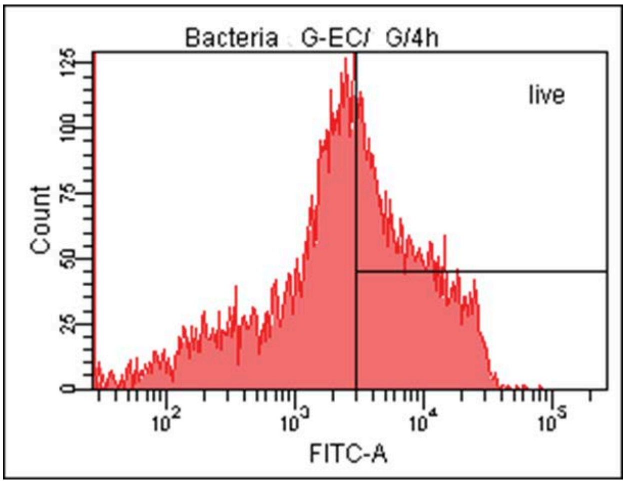

Fig. 13. Histogram of the proportion of live bacteria emitting green fluorescence after staining with cFDA (FITC-A), in the exposed sample after 4 h

DISCUSSION

The router works by constantly emitting electromagnetic radiation, but only in standby mode, which is characterized by low radiation power. Only when it receives a request from a device that wants to automatically or manually connect to the Wi-Fi network is this connection established, and the router then switches to the standard transmission power, which, however, depends on the speed of the transmitted data, i.e., it is not constant. However, these posed a problem in carrying out the experiment, because in order to assess the impact of electromagnetic radiation on bacteria, it was necessary for the radiation to be constant, at a standard wavelength. This problem was solved in cooperation with experts from the Faculty of Electrical Engineering and Informatics (FEI) of the Technical University of Košice (TUKE), who developed the Network Flood software.

RF-EMF can have different effects on bacteria depending on the frequency, intensity, length of exposure, and type of bacteria. The main effects of RF-EMF on bacteria include changes in growth and proliferation, with some studies suggesting that RF-EMF can decelerate bacterial growth [21], while others show its acceleration [22].

In several studies, changes in structure and morphology have been observed, where it has been shown that RF-EMF can disrupt the cell wall and membrane of bacteria, which can lead to cell weakening or death [23, 24]. In other cases, changes in the size and shape of bacteria, oxidative stress, and the formation of reactive oxygen species (ROS) were registered, where RF-EMF can induce the formation of ROS, which causes oxidative stress and can lead to damage to DNA, proteins, and lipids [25]. Some research suggests that RF-EMF can affect the expression of genes associated with bacterial resistance to antibiotics. RF-EMF has been shown to alter the sensitivity of some microorganisms to various antibiotics [26]. An effect on biofilm formation has also been observed, where RF-EMF can affect the ability of bacteria to form biofilms that provide them with protection from adverse conditions. In some cases, a weakening of biofilms has been noted, while in others, their formation has increased [15, 17, 27]. The effects of RF-EMF on bacteria are therefore complex and depend on many factors. Some studies point to negative effects such as cell damage and growth inhibition, while others suggest the possibility of bacterial adaptation and increased resistance. The results of research in this area are still ambiguous and require further detailed studies.

Key exposure parameters include exposure duration, frequency band (Hz), and power density (Watt). These variables determine the reaction of bacteria to RF-EMF, and, in general, whether the thermal or non-thermal effect of this type of radiation will be the priority depends primarily on the power density and water content in the living organism [28]. The most commonly used Wi-Fi transmitters (routers) currently use the permitted frequency bands of 2.4 GHz and 5 GHz, which are characterized by a very low level of power density. For example, the router used in our experiment is capable of operating in two frequency bands, either simultaneously or only in one of them. The first frequency band at 2.4 GHz has a radiated power density of 0.062 W and the second frequency band at 5 GHz has a density of 0.077 W [6].

From the above, it follows that in our study we can with great probability exclude the thermal effect of RF-EMF, for which many times higher power density values are required. When comparing the effect of RM-EMF exposure on *E. coli* in our experiment with the results of other authors, we could only work with a limited number of relevant professional publications, both due to the absence of extensive research in this area and because other authors had different exposure parameters set in their experiments.

When setting up the experiment, we tried to determine the so-called exposure window, with statistically significant growth inhibition compared to the control. For this reason, we also performed measurements after 2 and 4 hours, and subsequently, after evaluation, we found that the exposure window was located somewhere in the time interval from the beginning of exposure to 4 hours of exposure. Only in measurements after 2 hours of exposure did we observe a significant difference in the number of bacteria (about 0.5 log) between the experimental and control samples, while in measurements after 4 hours and 24 hours of exposure, the numbers between the experimental and control samples were approximately the same. This result can be explained by the fact that resistant bacteria were probably selected in the further course of the experiment, which is related to the fast life cycle of *E. coli* [29, 30]. This property allows for rapid adaptation to the environment, and therefore, in subsequent multiplication cycles, the numbers in the control and experimental samples equalized and after 24 hours were even slightly higher than in the control sample. The exposure windows where specific RF-EMF parameters cause maximum biological effects are also mentioned in Pall's work [14]. There are several mechanisms that could have contributed to the inhibition of *E. coli* growth, and given the aforementioned inconsistency of the study results, we cannot clearly state which one was decisive. However, they were most likely related to changes in cell membrane morphology, where the study results are best documented [31, 32, 33].

Our observations also confirm the results of similar experiments with *E. coli* after exposure to RF-EMF, which revealed that 88% of the cells survived [23]. The authors conclude that one of the effects of exposing *E. coli* cells to RF-EMF under sublethal temperature conditions is that the cell surface undergoes a modification that is electrokinetic in nature, leading to reversible RF-EMF-induced porosity of the cell membrane. Scanning electron microscopy (SEM) analysis performed immediately after RF-EMF exposure revealed that *E. coli* cells displayed a cell morphology significantly different from that of negative controls. However, this effect appeared to be temporary, as after another 10 minutes, the cell morphology appeared to return to a state that was identical to that of the control sample. Confocal laser scanning microscopy (CLSM) revealed that dextran (150 kDa) conjugated with fluorescein isothiocyanate (FITC) was taken up by *E. coli* cells after exposure to RF-EMF, indicating that pores were formed in the cell membrane [23].

The effect of RF-EMF on cell membrane integrity was also demonstrated by the results of another study conducted on *E. coli*. The aim of this study was to investigate the effects of RF-EMF at a frequency of 2.45 GHz on *Escherichia coli* cell membranes under different conditions with the average temperature of the cell suspension maintained at 37 °C in order to investigate possible thermal versus non-thermal effects of short-term exposure. For this purpose, exposure of bacteria to RF-EMF was carried out under well-defined and controlled parameters, such that the average temperature of the bacterial cell suspensions was maintained at 37 °C. *Escherichia coli* cells were exposed to discontinuous radiation for different periods of time. For each experiment, conventional heating in a water bath at 37 °C was performed as a control. The effects of RF-EMF exposure on cell membranes were investigated using flow cytometry after staining the cells with propidium iodide and also by assessing intracellular protein release in bacterial suspensions. Thermal characterization indicated that the temperature reached by the samples exposed to RF-EMF during the specified contact time was not high enough to explain the measured modifications in cell membrane integrity [24]. In another study with *E. coli*, where a mobile phone simulator emitting radiation at a frequency of 900 MHz and a router emitting radiofrequency radiation at a frequency of 2.4 GHz were used as sources of electromagnetic radiation, RF-EMF was shown to alter the sensitivity of microorganisms to various antibiotics. Pure cultures of *Listeria monocytogenes* and *Escherichia coli* were exposed to RF-EMF, and the results showed that exposure to RF-EMF within a narrow exposure level (exposure window) renders microorganisms resistant to antibiotics. The findings of this study demonstrated that exposure to RF-EMF can significantly alter the inhibition zone diameters and growth rates for *L. monocytogenes* and *E. coli*. These findings may have implications for the management of serious infectious diseases [26]. Therefore, this adaptive phenomenon and its potential threats to human health should be further investigated in future experiments.

CONCLUSIONS

The study investigates the various biological effects of Wi-Fi radiation (RF-EMF) on microorganisms, emphasizing that these effects depend on the type of bacteria and exposure parameters such as duration, frequency, and power density. In our experiment, we investigated the effect of Wi-Fi radiation on *Escherichia coli*. The experiment with measurements after 2, 4, and 24 hours showed that after 2 hours of exposure there was a growth inhibition (0.5 log), which was not detected in later measurements, probably due to the selection of resistant bacteria. Our results showed the existence of an exposure window at low power densities and excluding the thermal effect, in which RF-EMF causes maximum biological effects.

The exact mechanisms of action of radiofrequency electromagnetic fields on microorganisms are not yet fully understood, and the results of studies are sometimes inconsistent. Research in this area is continuously improving our understanding of adaptive responses of microorganisms and potential health risks in the form of increased bacterial resistance to antibiotics, which can have a significant impact on healthcare.

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Conflict of Interest

We declare that all listed authors are without a Conflict of Interest.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

This study did not require any Ethical approval (Directive 2010/63/EU).

Authors' Contributions

All authors contributed to the study revision. Material preparation, data collection and analysis were performed by R.G., D.M., L.S., M.M. and J.M. All authors read and approved the final manuscript.

Generative AI Statement

The authors declare that no generative AI was used in the creation of this manuscript.

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