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# Control of *Mycobacterium tuberculosis* and activity by ELF-EM pulses at Resonance Frequency

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## ABSTRACT

Tuberculosis as a major worldwide human disease, caused by *Mycobacterium tuberculosis* (MTB), is the second most fatal infectious disease after AIDS and one of the most prevalent pathogen that is responsible for socioeconomic disaster for millions of people than any other microorganism.

For this purpose, MTB strain H37Rv was exposed to different square electric pulses of extremely low frequency (ELF) generated between two parallel plate electrodes, at different frequencies in the range 0.1 to 1Hz for a period of 30 minutes. The field strength between the electrodes was 400V/m. From the growth characteristics of the microbe for control (non-exposed) and exposed samples, it was found that maximum inhibition in the growth characteristic of the microbe occurred for samples exposed to 0.6 Hz for 45minutes.

Transmission electron microscope (TEM) examination of the treated samples as compared with control indicated remarkable morphological changes in the cell walls and inter-constituents of microbe. Antibiotic Sensitivity test indicated significant increase in the inhibitors for protein synthesis susceptibility, cell wall biosynthesis and RNA as the result of exposure. It was concluded that this new non-invasive treatment of MTB is a promising technique to treat and resist this disease.


**Keywords:** Electromagnetic Fields, Resonance Frequency, *Mycobacterium tuberculosis*, Extreme Low Frequency.

## 1. INTRODUCTION

Tuberculosis (TB), a devastating chronic infectious disease caused by the gram-positive, acid-fast *Mycobacterium tuberculosis*, was classified as a global health emergency by the World Health Organization (WHO) in 1993. Tuberculosis is still a highly pathogenic disease, estimated at 10.4 million new cases and 1.4 million deaths in 2015 (WHO, 2016).

Treatment with optimum chemotherapy is the key to triumphant elimination of TB (Joshi, 2011). Drug-resistant *M. tuberculosis* strains are a major global health concern because treatments of these cases require second-line drugs, as well as sophisticated infrastructure for drug susceptibility testing which are more toxic, more expensive and less effective, and not readily available in resource-limited settings (WHO, 2016). The Multi Drug-Resistant (MDR-TB) and Extensive Drug-Resistant (XDR-TB) represents a threat to national TB control efforts (Alene et al., 2017).

The peerless use of different technologies results in an increased exposure to Extremely Low-Frequency (ELF) Electromagnetic Fields (EMFs) generated by structures and appliances such as power lines and ordinary apparatuses used in house and workplace. There upon, the effects of ELF-EMFs on the biological functions of living organisms are coming to light. Many researches have been conducted recently to authenticate direct effects exerted by ELF-EMF on cell functions (Segatore et al., 2012). Recently several trials have been carried out to control cellular activities by using non-invasive technique of ELF-EMF and frequencies which resonates the bioelectric signals generated during a particular metabolic activity. These trials succeeded to control the growth of bacterial cells (Fadel et al., 2005 & 2013 & 2014a; Motaweh et al., 2014), viruses (Fadel et al., 2014b), and fungi (Fadel et al., 2009). Therefore it seems necessary to apply this non-invasive technique that studies the possibilities of application for treatment of TB in vitro and Vivo.

## 2. MATERIALS AND METHODS

### Characteristics of TB bacteria

In this work the used strain of *M. tuberculosis* H37Rv (ATCC 27294) was obtained from the Veterinary Serum and Vaccine Research Institute, Cairo, Egypt. The microorganisms were prepared and the growth characteristics of *M. tuberculosis* were measured by optical density at 600nm (OD600) following Peñuelas-Urquides et al., 2013.

### ELF-EMF Exposure Facility

Direct current (9v-DC) produced from a power supply was interrupted through an electronic device then amplified to + 400V through DC/DC converter (block diagram figure-1) was connected to square Copper electrodes in the form of a capacitor of 20 cm side and one meter separation distance. The plates were covered by electrical insulating thin film to prevent direct contact with the electrodes. The field strength between the electrodes, as measured by field meter, was 400V/m and the pulse shape was displayed through the use of a memory scope.

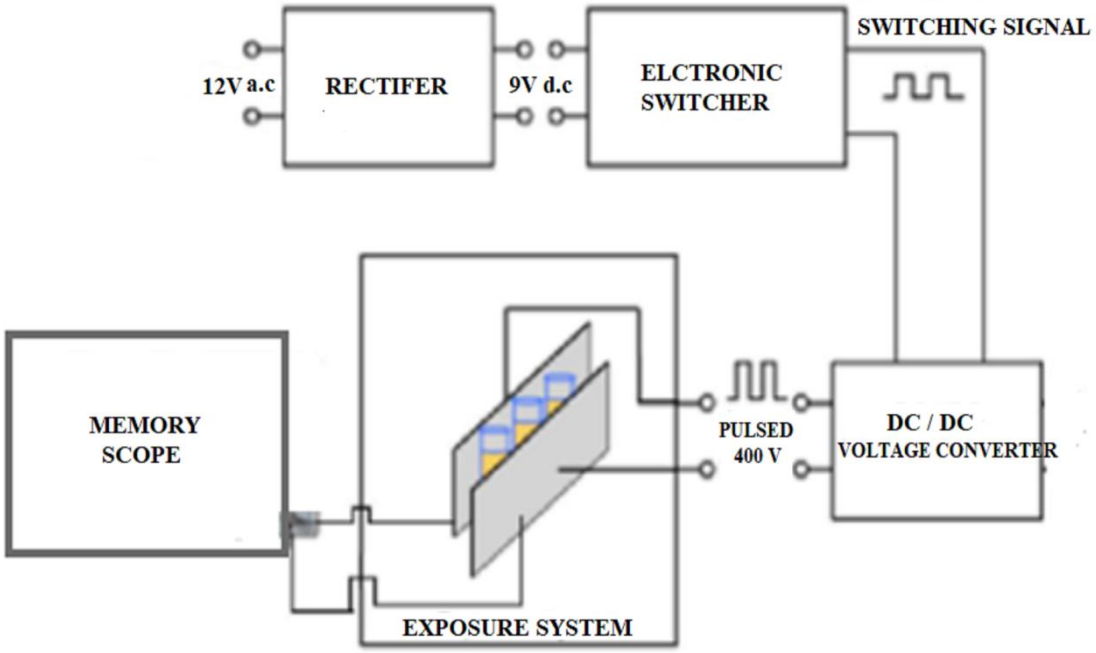



**Figure 1** Schematic illustration of the experimental design used for exposure system

**Safety of Experimental techniques and radiation facilities**

For safe handling of the microbe, all the experiments were done in a safety cabinet of level three. The exposure of the microbe was done in field strength of 400 V/m which is within the safe limit of exposure recommended by the International Commission on Non-Ionizing Radiation Protection (ICNIRP 1998; 2010)

**Inhibiting Resonance Frequency (Fr) Determination:**

Suspension of TB bacteria was equally divided in 30 tubes. Each three tubes formed a group which was exposed in the field at specific frequency in the range of 0.5 Hz to 1.0 Hz for a period of 30 minutes. The growth characteristics of the exposed bacteria in each sample were then done and the averages of the 3 sample readings were considered.

**Exposure Time Dependence**

The most effective exposure time (Tr) was determined from the data obtained from OD measurement of samples of TB were equally divided into eighteen cuvettes which were continuously exposed to Fr for periods of 30, 45, 60, 75 and 90 min, and then 3 samples were removed from the field each 15 minutes till 90 minutes. The three samples were not exposed to any radiation and used as reference control.

**Transmission Electron Microscope (TEM) and Antibiotic Susceptibility Test**

Samples were prepared and examined with a JEOL JEM-1400 TEM transmission electron microscope (Rodríguez et al., 2014) and antibiotic susceptibility test by The MGIT 960 system (Mycobacteria Growth Indicator Tube, Becton Dickinson Co.); (Diriba et al., 2017).

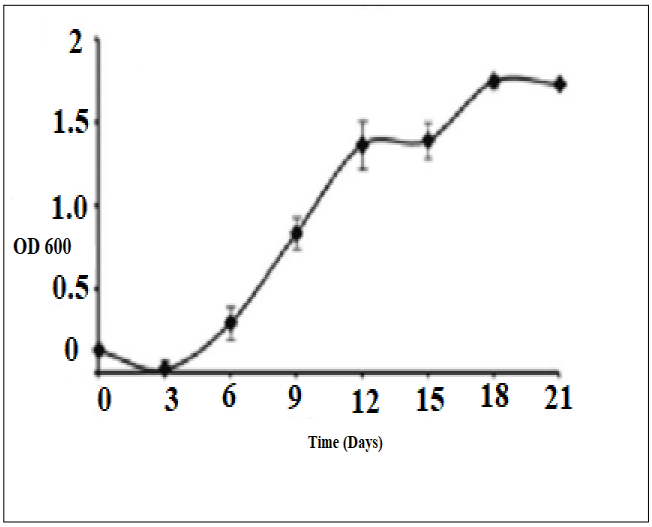
**Statistical Analysis**

The statistical analysis was conducted by using statistical SPSS Package program version 25 for Windows (SPSS, Inc., Armonk, NY, USA). Unpaired (Independent) t-test to compare between mean values of control versus exposed group B at 0.6 and 0.7 Hz. All statistical analyses were significant at 0.05 ($P \leq 0.05$).

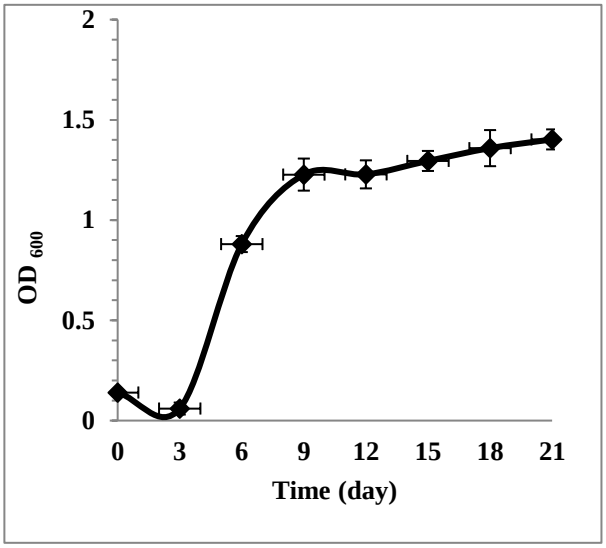
## 3. RESULTS

### Growth Characteristics

Figure (2) illustrates the growth characteristics of TB microbe as measured in the present work and published work by Peñuelas-Urquides et al., 2013. The results indicate similarity in the growth characteristics of TB for the measurement and previously reported.




(A)




(B)

**Figure 2** the growth characteristic of TB microbe where (A) represent the published work and (B) represent the present work

Figure 3 represent a histogram growth for the inhibition percentage of the TB microbe resulting from exposure to different frequencies of Ep for 30 minutes as measured at day 18 of incubation. The data indicates that 0.6 Hz is the most effective frequency.

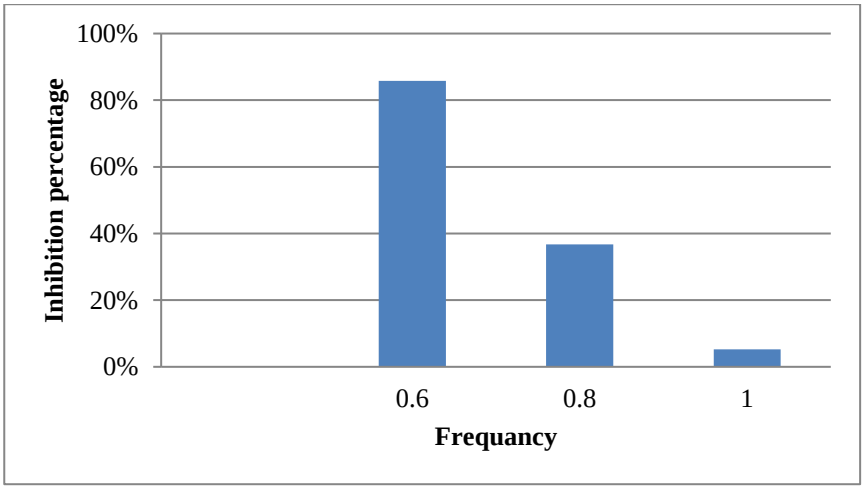



**Figure 3** the inhibition percentage of the TB microbe at day 18 of incubation

### Most Effective Exposure Time

The histogram in figure 4 represents the inhibition percentage of TB growth, measured at day 18 after being exposed to 0.6 Hz Ep for different periods. The data indicates that the most effective conditions for cell growth inhibition are at 0.6 Hz Ep for 45 minute.

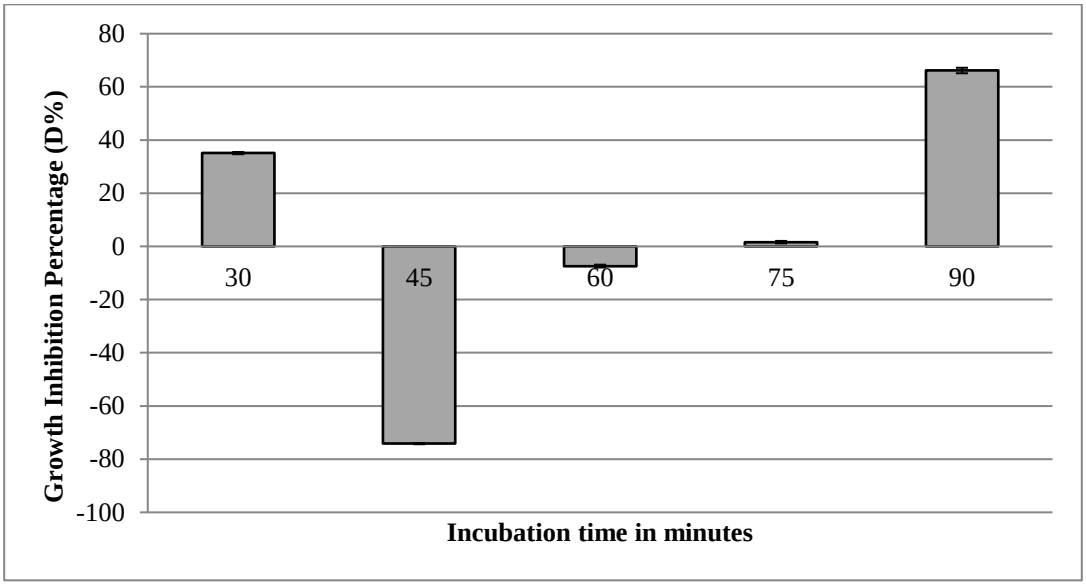



**Figure 4** Exposure Time Dependence

**Antibiotic Susceptibility Test**

It is clear from table (1) that exposure to ELF-EMF at 0.6 Hz showed significant increase in susceptibility to protein synthesis inhibitors, Streptomycin (STR), cell wall biosynthesis inhibitor, Isoniazid (INH)-Ethambutol (ETM), and RNA inhibitors, Rifampicin (RIF).

**Table 1** Antibiotic Susceptibility Test

| Sample/ Antibiotic | Cell Wall inhibitors | | Protein inhibitors | RNA inhibitors |
|---|---|---|---|---|
| Antibiotic | Isoniazid (INH) | Ethambutol (ETM) | Streptomycin (STR) | Rifampicin (RIF) |
| Control (unexposed) | Resistive | Resistive | Resistive | Resistive |
| Exposed to ELF-EMF at 0.6 Hz | Sensitive | Sensitive | Sensitive | Sensitive |

**Morphological change**

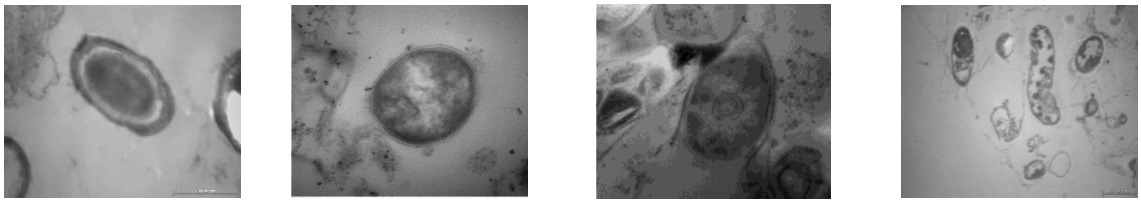



**Figure 5 (A)** TEM photo for positive control microbe and (**B-C-D)** TEM photoes for treated strains with 0.6 Hz for 45 minutes

Figures 5(A-B-C-D) illustrate TEM images for *M. tuberculosis* for control and treated strains with 0.6 Hz for 45 minutes respectively. Figures (5-A) show normal morphological form of *M. tuberculosis* (control). In Figure (5-B) Early stage of destruction to the outer surface of the bacilli with no electron-dense molecules appear in this section which reflects the early stage of cell injury and the response did not appear yet. Figure (5-C) severs destruction to the exoskeleton of the cells although it still retains its ovoid shape. Accumulation of electro-dens materials near the outer plasma membrane denotes over-expression of some proteins sharing the defence mechanism of the bacilli against the damage caused by the exposure. Figure (5-D) Severe damage and destruction of the exoskeleton with escaping of the intracellular components at position begin to appear the accumulation of the electro-dens molecules appears to increase especially in the center of the bacilli may as a method for repairing the damage

## 4. DISCUSSION

The finding presented in this work can be discussed depending on the interaction mechanism of ELF-PE at resonance frequency with the cellular structure of the TB microbe.

This work was designed according to the Metabolic Bio-magnetic Resonance Model (BMRM), suggested by Fadel (1998), who reported that a metabolic biomagnetic resonance interaction could only occur when an external applied ELF-PE has the same frequency of a certain bioelectric signal that means it will be selective for a certain metabolic process and ineffective for the others. The present results indicated that resonance inhibition for microbial growth at frequency 0.6 Hz as shown in (figure-3) and the maximum inhibition of microbial growth were achieved at exposure time of 45 min (figure-4).

Since the outer microbial membrane is formed of phospholipid bilayer macromolecules packed together by Van der Waals weak electro static forces which can be disturbed by the applied electric pulses when their frequencies are at resonance with the natural macromolecular motions.

These changes in the biophysical properties of cell wall can be uncontrollable gates for the permission of antibiotics to flow inside the cell. This understanding can be supported by the antibiotic sensitivity test and TEM data. One more important point is that exposure to the electric pulses caused pronounced rearrangement of the inter-constitutions of the cell in a way that something like separate Islands. These internal cellular changes may cause deterioration of the microbial metabolic activities and hence secretion of toxins.

## 5. CONCLUSION

From the present findings it may be concluded that this new non-invasive technique for the treatment of TB microbe is a promising, fast, safe and non-expensive technique. It can be of global economic impact for the treatment of this dangerous microbe. However; in vivo studies are is needed to be carried out to evaluate the applicability of new technique as a step forward to be used for human.

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**Conflict of interest**

This work is free of conflicts of interest; financial support is exclusive to the authors.

**Ethical approval**

In this work, all the ethical principles for the investigation were respected. It has authorization from the Veterinary Serum and Vaccine Research Institute, Cairo, Egypt.

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