Original

Evaluation of Wi-Fi Radiation Effects on Antibiotic Susceptibility, Metabolic Activity and Biofilm Formation by Escherichia *Coli* 0157H7, *Staphylococcus Aureus* and *Staphylococcus Epidermis*

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ABSTRACT

Background: The radiation emitted from electromagnetic fields (EMF) can cause biological effects on prokaryotic and eukaryotic cells, including non-thermal effects.

Objective: The present study evaluated the non-thermal effects of wireless fidelity (Wi-Fi) operating at 2.4 GHz part of non-ionizing EMF on different pathogenic bacterial strains (*Escherichia coli* 0157H7, *Staphylococcus aureus, and Staphylococcus epidermis*). Antibiotic resistance, motility, metabolic activity and biofilm formation were examined.

Material and Methods: In this case-control study, a Wi-Fi router was used as a source of microwaves and also bacterial cells were exposed to Wi-Fi radiation continuously for 24 and 48 hours. The antibiotic susceptibility was carried out using a disc diffusion method on Müller Hinton agar plates. Motility of *Escherichia coli* 0157H7 was conducted on motility agar plates. Cell metabolic activity and biofilm formation were performed using 3-(4, 5-Dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT) assay and crystal violet quantification, respectively.

Results: The exposure to Wi-Fi radiation altered motility and antibiotic susceptibility of *Escherichia coli* 0157H7. However, there was no effect Wi-Fi radiation on antibiotic susceptibility of *Staphylococcus aureus and Staphylococcus epidermis*. On the other hand, the exposed cells, as compared to the unexposed control, showed an increased metabolic activity and biofilm formation ability in *Escherichia coli* 0157H7, *Staphylococcus aureus and Staphylococcus epidermis*.

Conclusion: These results proposed that Wi-Fi exposure acted on bacteria in stressful manner by increasing antibiotic resistance and motility of *Escherichia coli* 0157H7, as well as enhancing biofilm formation by *Escherichia coli* 0157H7, *Staphylococcus aureus and Staphylococcus epidermis*. The findings may have implications for the management of serious diseases caused by these infectious bacteria.

Citation: Said-Salman I. H, Jebaii F. A, Yusef H. H, Moustafa M. E. Evaluation of Wi-Fi Radiation Effects on Antibiotic Susceptibility, Metabolic Activity and Biofilm Formation by Escherichia Coli 0157H7, Staphylococcus Aureus and Staphylococcus Epidermis. *J Biomed Phys Eng*. 2019;9(5):579-586. https://doi.org/10.31661/jbpe.v0i0.1106.

Keywords

Non-thermal Effect; EMF; Wi-Fi; Disc Diffusion; Motility; MTT; Biofilms; Escherichia coli; Staphylococcus

Introduction

Over the past century, the natural environment has been altered by greater the use of telecommunication, such as global system for mobile communication (GSM) and wireless fidelity (Wi-Fi). Wi-

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Received: 8 January 2019
Accepted: 6 February 2019

Fi waves are a part of the non-ionizing radiation of the electromagnetic spectrum and they use radiofrequency typically operating at 2.4 GHz [1]. Non-ionizing electromagnetic radiation affects biological system through either thermal or non- thermal effects [1,2]. Many scientific studies have investigated biological effects of electromagnetic fields (EMF) [1–3]. Non-thermal EMF induced different responses on bacteria depending on frequency intensity, exposure time and organism model [4].

Several studies investigated the non-thermal effects of high frequency electromagnetic fields (GSM and Wi-Fi) on different strains of bacteria [5–11]. Exposure of *Staphylococcus aureus, Staphylococcus epidermis*, and *Pseudomonas aeruginosa* to GSM revealed no effects on their bacterial growth rate or antibiotic susceptibility [7]. However, others reported that exposure to Wi-Fi and GSM altered the inhibition zone diameters and growth rate for of *Escherichia coli* and *Listeria monocytogenes* [8]. In addition, Wi-Fi radiation significantly increased the sensitivity of *klebsiella pneumoniae* to different antibiotics followed by a decrease suggesting an adaptive response [9].

The aim of this study was to analyze the influences of electromagnetic fields (2.4 GHz) emitted by a WI-FI router on the antibiotic resistance, cell metabolic activity and ability to form biofilm by different pathogenic strains (*Escherichia coli* 0157H7, *Staphylococcus aureus, and Staphylococcus epidermis*).

Material and Methods

Microwave exposure system

The microwave (MW) source was generated continuously by 54 M wireless router Tp-Link extended range (TL-WR524G) corresponding to the appropriate frequency 2.4 GHz connected to an amplifier and monopole antenna, then mounted in an incubator which was kept at 37°C at 30 cm of distance from bacterial culture. The exposure protocol consisted of a phase of continuous exposure to the MW for 24 or 48 hours. For each experimental condition, a control was performed in Faraday bag at the same temperature to limit any exterior radiation.

Bacterial strains

The bacterial strains used in the current *in vitro* case-control study were *Escherichia coli* 0157H7, *Staphylococcus aureus and Staphylococcus epidermis*. They were provided by the Microbiology Laboratory, Faculty of Science, Beirut Arab University (BAU), Lebanon. The isolates were checked for their purity and identity. The growth of colorless transparent colonies on sorbitol MacConkey agar medium (Oxoid) confirmed the inability of *E. coli* 0157H7 to ferment sorbitol [12]. API Staph was used to confirm the identification of *S. aureus* and *S. epidermis*.

Antimicrobial Susceptibility

Müller Hinton agar medium (Oxoid) was used for susceptibility testing based on the criteria of the National Committee for Clinical Laboratory Standards [13]. The isolated *E. coli* 0157H7 was tested for sensitivity to the antimicrobials (Oxoid), including Meropenem (MEM) 10 µg, Imipenem (IMI) 10 µg, Rifampicin (RD) 30 µg, Levofloxacin (Lev) 5 µg, Oflaxacin (OFX) 5 µg, and Chloramphenicol (C) 10 µg, Azithromycin. (AZM) 15 µg and Gentamicin (CN) 120 µg. *S. aureus* and *S. epidermis* strains were tested for sensitivity to antimicrobials, including Gentamicin (CN) 120 µg, Penicillin G (P) 10 µg, Oflaxacin (OFX) 5 µg, and Chloramphenicol (C) 10 µg. For both the control and exposed to 2.4 GHz, standard inocula of 0.5 McFarland was swabbed on Müller Hinton agar then incubated 24 hours at 37°C. The mean diameter of inhibition zones was recorded in mm.

Bacterial motility

Among the studied pathogenic strains, *Escherichia coli* 0157H7 was the only motile bacterium. Therefore, a single colony of *E. coli*

0157H7 was inoculated in 5 ml of Luria-Bertani (LB) broth (Sigma-Aldrich) and incubated at 37°C overnight with agitation at 200 rpm. Bacterial overnight culture (1µl) was injected into three spots on freshly prepared motility agar plates containing 0.5% agar (Motility Test Medium Himedia) [14]. The plates were incubated at 37°C for 24 and 48 hours under exposure of 2.4 GHz. The diameters of the bacterial growth rings were measured.

MTT and crystal violet assays

One colony forming unit (CFU) of each strain was transferred into 5 mL of Tryptic Soy Broth (TSB Oxoid) and incubated for 24 hours at 37 °C with shaking. The cultures were diluted to 1 /100 in TSB to obtain 0.1 optical density (OD), at 595 nm for all bacterial inocula. Two hundred microliters of each bacterial inocula were transferred into 96 well plates in duplicate (control and exposed to 2.4 GHz) and incubated for 24 hours at 37 °C; Biofilms were washed three times and subjected to 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT) assay to evaluate the viability of the biofilms and crystal violet based on the quantification of biofilm biomass.

The MTT reduction assay, which determines the metabolic activities of bacteria, was done as previously described with minor modifications [15,16]. Upon washing step, 10 µl of 5 mg/ml MTT (Sigma-Aldrich) was added to 100 µl phosphate buffered saline (PBS) into each well, and the plates were incubated for 1 hour at 37 °C. A 100 µl of solubilizing isopropanol was added to each well, and the plates were vigorously shaken. The amount of MTT formazan formed was measured by the ELx800 Universal Microplate Reader at a wavelength of 570 nm. The pure TSB was used as blank. The results are shown as an index of cell metabolic activity and calculated by the formula: Index of cell metabolic activity = (OD sample − OD background)/ (OD control − OD background) [17].

The quantitative assessment of biofilm formation was determined as previously described with minor modification [18,19]. Biofilms were stained with 200 µL of 0.1% crystal violet. Excess stain was removed, and the wells were air-dried for 15 minutes and then the dye bounded to the adherent cells was solubilized with 200 µL of 33% acetic acid. The OD of each solubilized liquid was measured at wavelength of 595 nm. The pure TSB was used as blank. The results were shown as an index of biofilm formation and calculated by the same formula described above.

Statistical analysis

The data of this study are presented as means ± standard errors of the means calculated from three repetitions of each experiment. The statistical analysis was conducted using Graphpad Prism 8 by mean of paired t test. P values <0.05 was statistically significant.

Results

Effect of 2.4 GHz Wi-Fi exposure on antibiotic susceptibility

The susceptibility of *Escherichia coli* 0157H7, *Staphylococcus aureus and Staphylococcus epidermis* to various antibiotics was evaluated in the presence of Wi-Fi radiofrequency radiation. The data obtained for exposed and non-exposed (control) bacteria are represented in Tables 1 and 2.

The continuous exposure to Wi-Fi radiofrequency radiation decreased significantly the inhibition zone diameters of *E. coli* 0157H7 (p value<0.05) (Table 1). However, it revealed no significant changes in diameters of inhibition zones of *S. aureus* and *S. epidermis* (Table 2).

Effect of 2.4 GHz Wi-Fi exposure on motility of E. coli O157H7

The effect of Wi-Fi radiofrequency radiation on the motility of *E. coli* 0157H7 was evaluated by measuring the diameter of bacterial growth rings on motility agar medium after

Table 1: Inhibition zone diameters (mm) for control and exposed Escherichia coli 0157H7 to 2.4 GHz Wi-Fi radiofrequency radiation.

Antibiotic	Control	Wi-Fi Exposure	P-value
IPM 10	35.33±0.58	29.67±0.58	0.0002*
OFX 5	25.33±0.58	21.67±0.58	0.0014*
C 10	18.33±0.58	12.33±0.58	0.0002*
AZM 15	14.33±0.58	11.33±0.58	0.0031*
RD 30	16.67±0.58	14.67±0.58	0.0132*
MEM 10	29.33±0.58	23.67±0.58	0.0002*
LEV 5	31.67±0.58	28.33±0.58	0.0021*
CN120	30.33±0.58	28.33±0.58	0.0132*

Each value is the mean ±SD of three repetitions.
*statistically significant ($p<0.05$)

24 and 48 hours of Wi-Fi exposure. The data show significantly increased motility by 28 % and 29 % for 24 and 48 hours of 2.4 GHZ Wi-Fi exposure, respectively, as compared to un-exposed *E. coli* 0157H7 (Figure 1).

Effect of 2.4 GHz Wi-Fi exposure on cell metabolic activity and biofilm formation

In order to explore the effects of Wi-Fi radiofrequency on the bacterial metabolic activity and biofilm formation, the MTT assay and crystal violet quantification were performed. The MTT test is based on the reduction of the yellow MTT dye by dehydrogenase in living cells to purple MTT formazan, which can be solubilized and quantified by spectrophotometric measurements. The crystal violet assay is based on the values of optical density of the crystal violet stain bonded to biofilm. Data obtained from bacterial metabolic activity and biofilm formation index are summarized in Table 3. As consequence of 24 hours exposure to 2.4 GHz Wi-Fi radiofrequency radiation, results showed statistically significant 3 fold increase in cell metabolic activity and 1.9 fold raise in biofilm formation by exposed *E. coli, S. aureus* and *S. epidermis* as compared to controls (Table 3).

Discussion

The results of this study showed that 2.4 GHz Wi-Fi radiofrequency radiation induced a rise in the antibiotic resistance of *E. coli* 0157H7 but revealed no significant changes in that of *S. aureus* and *S. epidermis*. This observation coincides with the previous reports shown that exposure to electromagnetic fields (EMF) makes some bacteria resistant to antibiotics [8] and the effects of EMF exposure depend on the morphology of exposed cells [20]. Moreover, continuous 24 hours exposure to mobile 1.8 GHz waves showed the presence of persisters *Pseudomonas aeruginosa* cells with enhanced antibiotic resistance [11]. In contrast, no significant effects of high frequency electromagnetic fields were revealed on anti-

Table 2: Inhibition zone diameters (mm) for control and exposed S. aureus and S. epidermis to 2.4 GHz Wi-Fi radiofrequency radiation.

Antibiotic	S. aureus			S. epidermis		
	Control	Wi-Fi Exposure	P-value	Control	Wi-Fi Exposure	p-value
P 10	17.67±0.58	16±1	0.0667	13.67±0.58	14.33±0.58	0.2302
OFX 5	29.67±0.58	28.67±0.58	0.1011	28.33±0.58	28.67±0.58	0.5185
C 10	18.67±0.58	17.33±0.58	0.2302	15.33±0.58	15.67±0.58	0.5185
CN120	22.67±0.58	22.33±0.58	0.5185	23.33±0.58	23.67±0.58	0.5185

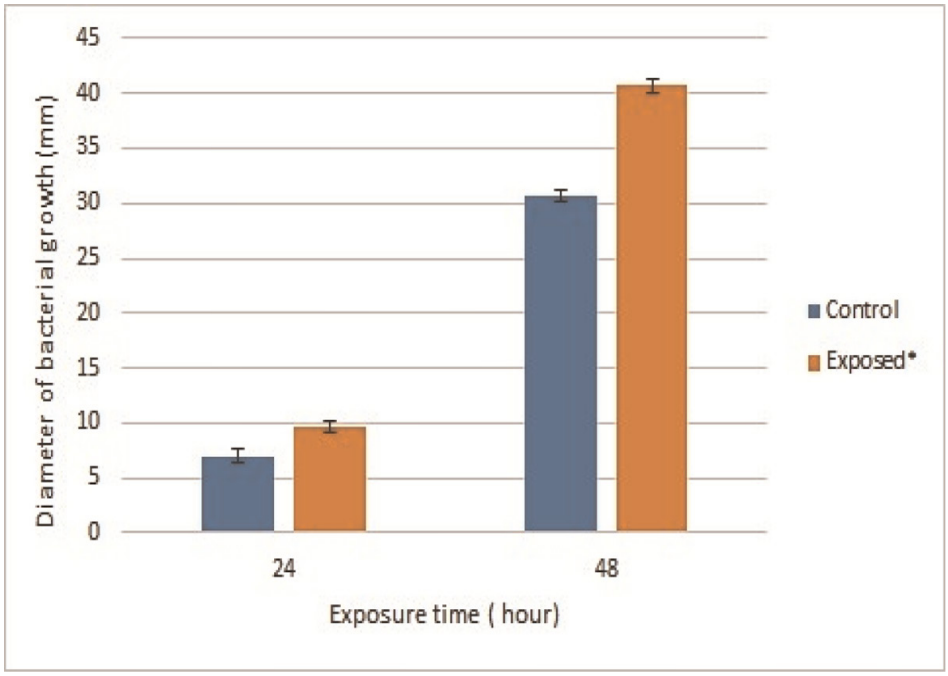

Figure 1: Motility of exposed E. coli O157H7 to 2.4 GHz Wi-Fi radiofrequency radiation. Each value is represented as mean ±SD (n=3). *Significance was considered at (p-value<0.05)

Table 3: Effects of 2.4 GHz Wi-Fi radiofrequency radiation on metabolic activity and biofilm formation of E. coli, S. aureus, and S. epidermis.

		control	Exposed	P-value
E. coli 0157H7	Cell metabolic activity	1.018±0.023	2.432±0.102	0.002*
	Biofilm formation	1.009±0.080	1.413±0.203	0.042*
Staphylococcus aureus	Cell metabolic activity	1.026±0.081	3.323±0.280	0.004*
	Biofilm formation	1.053±0.068	1.918±0.223	0.022*
Staphylococcus epidermis	Cell metabolic activity	1.327±0.304	3.024±0.267	0.036*
	Biofilm formation	1.020±0.027	1.506±0.062	0.007*

Each value is the mean ±SD. *statistically significant (p<0.05)

biotic sensitivity of exposed *Staphylococcus* [7]. The different effect seen in the present study between *E. coli* 0157H7 and *S. aureus* & *S. epidermis* may be due to the difference in cell wall composition and how microwaves affect gram-positive and gram-negative bacteria. Several studies reported that radiation induced changes in membrane fatty acids and murein composition of bacteria as well as influenced antibiotic sensitivity [9,21]. Different antibiotics that act through various mechanisms were used in the current study, including inhibition of protein, DNA and cell wall synthesis. The radiation may alter the sensitivity of the efflux pumps or ion channels by permitting the entrance of the molecules through the bacterial cell wall [8,22].

In addition to antibiotic resistance, there

was a significant increase in *E. coli* 0157H7 motility after 2.4 GHz Wi-Fi exposure, which may be a strategy of the pathogen's survival in the face of radiation stress. These results are in agreement with prior studies that showed promoting *E. coli* 0157H7 motility under heat and acid stress [23,24]. Flagella are critical virulence factors, that permit bacterial motility and promote adherence to mucins, the major component of the mucus that lines the gastrointestinal tract [25].

The current study examined the effects of exposing *E. coli* 0157H7, *S. aureus* and *S. epidermis* to Wi-Fi radiofrequency radiation 2.4 GHz on their cell metabolic activity and ability to form biofilms where bacterial cells treated with Wi-Fi radiation continuously for 24 hours. Previous reports studied the effects of extremely low frequency EMF exposure and short time Wi-Fi radiofrequency exposure on biofilm formation [10,17]. Based on the current study, the exposure of bacteria to 2.4 GHz Wi-Fi radiofrequency radiation for 24 hours showed the statistically significant increase in the cell metabolic activity and ability to form biofilm of exposed *E. coli, S. aureus* and *S. epidermis* as compared to controls. Such finding agreed with previous observations for the influences of rotating magnetic field (RMF) on bacteria [17]. In contrast, short time exposure of *Staphylococcus aureus* to mobile phone electromagnetic waves did not affect their ability to form biofilm [10].

Conclusion

Based on our results, it can be concluded that Wi-Fi radiofrequency radiation affects bacterial strains in the stressful manner. Increasing antibiotic resistance, motility and biofilm formation which are pathogenic traits of bacteria is a worldwide threat to public health. There are some ambiguities that need further investigations regarding answering questions such as which cellular mechanism is responsible for this stress induced by EMF. Moreover, gene expression experiments are performed to clarify many uncertainties.

Acknowledgment

This work has been supported by grants from the Lebanese University.

Conflict of Interest

None

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