JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

MODULATION OF *STAPHYLOCOCCUS AUREUS* BIOFILM BY ELECTROMAGNETIC RADIATION

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doi: 10.15414/jmbfs.2020.9.5.1020-1022

ARTICLE INFO

Received 12. 9. 2019
Revised 12. 11. 2019
Accepted 20. 11. 2019
Published 1. 4. 2020

Regular article

OPEN ACCESS

ABSTRACT

Mobile communication systems are undoubtedly an environmental source of electromagnetic radiation (EMR). Although the direct health effect of a cell phone to human is still elusive, the effect to unicellular organisms is rather apparent. The aim of this study was to examine the effects of EMR on development of bacterial biofilm. Microtitration plates with four strains of *Staphylococcus aureus* were exposed to electromagnetic field of frequencies 1-5 GHz, which are used in mobile phones. The results showed mostly the inhibition of the development biofilm activity at frequencies 1, 2 and 3 GHz, however the significant stimulation of biofilm development occurred at frequencies 4 and 5 GHz. Our observations demonstrate that EMR exposure produced modulation effects on bacterial biofilms, which are very important in commensal and pathogen bacteria.

Keywords: electromagnetic radiation, mobile, *Staphylococcus aureus*, biofilm

INTRODUCTION

Cell phones and electronic appliances and devices are inseparable from most people in modern society and the electromagnetic
field (EMF) from the devices is a potential health threat. Radio-frequency radiation (RF) (3 MHz to 300 GHz) is emitted from radio and TV broadcast antennas, Wi-Fi access points, routers, and clients (e.g. smartphones, tablets), cordless and mobile phones including their base stations, and Bluetooth devices. Extremely low frequency electric (ELF EF) and magnetic fields (ELF MF) (3 Hz to 3 kHz) are emitted from electrical wiring, lamps, and appliances. Very low frequency electric (VLF EF) and magnetic fields (VLF MF) (3 kHz to 3 MHz) are emitted, due to harmonic voltage and current distortions, from electrical wiring, lamps (e.g. compact fluorescent lamps), and electronic devices. The new fifth generation (5G) technologies will use frequencies between 30 and 100 GHz (**Pavlík, 2019**).
There is strong evidence that long-term exposure to certain EMFs is a risk factor for diseases such as certain cancers, Alzheimer's
disease, and male infertility (**Belyaev *et al.* 2016**). Mature rats were exposed to electromagnetic field of frequency 2.45 GHz for 3 h/d for 3 weeks and the presence of moderate hyperemia, dilatation of liver sinusoids, and small inflammatory foci in the center of liver lobules were found (**Holovská *et al.* 2015**). The whole body pulsed EMR on the juvenile Wistar albino rat testis at a frequency of 2.45 GHz caused an irregular shape of seminiferous tubules with desquamated immature germ cells in the lumen, a large number of empty spaces along the seminiferous epithelium and dilated and congested blood vessels in the interstitial tissue of the testis (**Simaiová *et al.* 2019**). Exposure of juvenile rats to EMR displayed locomotor hyperactivity and decreased risk assessment in adulthood (**Raček *et al.* 2018**).
Two types of microwaves effects have been recognized, thermal and non-thermal . Thermal effects relate to processes which generate heat as a result of the absorption of the microwave energy by water, or organic complexes marked by either constant or induced polarization. The microwave energy is transformed into heat derived from the internal resistance of rotation. Non-thermal effects (also known as 'athermal effects' or 'specific effects of electromagnetic irradiation') relate to several microwave induced phenomena unrelated to temperature rise (**Zielinski *et al.* 2007**).
Static and radiofrequency electromagnetic fields have significant yet variable effects on the growth of human skin *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* was unaffected, increased, or suppressed. Cell phone level RF-EMF disrupts human skin microbiota (**Crabtree *et al.* 2017**).
High-throughput RNA-sequencing of 2.4 GHz exposed (5 h) and non-exposed *Escherichia coli* K-12 DH5α revealed that 101 genes were differentially expressed. The up-regulated differencially expressed genes are involved in metabolic pathways, transposition, response to stimuli, motility, chemotaxis and cell adhesion. The downregulated genes are associated with metabolic pathways and localization of ions and organic molecules (**Said-Salman *et al.* 2019**). In particular, for prokaryotic systems, the exposure to electromagnetic fields produces stress effects causing phenotypic and transcriptional changes on free cells and affecting the surface adhesion on cells organized in biofilm. An exposure to ELF-EMF of H. pylori biofilm induces phenotypic changes on adhering bacteria and decreases the cell adhesion unbalancing the bacterial population therefore reducing the *Helicobacter pylori* capability to protect itself (**Di Campli *et al.* 2010**).
The objective of this article was to investigate the role of the exposure of five mobile radiation frequencies (1-5 GHz) on the development of *Staphylococcus aureus* biofilm.

MATERIAL AND METHODS

Bacterial strains

Staphylococcus aureus No. 12 and *Stapylococcus. aureus* No. 14 with *hla* (alpha-hemolysin), *isdA*- (iron-regulated surface determinant protein A*)* genes, were isolated from ewes' milk (Čuvalová, Kmeť 2018) ; S*taphylococcus .aureus* No. 2 with *icaAD*- (intracellular adhesion), *blaZ*- (betalactamase), *mecA* –(meticillin resistance) genes from small mammals (**Kmeť *et al.* 2018**) , *Staphylococcus aureus* No. 133 with *agrI* (accessory gene regulator) gene - reference biofilm strain from NRC – Culture collection of pathogenic microorganisms, Slovak Medical University in Bratislava.

Electromagnetic radiation

In this experiment, an EM field with a power of 52.7 mW and frequencies of 1, 2, 3, 4 and 5 GHz were used. The frequencies were selected from the frequency range based on the devices used in telecommunication networks (mobile phones and smartphones) for data transmission (Wi-Fi, LTE, EDGE).

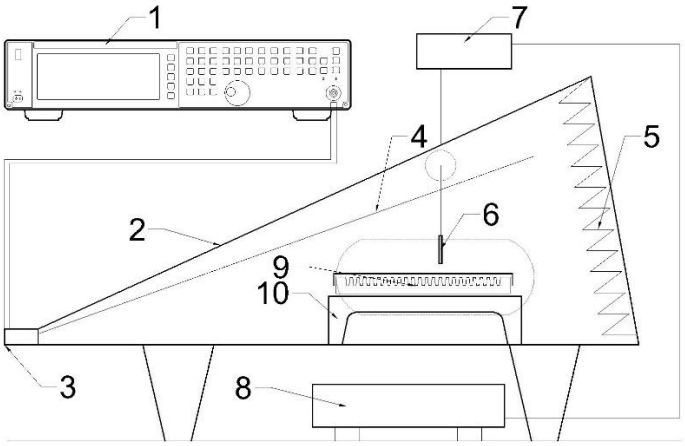

Figure 1 Diagram of experimental set-up
Legend: Agilent MXG Analog signal generator N5183A (Keysight Technologies, USA) with a frequency range of 100 kHz - 20 GHz (1) was used to generate the EM field (**Kosterec *et al.* 2016**). The generator output signal was fed to the GTEM-250 cell of AMETEK CTS Europe GmbH, Germany (2), which is adapted for testing electromagnetic compatibility, telecommunications applications, but also for biomedical and dosimetric applications (**Nicolae *et al.* 2014**). The GTEM-250 cell consists of input port (3) for connection of signal generator, septum (inner conductor) (4) and ferritefoam absorbers (5). The temperature was controlled and adjusted to 36 ° C (± 1 ° C) using a temperature sensor (6) connected to a regulator (7), which controled an external heater (8). The samples (microtiter plates with individual *Staphylococcus aureus* isolates) (9) was placed at a height of 15mm on a porcelain mat (10), each sample being exposed to an EM field at a given frequency for 3 hours.

Microtiter plate assay

Quantification of the biofilm production was performed using MaxiSorp polystyrene U-bottomed 96-well microtiter plates with a high protein binding capacity and hydrophilic surface (Nunc, Roskilde, Denmark) by a previously published method **Čuvalová and Kmeť (2018)** with slight modifications. In brief, staphylococci were grown on BHI agar (Oxoid), colonies were transferred to BHI broth (Oxoid) to reach the density equivalent to McFarland standard 0.5. Volumes of 200 µl of these cell suspensions were transferred to wells of the microplates and incubated statically for 24 h at 37 °C. Following incubation, the content of each well was removed and the wells were washed three times with 250 µl of Phosphate Buffered Saline solution (PBS, Thermo Fisher Scientific). Adherent cells were stained with 0.1% crystal violet (Mikrochem, Pezinok, Slovakia) solution for 15 min. Afterwards, excess stain was rinsed off by filling the wells with sterile distilled water. The adhering dye was dissolved with 30% acetic acid. The optical density of wells was measured at 570 nm using Synergy HT Multi-Mode Microplate Reader (BioTek, Winooski, Vermont, USA).

Statistical analysis

All assays were performed in six replicates and mean as well as standard deviation were calculated by Statistica 9.0 software (StatSoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

To verify the effect of 1-5GHz EMF on the development of *Staphylococcus aureus* biofilm formation, bacterial cultures in microtiter plates were exposed during 3 hours with comparison to the respective non-exposed controls. The results showed mostly the inhibition of the development biofilm activity at frequencies 1,2 and 3 GHz, however the significant stimulation of biofilm development occurred at frequencies 4 and 5 GHz (Table 1). For example there was the significant inhibition of biofilm development at frequency 2 GHz with human strain *Staphylococcus aureus* 133 from value of absorbance 0,670 ± 0,36 (SD) in control to value 0,386 ± 0,11. The development of biofilm formation in milk *Sstaphylococcus aureus* 14 was significantly inhibited by 4GHz from value 0,114 ± 0,048 in control to value 0,066 ± 0,016 in exposed one. The frequency 5GHz decreased biofilm formation in milk strain S*taphylococcus aureus* 12 from value 0,100 ± 0,05 to value 0,033 ± 0,018. Biofilm of meticillin resistant *Staphylococcus aureus* 2 from small mammal was statistically significantly inhibited oat 1GHz and 5 GHz frequencies, while at 4GHz biofilm development was significantly stimulated from value 0,100 ± 0,027 to 0,161 ± 0,05 in irradiated group.
Many published studies aimed at determining whether RF induces nonthermal effects on biologics fail to provide accurate maintenance of sample temperature thus preventing differentiation between thermal and nonthermal (**Fortune *et al.* 2010**). We eliminated the thermal effects of mobile frequencies exposure through the use a regulator and an external heater capable of maintaining microplate temperature with staphylococci in the range 36 ° C (± 1 ° C). This is very important for a proper evaluation of biofilm formation, which is based on absorbance measurement of bacterial culture, adhered to the microplate. **Fortune *et al.* (2010)** found that none of the RF frequencies (2.45 GHz, 915 MHz and 13.56 MHz during 4 hours) investigated appreciably affects the viability of *Staphylococcus aureus* in 0.85% aqueous NaCl. However, **Zielinski and Krzemieniewski (2007)** found microwave radiation (2.45 GHz at 18 W) can affect the structure and function of bacterial sludge communities in bioreactor (21° C ± 1° C) independent of thermal effects. Moreover, bacterial richness measured by Shannon index was significantly higher in the microwave treated samples.
Mohd-Zain *et al.* (2012) found that electromagnetic field (2G mobile phone, 900/1800 MHz) from the standby-mode has enhanced the growth of *Staphylococcus aureus* suspension (10^9 CFU/ml) but during on-call (during 15,30,45 and 60 min), the growth was suppressed. No significant difference in the amount of biofilm produced in both modes of exposure was observed. **Di Campli *et al* (2010)** reported that EL-EMF (50 Hz) exposed to *Helicobacter pylori* for 2 h was able to interfere with cell adhesion during biofilm formation.

Table 1 The modulation effect of electromagnetic radiation (1-5 GHz) on *Staphylococcus* aureus biofilm formation.

1 GHz		
		Mean ± SD
Staph. aureus 12	control	0,075 ± 0,036
	irradiated	0,053 ± 0,003 n.s. ↓
Staph. aureus 14	control	0,071 ± 0,027
	irradiated	0,049 ± 0,027 n.s. ↓
Staph. aureus 2	control	0,049 ± 0,009
	irradiated	0,036 ± 0,005 *** ↓
Staph. aureus 133	control	0,230 ± 0,120
	irradiated	0,195 ± 0,075 n.s. ↓

2 GHz		
		Mean ± SD
Staph. aureus 12	control	0,123 ± 0,069
	irradiated	0,117 ± 0,056 n.s. ↓
Staph. aureus 14	control	0,090 ± 0,027
	irradiated	0,105 ± 0,075 n.s. ↑
Staph. aureus 2	control	0,103 ± 0,046
	irradiated	0,078 ± 0,019 n.s. ↓
Staph. aureus 133	control	0,670 ± 0,360
	irradiated	0,386 ± 0,110 * ↓

3 GHz		
		Mean ± SD
Staph. aureus 12	control	0,094 ± 0,030
	irradiated	0,053 ± 0,030 * ↓
Staph. aureus 14	control	0,078 ± 0,031
	irradiated	0,053 ± 0,016 * ↓
Staph. aureus 2	control	0,12 ± 0,05
	irradiated	0,095 ± 0,043 n.s. ↓
Staph. aureus 133	control	0,316 ± 0,07
	irradiated	0,25 ± 0,09 n.s. ↓

4 GHz		
		Mean ± SD
Staph. aureus 12	control	0,057 ± 0,018
	irradiated	0,05 ± 0,009 n.s. ↓
Staph. aureus 14	control	0,114 ± 0,048
	irradiated	0,066 ± 0,016 * ↓
Staph. aureus 2	control	0,100 ± 0,027
	irradiated	0,161 ± 0,050 * ↑
Staph. aureus 133	control	0,196 ± 0,100
	irradiated	0,150 ± 0,060 n.s. ↓

5 GHz		
		Mean ± SD
Staph. aureus 12	control	0,100 ± 0,050
	irradiated	0,033 ± 0,018 * ↓
Staph. aureus 14	control	0,110 ± 0,060
	irradiated	0,260 ± 0,150 * ↑
Staph. aureus 2	control	0,056 ± 0,024
	irradiated	0,039 ± 0,008 * ↓
Staph. aureus 133	control	0,280 ± 0,220
	irradiated	0,420 ± 0,260 n.s. ↑

Legend: In green-inhibition of staphylococci biofilm formation

CONCLUSION

The results showed mainly the inhibition of the development biofilm at EMR frequencies 1, 2 and 3 GHz, however the significant stimulation of biofilm development occurred at frequencies 4 and 5 GHz. Additional work need to be performed to gather more information on other biological changes in bacteria that may occur due to the exposure to electromagnetic field of mobile phones.

Acknowledgments: This work was supported by Slovak grant VEGA 2/0085/18 and APVV – 16 – 0171.

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