Effects of Radiofrequency Electromagnetic Fields Emitted from Mobile Phones and Wi-Fi Router on the Growth Rate and Susceptibility of *Enterococcus faecalis to Antibiotics*

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ABSTRACT

Background: During the last decade, people have been dramatically exposed to radiation emitted from widely-used radiofrequency electromagnetic fields (RF-EMF) generating devices.

Objective: This study aimed to evaluate the effects of exposure to RF-EMF emitted from smart phones and Wi-Fi routers on the growth rate and antibiotic sensitivity of *Enterococcus faecalis (E. faecalis)* as a pathogen in the root canals of teeth.

Material and Methods: In this experimental study, *E. faecalis* ATCC 19115 was used, characterized and confirmed by morphological and biochemical tests. Antibiotic susceptibility test was measured for several common antibiotics. To perform antibiotic susceptibility tests, disk diffusion (Kirby-Bauer) method on Mueller-Hinton agar plates was used before and after exposure to RF-EMFs emitted from a commercial Wi-Fi router or a mobile phone simulator. Moreover, we measured the optical density at 625 nm after different exposure times using a calibrated UV-visible spectrophotometer to evaluate the effect of RF-EMF exposure on the bacterial growth rate.

Results: Exposure to RF-EMF significantly altered the antimicrobial sensitivity of the *E. faecalis*. While, the susceptibility of the bacteria decreased significantly after 6 h of exposure, longer exposure time (e.g. exposure for 24 h) increased the susceptibility of the bacteria to all antibiotics. Furthermore, it was found that the bacteria tended to regress to their early state. Moreover, the non-exposed *E. faecalis* showed a slower growth rate than the bacteria exposed to RF-EMFs.

Conclusion: Exposure to RF-EMF emitted by Wi-Fi routers or mobile phone simulator can significantly change the antibiotic susceptibility and growth rate of *E. faecalis*.

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Keywords

Anti-Bacterial Agents; *Enterococcus faecalis*; Radiofrequency; Electromagnetic Fields

Introduction

During the last decade, dramatic exposure to electromagnetic fields (EMF) generated by various satellite, telecommunication systems, cellular phones, microwave ovens, and military radars has been observed due to greater use of technology. It has been demonstrated that very small doses of EMF can affect the biological functions of living organisms such as bacteria. The external EMF can change the membrane processes and metabolic state of bacteria and their response to chemical factors and antibiotics. The bacterial resistance to antibiotics is on increase. Therefore, evaluation of the EMF effects on bacteria seems to be necessary to investigate

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the antibiotic resistance pattern correlation with the possibility of controlling bacteria in the environment or in the clinical laboratories. EMF may be useful in therapeutic practices, controlling the sensitivity of bacteria toward antibiotics and also investigating the effect of environmental stress on biological systems.

The biological effects of EMFs usage were considered in 1976 for the first time. It has been clearly revealed that EMF can negatively or positively affect bacterial growth and antibiotic sensitivity depending on EMF wavelength, intensity, coherence, exposure duration, the type of bacterial cells, bacterial growth phase, and composition of growth media. *Enterococcus faecalis (E. faecalis)* is a microorganism detected in asymptomatic, persistent endodontic infections with a prevalence of 24% to 77% [1]. It has a pathogenic role in chronic endodontic treatment failure [2]. Several studies have been conducted on the biological effects of EMF on different microorganisms such as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, Klebsiella pneumoniae, Dictyostelium discoideum, Kaposi's sarcoma-associated virus, Paramecium, *Enterococcus hirae*, and *Entamoeba invadens* [3-12]. In spite of this, to the best of our knowledge, our experiment is the first study on the effects of RF-EMFs on *E. faecalis*. Given this consideration, the present study aimed at investigating the effects of exposure to RF-EMFs generated by mobile phones and Wi-Fi devices on the growth rate and antibiotic susceptibility of *E. faecalis*.

Material and Methods

Antibiotic Susceptibility Test

In this experimental study, *E. faecalis* ATCC 19115 was used, characterized, and confirmed by morphological and biochemical tests. Mueller-Hinton Broth (MHA-Biolife, Italy) was used to dilute the pure culture of *E. feacalis* and then grown to reach 0.5 McFarland turbidity standards. The bacterial suspension was cultured on the plates, treated with a set of 10 antimicrobial substance disks, and incubated at 35 °C for 24 h (overnight condition). The test was carried out by the disk diffusion method (Kirby-Bauer method) on Mueller-Hinton agar plates according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2016).

Antimicrobial Agents

Antibiotic disks used for *E. faecalis* tests were Imipenem (IMI 10 µg), Levofloxacin (LEV 5 µg), Ciprofloxacin (CIP 5 µg), Piperacillin (PIP 100 µg), Doxycycline (DOX 30 µg), Azithromycin (AZI 30 µg), Vancomycin (VAN 30 µg), Ampicillin (AMP 10 µg), Amoxicillin (AMOX 30 µg), and Tetracyclin (TET 30 µg).

All antibiotic disks were purchased from ROSCO Diagnostica (DK-2630 Taastrup, Denmark). We measured and analyzed the antibiotic susceptibility both before and after exposure to RF-EMFs generated by Wi-Fi and RF simulator. The diameter of the inhibition zone for each antibiotic was calculated as the average of at least 2 different measurements. Moreover, for each regime, three replicate agar plates were used.

Wi-Fi Router

The exposure source in this study was a D-Link Wi-Fi router (D-Link, D-Link Corporation, Taiwan). During the exposure time, data were exchanging at a constant rate between the modem and a laptop computer. The laptop was placed in another room 5 meters away from the Wi-Fi router. The Wi-Fi router was operating at 1W and the specific absorption rate (SAR) was 0.13 W/kg. Each bacterial sample was collected after a specific exposure time (2, 4, 6, 8, 10, and 24 h after the start of exposure), and the antibacterial susceptibility tests were carried out.

Radiofrequency Simulator

A GSM 900/1800 MHz mobile simulator was used in this study as the radiation source. This simulator was designed and manufactured at the Department of Medical Physics and Biomedical Engineering of Shiraz Medical School by the active cooperation of the private sector.

Outgrowth Curve

To evaluate the effects of RF-EMF exposure on the growth rate of bacteria, the optical density (OD) at 625 nm [13] was measured using a UV-visible Spectrophotometer (UNICO UV-2100 Spectrophotometer, UNICO, USA) and the

growth curve was drawn.

Statistical Analysis

For both exposure and control groups, all experiments were replicated three times. The non-parametric Mann-Whitney U test was used to compare the means. Moreover, the statistical significance of the differences observed among the means was determined using SPSS (version 18). P-values below 0.05 were considered statistically significant.

Results

In the present study, antimicrobial susceptibility of *E. faecalis* to several common antibiotics after exposure to 900 MHz and 2.4 GHz radiofrequency radiation was carried out. The antimicrobial susceptibility was recorded as an inhibition zone diameter for each antibiotic disk. Besides, Figures 1 and 2 showed the effects of radiofrequency (RF) radiation on the growth rate of the bacteria for the exposed and non-exposed bacteria. The antimicrobial sensitivity of the bacteria was altered during exposure to RF, but at the 6th h of exposure, a significant decrease in the susceptibility of *E. faecalis* was seen ($P < 0.05$).

According to Tables 1 and 2, the antimicrobial susceptibility of the bacteria was alterd after exposure to radiofrequency radiation (2.4 GHz), which may be because of changes in their physicochemical characteristics. Hence, the inhibition zone diameters of the exposed and non-exposed *E. faecalis* for each antibiotic were determined at different times after exposure.

As shown in Figures 3 and 4, there were no changes significantly in the bacterial sensitivity after the 3rd and until the 6th h of exposure; however, after the 6th h of exposure, a maximum significant fall in the antibacterial sensitivity of the bacteria was showed. At the more exposure times (24 h exposure), the sensitivity of *E. faecalis* to all antibiotics increased and bacteria returned to the early state. In addition, the effects of radiofrequency radiation on the growth rate of bacteria were carried out (Figures 3 and 4). Based on Figures 3 and 4, bacteria in the non-exposed groups had a slower growth rate than those which were exposed to radiation in the exponential phase.

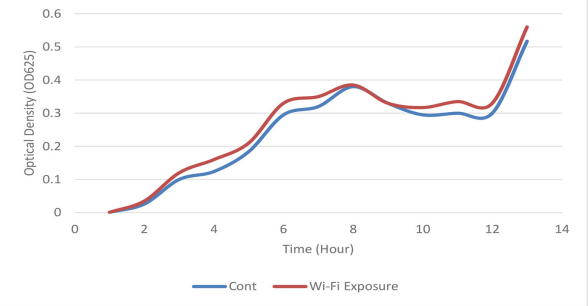

Figure 1: Growth curves of *E. feacalis* before and after exposure to Wi-Fi radiation

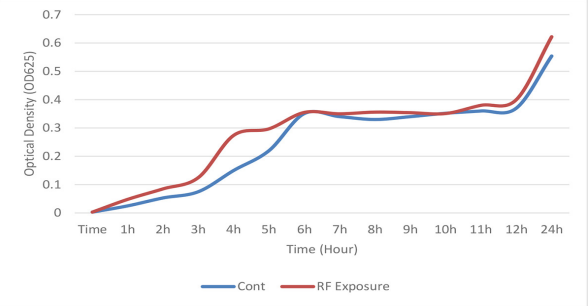

Figure 2: Growth curves of *E. feacalis* before and after exposure to radiofrequency (RF) simulator radiation

Discussion

Altogether the findings of this study are in line with the former studies that aimed at investigating the effects of RF-EMF on the susceptibility of *Klebsiella pneumoniae* [6, 14], *Listeria monocytogenes*, and *E. coli* [7] to antibiotics after irradiation with 2.4 GHz (Wi-Fi router radiation) and 900 MHz (mobile phone simulator) and confirm the existence of the so-called "window theory" [3, 14-18]. According to window theory, when the exposure level (ionizing or nonionizing radiation) lies within the window (lies between the lower and upper levels), exposure can lead to the occurrence of some stimulatory effects.

The effect of EMF is generally dependent on physical characteristics of the radiation applied (frequency, intensity, duration of exposure) and the biological characteristics of the bacteria (cell metabolic state, genotype, membrane proportion, bacterial growth phase). EMF interaction with bacteria can change the metabolic state of the bacteria and sensitivity to chemical agents such as antibiotics. Torgomyan et al. reported a decrease

Table 1: Inhibition zone diameters before and after exposure to Wi-Fi radiation for *E. faecalis*

Exposure Time	Wi-Fi Exposure			
	Drug	Control (Mean ± SD)	Exposure (Mean ± SD)	P Value
2h	PIP	28.6±0.58	28.3±0.58	0.456
	AZI	13±0	12.3±0.58	0.114
	LEV	21.3±0.58	21.3±0.58	1.000
	AMP	26.3±0.58	25.6±0.58	0.197
	VAN	15.3±0.58	14.6±0.58	0.197
	DOX	17.3±0.58	16±0	0.034
	CIP	20.3±0.58	19.3±0.58	0.099
	TET	14±0	14±0	1.000
	IMI	29.3±0.58	27.6±0.58	0.043
	AMOX	31.3±0.58	30.3±0.58	0.099
4h	PIP	28.6±0.58	25.6±0.58	0.043
	AZI	13±0	12.3±0.58	0.114
	LEV	21.3±0.58	20.3±0.58	0.099
	AMP	26.3±0.58	25.3±0.58	0.099
	VAN	15.6±0.58	16±0	0.114
	DOX	17.3±0.58	15.6±0.58	0.043
	CIP	20.3±0.58	18.6±0.58	0.043
	TET	14±0	13.6±0.58	0.317
	IMI	29.3±0.58	27±0	0.034
	AMOX	31.3±0.58	28.3±0.58	0.043
6h	PIP	28.6±0.58	25.6±0.58	0,043
	AZI	13±0	10.6±0.58	0.034
	LEV	21.3±0.58	17.3±0.58	0,043
	AMP	26.3±0.58	23.3±0.58	0.043
	VAN	15.6±0.58	15±0	0.317
	DOX	17.3±0.58	14.6±0.58	0.043
	CIP	20.3±0.58	20.6±0.58	0.456
	TET	14±0	12.3±0.58	0.034
	IMI	29.3±0.58	24.6±0.58	0.043
	AMOX	31.3±0.58	30.3±0.58	0.099
8h	PIP	28.6±0.58	25.6±0.58	0.043
	AZI	13±0	13±0	1.000
	LEV	21.3±0.58	20.3±0.58	0.099
	AMP	26.3±0.58	25.6±0.58	0.197
	VAN	15.6±0.58	14.6±0.58	0.197
	DOX	17.3±0.58	15.6±0.58	0.043
	CIP	20.3±0.58	20±0	1.000
	TET	14±0	14.6±0.58	0.114
	IMI	29.3±0.58	26.3±0.58	0.043
	AMOX	31.3±0.58	26.3±0.58	0.043

Exposure Time	Wi-Fi Exposure			
	Drug	Control (Mean ± SD)	Exposure (Mean ± SD)	P Value
10h	PIP	28.6±0.58	25.6±0.58	0.043
	AZI	13	13	1.000
	LEV	21.3±0.58	20.3±0.58	0.099
	AMP	26.3±0.58	25.3±0.58	0.099
	VAN	15.6±0.58	14.6±0.58	0,197
	DOX	17.3±0.58	16.3±0.58	0.099
	CIP	20.3±0.58	20±0	0.317
	TET	14±0	14.3±0.58	0.317
	IMI	29.3±0.58	27.3±0.58	0.043
	AMOX	31.3±0.58	29.3±0.58	0.043
24h	PIP	28.6±0.58	28.6±0.58	1.000
	AZI	13±0	13±0	1.000
	LEV	21.3±0.58	21.6±0.58	0.456
	AMP	26.3±0.58	26.6±0.58	0.456
	VAN	15.6±0.58	16.6±0.58	0.068
	DOX	17.3±0.58	16.6±0.58	0.197
	CIP	20.3±0.58	18.3±0.58	0.043
	TET	14±0	15±0	0.025
	IMI	29.3±0.58	28.6±0.58	0.197
	AMOX	31.3±0.58	30±0	0.034

PIP: Piperacillin, AZI: Azithromycin, LEV: Levofloxacin, AMP: Ampicillin, VAN: Vancomycin, DOX: Doxycycline, CIP: Ciprofloxacin, TET: Tetracyclin, IMI: Imipenem, AMOX: Amoxicillin

in the bacterial growth specific rate of *E. coli* and *E. hirae*, and an increase in the bacterial sensitivity towards antibiotics [8]. Kamel et al. found that the high-frequency magnetic fields caused a significant decrease in the number of *S. aureus* and increased sensitivity of *S. aureus* to the antibiotic [10]. The inhibitory effect of extremely high-frequency EMF on the growth properties of *E. hirae*, *E. coli,* and *L. acidophilus* has been found [3-8]. The present study showed that EMF significantly decreased the bacterial growth rate and changed the antibiotic resistance. Therefore, EMF can be used in biotechnology and therapeutic practices.

The depression of bacterial growth after EMF exposure has been related to the effect on three different targets, including bacterial membranes, water in the surrounding media and nucleotides. Previous studies demonstrated that extremely low-frequency EMFs can cause physiological outcomes in living organisms. These fields can cause the alteration in the growth rate and morphology of bacteria. Most previous studies showed a decrease in the growth of bacteria. Therefore, these investigations have considered that the alteration caused by EMFs could be exploited for beneficial purposes and controlling bacterial infections. Nowadays, EMFs have been applied for therapeutic application as a monotherapy or combined with antibiotic treatment. Furthermore, EMF is used in disinfecting applications in meat, rice, and agriculture treatments.

Conclusion

Exposure to RF-EMF emitted by Wi-Fi routers or mobile phone simulator significantly altered the antimicrobial sensitivity of the *E. faecalis*. The susceptibility of the bacteria decreased significantly after 6 h of exposure, longer exposure times (e.g. exposure for 24 h) increased the sensitivity of the bacteria to all antibiotics. Furthermore, bacteria exposed to radiation had a faster growth rate compared to non-exposed bacteria. These findings may have implications for the management of endodontic infections.

Table 2: Inhibition zone diameters before and after exposure to radiofrequency (RF) simulator radiation for *E. faecalis*

Exposure Time	RF Radiation			
	DRUG	Control (Mean ± SD)	Exposure (Mean ± SD)	P Value
2h	PIP	28.6±0.58	27.3±0.58	0.068
	AZI	13±0	12.3±0.58	0.114
	LEV	21.3±0.58	20±0	0.034
	AMP	26.3±0.58	26.6±0.58	0.456
	VAN	15.6±0.58	15.6±0.58	1.000
	DOX	17.3±0.58	16±0	0.034
	CIP	20.3±0.58	19.3±0.58	0.099
	TET	14±0	14±0	1.000
	IMI	29.3±0.58	26.6±0.58	0.043
	AMOX	31.3±0.58	29.6±0.58	0.043
4h	PIP	28.6±0.58	26.6±0.58	0.043
	AZI	13±0	12.3±0.58	0.114
	LEV	21.3±0.58	20.3±0.58	0.099
	AMP	26.3±0.58	28.6±0.58	0.043
	VAN	15.6±0.58	15.6±0.58	1.000
	DOX	17.3±0.58	15.6±0.58	0.043
	CIP	20.3±0.58	18.6±0.58	0.043
	TET	14±0	13.6±0.58	0.317
	IMI	29.3±0.58	26.6±0.58	0.043
	AMOX	31.3±0.58	30±0	0.034
6h	PIP	28.6±0.58	25.6±0.58	0.043
	AZI	13±0	11.6±0.58	0.034
	LEV	21.3±0.58	20.3±0.58	0.099
	AMP	26.3±0.58	28.3±0.58	0.043
	VAN	15.6±0.58	15.6±0.58	1.000
	DOX	17.3±0.58	14.6±0.58	0.043
	CIP	20.3±0.58	20.6±0.58	0.456
	TET	14±0	12.3±0.58	0.034
	IMI	29.3±0.58	26.6±0.58	0.043
	AMOX	31.3±0.58	30.3±0.58	0.099
8h	PIP	28.6±0.58	25.6±0.58	0.043
	AZI	13±0	13±0	1.000
	LEV	21.3±0.58	17.3±0.58	0.043
	AMP	26.3±0.58	25.6±0.58	0.197
	VAN	15.6±0.58	15.3±0.58	0.456
	DOX	17.3±0.58	15.6±0.58	0.043
	CIP	20.3±0.58	20±0	0.317
	TET	14±0	14.6±0.58	0.114
	IMI	29.3±0.58	25.3±0.58	0.043
	AMOX	31.3±0.58	29.6±0.58	0.043

Exposure Time	RF Radiation			
	DRUG	Control (Mean ± SD)	Exposure (Mean ± SD)	P Value
10h	PIP	28.6±0.58	24.6±0.58	0.043
	AZI	13±0	13±0	1.000
	LEV	21.3±0.58	20±0	0.034
	AMP	26.3±0.58	26±0	0.317
	VAN	15.6±0.58	15±0	0.114
	DOX	17.3±0.58	16.3±0.58	0.099
	CIP	20.3±0.58	20±0	0.317
	TET	14±0	14.3±0.58	0.317
	IMI	29.3±0.58	24.6±0.58	0.043
	AMOX	31.3±0.58	25.6±0.58	0.043
24h	PIP	28.6±0.58	28.6±0.58	1.000
	AZI	13±0	16.6±0.58	0.034
	LEV	21.3±0.58	21.3±0.58	1.000
	AMP	26.3±0.58	31.6±0.58	0.043
	VAN	15.6±0.58	16±0	0.317
	DOX	17.3±0.58	16.6±0.58	0.197
	CIP	20.3±0.58	21.3±0.58	0.099
	TET	14±0	16.6±0.58	0.034
	IMI	29.3±0.58	27.6±0.58	0.043
	AMOX	31.3±0.58	34.6±0.58	0.043

RF: Radiofrequency, PIP: Piperacillin, AZI: Azithromycin, LEV: Levofloxacin, AMP: Ampicillin, VAN: Vancomycin, DOX: Doxycycline, CIP: Ciprofloxacin, TET: Tetracyclin, IMI: Imipenem, AMOX: Amoxicillin

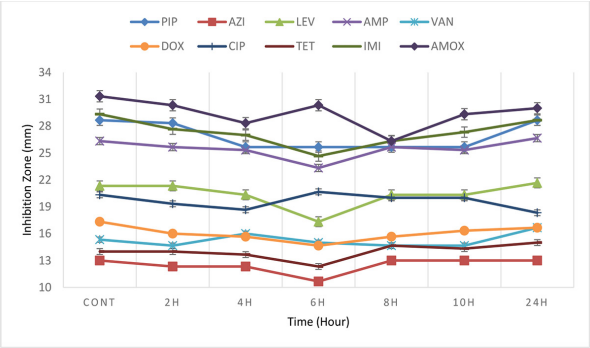

Figure 3: Inhibition zone diameters pre and post-exposure to Wi-Fi radiation for *E. feacalis*

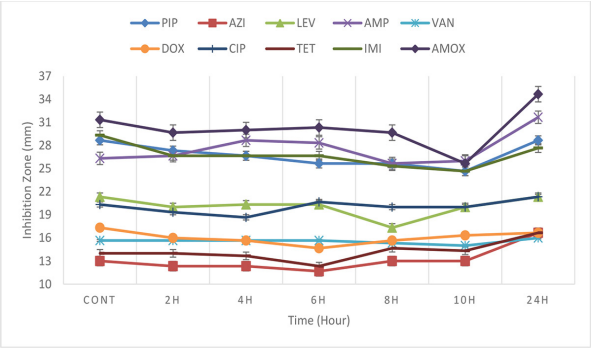

Figure 4: Inhibition zone diameters pre- and post-exposure to radiofrequency (RF) simulator radiation for *E. feacalis*

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Authors' Contribution

SMJ. Mortazavi conceived the idea. Introduction and manuscript of the paper was written by

SMJ. Mortazavi, M. Paknahad. The method implementation and experimental studies was carried out by M. Taheri and Analysis was carried out by S. Khandadash. All the authors read, modified, and approved the final version of the manuscript.

Ethical Approval

The Ethics Committee of Shiraz University of Medical Sciences approved the protocol of the study (Ethic cod: IR.SUMS.REC.1395.S1167).

Conflict of Interest

None

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