UDC 579.0:537.868

**Ali F. M.[a], El-Khatib A. M.[b], Sabry S. A.[c], Abo-Neima S. E.[d], Motaweh H. A.[d]**
[a]Biophysics Department, Faculty of Science, Cairo University, Egypt
[b]Physics Department, Faculty of Science, Alexandria University, Egypt
[c]Botany & Microbiology Department, Faculty of Science, Alexandria University, Egypt
[d]Physics Department, Faculty of Science, Damanhour University, Egypt
E-mail: prof_motaweh@yahoo.om

# CONTROL OF STAPHYLOCOCCUS AUREUS GROWTH BY ELECTROMAGNETIC THERAPY

**The present work aimed to study the biological effects of Extremely Low Frequency Electromagnetic Field (ELF-EMF) on the growth rate, morphology and antibiotic sensitivity patterns of the Gram-positive bacterium Staphylococcus aureus to determine any morphological and metabolic changes that might have been caused by ELF-EMF. In order to compare cell viability, number of colony-forming units (CFU) and growth rate (optical density at 600nm) was determined. The results showed that a highly significant inhibition effect occurred when S.aureus was exposed to resonance of 0.8 Hz QAMW for a single exposure (120min). Moreover, exposed cells became more sensitive to the tested antibiotics compared to control. Significant ultra structural changes occurred as observed by Transmission Electron Microscope (TEM). Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) revealed genetic fingerprinting variation as observed in the electrophoresis patterns between exposed and unexposed cells of S.aureus.**

**Key words: Staphylococcus aureus, ELF-EMF, bacterial growth rate, antibiotic sensitivity, morphological changes, TEM, RAPD– PCR.**

## *[I] NTRODUCTION*

In the modern society, greater use of technologies leads to increasing exposure to extremely low frequency electromagnetic fields (ELF-EMFs) generated by structures and appliances such as power lines and ordinary devices used inside house and work places. As a consequence, the effect of ELF–EMFs on the biological functions of living organisms represent an emerging area of interest with respect to environmental influences on human health.In latest years, several studies have been performed to study the variety of cell responses observed due to exposure to ELF-EMFs involving proliferation and differentiation [1, 2],gene expression[3, 4],modulation of the membrane receptors functionality[5, 6], apoptosis[7–8], alteration in ion homeostasis[1, 9, 10, 11, 12], and free radical generation [13, 14, 15]. Fadel et al.,2003 [16] reported that the main damaging role of 50 Hz magnetic field may be on the cellular membrane that strongly affects, not only the cellular physiological functions, but also the cell-to-cell communications.

Effects of ELF-EMFs on bacterial cells have been also studied **[**17, 18].In particular, it has been demonstrated that ELF-EMFs can negatively [18, 19, 20, 21, 22], or positively [21, 22, 23, 24, 25] affect functional parameters (cell growth and viability). Inhan-Garip et al., 2011[18] studied the effect of extremely low frequency (<300 Hz) electromagnetic fields (ELF-EMF) on the growth rate of Gram-positive and Gram-negative bacteria and determined morphological changes that might have been caused by ELF-EMFs. Over the last few years, bacteria were subjected to many experimental procedures to evaluate how such unicellular systems may respond to EMFs [16, 20, 26, 27, 28, 29, 30, 31, 32].

Studying the effects of ELF-EMFs on bacteria is essential not only for investigation the environmental stress, but also to explore the possibility of controlling the sensitivity of bacteria at resonance frequency. Therefore, considerable efforts have been made towards the development of alternative method for the treatment of bacterial infections. Recently, efforts were devoted to control cellular activities by using electromagnetic waves of very low field intensity and frequencies which resonates the bioelectric signals generated during a particular metabolic activity. These trials succeeded to control the growth of Erlich tumors [33], bacterial cells [34], and fungi [35]. Some studies showed that electromagnetic waves may affect microbial growth which may be used to control the growth of bacteria [36, 37].*Obermeier et al., 2009*[38] studied the effect of different electric and electromagnetic fields on the growth of *S.aureus*. The results indicated that no significant difference between colony-forming units of exposed samples and non-exposed.

This study is focused on investigating the possible influence of ELF-EMF on a strain of *Staphylococcus aureus (S.aureus*) (ATCC# 25923). This bacterium was chosen as example of well-characterized Gram-positive widely distributed in the environment and clinically relevant in nosocomial infections. Therefore, in particular, this work evaluated the in

vitro effect of ELF-EMF on the growth rate and antibiotic sensitivity of this strain, as well as the cellular and molecular changes that might have occurred.

### *[II] MATERIALS AND METHODS*

#### *2.1. Exposure Facility System*

Fig.1 indicates sketch diagram for synthesized function generators of the exposure facility of the bacterial culture, the modulating waveform was square and the carrier frequency was 30 MHz sine wave. The carrier and modulating wave carrier was generated by synthesized arbitrary function generator type DS345manufactured by Stanford Research System. The samples in autoclaving tube were exposed to the QAMW through two parallel cupper disk electrodes, each of diameter 8 cm and the distance between two electrodes 1.5 cm. During our trials to find out the resonance frequency of the modulated waves, samples were exposed different frequencies in the range 0.1 to 1 Hz in steps of 0.1 Hz for 60 min in order to determine the resonance frequency of growth inhibition. After finding the resonance frequency an experiment was carried out to determine the most effective exposure time. The unexposed bacteria cells were left to grow in similar conditions, but without electric field.

#### *2.2. Microorganism Growth Conditions*

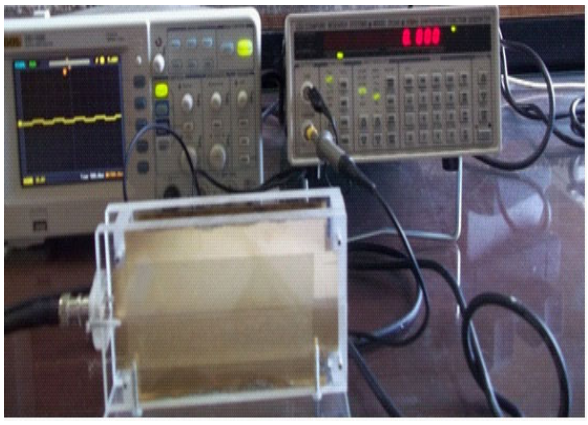
The bacterium used in this work is a strain of *S.aureus* (ATCC# 25923) provided from the microbiological Lab, Faculty of Science, Alexandria University. A broth subculture was prepared by inoculating a test tube containing 5ml of sterile nutrient broth of pH 7.1 with two single colonies of bacteria from nutrient agar Plate, followed by incubation at 37°C for 24 h. After mixing (time t=0 h) the new culture was divided into eleven groups, one control, the others were exposed to QAMW for different frequencies in the range 0.1 to 1 Hz in steps of 0.1 Hz for 60 min in order to determine the resonance frequency of growth inhibition. The cultures were then incubated at 37°C.The number of colony forming units (CFU) was used to quantify our results and was determined by plate counting technique [39].Appropriate dilutions of the bacterial cells were used to inoculate nutrient agar plates. Fresh bacterial cultures were used throughout the experiments. Control cultures were kept in the same conditions as the exposed ones.Each experiment was made in triplicates and the average was considered.

#### *2.3. Effect of Exposure Time*

Fresh bacterial cultures were used and exposed to 0.8 Hz QAMW for different times (15, 30, 45, 60, 75, 90, 105 and 120 min).After exposure, 100 μL of each culture was used to inoculate agar plates of the same medium. Plates were then incubated aerobically for 24h at37°C, and then numbers of viable cells were expressed as colony-forming units (CFU/mL). Each experiment was repeated three times alongside control groups, which were kept under identical conditions.

#### *2.4. Antibiotic Susceptibility Test*

*S.aureus* cells were tested for their in vitro susceptibility to various antibiotics using the agar diffusion method.The antibiotics used in this study were chosen to represent different modes of action. These discs were Amicacin [AK (30μg)], Gentamicin [CN (10μg) ], Streptomycin [S (10μg) ], Kanamycin [K (30μg)], Penicillin [P (10μg)], Ampicillin [AM (10μg)] and chloromphenicol [C (30μg)] which inhibit protein synthesis. Also, Ciprofloxacin [CIP (5μg)], trimethoprim–sulfam-



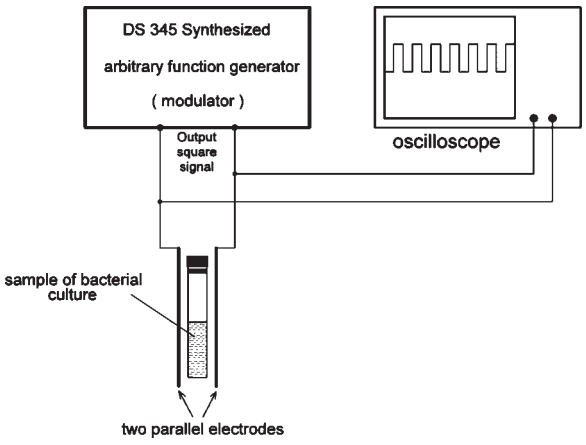



Fig: 1. Synthesized function generator for the exposure facility of the bacterial culture

ethoxazole [SXT (25μg)], Levofloxacin [LEV(5μg)] and Norfloxacin [NOR (10μg)] which are inhibitors for bacterial DNA. In addition to ceftazidime [CAZ (30μg)], Amoxicillin/Clavulanic acid [AMC (30μg)], Nitrofuran [F (300μg)] and Rifampin [RA (5μg)] which are inhibitors for the bacterial cell wall. After plate inoculation and incubation at 37 C for 24 h, the diameters of the inhibition or stimulation zone of exposed and unexposed cells were measured in mm.

### *2.5. Transmission Electron Microscopy*

The morphological changes of control group and group exposed to 0.8 Hz have been determined using Transmission Electron Microscope (TEM). TEM investigation was done in TEM Unit, Medical Research Institute, Alexandria University. Bacterial cells (10–100мl) were fixed in 300мl of glutaraldehyde (Merck, Darmstadt, Germany) and 3% 0.1 M phosphate buffer saline (PBS) (Sigma, Steinheim, Germany), pH 6 in a micro-centrifuge (Hettich Universal 30 RF, Hettich, Tuttlingen, Germany), so that pellets no larger than 0.5 mm thick were obtained. The supernatant fluid was decanted and aspirated with a Pasteur pipette. The pellet was resuspended in same fixative and allowed to stand for 4 h. The fixative was replaced with a phosphate buffer solution (0.1 M PBS, pH 7.2). Centrifugation was followed by three washes in phosphate buffer; each involved gentle suspension of the bacterial cells with a Pasteur pipette and then prompts sedimentation in the centrifuge. For post-fixation the pellet was then suspended in the microfuge tubes with 2% $Os0_4$(Merck, Darmstadt, Germany) in 0.1 M PBS,pH 7.2, for 2 h and then progressively dehydrated with ethyl alcohol. After dehydration, intact pellets were easily removed from the microfuge tubes for embedding in Epon 812 (FlukaChemie, Buchs, Switzerland). Thin sections from Eponblocks were cut with anultramicrotom (Leica UltracutR,Vienna, Austria) stained with 2% uranyl acetate (SPI-CHEM, West Chester, PA, USA) for 15 min at 60°C, then washed, and stained for 10 min at room temperature with saturated lead citrate (FlukaChemie, Buchs, Switzerland). The sections were examined by TEM [40].

### *2.6. Isolation of DNA*

DNA was extracted from 50 mg of fresh cultures of bacteria (either control or exposed cultures) according to this method which is an improved method of the standard phenol/chloroform method [41].To extract the DNA from Gram-positive bacteria, 1 ml cell suspension was centrifuged at 8000 g for 2 min, to pellet cells. After removing the supernatant, the cells were washed with 400 μl STE Buffer (100 mM NaCl, 10 mM Tris/ HCl, 1 mM EDTA, pH 8.0) twice. Then the cells were centrifuged at 8000g for 2 min. The pellets were resuspended in 200μl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0). Then 100μl Tris-saturated phenol (pH 8.0) was added to these tubes, followed by a vortex-mixing step of 60 second to lyse cells. The samples were subsequently centrifuged at 13000 g for 5 min at 4°C to separate the aqueous phase from the organic phase. 160 μl upper aqueous phases was transferred to a clean 1.5 ml tube. 40 μl TE buffer was added to make 200 μl and mixed with 100μl chloroform and centrifuged for 5 min at 13000 g at 4°C Lysate was purified by chloroform extraction until a white interface was no longer present; this procedure might have to be repeated two to three times. 160 μl upper aqueous phases was transferred to a clean 1.5 ml tube. 40 μl TE and 25μl Proteinase K (modified instead of 5μl RNAs) were added and incubated at 37°C for 10 min to digest RNA. Then 100μl Chloroform was added to the tube, mixed well and centrifuged for 5 min at 13000 g at 4°C. 150μl upper aqueous phase was transferred to a clean 1.5 ml tube. The aqueous phase contained purified DNA and was directly used for the subsequent experiments or stored at) 20°C. The purity and yield of the DNA were assessed spectrophotometrically by calculating the $A_{260}/A_{280}$ ratios and the $A_{260}/A_{280}$ values to determine protein impurities and DNA concentrations [42].

### *2.7. Random Amplified Polymorphic DNA Technique (RAPD-PCR)*

Eight primers were tested.Codes, nucleotide sequences and G+C percentages of tested primers used in the RAPD reaction are shown in table (1). The reaction was carried out in a DNA Thermocycler (MJ Research Inc.USA). Reactions without DNA were used as negative controls. A stock buffered solution containing 250 μl of 10×PCR buffer, 12. 5 μl at a concentration of 125 milimole (Mm) for each dATP, dTTP,dGTP, Dctp and 100μl of 25 Mm $MgCl_2$ was prepared in 1.5 ml eppendorf tube. The primers were used at a concentration of 20 icogram, and double distilled water was added to pring the volume of the stock buffer solution to 1.5 ml. A volume of 5μlof the target DNA was added to 44μl of the stock solution in PCR tubes and mixed by vortexing. A volume of 1μl of Taq DNA polymerase (Perkin Elmer, Amersham, USA) was

Table: 1. Code, nucleotide sequence and G+C (%) of arbitrary primers used in the random amplified polymorphic DNA (RAPD) reactions

| primer | Sequence 5/-3/ | G+C (%) | Annealing (°C) |
|---|---|---|---|
| P1 | ATGCCC CTG T | 60 | 32 |
| P2 | GGTGACGCAGGGGTAACGCC | 70 | 28 |
| P3 | AGGAGG TGA TCC AAC CGC | 60 | 30 |
| P4 | GAG CCA GTG TCT GCT TTG | 56 | 30 |
| P5 | TGAAGGGGGGAACCCCTGTG | 65 | 62 |
| P6 | CGC TGT CGC C | 80 | 28 |
| P7 | GGY GGY TGG AAT GAR GG | 53 | 30 |
| P8 | GAYTTAGATTGGGAATAYCC | 35 | 30 |

used at a concentration of 5.0 units. To avoid evaporation of the PCR mixture during the high temperature of the thermal cycling profiles. A drop of mineral oil was added to cover the reaction mixture. All PCR amplification reactions were carried out in a final volume of 50µl. The thermal cycling profiles were as follow: a 2-min incubation at 95°C, followed by 40 cycles of 94°C for 1 min, 55°C for 30 sec and72°C for 45 sec, and a final incubation at 72°C for 10 min. Thermal profiles were performed on a Techne PHC-2 thermal cycler (Techne, Princeton, N.J.U.S.A) FOLLOWING AMPLIFICATION, 20 µL from each PCR reaction containing amplified product were loaded onto gels of 1.5% Seakem agarose (FMC Bioproduct, Rockland ME.,U.S.A) and electrophoresed. The gels were stained with ethidium bromide and the PCR products were identified following visualization under UV light [43, 44, 45, 46].

### *2.8. Statistical Analysis*

All experiments were replicated at least three times and the statistical significance of each difference observed among the mean values was determined by standard error analysis. Data from bacterial growth studies were compared for statistical significance using Student t-test and ANOVA analysis, the level of significance was set at $p < 0.05$.

## [III] *RESULTS AND DISCUSSION*

The main objective of this work was to find out the frequency of the (ELF-EMFs) that resonates the bioelectric signals generated from *S.aureus* during cell division and studying the changes that may occur in its nuclear DNA and cell membrane structure. The bioelectric signals generated during metabolic activities of cells are known to be in the extremely low frequency range, therefore, to interfere with this signals; the applied electromagnetic wave should have the same frequency of the bioelectric signal.

Based on the Metabolic Bio-magnetic Resonance Model (BMRM) suggested by Fadel (1998) [47], an external applied electromagnetic signal can interfere with a bioelectric signal when both at resonance. The resultant of the interference is the algebraic summation of the two waves which may be instructive or destructive, i.e. enhancement or inhibition, respectively, for the running process. Based on these bases the present work was planed, [48].

The bioelectric signals generated during metabolic activities of cells are known to be in the extremely low frequency range, therefore, to interfere with these signals; the applied electromagnetic wave should have the same frequency of the bioelectric signal. Since the impedance of tissue (Z) is equal to $1/2\pi fc$ , where (f) is the applied frequency (C) is the capacitance of the electrodes with tissue, so «(Z) is inversely frequency (f) dependent, and will be of very high values for extremely low frequency (ELF) waves and equal to infinity for D.C. To allow ELF waves to penetrate biological tissue it was necessary to use amplitude modulated waves which carry (ELF-EM) waves by wave carrier of medium frequency through a biological system to render electrical impendence of the treated biological tissue to the minimum. In this work a wave carrier of 30MHz was used. The growth curve for *S.aureus* was measured for different samples after the exposure to square amplitude modulated waves (QAMW) for a period of 60 min different amplitude modulating frequencies. This experiment started with a pilot study where the frequency of the applied electric field was changed to cover the frequency ranges from 1.0 to 50 HZ in steps of 5 Hz. There was no change in the growth curve for the samples exposed to QAMW in the frequency range from 1.0 to 50 Hz as shown in fig 2. The study was then directed towards frequencies lower than 1.0 Hz in step of 0.1 Hz. It was noticed that exposure for 120 min of the samples to 0.8 Hz inhibited cellular division of the microorganism. The change of absorbance at 11 hr incubation time with respect to control as a function of QAMW frequency in the range 0 Hz up to 1.0 Hz is shown in figure (3).

### 3.1. Standard calibration curve

Figure (4) illustrates the variation of sample optical density measured at 600nm[38] as a function of *S.aureus* concentration (CFU/ml) in nutrient broth

medium. The plot shows a linear dependence of the absorbance on the microorganism concentration.

### 3.2. Effect of 0.8 Hz QAMW on Growth of *S.aureus*

A broth of bacteria was subjected to the resonance frequency 0.8 Hz QAMW for one hour. Broth was then placed in the incubator at 37C° and samples were then taken every hour to measure optical density at 600nm and inoculate plates to determine viable count. ELF-EMF was seen to cause a statistically significant ($p \leq 0.05$) decrease in growth rate of *S.aureus* that persisted until the stationary phase was reached as shown in Fig (5-a). The decrease was exponential. Our results are in accordance with the results of previous studies [Strasak et al., 2002[19], Fojt et al., 2004[20], El-Sayed et al., 2006[22]. Strasak et al. (2002) [19] applied ELF-EMF up to 120 min. They concluded that the field effect increased with exposure time. El-Sayed et al. (2006)[22] applied ELF-EMF for 6 and 16 h to *E. coli* and their results are comparable to ours. This suggests that the field effect is maximal in the first hours and then decreases, implying an adaptive response of the exposed cells to field stress. Such an adaptive response can be due to an increase of heat shock, Obermeier et al., 2009[38] study the effects of EMF(5mT, 20Hz)+ maximum a.c electric field (470mV/cm) on staphylococcus aureus, the results show decreased in CFU shown in fig (5-B).

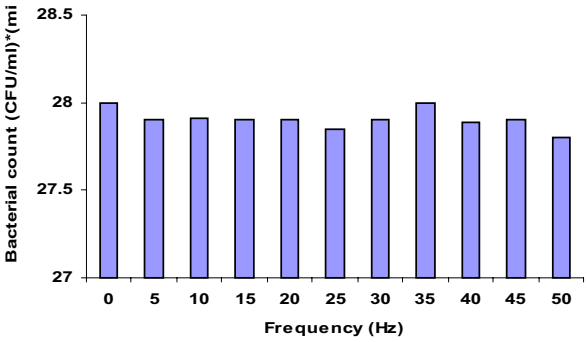



Fig 2. Bacterial count (CFU/ml) of *S.aureus* as a function of frequency (Hz) from 1 -50 Hz with step 5 Hz.

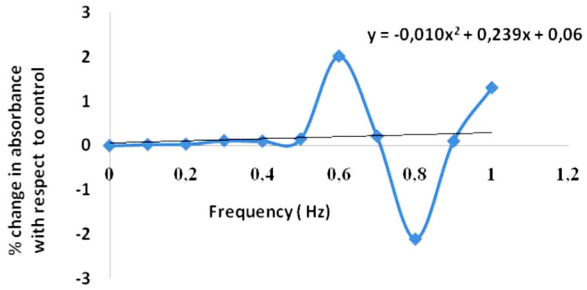



Fig: 3. Changes in the absorbance post 11 hour incubation with respected to control as a function of frequency

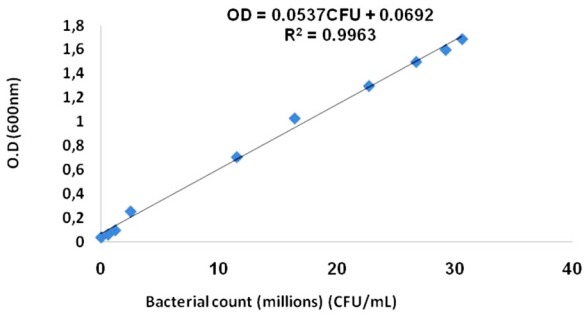
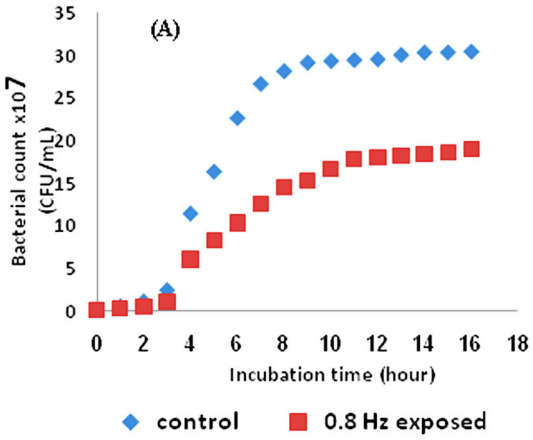



Fig: 4. Count –Absorbance calibration curve for S.aureus



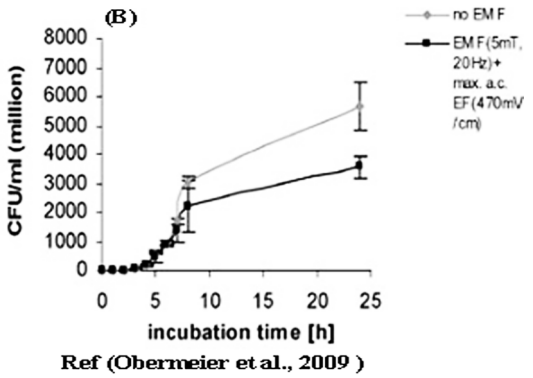




Fig:5.(A) Growth curve of *S. aureus* exposed to 0.8 HZ QAMW for a period of one hour as compared with control.(B) Growth curve of *S. aureus* exposed to EMF(5mT, 20Hz)+ maximum a.c electric field (470mV/cm) (Obermeier et al., 2009)

### *3.3. Growth Curve Dependence on Exposure Time*

As the bacteria cells exposed on the agar plates to 0.8 QAMW. One found that the number of CFU decreases with the exposure time and this decrease was exponential.

The significant decrease in growth rate of exposed bacteria let us to study whether the effect of ELF-EMF persist following the exposure. In order to determine the post exposure effect of ELF-EMF, bacterial strain was exposed at resonance frequency collected at mid-log phase, transferred to fresh nutrient broth and growth rates at 37$^0$C. Figure(6) indicates the concentration of *S.aureus* measured in CFU/mL as a function of exposure time (min) to find out the most effective time for irradiation. It was found that as the exposure time increased the concentration of microorganism will be decreased. There was inhibition of about 87% when the bacteria exposed to 120 min single continuous exposure at 0.8 Hz QAMW. There are several parameters involved and can affect in the cellular division of the microorganism such as cell membrane structural properties and DNA.

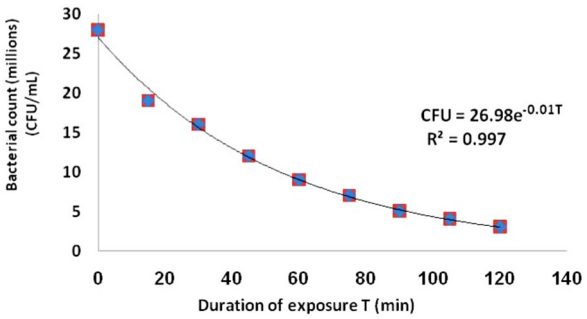


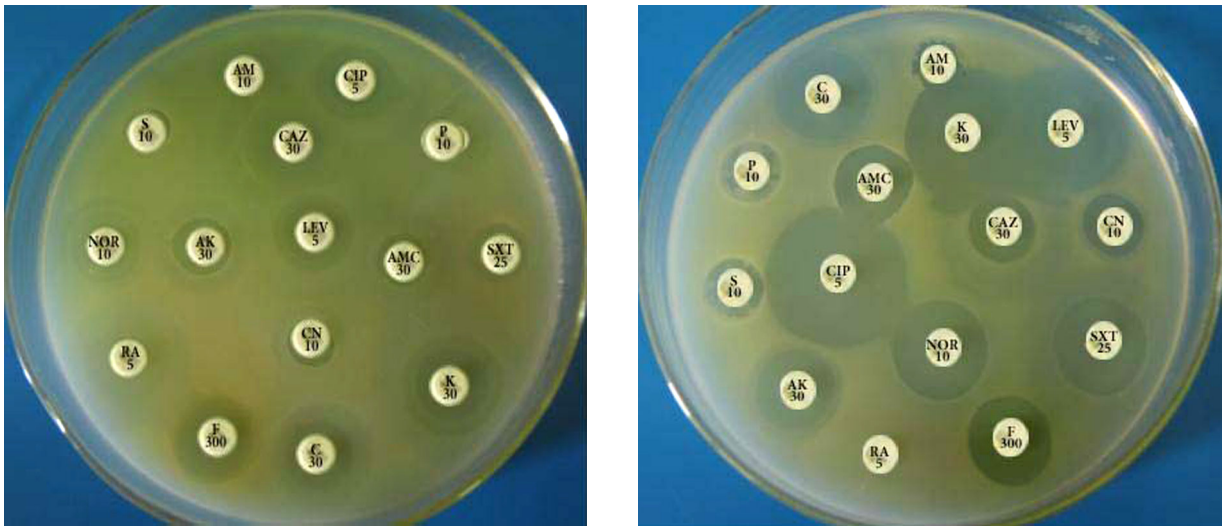

Fig: 6. Viable count of *S.aureus* as a function of exposure time

### *3.4. Effect of o.8 Hz QAMW exposure on Antimicrobial Sensitivity*

Figs (7&8) illustrate an increase in the sensitivity of bacteria to the antibiotics used speciallyCiprofloxacin,Streptomycin,trimethoprim–sulfamethoxazole,LevofloxacinNorfloxacin, Kanamycin and Amoxicillin as revealed in the increase of the zone diameter of the microorganism. These results indicate that the exposure to 0.8 Hz QAMW affect the sensitivity of the bacterial cells to the used antibiotics this is obvious through inhibition of, cell wall synthesis, protein synthesis, nucleic acids, essential enzymes and change in membrane permeability [49]. Moreover, Stansell *et al.* (2001) [50] stated that exposing the bacteria to medium strength magnetic field could significantly alter antibiotic sensitivity. Similar results were obtained with *E.coli* [22].

### *3.5. Effect of ELF-EMF on Morphology of S.aureus*

Fig.(9.A).The dark area show where the sample had a high electron density. The morphological investigation of the samples treated with 0.8 Hz QAMW were greatly different to those of the un treated cells revealed a disintegration of the cell wall, Fig (9.B), the light area show where the sample had a low electron density, extrusion of




A B

Fig:7. Inhibitory zone diameter for bacterial sample un exposed (A) and exposed to 0.8 HZ QAMW for 120min (B)

the cytoplasmic contents, Fig(9.C),retraction of the cytoplasmic membrane (Fig9D&E)resemble the results reported by Yenugu et al.,(2004) [51]who applied cationic HE2 protein (human epididymis 2protein) isoforms to *E. coli*. They suggested that the cationic peptides disrupted anionic target membranes. In case of gram-positive bacteria, the structural changes in *E. faecalis*– heterogeneous appearance of the cytoplasm, condensed spots resemble morphological changes produced by cationic peptides on *S. epidermidis* and *S. aureus*(Friedrich et al. 2000) [52]. The enlarged and less dense electron space indicative of dissolution of the cell wall which caused by a weaken association of cytoplasmic membrane and cell wall. Previously, Rodriguez et al. (2004) [53] observed abnormal septation in *S. aureus* when a cationic preservative was applied which is similar to our observation. Electric field has altered the electrostatic balance of the membrane components such morphological changes caused by ELF-EMF are in accordance with the observations of Fang et al. (2006) and Cellini et al.(2008). Fang et al. (2006) demonstrated that application of a high voltage pulsed electric field to Pseudomonas– aeruginosa resulted in disintegration of the cell wall and leakage of cell material, Ayse Inhan et al.(2011) [54].

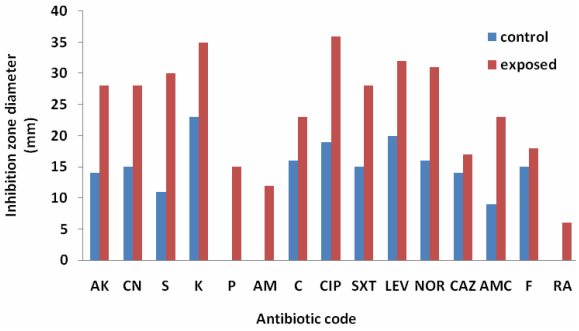



Fig: 8.Histohraph for inhibitory zone diameter for bacterial sample unexposed (control) and exposed to 0.8 HZ QAMW for 120 min

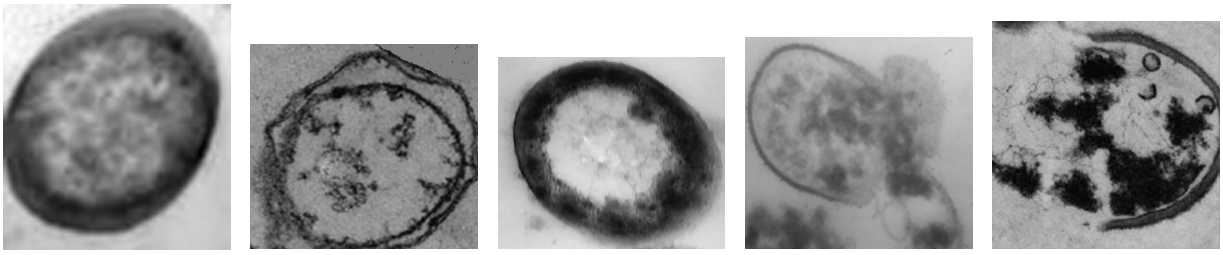
***Table (2)*** *Antibiotic sensitivity of Staphylococcus aureus before and after exposure to 0.8 HzQAMW (mean inhibition* zone diameter in mm)

| Antibiotics | Inhibition Zone diameter (mm) | |
|---|---|---|
| | Before treated | After treated |
| ***Protein inhibitors*** | | |
| AK | 14±0,91 | 28±0,71 |
| CN | 15±0 | 28±0,14 |
| S | 11±0,35 | 30±0,49 |
| K | 23±0,7 | 35±0,7 |
| P | No inhibition zone | 15±0.48 |
| AM | No inhibition zone | 12±0,4 |
| C | 16±0 | 23±0,34 |
| ***DNA inhibitors*** | | |
| CIP | 19±0,32 | 36±0,12 |
| SXT | 15±0,6 | 28±0,18 |
| LEV | 20±0,45 | 32±0,12 |
| NOR | 16±0,13 | 31±0 |
| ***Cell wall inhibitors*** | | |
| CAZ | 14±0,18 | 17±0,04 |
| AMC | 9±0 | 23±0,08 |
| F | 15±0,5 | 18±0,4 |
| RA | No inhibition zone | 6±0,3 |

Since the biological cellular membrane is composed of phospholipids bilayer molecules in addition to the protein molecules (intrinsic and extrinsic) imbedded on the surface of the membrane, one may illustrate that the increase in the electric charge is due to changes in the charge distribution upon the protein molecules of the cellular membrane as a result of exposure to 0.8 Hz QAMW. This analysis is supported by the data obtained from antibiotic sensitivity test.

### *3.6. RAPD Analysis*

Genetic fingerprinting between the control cells of *S,aureus* and those exposed to 0.8 Hz QAMW were determined. A noticeable variation was observed depending on the primer used (Fig.10).Genomic variability in the *S. aureus* strain became evident in the RAPD-PCR analysis. The results indicated the appearance of new bands in the amplified DNA for primers, P2, P4and



A B C D F

*Fig: 9.transmission* electron microscope images for s.aureus cells Control (unexposed A) and (B, C, D and E) exposed to 0.8 HZ QAMW (magnification × 20000)

P5, after exposure to 0.8 Hz QAMW for 120 min. Upon using primer 6 a band at 80pb disappeared in exposed cells. This could be considered as a marker for genetic alterations in the DNA as a result of exposure to 0.8 Hz QAMW. Primers 7 and 8 didn't show any differentiation between exposed and unexposed cells. This means primer1 of sequence 5/– ATGCCC CTG T-3/ is the most efficient in differentiation between exposed and unexposed samples (60% of bands are monomorphic to each other and 40% are polymorphic). These results are supported by the highly significant increase in the sensitivity of *S.aureus* to all antibiotics which are inhibitors for DNA, proteins and cell. Most of the experimental work found in the literature used electromagnetic fields of high field intensity in the range from 10Kv/cm up to several Mv/m the aid of very high temperature for inactivation of bacterial growth [30, 31, 32, 34, 55-56]. These conditions hampered the application of this experimental work in practice either for the treatment of human infections or pasteurization and sterilization of food products.

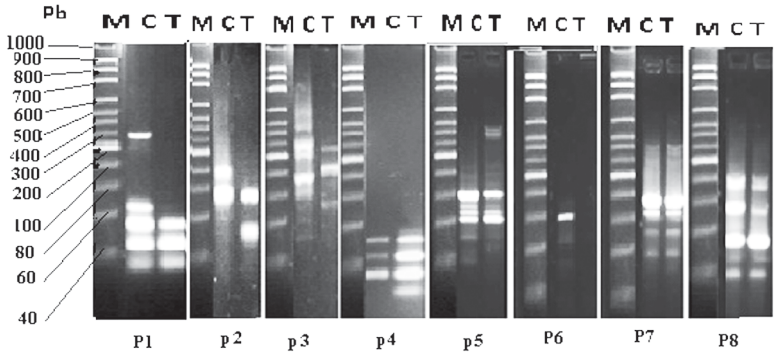



Fig: 10 electrophoretic RAPD patternes for the nuclear DNA extracted from s.aureus before and after exposure to 0.8 HZ QAMW for 120min.M=DNAladder (DNA marker). C=DNA of control sample. T=treated sample exposed to 0.8 HZ QAMW.

DNA fingerprinting patterns

## [IV] CONCLUSION

In conclusion, our study has shown that ELF-EMF application to cells of *S.aureus* resulted in a decrease in growth rate, increase in antibiotic sensitivity, in addition to morphological alterations. Moreover RAPD –PCR revealed variation in genetic fingerprints between exposed and unexposed cells. This finding seems interesting from the point of view that the extremely low frequency electromagnetic waves (ELF-EMW) could be a promising method for the treatment of *S.aureus* infection either in vivo or in vitro, and make this new technique applicable to control such pathogenic bacteria. In addition, although ELF-EMF, is a weak stressor, it may act synergistically with other decontaminating agents and thus be considered as an alternative method for industrial applications. It is reasonable to speculate that ELF-EMF has altered the bacterial growth after ELF-EMF application. Therefore, the use of 0.8 Hz QAMW in controlling the biological activity of *S.aureus* seems to be a new and promising medical activity.

22.03.2013

References:
1. C. Crassi,M.D Ascenzo, A. Torsello et al, [2004] Effects of electromagnetic fields on voltage-gated $Ca^{2+}$ channels and their role in modulation of neuroendocrine cell proliferation and death cell calcium,vol.35,No.4,pp.307-315.
2. A. Foletti,A.Lisi,M.Ledda,F.Decarlo,andS.Grimaldi[2009] Cellular ELF signals as a possible tool in informative medicine, Electromagnetic Biology and Medicine, vol. 28, no.1, pp. 71-79.
3. R. Goodman and M.Blank, [1998] Magnetic field stress induces expression of hsp 70, Cell stress and chaperones, vol.3, No. 2, pp. 79-88.
4. R. Goodman,A. Lin-Ye,M.S. Geddis et al, [2009] Extremely low frequency electromagnetic fields activate the ERK cascade, increase hsp 70 protein level and promote regeneration in planaria, International Journal of Radiation Biology, vol. 85, No. 10, pp. 851-859.
5. F. Bersani, F. Marinelli, A. Ognibene et al, [1997] Intramembrane protein distribution in cell cultures is affected by 50 Hz pulsed magnetic fields electromagnetics, vol. 18, No. 7, pp. 463-469.
6. M. de Mattei, K. Varani, F.F. Masieri et al. [2009] Adenosine analogs and electromagnetic fields inhibit prostaglandin E2 release in bovine synovial fibroblasts, Osteoarthritis and cartilage, vol. 17, No. 2, pp. 252-262.
7. F. Tian, T. Nakahara, K. Wake, M. Taki, and J. Miyakoshi, [2002] Exposure to 2.45 GHz electromagnetic fields induced hsp 70 at a high SAR of more than 20 W/Kg in human glima MO54 CELLS, International Journal of Radiation Biology, vol. 78, No. 5, pp. 433-440.
8. M.T. Santini,A.Ferrante,R.Romano et al, [2005] A 700MHz 1H-NMR study reveals apoptosis-like behavior in human K562 erythroleukemic cells exposed to a 50 Hz sinusoidal magnetic field, International Journal of radiation Biology, vol. 81, No. 2, pp. 97-113.
9. A. Lisi, M. Ledda, E. Rosola et al. [2006]Extremely low frequency electromagnetic field exposure promotes differentiation of pituitary corticotrope-derived At T20 D16V cells, electromagnetics, vol. 27, No. 8, pp. 641-651.

10. R.Piacentini,C.Ripoli,D.Mezzogori,G.B.Azzena,and C.Grassi, [2008] Extremely low– frequency electromagnetic fields promote in vitro neurogenesis via upregulation of cavl-channel activity,Journal of cellular physiology, vol. 215, No. 1, pp.129-139.
11. J.Zhou.G.Yao,J. Zhang, and Z.Chang, and Z. Chang, [2002]CREB DNA binding activation by a 50Hz magnetic field in HL60 cells is dependent on extra-and intracellular $Ca^{2+}$ but not PKA,PKC,ERK, or p38 MAPK, Biochemical and Biophysical Research Communications, vol. 296, No. 4, pp. 1013-1018.
12. R.Iorio,S.DelleMonache,F.Bennato et al. [2011] Involvement of mitochondrial activity in mediating ELF-EMF stimulatory effect on human sperm motility, Bioelectromagnetics, vol. 32, No. 1, pp. 15-27.
13. C.Morabito,F.Rovetta,M.Bizzarri,G.Mazzoleni,G.Fano,and M.A.Mariggio, [2010]Modulation of redox status and calcium handling by extremely low frequency electromagnetic fields in C2C12 muscle cells: a real – time,single-cell approach,Free Radical Biology and Medicine, vol. 48, No. 4, pp. 579-589.
14. K.Zwirska-Korczala,J.Jochem,M.Adamczyk-sowa et al, [2005] Effects of extremely low frequency electromagnetic fields on cell proliferation, antioxidative enzyme activities and lipid peroxidation in 3T3-L1 preadipocytes an invitrostudyJournal of physiology and pharmacology, vol. 56, No. 6, pp. 101-108.
15. S.DiLoreto,S.Falone,V.Caracciolo et al, [2009] Fifty hertz extremely low frequency magnetic field exposure elicits redox and trophic response in rat-cortical neurons, Journal of cellular physiology, vol. 219, No. 2, pp. 334-343.
16. Fadel, M.A., S.M. Wael, R. Mostafa, [2003] Effect of 50Hz, 0.2 mT magnetic fields on RBC properties and heart functions of albino rats, Bioelectromagnetic, vol.24,No.1,pp.535-545.
17. Y.D.Alipov,I.Y.Belyaev, and O.A.Aizenberg, [1994] Systemic reaction of Escherichia coli cells to weak electromagnetic fields of extremely low frequency, Bioelectrochemistry and Bioenergetics,vol.34,No.1,pp.5-12.
18. A.Inhan-Garip,B.Aksu,Z.Akan,D.akakin,A.N.ozaydin, and T.San, [2008]Effects of extremely low frequency electromagnetic fields on growth rate and morphology of bacteria,International Journal of Radiation Biology vol,87,No.12,pp.1155-1161.
19. L.Strasak,V.Vetterl, and J.Smarda, [2002] Effects of low frequency magnetic fields on bacteria Escherichia coli, Bioelectrochemistry,vol.55,No.1-2,pp.161-164.
20. L.Fojt,L.Strasak,V.Vetterl,and J.Smarda, [2004] Comparison of the low frequency magnetic field effects on bacteria Escherichia coli,Leclerciaadecarboxylata and staphylococcuaaureus, Bioelectrochemistry,vol.63,No.1-2,pp.337-341.
21. O.R.Justo,V.H.Perez,D.c.Alvarez, and R.M.Alegre, [2006] Growth of Escherichia coli under extremely low-frequency electromagnetic fields Applied Biochemistry and Biotechnology,vol.134,No.2,pp.155-163.
22. E.S. A.Gaafar, M.S. Hanafy, E.T. Tohamy, and M.H.Ibrahim, [2006] Stimulation and control of E.Coli by using an extremely low frequency magnetic field.Romanian Journal of Biophysics, vol.16, No.4, pp.283-296.
23. I.V.Babushkina,V.B. Borodulin,N.A.Shmetkova et al, [2005] The influence of alternating magnetic field on Escherichia coli bacterial cells, Pharmaceutical Chemistry Journal,vol.39,no.8,pp.398-400.
24. L.Cellini,R.Grande,E.DiCampli et al, [2008] Bacterial response to the exposure of 50Hz electromagnetic fields, Bioelectromagnetics,vol.29,No.4,pp.302-311.
25. I.Belyaev, [2011] Toxicity and SOS-response to ELF magnetic fields and nalidixic acid in E.coli cells, Mutation Research,vol.722,No.1,pp.56-61.
26. JayaramS.,Castle G.S. And Margaitis,A Biotech Bioeng, [1992] vol.40,No.1,pp.1412-1420.
27. Pothakamury U.,Monsalve-Gonzalez A.,Barbosa-Canovas G. and Swanson B, [1996] Journal.food prot.vol.59:1167-1171.
28. Jeantet R.,Baron F.,Nau F.,Rorgnant M. and Brule G. Journal.foodprot,[2000] vol.62,No.12,pp.1381-1386.
29. Alvarez I., Raso J.,PalopA.andSalaF, [2000] Influence of different factors on the inactivation of salmonella senftienberg by pulsed electric fields. International.Journal.Food Microbial. vol.13,No.1,pp.16-34.
30. Amiali M., Ngadi M., Smith J.andRaghavanV, [2007] Synergistic effect of temperature and pulsed electric field on inactivation of Escherichia coli O157:H7 and Salmonella enteritidis in liquid egg Yolk. Journal Food Eng. vol.79, No.1, pp.689-694.
31. JiW.,HuangH.,DengA.and Pan C, [2009] Effects of static magnetic fields on Escherichia coli.Micron. vol.40,No.1,pp.894-898.
32. Tagourti J., El May A.,Aloui A.,Chatti A.,AissaR.andLandoudoulsi A, [2010] Static magnetic field increases the sensitivity of Salmonella to gentamicin Ann Microbiol.vol.60,no.1,pp.519-522.
33. FadelM.Ali, ReemH.ElgebalyA.Ali,FakhryF.Ibrahim, [2005] Control of Ehrlich tumor growth in mice by electromagnetic waves at resonance frequency,in vivo, Electromagnetic biology and medicine, vol.24,No.1,pp.9-12.
34. El Hag,M, [2003] Effect of extremely low frequency electric fields on some biophysical and biological activity of some microorganisms, PHD thesis Faculty of Science, Cairo University.
35. FadelM.Ali,M.A.Ahmed and M.A.El Hag, [2009] Control of sclerotiumcepivorum (Allium White Rot) activities by electromagnetic waves at resonance frequency. Australian Journal of Basic and Applied Science,issn 1991-8178,vol.3,No.3,pp.1994-2000.
36. Aronsson,K.,U.Ronner and E.Borch, [2005] permeabilization and subsequent leakage of intracellular compounds due to pulsed electric field processing International.Journal. Food Microbial.,vol.99,No.1,pp.19-32.
37. Elez-Martinez,P.J.Escola-Hernandez,R.C.Soliva-Fortuny and O.Martin-Belloso, [2004] Inactivation of Saccharomyces cerevisiae suspended in orange juice using high-intensity pulsed electric fields.J.Foodprot., vol.67,No.11,pp.2596-2602.
38. Obermeier A., Matl F., Friess, [2009] Inactiviation of Escherichia coli, Listeria innocua and saccharomyces ceevisiae in relation to membrane W. and Stemberger A. Growth inhibition of staphylococcus aureus induced by low–frequency electric and electromagnetic fields. Bioelectromagnetics. vol.30,No.1,pp.270-279.
39. Atlas R, [1995] Principles of Microbiology Mosby-Year book,INC.
40. AyseInhan-GaripZafer Akan, BurakAksu, DilekAAkakin, NiluferOzaydin, TangulSan, [2011]"Effect of extremely low frequency electromagnetic fields on growth rate and morphology of bacteriaInternational Journal of Radiation Biology, vol. 87.no.12.pp.1155–1161.
41. Neumann B,Pospiech A, S chairrer HU, [1992]. Rapid isolation of genomic DNA from gram negative bacteria. Trends. 8:332-333.
42. Hai-Rong chheng & Ning, Jiang, [2006]. Extremely Rapid extraction of DNA from bacteria and yeasts Biotechnology letters vol (28): pp55-59.
43. Martinez,V.Alonso,A.Quispe,M.C.Thqmas,R.Alonso,J.E.Pineroi,.C.Gonzalezi,A.Ortega and B.Valladares, [2003] RAPD method useful for distinguishing Leishmaniaspecies design of specific primers for L. braziliensis.
44. Williams,J.G.,Kubelik,A.R.,Livak,K.J.,RafalskiI,J.A.&Tingeey,S.V, [1990] DNA polymorphins amplified by arbitrary primers are useful as genetic markers.Nucleic Acids Research18, 6531–6535.
45. Padmalatha K,Prasad M.N.V, [2006] Optimization of DNA isolation and PCR protocol for RAPD analysis of selected medicinal and aromatic plants of conservation concern from Peninsular India African Journal of Biotechnology vol. 5. No3, pp.230-234.
46. Kamaledin Eltoum, Imadeldin Aradaib, and Suliman El-Sanousi., (2003) PCR-based randomly amplified polymorphic DNA(RAPD) fingerprinting for detection of genetic diversity among sudanese isolates of Haemophilus somnus Veterinarski arhiv

Journal Vol. 73(6), PP.315-321.
47. Fadel, M.A,(1998)A new metabolic biomagnetic resonance model to describe the interaction of ELF-EM field with biological system,International school on Theoretical Biophysics,Moscow.
48. Fadel Mohamed Ali,Azza Mahmoud Gawish,Massarat Bakr Saddek Osman, Ashraf Mahmoud Abdelbacki, and Amira Hamdi El-Sharkawy.(2012) Control of Salmonella activity in rats by pulsed ELF Magnetic field (in vivo study). Journal of international Dental and Medical Research, Vol 5,No (2),pp.129-135.
49. Klachkova, Y.F., M.E. Kvitkina, [1993] Disinfecting cattle slurry by treatment with electromagnetic radiation, Problemy Veterinarnoi Sanitarii I., Ekologii,,vol 2, pp.14–18.
50. Stansell, M.J., W.D. Winters, R.H. Doe, B.K. Dart, [2001] Increased antibiotic resistance of E. coil exposed to static magnetic field, Bioelectromagnetics,, vol 22,No2, pp.129–137.
51. Yenugu S, Hamil KG, Radhakrishnan Y, French FS, Hall SH:TheAndrogen Regulated Epididymal Sperm-Binding Protein,ESC42, Is An Antimicrobial Beta-Defensin, [2004]. Endocrinology,vol.145.pp.3165-3173.
52. Friedrich, W., Galensa, R., Geisler, R. and Grlich, R, [2000] HPLC analysis of polyphenols withcoulometric electrode array detector. Eur. Comm., [Rep.] EUR, EUR 18169, Polyphenols in food.Vol 25. No 32. PP.2832:2855.
53. Rodriguez, A. B., J. B. Anderson, F. P. Siringan, and M. Taviani, [2004] Holocene evolution of the east Texas coast and inner continental shelf: Along-strike valiability in coastal retreat ratesJournal of Sedimentary Research, vol. 74, No, p. 405-421.
54. Ayse Inhan-Garip, Burak Aksu,Zafer Akan, Dilek Akakin, A. Nilufer Ozaydin & Tangul San.,(2011) Effects of extremely low frequency electromagnetic fields on growth rate and morphology of bacteria International Journal of Radiation Biology, vol. 87. No12,pp. 1155-1161.
55. Jaegu C.,Douyan W.,Takao N.,Sunao K.,Hidenori A., XiaofeiL.,Hiroshi S.,HarumichiS.,Hitoshi M.A.,XiaofeiL.,Hiroshi S.,Harumichi S., Hitoshi M. and Takeshi S, [2008] Inactivation of spores using pulsed electric field in a pressurized flow system. J.App.Phys. vol.104,No.1,pp.094701-094701.
56. Ruiz-Gomez M.,Sendra-PorteroF.and Martinez-Morillo M, [2010] Effects of 2.45 Mt sinusoidal 50 Hz magnetic field on Saccharomyces cerevisiae strains deficient in DNA strand breaks repair. International.Journal.Radiat.Biol.vol.86, No.1, pp.602-611.

**F. M. Ali**, Biophysics Department, Faculty of Science, Cairo University, Egypt. [Professor. Doctor], e-mail: fadelaga48@gmail.com, Tel:+201001429897.
**A. M. El-Khatib**, Physics Department, Faculty of Science, Alexandria University, Egypt. [Professor. Doctor], e-mail: Elkhatib60@yahoo.com, Tel:+201000230122.
**S. A. Sabry**, Botany & Microbiology Department, Faculty of Science, Alexandria University, Egypt. [Professor. Doctor], e-mail: sabrysoraya@yahoo.com, Tel:+2 01065527751.
**S. E. Abo-Neima**, Physics Department, Faculty of Science, Damanhour University, Egypt. [Assistance Lecturer], e-mail: sahar_amr2002@yahoo.com, Tel:+201229631153.
**H. A. Motaweh**, Physics Department, Faculty of Science, Damanhour University, Egypt. [Professor. Doctor], e-mail: Prof_ motaweh@yahoo.com, Tel:+201286133312.