Bacterial Adaptation to Radiofrequency Electromagnetic Fields Based on Experiences from Ionizing Radiation

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ABSTRACT

Bacteria, part of the three domains of life (Eukarya, Archaea, and Bacteria), are constantly exposed to man-made electromagnetic fields, which often exceed the intensity of natural electromagnetic sources. In response to this exposure, bacteria have developed various defensive and resistant traits. This article presents an overview of both historical and recent research on how bacteria adapt to common sources of Radiofrequency Electromagnetic Fields (RF-EMF. The widespread use of mobile phones and Wi-Fi, both utilizing Radiofrequency (RF) radiation, raises potential public health concerns, which have been addressed by international organizations like the World Health Organization (WHO). Understanding how bacteria adapt to EMF is important for mitigating the risk of increased pathogenicity of radio-resistant bacteria in the human environment.

Keywords

Radioadaptation; Bacteria; Adaptation, Physiological; Drug Resistance, Bacterial; EMF; RF; Radio-Resistance; Bacterial Adaptation; Bacterial Resistance

Introduction

Life evolved in a radiation environment that was either harmless or caused adaptation. Over the past 3.5 billion years, the forms of life evolved starting from the first cells to the emergence of mankind in an environment filled with different ionizing and non-ionizing radiations [1-3]. While the ambient dose rates at the early days of life (~10 times higher than today) accounted for up to 33% of mutations of the first forms of life, current background radiation accounts only for 1–6% of the mutations [4, 5]. Due to higher concentrations of radionuclides, and the existence of "natural reactors" (e.g. those remaining found in the Oklo and Bangombédeposits of the Franceville basin in Gabon, Western Africa), the levels of ionizing radiation were generally higher on Earth in the early days of life than nowadays [6].

Adaptive Response (AR) can be defined as an increased resistance to high levels of a stressor (physical or chemical) after exposure to a low-level stress (either the same stressor or other types of stress) [7, 8]. The applications of this phenomenon in different fields, including, but not limited to, the treatment of tumors, risk management, and in particular

radiation protection are well-documented. Studies performed on the AR triggered by small doses of ionizing radiation show the initiation of several signalling pathways, change in gene transcription, specific protein synthesis, increased antioxidant production, free radical release and detoxification. As a consequence, these potential cellular mechanisms induce DNA repair systems and cell defenses that can be considered underlying the phenomenon of AR (Figure 1). In this mini-review, we address certain unresolved questions concerning adaptive response in bacteria focusing on the radiofrequency-induced adaptive response.

Effects of electromagnetic fields on bacteria

Electromagnetic Fields (EMF) can cause biological effects on exposed microorganisms, which can potentially induce either an inhibiting or a stimulating response. There are two main categories of biological effects: thermal and non-thermal effects. The manifestation of one type of these effects on microorganisms depends on the power and frequency of the electromagnetic field [9]. Ionizing radiations (such as gamma rays, X-rays, and particle radiation) are generally accompanied by thermal effects, which have energies high enough to ionize certain molecules. As a consequence, the temperature rises by more than 1 °C, causing intracellular changes, which might induce heat-stress-related responses [10]. For non-ionizing radiation, the effects do not cause an increase in temperature due to the lower frequency and are therefore non-thermal effects. Among non-ionizing radiation, the most common public sources are extremely low-frequency electromagnetic fields that are commonly used in the transmission of electric power and Radiofrequency (RF), which compromise mobile communication systems and Wi-Fi waves [11]. The mechanisms of RF-induced non-thermal effects on bacteria are not well-known and seem to have multiple origins. Some hypotheses evoke cellular and physiological changes at many levels: growth rate, metabolism, cell membrane integrity, antibiotic sensitivity, biofilm formation, gene expression, and others. Table 1 summarises the non-thermal effects of radiofrequency radiation (mobile phones, Wi-Fi routers, and mobile base stations) on various bacterial species.

Effects of RF on bacterial growth

EMF, and other environmental stressors, have been reported to affect the growth of bacteria (Table 1). The effects of EMF on

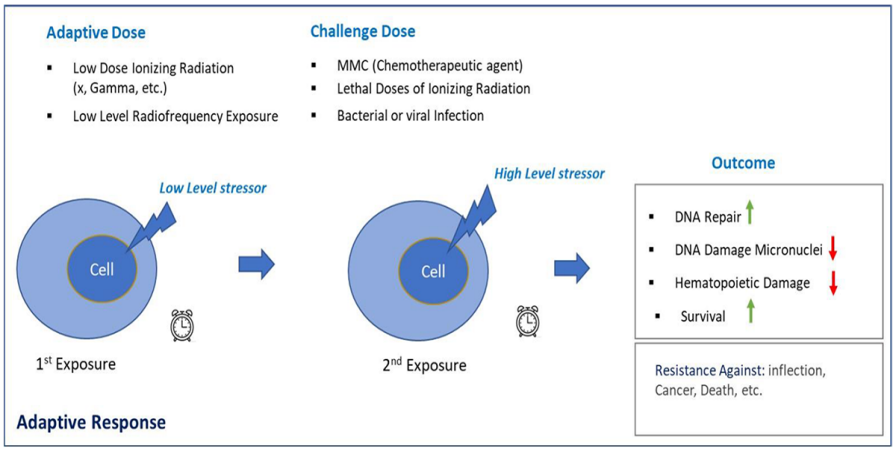

Figure 1: Schematic illustration of adaptive response. Pre-exposure of cells to a low-level stressor (e.g., low-dose ionizing radiation such as X-rays, and/or gamma rays, or non-ionizing radiations such as radiofrequency radiation) increases their resistance against a following high-level stressor (e.g., high dose radiation).

Table 1: Bio-effects of Radiofrequency Electromagnetic Fields (RF-EMF) on various cellular functions of bacteria.

EMF frequency	Time of exposure	Bio-effect	Bacterial strains	Reference/ year
GSM 0.835 GHz	Continuous for 48 hours	No mutagenic effect, no DNA degradation	*Escherichia coli and Salmonella typhimurium*	[12]/2005
GSM 0.9-1.8 GHz	15, 30, 45, and 60 min	Reducing the number of CFU of *S. aureus* by 14% to 33% depending on exposure time, with no change in biofilm production	*S. taphyloccoccus aureus*	[13]/2012
Wi-Fi 2.4 GHz	3, 4.5, and 8 hours	Significant increase in the susceptibility to 5 antibiotics (*AZT, CTR, IMI, PIPRA, and CTX*) after 4.5 hours by increasing the diameter zone, followed by a decrease of the antibiotic diameter zone after 8 hours of exposure	*Klebsiella pneumoniae*	[14]/2015
MW 2.4 GHz	Continuous Up to 24 hours	Presence of MW-induced persisters with increased antibiotic resistance to Tobramycin	*Pseudomonas aeruginosa*	[15]/2017
GSM/Wi-Fi 0.9–2.4 GHz	Continuous Up to 24 hours	Differences in the bacterial growth curve (faster reach in exposed bacteria of Log phase, lower CFU at 8 hours, higher CFU at 24 hours) and variable antibiotic-resistant patterns depending on exposure time, antibiotic, and strain.	*E. coli and Listeria monocytogenes*	[16]/2017
GSM 0.9/1.8 GHz	2 hours	Significant reduction in *P. aeruginosa* growth rate, increasing the susceptibly of *S. aureus* to Amoxicillin, no effect on growth and antibiotic susceptibility of the other bacteria.	*S. aureus, S. epidermis, and P. aeruginosa*	[17]/2018
Wi-Fi 2.4 GHz	Continuous for 24 and 48 hours	Increasing antibiotic resistance of *E.coli* to several antibiotics and motility up to 29%., increasing biofilm production and cell metabolic activity of studied bacteria (up to 3 fold),	*E. coli 0157H7, S. epidermis and S. aureus*	[18]/2019
Wi-Fi 2.4 GHz	Continuous for 5 hours	Alteration of 101 differentially expressed genes (DEGs) implicated in cellular and metabolic processes	*E. coli DH5α*	[19]/2019
Wi-Fi 2.4 GHz	15, 30, 45 and 60 minutes	Increased proliferation and lactic acid production (up to 30%)	*Lactobacillus acidophilus and Lactobacillus casei*	[20]/2020
0.75–0.9 GHz	24 hours	Average reduction of bacterial growth rate by 10%.	*S. aureus*	[21]/2020
Wi-Fi 2.4 GHz	Continuous for 24 hours	Nonlinear antibiotic susceptibility to Colistin and Gentamycin and greater biofilm formation (up to 2.1 fold)	*K. pneumoniae*	[22]/2021
Wi-Fi 5 GHz	3-24 hours (measurement every 3 hours)	Alteration of antibiotic susceptibility studied with 8 antibiotics	*E. Coli, K. pneumoniae, P. aeruginosa, and S. aureus*	[23]/2022
RF 1-5 and Wi-Fi 2.4 GHz	3 or 24 hours	Rise in biofilm formation at 1, 2, and 4 GHz and decrease at 2.4 GHz	*E. coli, K. oxytoca and P. aeruginosa*	[24]/2022
GSM 0.9/1.8 GHz and Wi-Fi 2.4 GHz	2, 4, 6, 8, 10, and 24 hours	Alteration of the antimicrobial susceptibility to 10 antibiotics and increased growth rate of the *Escherichia faecalis.*	*E. faecalis.*	[25]/2022

EMF: Electromagnetic Fields, GSM: Global System for Mobile Communications, CFU: Colony Forming Unit, AZT: Azidothymidine, CTR: Ceftriaxone, IMI: Imipenem, PIPRA: Piperacillin, CTX: Cefotaxime, MW: Microwave, RF: Radiofrequency

bacterial growth depend on numerous parameters: the frequency, wavelength, intensity, pre- or post-exposure, and duration of exposure [26]. Some studies on RF-EMF have shown an increase in the viability of various bacterial strains by triggering their growth rate [20, 25]. However, other reports showed that these radiations decrease bacterial cell growth [13, 17, 27]. The contradicting results have caught the interest of several researchers who have concentrated on the aseptic action of higher-frequency waves [28, 29]. The bactericidal effects of electromagnetic waves on oral bacterial pathogens have been investigated, and according to Yumoto et al. irradiation at 500 kHz may be used for disinfection and sterilization purposes [30].

Effect of RF on bacterial susceptibility to antibiotics

The prospective use of specific antibiotics with induced synergistic and/or antagonistic effects in response to EMF has gotten special attention given the threat that antibiotic resistance represents to public health. This has also been addressed by the World Health Organization (WHO) [31]. Global System for Mobile Communications (GSM) and Wi-Fi radiations appear to induce resistance to antibiotics in some bacteria [14, 15, 18, 22]. Moreover, continuous 24-hour's exposure to GSM mobile waves induced *P. aeruginosa* to become persisters bacteria (a subpopulation of transiently antibiotic-tolerant bacterial cells that are often slow-growing or growth-arrested, and are able to resume growth after a lethal stress [32]) with enhanced antibiotic resistance. Exposed bacteria were able to resume growth with transient antibiotic tolerance after radiofrequency radiation stress [15]. Conversely, 2 hours GSM exposure revealed no important change in the antibiotic susceptibility of exposed *S. epidermidis* [17]. Furthermore, Wi-Fi radiation has shown a significantly higher susceptibility of *Klebsiella pneumoniae* to several antibiotics (Aztreonam, Cefteriaxone, Imipenem, Piperacillin, and Cefotaxime) before they reached an adaptation stage (a stage in which bacteria became forced to adapt to its environment) [14]. Some studies have reported that altered antibiotic resistance is related to substantial changes in the bacterial membrane and cell wall composition due to radiation exposure [14, 22, 33]. RF radiation may influence the mechanisms of antibiotic efflux by pumping out the antibiotic to the external environment using transporter proteins and activating Save-Our-Soul (SOS) response in bacteria (Figure 2) [34]. In this study, the upregulation of the genes *ybhG, ampE*, and *some ABC transporters* was reported in *E. coli* after 5 hours of

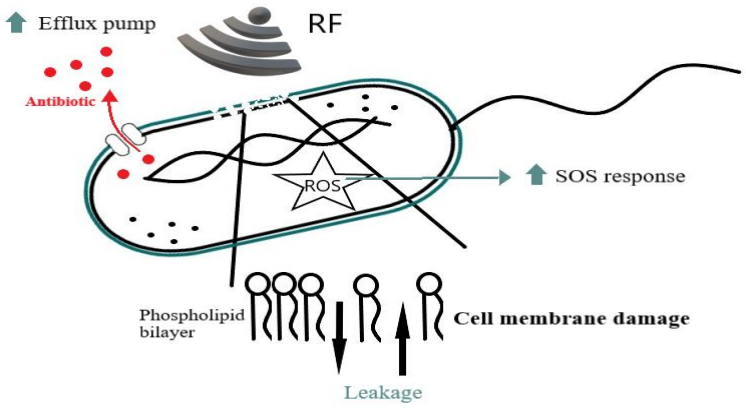

Figure 2: Potential effect of radiofrequency on bacterial antibiotic resistance. Radiofrequency (RF) Radiation damages the cell membrane and increases reactive oxygen species (ROS) in the cell. Alternatively, radiofrencency radiation activates Save-Our-Soul (SOS) response and antibiotic efflux pumps as mechanisms of defence and repair in bacteria.

Wi-Fi exposure where *YbhG* is implicated in the control of susceptibility to chloramphenicol through the efflux pathway [35] and AmpE is involved in peptidoglycan murein recycling by the expression of β-lactamase [36, 37]). Therapeutically, scientists validated that the effect of several antibiotics on bacteria could be enhanced synergically using Higher frequency of EMF from the ranges of 70-75 GHz [26, 38].

Effect of RF on bacterial DNA

DNA impairment and genotoxicity induced by EMFs have been intensively studied in organism models, such as for microorganisms, animals and plants [39]. The results revealed some possible mutagenic effects of low-frequency EMFs on *Salmonella typhimurium* [40]. Results obtained in this study showed a higher number of revertants in the presence of the magnetic field up to 18-fold compared to control plates. Furthermore, exposure of *E. coli* to magnetic fields can activate DNA repair by the induction of chaperone protein DnaK synthesis in response to the applied stress by 20 % repair improvement [41], while exposure of bacteria to RF at 0.835 GHz revealed no change in the reversion frequency neither in the DNA degradation *in vitro* using Ames method [12]. In parallel, 5 hours of Wi-Fi radiofrequency exposure upregulated *sulA, yjjQ, oxC* and *arsC* genes which are part of the defense system against DNA damage (SOS response) and 11 transposition-related genes as a response to the environmental radiation [34] (Figure 2).

Effect of RF on bacterial cell morphology

Microscopic analyses have revealed that bacterial cells exposed to EMF exhibited different cell morphology than unexposed controls. For example, *E. coli* cells exposed to microwave radiation at 18 GHz and at a temperature below 40 °C appeared dehydrated and shrunken compared to those not exposed, and even similar to those thermally heated (40 °C) [42]. The effect of these radiations was temporary and returned to the original state after 10 min. Similar effects were observed in Wi-Fi-exposed *K. pneumoniae* such as disruption of the protoplasm and plasma membrane [22]. The use of electrical and RF fields has been also applied in therapeutical treatments such as heart arrhythmias and tumor therapies [43].

Effects of RF on bacterial motility and chemotaxis

Motility is one of the strategies used by bacteria to escape environmental stressors. These prokaryotic cells can move by using their pili for gliding and twitching or their flagella for swimming [44]. Bacterial flagella are well-studied at the structural and molecular levels. Their movement has been shown to be powered by a rotational motor at their organizing centers, where 20 and 30 proteins are required to assemble and control the flagellar rotation [45]. Upon exposure to an environmental stimulus, bacteria can perceive the changes in their environment and therefore, they respond by deviating their motility that becomes directed toward a more favourable environment through a process known as chemotaxis [46].

In a recent study, the effect of RF on the motility of *E. coli* 0157H7 has been assessed by soft agar assay. As compared to non-exposed *E. coli*, the data demonstrates that motility has been dramatically enhanced by 28% and 29% over 24 and 48 hours of Wi-Fi exposure, respectively [18]. The outcomes of this study are consistent with previous research that demonstrated a substantial increase in E. coli motility under acid and heat stress [47, 48]. Furthermore, next-generation sequencing data revealed that exposure to Wi-Fi waves increased the expression of genes involved in chemotaxis and motility, including *fiA, fgM, motB, fiC, cheY, cheR, fiM, fiL, fgG,* and *fiT* with the higher Enrichment Score (ES) in DAVID functional clustering [19].

Effects of RF on bacterial biofilm formation

Another aspect of physiological changes

that the microorganisms undergo in response to different environmental stress factors is the biofilm formation. In this process, cells expressing a biofilm phenotype exhibit resistance to environmental stress conditions [49].

Biofilm formation occurs through at least three different mechanisms: (1) the attachment to the surface, (2) the multiplication and maturation of attached cells, and (3) the detachment and recruiting of cells from the bulk fluid [50]. In a biofilm environment, bacteria appear to be over a thousand times more resistant to a particular antibiotic than the same planktonic strains. It has been shown that RF at 10 MHz can increase the efficacy of antibiotics in *E. coli* biofilms [51]. However, short time exposure of *S. aureus* to GSM did not influence their biofilm production [13]. Rotating magnetic field increased biofilm formation by *S. aureus* and *E. coli* [52]. Similar results were revealed with exposure to RF showing an increase in biofilm formation up to 2.1-fold by some species of bacteria such as *E. coli*, *K. pneumoniae, S. aureus, and S. epidermidis* [16, 34]. In addition, exposure to Wi-Fi waves significantly increased the expression of the representative genes (*luxS, mrkA,* and *bcsA*) involved in biofilm formation and quorum sensing by 1 to 1.8 fold in *K. pneumoniae* [22].

Effects of RF on bacterial heat shock response

Heat Shock Proteins (HSPs) are present and conserved in all living organisms, from bacteria to humans [53]. They control and regulate cellular processes to protect the cell from environmental stress. Research laboratories have conceived numerous experimental models (*in-vivo* and *in-vitro*) to find possible biomarkers that are sensitive to physical stimuli and potential risks of EMF exposure. Henschenmacher et al. assessed the relationship between exposure to RF and oxidative stress through a meta-analysis study [54]. HSPs are known as "stress proteins" and are used as environmental biomarkers [55]. The level of expression of *Dnak* (equivalent to human HSP70) in *E. coli* was significantly raised after exposure to RF exposure. The study conducted by Aoude et al. using RF non-thermal effect revealed that *Dnak* and *lacZ* gene expression in exposed samples were higher at the level of mRNA using Reverse Transcription Polymerase Chain Reaction (RT-PCR) [56].

Effects of RF on bacterial gene expression

There are only a few studies of EMF effects on gene and protein expression in bacteria. Exposure to low-frequency EMF modifies slightly the global protein expression of *Chromobacterium violaceum* [57]. The results of the study conducted by El May in 2009, using a Static Magnetic Field (SMF) on cell growth, viability, and differential gene expression in *Salmonella* showed that the involved proteins were associated with protection against DNA damage and cellular metabolism. Moreover, exposure of *Salmonella hadar* to SMF showed a stress response mediated by an up-regulation of the *rpoA*, *katN*, and *dnaK* genes [58]. Next-generation RNA sequencing experiment conducted by Said-Salman et al. revealed that the exposure of *E. coli* DH5α to RF waves influenced 101 genes that are implicated in different metabolic and cellular mechanisms, stress adaptation, transposition, response to stimuli, and matrix adhesion [19]. In this research, 52 upregulated genes were mainly involved in stress adaptation such as motility and chemotaxis while the downregulated genes were essentially related to metabolic processes [19].

Bacterial Radio-adaptation

Adaptation of bacteria to ionizing radiation

To investigate the susceptibility of microorganisms to antibiotics after exposure to gamma radiation, Mortazavi et al. exposed different bacterial samples of *S. typhimurium*, *S. aureus*, and *K. pneumoniae* to gamma rays emitted from soil collected from the high background radiation areas of Ramsar in Northern Iran [59]. While the mean diameter of no growth

zone, in the standard Kirby-Bauer test, was 20.3±0.6 mm in non-irradiated *K. pneumoniae* control samples; it was only 14.7±0.6 mm in irradiated bacteria. The authors concluded that exposure to gamma rays significantly changed bacterial susceptibility to antibiotics. They hypothesized that natural background radiation was able to induce adaptive phenomena that helped bacteria better cope with the inhibitory effects of antibiotics.

Adaptation of bacteria to non-ionizing radiofrequency radiation

As shown in Figure 3, besides mechanical waves, such as diagnostic ultrasound [60], and pre-exposure to low-level ionizing electromagnetic radiation (e.g., gamma rays) [59], evidence shows that bacteria develop different mechanisms of adaptation to non-ionizing electromagnetic Radiofrequency (RF) such: antibiotic resistance, biofilm formation, altered growth, differentiated gene expression, and cell membrane impairment (Table 1). Bacteria that have become radioadapted not only developed more resistance to higher doses of radiation (such as mobile phone and Wi-Fi) but also resistance to any other factor that can be fatal for bacteria (e.g. antibiotics) [14, 18, 22, 61]. There are more than 8 billion mobile subscriptions in use worldwide in 2022 [62]. Bacteria have developed over the years around 800 proteins that contribute to antibiotic resistance as stated by the Centers for Disease Control and Prevention (CDC) Antimicrobial Threats Report [63], which correlate with the increased number of RF-EMF (mobile phone, Wi-Fi, base stations,…) (Figure 4).

EMF Biohazard

Due to the difficulties of reproducing the exact parameters of published experiments, the effects of EMF on biological systems (bacteria, plants, or cellular cultures…) have generated several disagreements. There is a substantial debate about whether the EMFs are detrimental or advantageous to human health; several *in vivo* and *in vitro* studies revealed that non-ionizing radiation might have a negative impact on human health [64];

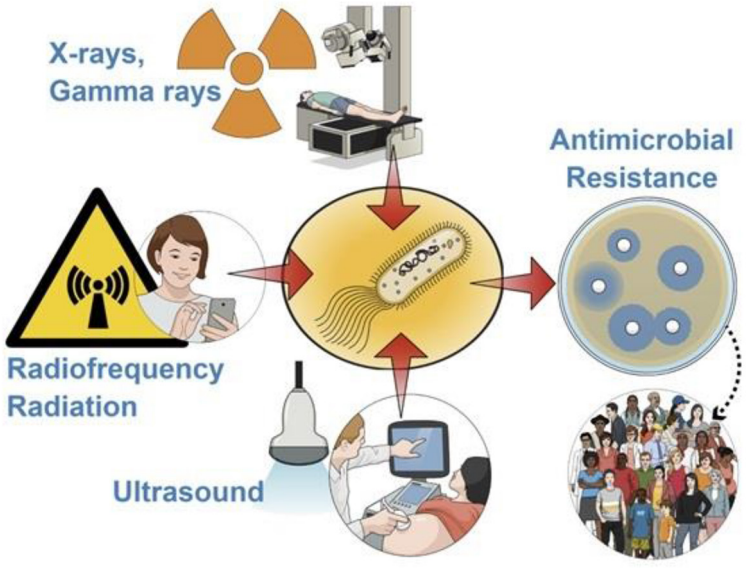

Figure 3: In addition to mechanical waves like diagnostic ultrasound, and exposure to ionizing radiations (e.g., gamma rays), research indicates that bacteria exhibit various adaptation mechanisms to non-ionizing electromagnetic radiation (e.g., radiofrequency) observed as antibiotic resistance, biofilm formation, changes in growth patterns, modified gene expression, and impairment of cell membranes.

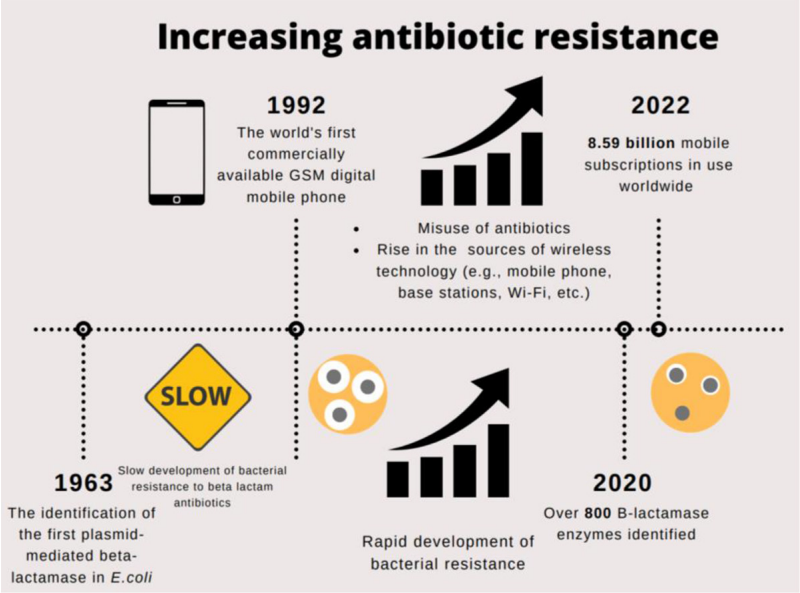

Figure 4: Cumulative number of beta-lactamase enzymes identified in bacteria in correlation with the estimated number of mobile phone subscriptions over the past years.

however, others suggested a beneficial effect of EMF [65]. The WHO has advised the assessment of the biological effects of the prevailing EMF radiation in inhabitants before authorizing the settlement of new EMF networks [66]. Another concern is that the exposure limits that have been set by regulatory agencies based on experiments using radiation in isolated spaces not taking into consideration other environmental toxic stimuli (biological and chemical). The set exposure limits would be in this case much lower for risk-free use. In a toxicology letter, Kostoff et al. reported that under real-life conditions, other toxic stimuli should be considered in combination with the new 5G wireless networking technology, which will increase the hazardous effects related to only RF exposure [67].

Mechanisms of bacterial adaptation to electromagnetic radiation

Based on screened data revealed in previously published studies conducted with the aim to evaluate the effects of radiofrequency radiation on microorganisms (Table 1), we hereby propose a potential mechanism for bacterial adaptation to surrounding radiation. Pre-exposure of bacteria to a low-level radiation might increase their resistance against a high-level radiation by activating a bacterial regulatory network in response to EMF, as shown in Figure 5-a. Under environmental radiation stress, a microorganism may alternate between the stages of proliferation and slow proliferation to resist the applied environment. Some of the bacterial communities go into a dormant state (Figure 5-b). At this stage, bacteria preserve a part of their metabolic activity but they become unable to replicate as a consequence of their slight adjustment ability. Modifications in the bacterial gene expression enable bacteria to counter the stress circumstances by shutting down their metabolism and arresting their growth in order to survive [19]. Other persistence mechanisms could be generated in response to environmental stress such as SOS repair, efflux pump and ability to form biofilm [68-71].

Discussion

It has been shown that the range of antimicrobial concentrations known as “the mutant

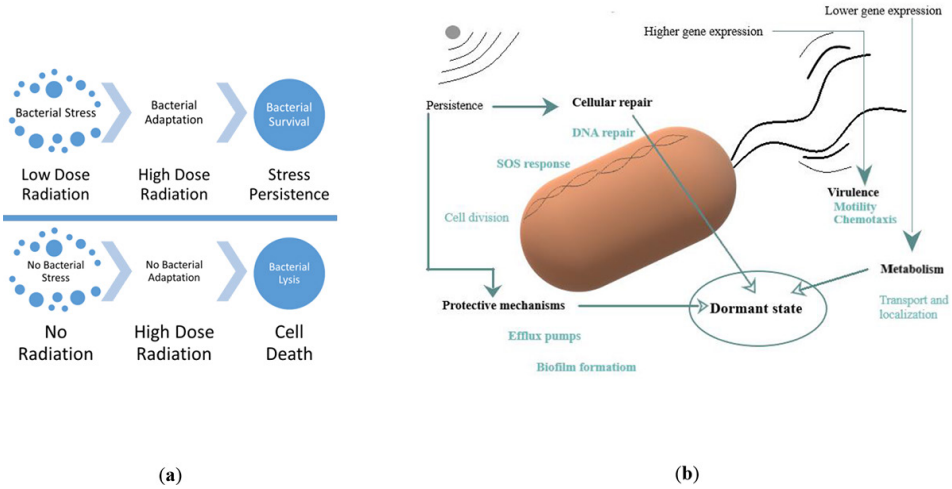

Figure 6: Suggested mechanisms of bacterial resistance to electromagnetic radiation. (**a**) Schematic representation of bacterial adaption to high level radiation after pre-exposure to low-level radiation (**b**) Schematic representation of bacterial regulatory network activated in response to radiation.

selection window" extends from the lowest concentration needed to prevent the growth of wild-type bacteria to the highest concentration needed to prevent the growth of the least susceptible mutant [72]. Limiting the enrichment of mutants is achieved by maintaining antimicrobial concentrations above the window [73]. The idea was also adapted for radiation biology. The exposure to radiofrequency should be within a narrow level of exposure and exposure rate (the so-called "exposure window theory") to turn microorganisms resistant to antibiotics. This type of multi-phasic response is similar to the responses induced by ionizing radiation. Mortazavi has previously shown that the findings of some experiments on pre-exposure to radiofrequency radiation support the existence of a minimum level of damage to trigger an adaptive response [74]. According to the exposure window theory, the induction of adaptive responses only occurs when the exposure(s) rates are within a specific window [14]. Given this consideration, Mortazavi has reported that these responses are similar to those frequently reported for induction of adaptive response by ionizing radiation [59]. Regarding adaptive responses induced by ionizing radiations, Mitchel has previously reported that "*the adaptive response in mammalian cells and mammals operates within a certain window that can be defined by upper and lower dose thresholds, typically between about 1 and 100 mGy for a single low dose rate exposure*" [75].

Conclusion

The exposure of bacteria to EMF can be appreciated when altering with bacterial survival mechanism and may compromise therapeutic success. However, continuous exposure of microorganisms to common sources of EMF such RF may emerge super-pathogens with high resistance to treatments.

Authors' Contribution

I. Said-Salman provided the conceptualization, original draft preparation, review and editing. SMJ worked on the original draft preparation, review, and editing. SAR. Mortazavi did review and editing. S. El Khatib did review, and editing. L. Sihver wrote the draft, reviewed the manuscript, edited and

submitted the final version of the paper. All authors reviewed the final version of the manuscript.

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Conflict of Interest

SMJ. Mortazavi and L. Sihver, as the Editorial Board Members, were not involved in the peer-review and decision-making processes for this manuscript.

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