Evaluation of the Effect of Extremely Low-Frequency Electromagnetic Fields on the Growth of *Escherichia coli*

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Abstract

The effect of the extremely low frequency electromagnetic waves (LF-EMW) on the bacterial growth of *Escherichia coli* (ATCC 25922) was studied in *Vitro* and in *Vivo*.

Both the most potent inhibition frequency and irradiation interval were determined in terms of colony forming unites (CFU) viability. The results of the exposure of standard bacterial suspensions to LF-EMW at different frequencies indicated that maximum inhibition was attained at 0.6 Hz irradiation frequency and 30 mins exposure time. Morphological deformations and genetic alterations were detected in the irradiated bacterial samples by using transmission electron microscope (TEM) and DNA fragmentation studies. In *Vivo* experiment was preceded using rat models where approximately 10^7 CFU were inoculated into wounds measuring 1.5×1.5cm on its dorsal surface, the results obtained revealed a decrease in the bacterial count by 70.1% , 94.3% and nearly 100% in the 1^{st} ,2^{nd} and 3^{rd} days respectively post irradiation in comparison to the untreated wounds.

The detected results reflect the beneficial use of LF-EMW which is a new un-thermal promising technique, and supports the integration of this form of therapy in inhibiting *E.coli* wound infections.

Keywords: Low frequency electromagnetic waves; Bacterial growth, *Escherichia coli.*

1. Introduction

The rates of development of multi-antibiotic resistance among bacteria that infect wounds and burns are constantly on the rise [1- 3]. Bacteria seek to establish themselves in open wounds to ensure their own survival and evolution [4]. Consequently, rapid control of wound infections and monitoring of new therapeutic strategies that selectively destroys the bacterial cells without destroying the host tissues have recently been proposed. Recent efforts were established to control microbial activities by using low field intensity electromagnetic waves (LF-EMW) which resonates the bioelectric signals generated during particular metabolic activities [5-7]. This type of waves produce significant effects on the growth of microbial cultures ,the nature and extent of the waves' effect depends on their frequency and the total energy absorbed by the microorganisms [8,9]. In addition studies have shown that extremely low-frequency (ELF) pulsed electromagnetic field (PEMF). Exposures can accelerate re-establishment of cells' normal potentials, promote cell proliferation, increase the rate of healing, and reduce swelling and bruising in damaged skin tissue [10 ,11] .The model system (micro-organism) selected for the above-mentioned experiments was the gram-negative bacterium *E. coli*. Infections caused by *E. coli* can easily be acquired by contact with the feces, or stool, of humans or animals . *E. coli* has been described as the second most common single pathogen involved in post-operative wound infections [12-14].

The aim of this work is to find out the resonance frequency of the extremely low field intensity electromagnetic waves (LF-EMW) that can inhibit the activity of *E-coli* and its ability to make division. Moreover, to investigate the genetic alterations that may occur as a result of exposure to the inhibiting resonance frequency.

2. Materials and methods

2.1.Microorganism:

The international reference strain *Escherichia coli* (ATCC 25922) isolate was kindly obtained from the, Faculty of pharmacy, Cairo University, Egypt. The bacterial strain was maintained by weekly subculture on nutrient agar medium (OXIOD.Ltd,UK).

2.2.Positive Square Electric Impulses Source:-

Direct current power supply (9V-DC) through an electronic switching device is maintained to produce a square pulsed current with different frequencies. The square pulses then directed into DC/DC voltage converter that produces the voltage field intensity (400 ± 25) v through squared copper plates 5×5 cm and 1 cm apart at which the samples suspensions are placed and the pulse shape was displayed using oscilloscope GOS-620. The system fabricated and manufactured locally in the Biophysics department, Faculty of Science, Cairo University.

2.3.Bacterial Growth:

A-Pre-Exposure

The bacterial strain was grown aerobically in nutrient broth (at 37°C,100 rpm) .Overnight culture was centrifuged and the pellet resuspended in Phosphate buffered saline (PBS).The optical density for the suspended bacterial culture was measured at 560 nm and adjusted at 0.05A (approximately15 x 10^9 colony forming unit per ml (CFU /ml)) . This step was necessary for maintaining the standardization for all conducted experiments.

B-Exposure

In a trail to find out the most potent resonance frequency of growth inhibition, 20µl of the standardized bacterial suspension was dispensed into tubes containing 2 ml nutrient broth media. The prepared bacterial samples were exposed to different frequencies in the range of 0.4 to 1 Hz for 30 mins. Control groups (unexposed) were kept at the same conditions as the exposed ones . Experiments were made in triplicates and the average were considered [15].

3-Post-Exposure:

After 24h of incubation at 37 °C, the effects of radiation on the viability of *E.coli*, was detected in terms of CFU/ml .Serial dilutions of the bacterial culture suspension were performed, a volume of 100 µl from the last tube was inoculated in the agar plates by spread plating [7].Finally, after incubation (24 h at 37 °C) , the colonies formed on the solid media (nutrient agar) were visually counted, by its multiplication with the dilution factor the number of viable cells in the initial population is determined, ensuring that we are not counting any bacterial cells that did not survive .

2.4. Determination of the most Optimum Exposure Time:

To study the effect of exposure time,20µl of the standardized bacterial suspension (15 x 10^9) was dispensed into tubes containing 2 ml nutrient broth media and exposed to 0.6 Hz (most potent frequency) for different time intervals (20, 30, 60, 90, and 120 mins), 100 µL of each culture was used to inoculate nutrient agar plates, after 24 h of incubation at 37°C the number of viable cells were expressed as CFU/ml and consequently the percentage of inhibition was determined. Each experiment was repeated three times along-side control groups, which were kept under identical conditions [7].

2.5. Determination of the Optimum Exposure Regime:

After determining of the optimum exposure time, three groups of *E.coli* (ATCC 25922) suspension were used in order to get the best regime of exposure, one group kept as control, the other group exposed continuously to resonance frequency of growth inhibition for the optimum exposure period (one shoot exposure) and the last group exposed to resonance frequency of growth inhibition in accumulative mode where the optimum exposure period divided into four equal periods interrupted by 30 mins of incubation (accumulated exposure). The number of viable cells were determined as in the previous experiment.

2.6. Transmission Electron Microscope (TEM) Examination:

The morphological changes of control group and the irradiated group for the most effective time have been determined using Transmission Electron Microscope (TEM).

In order to prepare the bacterial sample to be examined by TEM, the sample should undergo some processing according to [16]. The processing of bacterial cells (control and exposed) began after 30 minutes post exposure, where the bacterial cells were collected and washed three times in Phosphate buffered saline (PBS). The pellet was suspended in 2% glutaraldehyde in phosphate buffer, pH 7.2, and fixed overnight in the refrigerator. After fixation, the pellet rinsed three times in phosphate buffer, pH 7.2. The resulting pellet containing the cell was fixed in 1% osmium-tetroxidein phosphate, pH 7.2, for 3 h at 4 °C and dehydrated with increasing concentrations of ethanol. After the100% ethanol washes, cells were washed with 100% acetone and infiltrated with resin.

Semi thin sections were prepared on glass slides through cutting at 1 µm using the ultramicrotome. Sections were stained with Toludine blue for 5 minutes examined by light microscope model M-200M. Ultra-thin sections were cut using ultramicrotome Leica model EM-UC6 at

thickness 90nm, mounted on copper grids (400 mish). Sections were stained with double stain (Uranyl acetate 2% for 10 mins followed by Lead citrate for 5 mins) and examined by transmission electron microscope JEOL (JEM-1400) at the candidate magnification. Images were captured by CCD camera model AMT, optronics camera with 1632 x 1632 pixel format as side mount configuration. This camera uses a 1394 fire wire board for acquision.

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

DNA was extracted from 50 mg of fresh *E. coli* suspension (either control or exposed) according to the method developed by Eltoum *et al*., 2003[17]. The quantity of extracted DNA was measured by means of agarose gel electrophoresis and confirmed by spectrophotometer [17]. The extracted DNA subjected to amplification reaction via the polymerase chain reaction (PCR) manufactured by Thermocycler T1, Biometra, Germany. The PCR mixture consists of a PCR beads tablet (manufactured by Amessham Pharmacia Biotech). The specific primer The sequence of the L340GCF primer, which included a 40 base pair GC-clamp attached to the 5' end, was 5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGACTCCTACGGGAGGCAGCAG-3′, and the sequence of the K517R primer was 5′- ATTACCGCGGCTGCTGG -3′ used in the amplified polymorphic DNA reactions. The amplified DNA of all groups was electrophoreses using electrophoresis unit (wide mini-sub-cell GT Bio-RAD) on 2% agarose containing 0.5 µg/ml of ethedium bromide, at a constant 75 volt and 60 mA, and visualized with UV trans-illuminator. Then DNA gel was scanned for band, using gel documentation system (AAB Advanced American Biotechnology 1166 E. Valencia DP. Unit 6 C, Fullerton, CA 92631). The different molecular weights of bands were determined against a DNA standard (Mid Range DNA Ladder Jena Bioscience) with molecular weights 80, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1030 bp. The similarity level was determined by un-weighted pair group method based on the arithmetic mean (UPGMA).

2.8. In *Vivo* experimental procedure:

Twenty rats of either sex,6-8 weeks of age, weighing 250 ± 2 gms were housed one per cage (to prevent attacks on wounds), rates were anesthetized by intra peritoneal injection of Ketamine-Xylazine mixture and then shaved on the dorsal surfaces using an electric fur clipper. Rat skin was then scraped with No 15 scalped blades until a reddened area appeared . This procedure resulted in first degree skin abrasions each wound measured approximately 1.5x1.5 cm ,an inoculum of 100 µl of a

prepared bacterial suspension containing 10^7 CFU was inoculated into the rate wound using a micropipette.and smeared uniformly with the micropipette tip (The minimum bacterial inoculum required to develop infections in mice was determined by preliminary experiments).One group (n=10) was irradiated (0.6 Hz for 30 mins).In addition the same number of animals without irradiation were used as the controls.The infected wounds were irradiated daily for 3 consecutive days, starting from the first day post-induction, and always in the mornings [18].

Bacteriological analysis

A skin tissue sample (1 cm × 2 cm), including the wound, was homogenized in 1ml phosphate-buffered saline and was then centrifuged (3500 rpm, 5 min). The supernatant was collected and plated on nutrient agar plates. After incubation at 37°C for 48h, the number of colonies was counted ,samples were taken immediately ,24h, 48h and 72h after irradiation [19].

2.9. 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. The Sigma Stat 2.03 (SPSS, Chicago, IL) was used to test the statistical significance of differences between groups means (one-way ANOVA followed by Turkey's test) was considered to be statistically significant.

3. Results :

From the obtained results (Table 1) it was seen that there is a decrease in the viable bacterial cell count of *E.coli* after exposure to 0.6 Hz for 30 mins at different incubation intervals, while the exposure to other frequencies almost have slight effect on the bacterial growth.

Table 1:
Effect of different LF-EMW frequencies on the viable bacterial count

Incubation Time (hrs)	Viable Bacterial Cell Count (CFU/ml)						
	Control	0.4Hz	0.5Hz	0.6Hz	0.7Hz	0.8Hz	0.9Hz
1	7.50×10^{10}	1.80×10^{10}	9×10^{10}	0.80×10^{10}	6.6×10^{10}	6.0×10^{10}	2.40×10^{10}
2	1.35×10^{11}	1.59×10^{11}	1.11×10^{11}	3.80×10^{10}	8.1×10^{10}	6.90×10^{10}	5.10×10^{10}

3	1.89×10^{11}	1.83×10^{11}	1.38×10^{11}	5.30×10^{10}	9.0×10^{10}	1.11×10^{11}	8.70×10^{10}
4	1.95×10^{11}	1.56×10^{11}	1.41×10^{11}	6.50×10^{10}	1.02×10^{11}	1.26×10^{11}	1.05×10^{11}
5	2.19×10^{11}	1.56×10^{11}	1.98×10^{11}	7.40×10^{10}	1.05×10^{11}	2.04×10^{11}	1.56×10^{11}
6	2.16×10^{11}	1.89×10^{11}	2.46×10^{11}	8.00×10^{10}	2.10×10^{11}	2.19×10^{11}	1.56×10^{11}
7	2.13×10^{11}	2.49×10^{11}	2.58×10^{11}	0.23×10^{10}	1.71×10^{11}	2.16×10^{11}	1.92×10^{11}
8	2.01×10^{11}	2.55×10^{11}	1.89×10^{11}	8.90×10^{10}	1.86×10^{11}	2.04×10^{11}	1.65×10^{11}
9	1.98×10^{11}	2.40×10^{11}	1.68×10^{11}	9.00×10^{10}	1.74×10^{11}	1.86×10^{11}	1.59×10^{11}
10	1.71×10^{11}	2.07×10^{11}	1.41×10^{11}	8.40×10^{10}	1.68×10^{11}	1.80×10^{11}	1.50×10^{11}
11	1.74×10^{11}	2.04×10^{11}	1.37×10^{11}	7.80×10^{10}	1.65×10^{11}	1.74×10^{11}	1.35×10^{11}
12	6×10^{10}	1.77×10^{11}	1.28×10^{11}	7.80×10^{10}	2.01×10^{11}	1.71×10^{11}	1.29×10^{11}

3.2. Determination of the optimum exposure time:

The percentages of growth inhibition at the 14 th hr post incubation were represented in figure (1), it was clear that the exposure of *E.coli* suspension to 0.6 Hz for 30 mins causes maximum growth inhibition by 71.3% while for 20,60,90 and 120 mins exposure periods the percentages of growth inhibition were 10.70, 24.30, 26.12 and 31.35% respectively.

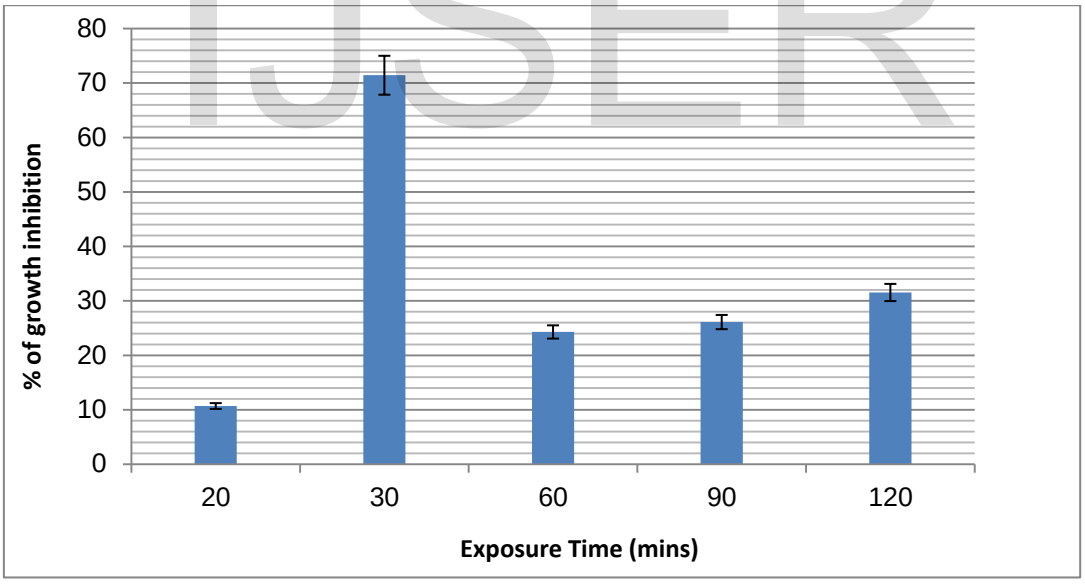


Fig.1. The percentage of growth inhibition after different exposure periods to 0.6 Hz at 14th hr of incubation.

3.3. Determination of The Optimum Exposure Regime:

The effect of different exposure regimes on the growth inhibition was illustrated in Fig. (2), where one group kept as control and the two other groups exposed 0.6 Hz for 30 min through two different regimes; one shoot exposure regime and accumulated exposure regime. It is clear

from the results that both exposure regimes have almost the same effect on growth inhibition of *E. coli* where the difference between their results is non-significant (P < 0.70).

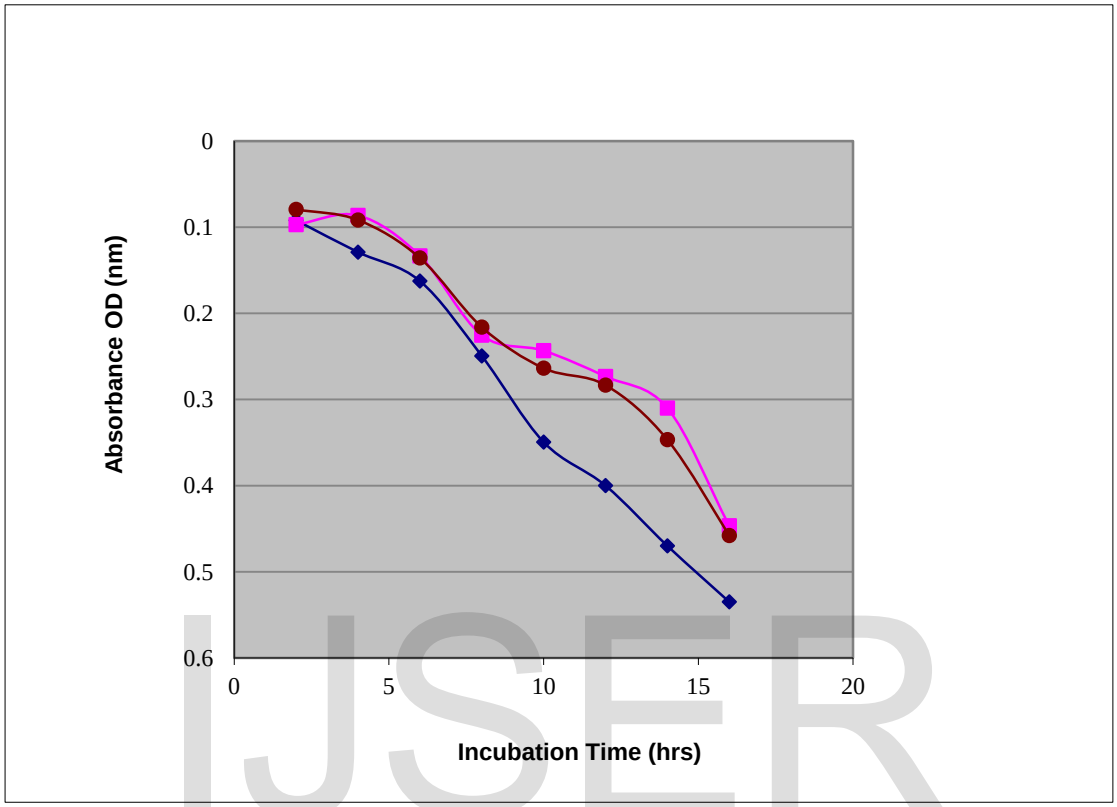
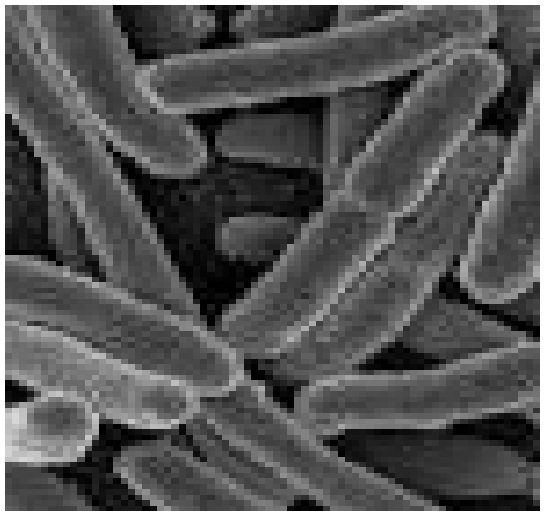


Fig. 2. Effect of different exposure regimes to 0.6 Hz on the growth inhibition of *E coli*:(Blue line) Control, (Brown line) one shoot exposure regime and (Rose line) accumulated exposure regime.

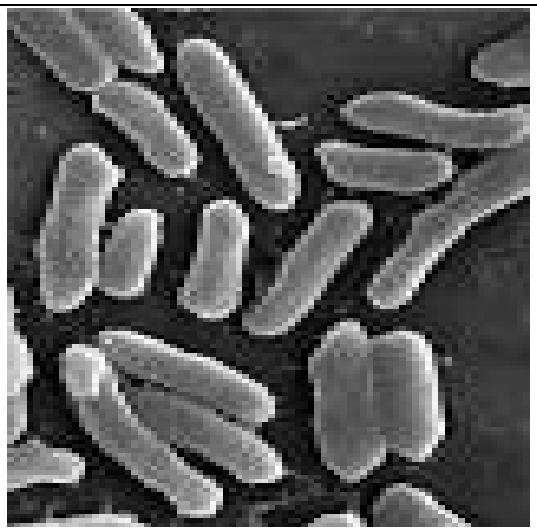
3.4. Effect of LF- EMW on the morphology of *E.coli*

The bacterial cells of *E.coli* were examined using TEM. This examination included both control and irradiated groups at 0.6 Hz for 30 mins. The normal *E.coli* cells are viewed by TEM (Fig. 3A).It is clear from the figure the typical rod shapped cells with the appearance of cell wall, cell membrane, ribosomes which give the cytoplasm of bacteria a granular appearance. Significant ultra structural changes were observed on the morphological shape of the bacterial cells after irradiation such as elongation and deformation of the bacterial cell in addition to fragmentation of DNA (Fig.3B), a less dense electron space and a heterogeneous appearance of the cytoplasm which is an indicative to the dissolution of the cell wall, and abnormal septation, disruption and disintegration of cell wall, retraction of cytoplasmic membrane (may have been one of the reasons leading to disintegration of cell wall) and

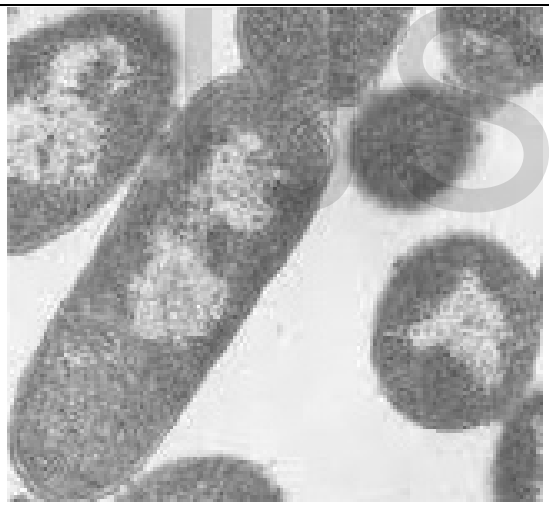
presence of *E.coli* ghost cell representative of retracted cytoplasmic material are seen in Fig. (3 C), an extrusion of cytoplasmic contents from cell wall and almost completely dissolution of the cytoplasm are also observed (Fig. 3 D).



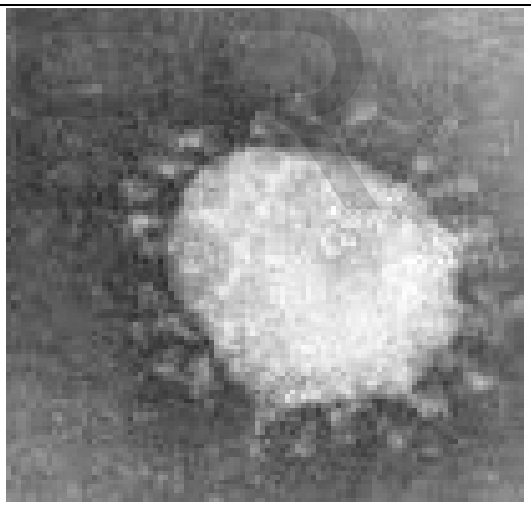
(A):TEM image of normal *E.coli* cells (Magnification 30000x).



(B):TEM image of exposed *E.coli* cells; showing elongation and deformation of bacterial cells (Magnification 30000x).



(C): TEM image of exposed *E.coli* cells. indicating retraction of cytoplasmic membrane and *E.coli* ghost cell (Magnification 30000x).



(D): TEM image of exposed *E.coli* cells showing dissolution of the cytoplasmic membrane and extrusion of the cytoplasmic contents . (Magnification 60000x).

Fig. 3. TEM images for control (A) and irradiated (B,C and D) *E.coli* cells.

3.5. RAPD-PCR analysis:

Genetic finger printing of the control and irradiated cells of *E.coli* were determined (fig 4),The appearance of new bands in the DNA pattern of the exposed bacteria proved that the DNA sequences have been changed

under the effect of irradiation in a way that the primer used find a new binding sequences which not present in the control bacteria.

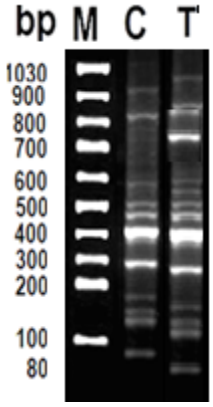


Fig. 4. Electrophoretic RAPD patterns for the nuclear DNA extracted from E.coli before and after exposure to low field intensity EMW of 0.6Hz for 30 min. *M= DNA Ladder (DNA Marker). C= DNA of control sample. T= DNA of the irradiated sample.

3.6. *In Vivo* Results:

The bacterial count decreased in the treated wound by 70.1% , 94.3% and nearly 100% in the 1st ,2nd and 3rd days respectively post irradiation in comparison to the untreated wounds ,in addition it can be seen that LF-EMW had promoted the wound healing rate.

4.Discussion:

Considering the appllication of LF-EMW as a new theraputic protocol to control the formation of bacterial biofilms and recovery from chronic and acute wound infections . The present work aimed to demonstrate the frequency of the electromagnetic waves that interferes with the bioelectric signals generated from bacterial cells during cell division ,also studying the changes that may occur in its nuclear structure leading to growth inhibition .This bioelectric signals are known to be in the low frequency range ,therefore to interfere with it the applied waves should have similar frequencies [20-22].Since the irradiation effect depends on the type of the microorganism, the exposure frequency and duration time [23,24],so our study was directed towards the application of a range of frequencies lower than 1.0 Hz. The experimental evaluation was defined in terms of colony forming units (CFU),where the decrease in its number after exposure is correlated with bacterial death[25,26].The obtained results indicated the significant growth reduction at 0.6 Hz after 30 mins of irradiation. Fojt *et al.* [27] showed that the electromagnetic waves of mT and frequency Hz affect the viability of different types of bacteria including *E.coli* and *S. aureus*, also our data supports the previous observations that indicates the ability of LF-EMW to induce changes in

the cell growth of *E. coli* [28-30]. Studying the exposure time is a very important factor in the determination of growth inhibition ,it was found that the reduction in the growth rate of *E.coli* and *S. aureus* was detected after 24 mins of exposure to an electromagnetic waves at 50Hz and 10mT [31].while in another study the most optimum exposure time for *E.coli* was found to be 20mins [32].In consistent with the findings mentioned above the highest reduction in the bacterial growth at 0.6 Hz was achieved after 30mins of irradiation (Fig 1).The inhibition of the bacterial growth can be attributed to the morphological or the metabolic changes induced by field application .The morphological investigation of the irradiated sample was greatly different from the non-irradiated one (Fig 3) revealing deformation of the bacterial cells , the less dense electron space is an indicative of the disintegration of the cell wall and extrusion of the cytoplasmic contents [22,33,34]. Since the biological cellular membrane is formed of two phospholipid layers imbedded on them protein molecules ,exposure to electromagnetic waves lead to changes in the charge distribution in the protein molecule of the cellular membrane as a response to irradiation [35].

Genetic finger printing of the control and the exposed samples (0.6Hz) estimated a noticeable variation between them (Fig 4) ,this indicated that the genetic sequence of the bacterial DNA was modified due to irradiation ,consequently protiens and enzyme synthesis expressed from these modified sites which may affect bacterial growth [36-38].

For a long time, wound infections were always successfully treated with topical and systemic antibiotics, however the rapid emergence of multidrug resistant strains of bacteria has become a considerable concern [39] .

Results obtained after wound treatment with LF EMF (0.6Hz) showed a significant decrease in the bacterial count from day one of irradiation, also it promotes faster wound healing, similar results indicated that application of EMW at low frequencies has an effect in reducing biofilm forming cell count, moreover it helps in the treatment of chronic wounds, and may reduce the costs related with antiseptic and antibiotic therapy[40].

Conclusion

Recent clinical studies show beneficial uses of electromagnetic therapy, and thus suggest the integration of this form of therapy in wound healing and controlling bacterial biofilms, the selection of the proper exposure frequency and optimum exposure time helps in enhancing the treatment process. Our study has shown that application of low frequency electromagnetic waves (0.6 Hz, 30 mins) resulted in morphological alterations and variation in the genetic finger print of *E.coli* cells, consequently significant inhibition of growth.

The obtained results show the beneficial use of LF- EMW which is a new un-thermal promising technique as an aid to avoid the use of antibiotics, and thus suggest integration of this form of therapy in inhibiting *E.coli* wound infections.

References

[1]-J.M Blondeau, D. Vaughan, " In vitro activity of 19 antimicrobial agents against 3513 nosocomial pathogens collected from 48 Canadian medical centers," Int. J. Antimicrob. Agents, vol. 15, pp. 213-19, 2000.

[2]-L. Balazs, J. Okolicany, M.Ferrebee, B.Tolley,G. Tigyi," Topical application of the phospholipid growth factor lysophosphatidic acid promotes wound healing in vivo," Am. J. Physiol. Reg. Integr. Comp. Physiol ,vol. 280,pp.466-72,2001.

[3]- K. Bush, P. Courvalin , G. Dantas ," Tackling antibiotic resistance," Nat Rev Microbiol ,vol. 9,no. 12, pp. 894-96, 2011.

[4]- S.Jawhara, S.Mordon , " In vivo imaging of bioluminescent *Escherichia coli* in a cutaneous wound infection model for evaluation of an antibiotic therapy," Antimicrob Agents Chemo, vol.48, no. 9, pp.3436-41,2011.

[5]- j. Milgram, R.Shahar, T.Levin-Harrus,P. Kass ," The effect of short, high intensity magnetic field pulses on the healing of skin wounds in rats," Bioelectromagnetics, vol.25,no.4,pp. 271-77, 2004.

[6]- A. Athanasiou, S.Karkambounous, A.Batistatou, E.Lykoudis, A. Katsaraki, T. Katsiouni, A.Papalois,A. Evangelou ," The effect of pulsed electromagnetic fields on secondary skin wound healing: an experimental study,"Bioelectromagnetics ,vol. 28,no.5,pp. 362-68,2007.

[7]- F.M.Ali, A.M.El-Khatib, A.S. Sabry, S.E. Abo-Neima , and H.A.Motaweh,"Control of *Staphylococcus aureus* growth by electromagnetic therapy," ,BECTHNK ORY vol.155 ,pp. 169-78, 2013.

[8]- N.M.Shupak , F.S.Parto, A.W.Thomas ," Therapeutic uses of pulsed magnetic-field exposure: a review," Radio Science Bulletins ,vol.307 ,pp. 9-32, 2003.

[9]- A. Istiaque, I. Taghrid , C. Irena , P. Elena ," Evaluation of the effects of extremely Low frequency (ELF) Pulsed electromagnetic fields
(PEMF) on survival of the bacterium *Staphylococcus aureus*," Nonlinear Biomed Phy, vol.1, no.5, pp. 2-17, 2013.

[10]- S.Goudarzi, M.E. Hajizadeh and K.A. Salmani ," Pulsed electromagnetic fields accelerate wound healing in the skin of diabeticrats," Bioelectromagnetics , vol.31, pp. 318-23,2010.

[11]- A.Istiaque , I.Taghrid and P. Elena ," Irradiation of *Escherichia coli* by extremely-low frequency (ELF) pulsed electromagnetic fields (PEMF): evaluation of bacterial survival," J. Electrom. Waves Appl., vol.3,no.56, pp. 1-12, 2014.

[12]- M.Ahmed,S.N. Alam,O. Khan andS. Manzar ," Post-operative wound infection: a surgeon' sdilemma," Pakistan J. surgery ,vol. 23 ,pp.41-47,2007.

[13]- S.J. Landis," Chronic wound infection and antimicrobial use,"Adv Skin Wound Care, vol. 21, pp. 531-540, 2008

[14]- B. Segatore, D. Setacci , F. Bennato ,R. Cardigno , G. Amicosante and R. Iorio," Evaluations of the Effects of Extremely Low-Frequency Electromagnetic Fields on Growth and Antibiotic Susceptibility of *Escherichia coli* and *Pseudomonas aeruginosa*," Int J Mirobiol., pp. 1-7, 2012.

[15]- E.S. Gaafar, M.S. Hanafy, E.T. Tohamy and M.H. Ibrahim ," Stimulation and control of by using an extremely low frequency magnetic field," Romanian Journal of Biophysics, vol. 16,no.4, pp.283-96,2006.

[16]- G.Z. Akan, B. Akusu , D.A. Akakin , N. Ozaydin and T. San ," Effect of extremely low frequency electromagnetic fields on the growth

rate and morphology of bacteria," Int J Rad Biol., vol.87,no. 12, pp. 1155- 61,2006.

[17]- K.Eltoum , I.Aradiab and S.El-sanousi ,"PCR-based randomly amplified polymorphic DNA(RAPD) fingerprinting for detection of genetic diversity among Sudanese isolates of *Haemophilus sommnus*," J Veterinarki arhiv., vol.73, no. 6, pp. 315-21,2003.

[18]- T. Dai , B. Garcia , C.K. Murray ,M.Vrahas and M.R.Hambin ," UVC light prophylaxis for cutaneous wound infections in mice," Antimicrob Agents Chemother., vol.56, no. 7,pp. 3841-8, 2012.

[19]- R. Ranjbar and M.A. Takhtfooladi ," The effects of low level laser therapy on *Staphylococcus aureus* infected third-degree burns in diabetic rats," Acta Cir. Bras ., vol 31, no. 4,pp. 250-5, 2016 .

[20]- F.M. Ali , M.A. Ahmed and M.A. El Hag ," Control of *Sclerotium cepivorum* (Allium White Rot) activities by electromagnetic waves at resonance frequency,"Aust. J. Basic Appl. Sci., vol. 3,pp. 1994-2000, 2009.

[21]- F.M. Ali, A.M Gawish, M.B.S.Osman, A.M.Abdelbacki and A.H.El-sharkawy," Control of *Salmonella* activity in rats by pulsed ELF Magnetic field (in *Vivo* study)," Int.J. Dental Med Res.,vol. 5, no. 2, pp.129-135,2009.

[22]- F.M. Ali, R.H. Elgebaly , M.S. Elneklawi and A.S. Othman , " Role of duty cycle on *Pseudomonas aeruginosa* growth inhibition mechanisms by positive electric pulses," Bio-Medical Materials and Engineering, vol.27, pp. 211-225,2016.

[23]- L. Strasak, V. Vetterl and L. Fojt ," Effects of 50Hz Magnetic Fields on the Viability of Different Bacterial Strains,"Electromagnetic Biol Med.,vol. 24, no.3, pp. 293-300, 2005.

[24]- M. Kohno, I. Yamazaki and M. Wada," Effect of static magnetic fields on bacteria: *Streptococcus mutans*, *Staphylococcus aureus*, and *Escherichia coli*," Pathophysiology, vol.7, no. 2, pp. 143-8, 2000.

[25]- T.W. Mak and M. Saunders ," The Immune Response: Basic and Clinical Principles," Elsevier Academic Press, Burlington, MA.,pp. 641–694,2006.

[26]- L. Fojt, L. Strasak and V. Vetterl ," Extremely-low frequency magnetic field effects on sulfate reducing bacteria viability," Electromagnetic Biology and Medicine, vol. 29. No. 4, pp. 177- 85, 2010.

[27]- L. Fojt, L. Strasak and V. Vetterl and J. Šmarda ," Comparison of the low-frequency magnetic field effects on bacteria *Escherichia coli*, *Leclerciaa decarboxylata* and *Staphylococcus aureus*," Bioelectro chemistry, vol. 63, no. 1-2, pp. 337- 41,2004.

[28]- O.R. Justo ,V.H. Perez , D.C. Alvarez and R.M. Alegre," Growth of *Escherichia coli* under extremely low-frequency electromagnetic fields,".Appl. Biochem .Biotechnol., vol. 134, no. 2, pp. 155-63, 2006.

[29]- L. Cellini, R. Grande and E. Di Campli E. Bacterial response to the exposure of 50Hz electro magnetic fields. Bioelectromagnetics 2008; 29 (4): 302-11,2008.

[30]- I. Belyaev ," Toxicity and SOS-response to ELF magnetic fields and nalidixic acid in *E. coli* cells," Mutation Research , vol. 722, no. 1, pp. 56-61,2011.

[31]- L. Strasak , V, Vetterl and J. Smarda," Effect of low frequency magnetic fields on bacteria *Escherichia coli*," Bioelectrochemistry, vol. 55, no. 1-2, pp.161-164, 2002.

[32]- M.Aslanimehr , A.Pahlevan , F.Fotoohi-Qazvini and H. Jahani-Hashemi H," Effect of extremely low frequency electromagnetic fields on the growth and viability of bacteria," Int J Res Med Heal Sci., vol. 1, no. 2 , pp. 8-15, 2013.

[33]- A.G.El-Sayed, H.S. Magda, Y.T.Eman and H.I. Mona," Stimulation and control of *E.coli* by using an extremely low frequency

magnetic field," Romanian Journal of Biophysics,vol. 16, no. 4, pp. 283- 96,2006.

[34]- A.Garip, B.Aksu, Z.Akani, D.Akakin , A. Ozaydin and T. San ," Effect of extremely low frequency electromagnetic fields on growth rate and morphology of bacteria," Int. J. Radiat. Biol., vol. 87, no. 12, pp. 1155- 61, 2011.

[35]- Y.Teow, P.V. Asharani , M.P. Hande and S. Valiyaveettil ," Health impact and safety of engineered nanomaterials,"Chem. Commun. (Camb)" vol. 47, no. 25, pp. 7025-38,2011.

[36]- F.I.Wolf, A.Torsello and B.Tedesco,"50 Hz extremely low frequency electromagnetic fields enhance cell proliferation and DNA damage: possible involvement of a redox mechanism. Biochimicaet Biophysica. Acta., 2005; 1743(1-2) : 120-9.

[37]- A.R. Ward, V.J. Robertson and H. Ioannou," The effect of duty cycle and frequency on muscle torque production using kilohertz frequency range alternating current," Med. Eng. & Phys., vol. 26, pp. 569-79, 2004.

[38]- G.Giorgi, P. Marcantonio, F. Bersani, E. Gavoci and B. Del Re," Effect of extremely low frequency magnetic field exposure on DNA transposition in relation to frequency, wave shape and exposure time," International Journal of Radiation Biology, vol. 87, no. 6, pp. 601-606,2011.

[39]- J.I. Ross," Antibiotic-resistant acne: lessons from Europe," J. Dermatol., vol. 148, pp. 148 :467- 78,2003.

[40]- A.F.Junka, R. Rakoczy, K.P. Szymczy, M. Bartoszewicz ,P.P. Sedghizadeh and K. Fijałkowski ," Application of Rotating Magnetic Fields Increase the Activity of Antimicrobials Against Wound Biofilm Pathogens," Scientific Reports, vol. 8, no. 167, pp. 1-12, 2018.