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IMPACT OF HIGH FREQUENCY ELECTROMAGNETIC FIELD ON SURVIVAL RATE AND THE MORPHOLOGY OF PATHOGENIC BACTERIA

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ABSTRACT

This study aimed to evaluate of impact of exposure to high frequency-electromagnetic fields on the survival rate and the morphology of bacteria. Three bacterial strains namely *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 25923), and *Pseudomonas aeruginosa* (ATCC 27853) were exposed to HF-EMF irradiation at 900MHz and 1800 MHz for 2 hour. After exposure, the viable counts (CFU/ml) of exposed bacteria were enumerated. The results showed that exposure to radiations at 900 MHZ and 1800 MHz led to reduction in viable counts compared to controls which were not subjected to exposure to irradiation. Further the impact of irradiation on cell morphology was evaluated by scanning electron microscopy (SEM). Results obtained for the SEM studies clearly showed that the HF-EMF irradiation resulted in changes in cell shape and size when compared to those cells which were not exposed (control). Cell diameters were observed to be reduced compared to that of unexposed cell. The SEM results also indicated shrinkage of bacterial cells resulting in changes in cell shape. It is concluded that HF-EMF irradiations emitted by mobile phones affect the survival of viable bacteria in the environment.

Keywords: High frequency electromagnetic field, Bacteria, Survival rate and Morphology.

INTRODUCTION

The effects of low-frequency electromagnetic fields (LF-EMF) on microorganisms, particularly bacteria have been reported by several workers (Fojt *et al*., 2004; Ludek *et al*., 2002; Mittenzwey *et al*., 1996, Novak *et al*., 2007) compared to the impact of high electromagnetic field (EMF). In fact earlier investigators have suggested that microorganisms including bacteria are good candidates for understanding the impact of electromagnetic fields (EMF) on living organisms (Berg, 1999; Markov *et al*., 2004).Numerous studies in the literature have reported that the magnetic fields influence functional changes in cell membrane proteins (Liburdy *et al*., 1993; Baureus Koch *et al*., 2003), mechanisms of ion exchange (Liburdy *et al*., 1993), and activities of enzymes (Blank and Soo,2001; Mihoub *et al*., 2012; Grassi *et al*.,2004; Ravera *et al*., 2004) in microorganisms. In this context the present study examined the impact of high frequency electromagnetic fields (HF-EMF) at 900 MHz and 1800 MHz, used in mobile phone on the survival rate and on the morphology of bacterial cells.

MATERIALS AND METHODS

Electromagnetic filed exposure system: Electromagnetic filed exposure system employed in the present study included a dummy voice call established between Radio Communication Tester CMU 200 by Rhode and Schwarz and the mobile phone handset. CMU 200 was considered as the base station emulator in this case. Bacterial cells were exposed to radiofrequencies (RF) by means of a mobile handset (Nokia, 6120). For this experiment, the mobile was operated in GSM 900 and 1800 band for two hours. Cultured bacteria on petri dishes were put in 0touching proximity of the mobile handset for RF exposure.

Bacteria used: Three standard bacteria were used ,namely *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228), and *Pseudomonas aeruginosa* (ATCC 27853) were used in the study. They were obtained from the Microbiology Laboratory of the College of Medicine, King Khalid University Hospital, Riyadh, Saudi Arabia.

Effect of HF-EMF on the Survival rate of bacteria: The effect of HF-EMF emitted by mobile phone set on the survival rate of bacterial cells was studied by exposing the three standard bacteria mentioned above to frequencies of 900 and 1800 MHz and determining the number of viable colony forming units (CFU/ml) employing plate count method. After exposure plating was done in triplicate, with 0.1 ml of 10-fold concentrated broth cultures after serial dilutions of culture samples, on Nutrient agar (OXOID LTD, Basingstoke, Hampshire, England). Inoculated plates were incubated at 37°C for 24hour and viable colony counts were determined. Controls were also done for all three bacteria without subjecting them to exposure to radio frequencies. Survival rate was calculated by

subtracting the CFUs obtained for the experimental cultures from the controls and expressed in terms of percent survival

Scanning Electron Microscopy: Scanning electron microscopy (SEM) studies were carried out with the bacterial cells, both unexposed to radio frequencies and those which were exposed to HF-EMF radio frequencies 900 MHz and 1800 MHz, for the purpose of studying their impact on the morphology of exposed bacteria. After exposure, bacterial cells were fixed in buffered aldehyde for 3 hours (Buffered Aldehyde for SEM consists of 2.5 % glutaraldhyde in phosphate buffer). Later glutaraldehyde was drained and rinsed 3 times, each time for 5 minutes, in sodium cacodylate solution buffer. Post fixing was done with Osmium tetroxide2Oso_4 prepared as 2% aqueous solution (1 g of Oso_4 in 50 ml D. Water),for 1 hour, after aldehyde fixation. Then, the specimen was rinsed in distilled water and dehydrated using a graded ethanol series 25%, 50%, 75%, and 100% (two times) each for 10 min. Again, rinsed in distilled water. Later the specimen was removed, mounted on specimen stabs, coated with gold and viewed in SEM (JSM-6380 LA) at 20 kv and t1 garin size 40 work distance 15.

Statistical analysis: Results obtained for the survival rate studies were statistically treated and the results were presented as mean ± standard deviation of three independent experiments. Data were analyzed using one-way ANOVA and Dunnett' test was used for statistical significance at $p<0.05$.

RESULTS AND DISCUSSION

Effect of HF-EMF on the Survival rate of bacteria: Exposure of bacterial cells of *S. aureus, S. epidermidis* and *P. aeruginosa* to high frequency-electromagnetic field (HF-EMF at 900 MHZ and 1800 MHZ), led to reduction in viable counts since the number of CFU/ml of exposed bacteria was observed to be significantly different ($p<0.05$) after exposure when compared to controls which were not exposed to radiation. Interestingly from the data presented in Figures 1-3, it was noted that the number of viable counts (CFU/ml) of bacteria were not significantly different for exposures between 900 MHZ and 1800 MHZ indicating the increase in frequency did not enhance lethal activity. Even radiation at 900 MHZ was sufficient enough to influence the survival percentage of bacterial cells irrespective of their species.

Results obtained from the present study clearly indicated negative effect of HF-EMF radiation on the growth and survival of both gram positive and gram negative bacteria tested during the course of the experiment. The viable counts estimated in terms of CFU/ml for *S. aureus, S. epidermidis* and *P. aeruginosa* were affected by the high frequency- electromagnetic field (900 MHZ and 1800 MHZ).

Similar results were reported earlier where increase in power of magnetic field caused death in *E. coli* suggesting that the bacteria lost its activity to procedure colonies and consequently, grew at a slower ratio compared to those which were not exposed to high power of magnetic field (Strasak *et al*., 2002). Further, results of the present study is also in agreement with the observations reported by Fojt *et al*., (2007) that the growth rate of *Paracoccusdenitrificans* is affected by the duration of exposure of the microorganism and the strength of the magnetic field.

From the literature it is clear that irradiation with electromagnetic fields (EMF) affect the ionic channels permeability in the membrane of cells, and consequently influence the transport of ions into the cells and cause biological changes in the organisms (Galvanoskis and Sandblom, 1998). Moreover radiation can results in the creation of free radicals when exposed to magnetic field (Fojt *et al*., 2004). Thus the reduction in survival percentage of the bacterial cells of the three known bacteria could be possibly due to similar negative effect caused by the tested HF-EMF radiation.

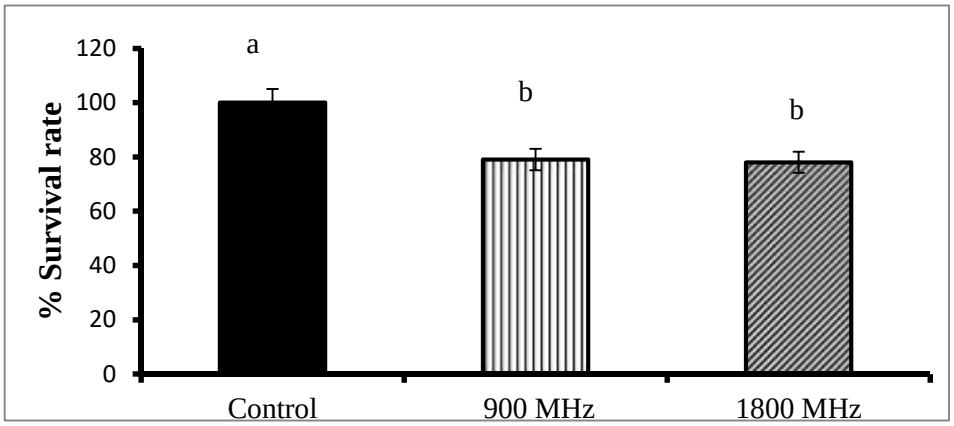

Figure1. The percentage of survival rates of EMF (900 MHz and 1800 MHz) for *S. aureus*.

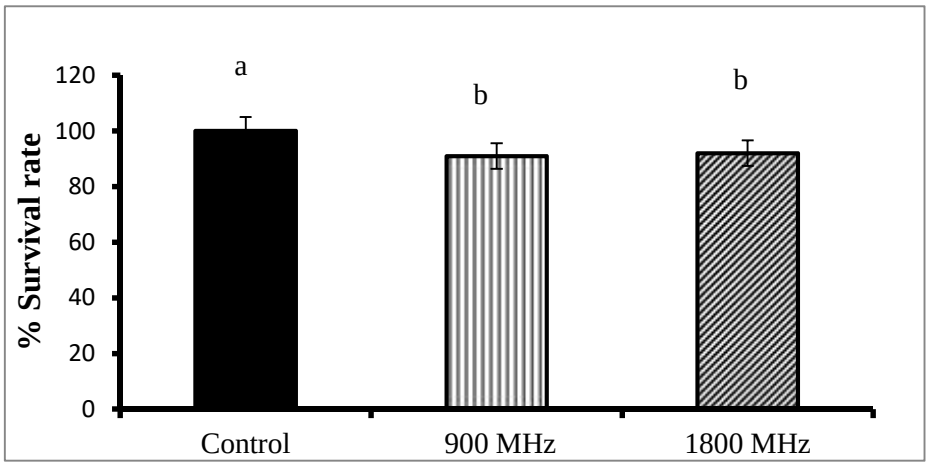

Figure2. The percentage of survival rates of EMF (900 MHz and 1800 MHz) for *S. epidermidis*.

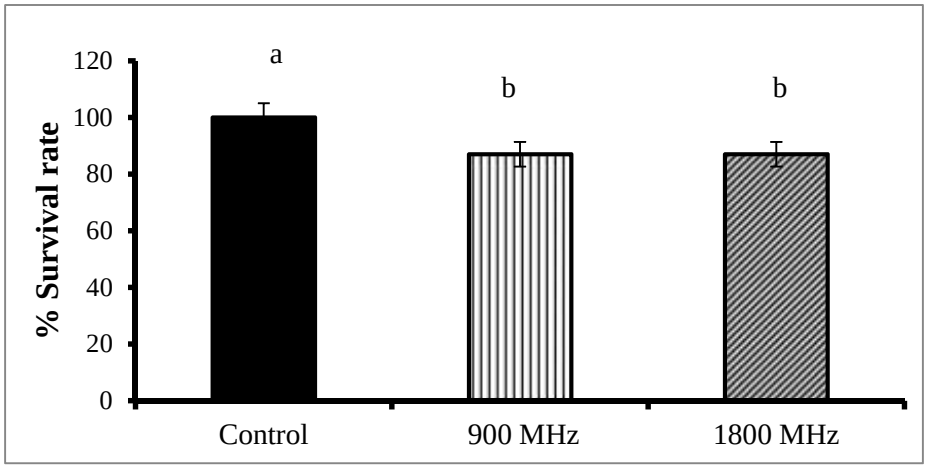

Figure 3. The percentage of survival rates of EMF (900 MHz and 1800 MHz) for *P.s aeruginosa*.

Effect of HF-EMF on the Morphology of bacteria: Potential impacts of high frequency-electromagnetic field at 900 MHz and 1800 MHz) on the shape and morphology of bacterial cells after exposure to radiation for a specific period of time was assessed with scanning electron microscopic (SEM) studies. Results obtained for the SEM observation are presented in Figure 4-6. Results recorded for *S.aureus* shown in Fig 4 indicated clearly that there was change in the cell shape and size when exposed to 900MHz and 1800 MHz compared to the respective. It was noted that the length and width of exposed bacterial cells were reduced compared to control cells. The average of width/length (nm/nm) of unexposed cells of *S. aureus* was 719.4/938.2 nm while it was 525.4/595.8 and 589/685.4 for 900 and 1800 MHz respectively (Table1). Similarly, the results of SEM observation for *S. epidermidis* shown in Fig.5 also showed significant reduction in size suggesting that the morphology of cells were affected leading to change and shrinkage. Average of width/length (nm/nm) of unexposed cells of *S. epidermidis* was 807.4/1006 nm while it was 438.8/517.2 and 438.8/517.2 for 900 and 1800 MHz respectively (Table2). The results obtained for SEM of *P. aeruginosa* shown in Fig.6 also recorded similar changes in morphology and size as it was noted with the *Staphylococci*. Average of width/length (nm/nm) of unexposed cells of *P. aeruginosa* was 556/1610 while 511/2260 and 784/1820 for 900 and 1800 MHz respectively (Table3).

SEM observations clearly show that HF-EMF radiations at 900 MHz and 1800 MHz resulted in change in cell morphology and size besides inflicting shrinkage and mortality when exposed to high frequency radiations

The results of the present study corroborates with the report of Cellini *et al*. (2008) who demonstrated that exposure to extremely low frequency-electromagnetic field at 50 Hz for short time (20–120 min) affected morphology and viability of *E. coli*. On the contrary, Fojt *et al*., (2007) have not detected any alteration in bacterial morphology of *E. coli* and *P. Denitrificans* when exposed to extremely low frequency-electromagnetic field at 50 Hz for 1 h, so they suggest that the shape of bacteria does not show any essential role in the interaction with magnetic field radiation.

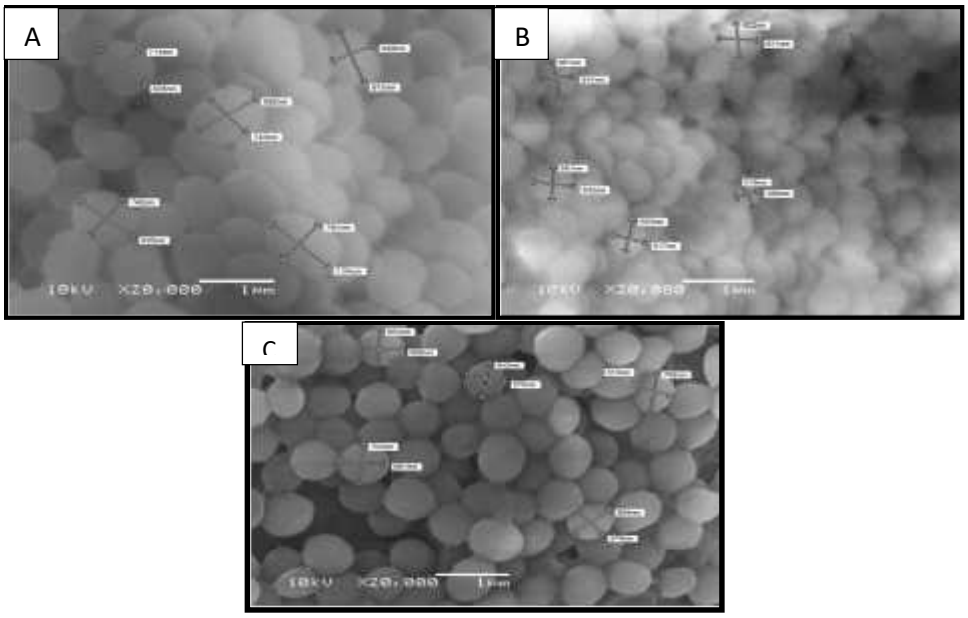

Figure 4.Scanning electron microscopy images of *S. aureus* (A) unexposed, (B) exposed to 900MHz and (c) exposed to 1800 MHz.

Table 1.Size of cells of *S. aureus* (width /length) after expose to 900 MHz and 1800 MHz.

Cells	Size of width /length (nm)		
	Control	900 MHz	1800 MHz
1	714/828	541/661	620/662
2	743/982	581/635	661/703
3	649/915	517/553	575/640
4	740/926	386/519	513/768
5	751/1040	602/611	576/654
Average	**719.4/938.2**	**525.4/595.8**	**589/685.4**

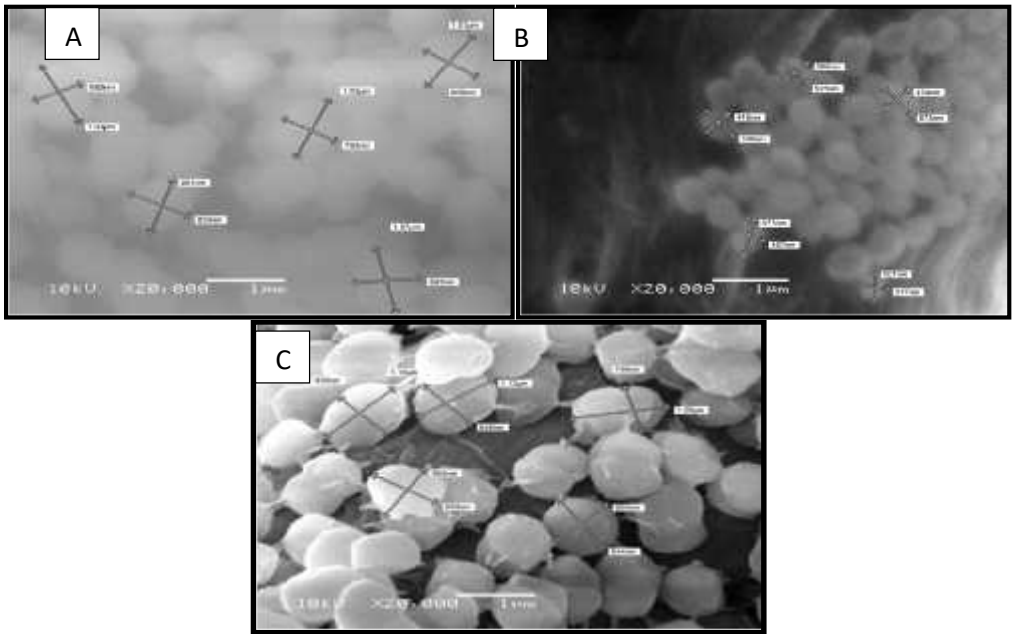

Figure5. Scanning electron microscopy images of *S.epidermidis* (A) unexposed, (B) exposed to 900MHz and (c) exposed to 1800 MHz .

Table 2.Size of cells of *S. epidermidis* (width /length) after expose to 900 MHz and 1800 MHz.

Cells	Size of width /length (nm)		
	Control	900 MHz	1800 MHz
1	685/1030	398/525	835/1180
2	859/901	418/530	849/1130
3	792/1020	577/457	746/1260
4	808/1010	424/573	944/955
5	893/1070	377/501	904/934
Average	**807.4/1006**	**438.8/517.2**	**855.6/1091**

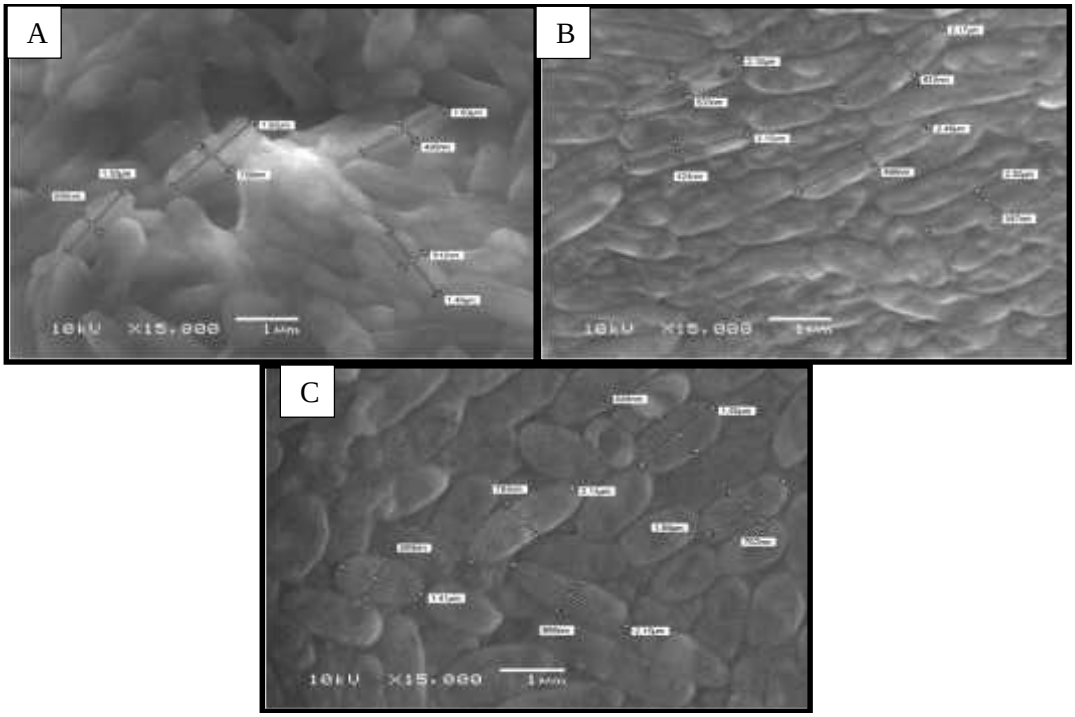

Figure 6. Scanning electron microscopy images of *P.aeruginosa*(A) unexposed, (B) exposed to 900MHz and (c) exposed to 1800 MHz.

Table 3.Size of cells of *P.aeruginosa* (width/ length) after expose to 900 MHz and 1800 MHz.

Cells	Size of width/ length (nm)		
	Control	900 MHz	1800 MHz
1	509/1530	533/2190	860/1410
2	707/1820	424/2010	785/2140
3	496/1630	499/2460	699/2150
4	512/1460	587/2300	792/1590
Average	**556/1610**	**511/2260**	**784/1820**

Conclusion: The effects of high frequency-electromagnetic fields (HF-EMF) on selected bacteria were studied to understand the impact of radiation stress in environment on biological systems. Bacteria were selected for the study since they grow quickly and have relatively short generation time when compared to other organisms. This study was conducted by exposure to bacteria to EMF at 900 MHz and 1800 MHz and evaluating the impact on morphology of bacterial cells and bacterial survival rate. SEM observation results of all bacteria showed that the shapes and sizes of bacterial cell were changed on exposure to 900MHz and 1800 MHz. The number of colony forming units (CFU) of all exposed bacteria showed significant difference when compared to with controls.

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