STIMULATION AND CONTROL OF *E. COLI* BY USING AN EXTREMELY LOW FREQUENCY MAGNETIC FIELD

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Abstract. The effect of a 50 Hz magnetic field of strength 2 mT on each of the growth characteristics, the antibiotic sensitivity and the ultra structure of *E. coli* bacteria cells have been studied. Equal volumes of *E. coli* cells were exposed to the magnetic field for different periods, the two most effective periods, namely, 6 h and 16 h were chosen for all our experimental studies. The results indicated that exposure of the microorganisms to the demonstrated magnetic field caused pronounced changes in the growth characteristic curves, where a suppressive effect was observed on the cell growth and the number of cells at stationary phase markedly decreased after exposure period of 6 h but there was a slight increase in the growth rate after exposure period of 16 h with increase in the number of cells. Further, changes in the antibiotic sensitivity was observed after exposure period of 6 h since *E. coli* cells became more sensitive to certain antibiotics such as amoxicillin, nalidixic acid and erythromycin as revealed in the increase in their zone diameters while, after a 16 h exposure period, it became more resistant to the same antibiotics. Furthermore, the results of the ultra structure showed that while exposure period 6 h decreased the cell length, the exposure period 16 h elongated the cell length with decreasing the thickness of the cell wall beside the disappearance of the majority of cytoplasmic components.

Keywords: electromagnetic field, *E. coli*, growth rate, antibiotic sensitivity, ultra structure.

INTRODUCTION

During the past few decades, due to the increasing consumption of electric energy in industry, medicine, research, communication systems and household electric appliances, the level of exposure of biological systems to electromagnetic fields has grown by orders of magnitude over a wide frequency range extending from 0 to 100 GHz. For example, hair dryers, electric shavers and electric hand tools may expose the user to magnetic fields of several times above the background.

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The extremely low-frequency (ELF) electromagnetic field (EMF) exists in all occupational and residential environments. Some scientists allege that exposure to magnetic fields generated by power delivery systems is responsible for certain diseases; therefore, it is both appropriate and important to evaluate the possible effects of man-made electromagnetic field on living organisms. Fadel *et al.* [7] reported that the main damaging role of the 50 Hz magnetic fields might be on the cellular membrane that strongly affects, not only the cellular physiological functions, but also the cell-to-cell communications.

Ma Haile *et al*. [15] studied the effect of pulsed magnetic field intensity and pulse number (PMF) on bactericidal property of PMF in sterilization of fresh watermelon juice. Their results showed that the overall bactericidal effect was strengthened as the magnetic field intensity and pulse number increased with the best effect observed when the magnetic field intensity was 2.53 T and pulse number was 20.

Piatti *et al*. [23] found that the exposure of *Serratia marcescens* to a static magnetic field of 80 ± 20 Gauss resulted in the inhibition of *S. marcescens* growth. Dacosta [5] and Barnickel [3] used new non-destructive decontamination technique to reduce the bacteria in milk, orange juice and also in cheese. Pulsed electric field, pulsed magnetic field and pulsed light were used.

Fojt *et al*. [8] found that *E. coli, Leclercia adecarboxylata* and *Staphyloccus aureus* viability was affected with the magnetic field (10 mT, f = 50 Hz) they also found that the decrease of the colony forming units (CFU) starts immediately after the magnetic field was switched on. Mei *et al*. [16] studied the inactivation of microorganisms by a pulsed magnetic field. It was reported that the application of electromagnetic pulses evidently causes a lethal effect on *E. coli* cells suspended in buffer solution.

Shengying *et al*. [31] studied the non-thermal sterilization by using the self-designed generator of magnetic field. The results showed that the magnetic flux density, which had the greatest effect on *E. coli,* was 1 T. The greatest destruction rate of *E. coli* was 78% under 8 hours of magnetic field (1 T) treatment.

Also, Mohamed *et al*. [18] reported that exposure of the microorganism *S. typhi* to the magnetic field (10, 20 G for a period of 2 hours) caused pronounced changes in the growth characteristics and the number of cells at the stationary phase increased.

Electromagnetic fields are also used in therapy to enhance the transdermal drug delivery [21]; in certain dairy industry to manipulate growth characteristics of yogurt culture, where the change in culture metabolism rather than an elevation in the overall bacterial population, was induced by a 60 Hz, 4.3 G EMF [17]; in soil studies and inactivation of indicator bacteria of cattle slurry by exposure of 400 – 700 g for 60 seconds to magnetic field (380 V, 50 Hz) [10]; in some food

preservation to control certain pathogenic bacteria such as *Salmonella* and *E. coli* contaminated meat samples [4]; in agriculture to improve soil fertility by increasing the nodulation process by exposure of Rhizobium sp. to a low strength (5×10^{-3} T) EMF before inoculation to mycorrhizal chick-pea seedlings [2].

Finally, EMF has been used either to inhibit or to stimulate the growth rate of microorganisms under appropriate conditions [10].

This work is concerned with the study of the biological effect of magnetic fields, as a component of the non-ionizing radiations, on a unicellular system. Pathogenic microorganisms, especially *Escherichia coli,* are chosen to be our experimental model for many reasons; it is widely distributed in the environment such as soil, water and air. *E. coli* is a member of the normal intestinal flora of humans. It causes several diseases such as urinary tract infection, wound infection, traveller's diarrhea. It reaches blood stream and causes sepsis and meningitis [25]. *E. coli* are rapidly growing, Gram-negative, rod-shaped cells measuring approximately 0.5×2 μm length [20].

In the light of the pathogenic effects of these bacteria, we aim, here, to study the effect of different exposure periods to a 20 G, 50 Hz magnetic field on the cell activity with the aim to control the activity through the exposure period. Moreover, we intend to take two exposure periods for investigating the effect of such a magnetic field on the growth rate, the antibiotic sensitivity and the ultrastructure of the exposed cells.

MATERIAL AND METHOD

BACTERIAL STRAIN

Escherichia coli ATCC ≠ 25992 was cultivated over night on Nutrient broth at 37 °C; each ml of bacterial suspension contained 13×10^3 CFU/ml.

SURVIVAL CURVE

To study the bacterial growth, a standard survival curve was plotted between the absorbance of volume A (unexposed cells) at 600 nm and the concentration of cells (number of cells / mL). For cell counting the plate count technique was used [28]. Appropriate dilutions of the bacterial cells were used to inoculate nutrient agar plates. Inoculated plates then incubated at 35 °C for 24 h by counting the number of colonies developed after incubation and multiplying it with the dilution factor the number of cells in the initial population is determined.

MAGNETIC FIELD EXPOSURE FACILITY

Bacteria volumes were exposed to a homogeneous magnetic field generated by a solenoid consisting of 320 turns from electrically insulated 2 mm copper wire

wound in a homogeneous way around a copper cylinder 1.5 mm thick, 40 cm diameter and 25 cm length. The cylinder wall was earthed to eliminate the electric field components effects. The magnetic field generator was temperature controlled during the exposure period by using a water pump as shown in Fig 1. The temperature during the exposure period was 37 °C. The tubes of the exposed bacteria were put in the middle of the coil by using supports inside it to get a homogeneous and higher magnetic fields strength. The ends of coil are connected to variac fed from the mains (220 V, 50 Hz). The field strength was 20 G and adjusted by changing the voltage through the coil.

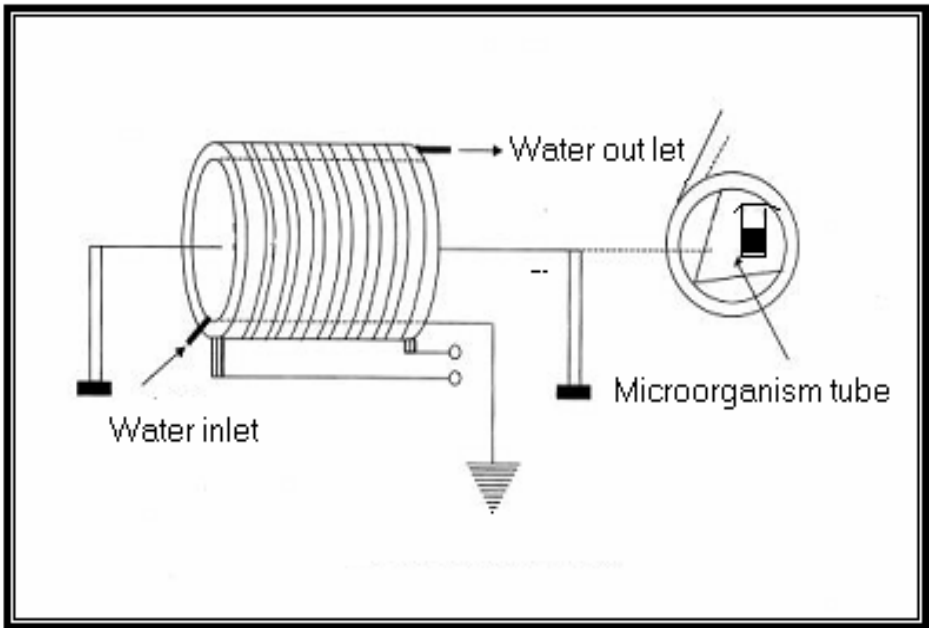

Fig. 1. Magnetic field exposure facility.

GROWTH CHARACTERISTICS

Ten volumes from the strain were incubated for 18 h and then exposed to different exposure periods. The first volume was exposed for two hours; the second volume was exposed for four hours; the third volume was exposed for six hours and so on until 20 h. For each exposure volume, there was a corresponding control volume. Through measuring the absorbance of every volume, the two volumes exposed to the two periods, 6 h and 16 h, were chosen for additional investigations concerning the growth rate, the antibiotic sensitivity and the ultra structure of the cells due to their high effects.

Four volumes were used in this study: A, B, C, and D. Volume A is the control of volume B exposed to 6 h magnetic field, volume C is the control of volume D exposed to a 16 h magnetic field.

GROWTH RATE

The growth rates of all volumes (A, B, C and D) were determined through measuring the absorbance of the viable cells after 2, 4, 6 until 24 h. The absorbance of the volumes was measured and then plotted as a function of time. Spectronic 20+ Series Spectrophotometer (USA) was used for this purpose.

ANTIBIOTIC SUSCEPTIBILITY TEST *IN VITRO*

The isolated *E. coli* cells were tested for their *in vitro* susceptibility to various antibiotics such as erythromycin 10 μg, chloramphenicol 30 μg, cefodoxil 30 μg, nalidixic acid 30 μg, garamycin 10 μg, and amoxicillin 25 μg by disk diffusion test according to Baker *et al*. [1].

The antibiotics used in this study were chosen to be with different modes of action. The diameters of the inhibition or stimulation zone of the volumes A, B, C, and D were measured after 24 h from the exposure process.

ULTRA STRUCTURE OF BACTERIA CELLS

Volumes A, B, C and D were prepared for the transmission electron microscope by the method recommended by Philipe [22] to define the changes in the morphological structure of *E. coli* cells.

RESULTS AND DISCUSSION

The results obtained in this work concern the induced changes in the structure and the characteristic behavior of *E. coli* resulting from the exposure to the demonstrated magnetic field. These results may be of a great importance for evaluating the benefits as well as the hazards of the exposure to the low frequency low-level magnetic field.

Also the importance of this work lays in the fact that *E. coli* as a microorganism is a unit cell behaving as a complete alive biological system.

SURVIVAL CURVE

Fig. 2 shows the variation of the number of microorganisms in CFU/ml as a function of the sample absorbance measured at 600 nm. The results show the linear dependence of the absorbance on the number of microorganisms in CFU/ml. By using this relation we can calculate the number of the microorganisms/ml (C) from

the measured value of its absorbance (*A*). The relation can easily express the linear dependence:

$$C = 9.7 \times 10^9 A \quad (1)$$

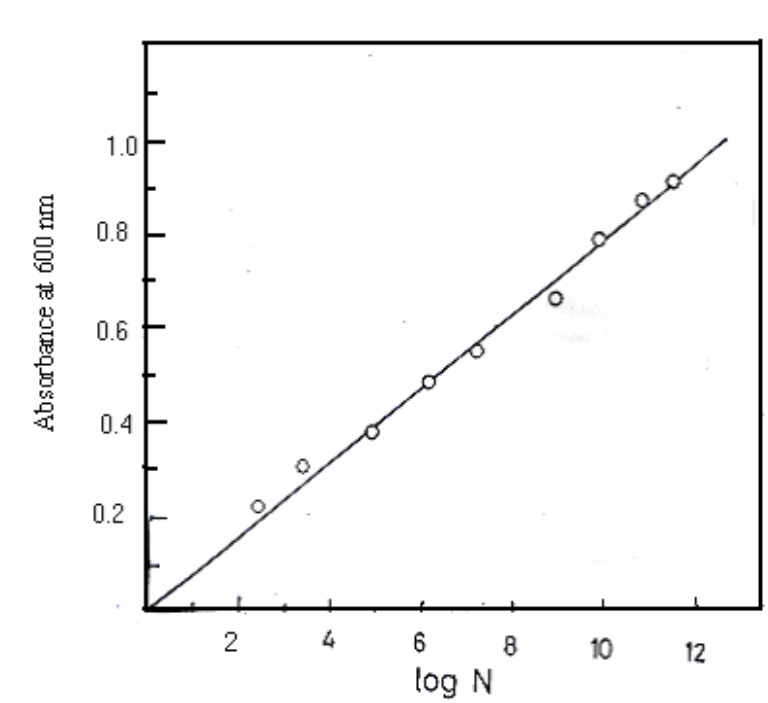

Fig. 2. Survival curve between log number of bacteria cells/ml and the absorbance at 600 nm.

GROWTH CHARACTERISTICS CURVE

Fig. 3 shows the change in the absorbance of bacterial strain as a function of the time of exposure to the magnetic field.

It is clear from this figure that the exposure periods 2, 4, 6, 8, 10 and 12 hrs decreased the absorbance and, in accordance with equation (1), indicate a decrease in the cells number and consequently an inhibition case for the bacteria. However, at the exposure periods of 14, 16, 18 and 20 h the increased absorbance relative to their control indicates an increase in the cells number and a stimulation case. These results are in a good agreement with Mohamed *et al*. [18] where the number of cells of *S. typhi* microorganism exposed to 20 G magnetic fields for 2 hours increased relative to those unexposed.

Also, Jaffe [10] reported that the electromagnetic field was used either to inhibit or to stimulate the growth of the microorganism under appropriate conditions.

For this reason we used the exposure period of 6 h (volume B) as an inhibition case where the number of cells was 10^8 and became 10^7 cells/ml also the exposure period of 16 hours (volume D) as stimulation case where the number of cells was 3.5×10^2 and became 3.5×10^4 cells/ml. Moreover, we intend to take the two exposure periods for investigating the effect of the magnetic field (20 G, 50 Hz) on the growth rate, the antibiotic sensitivity and the ultrastructure of the exposed cells.

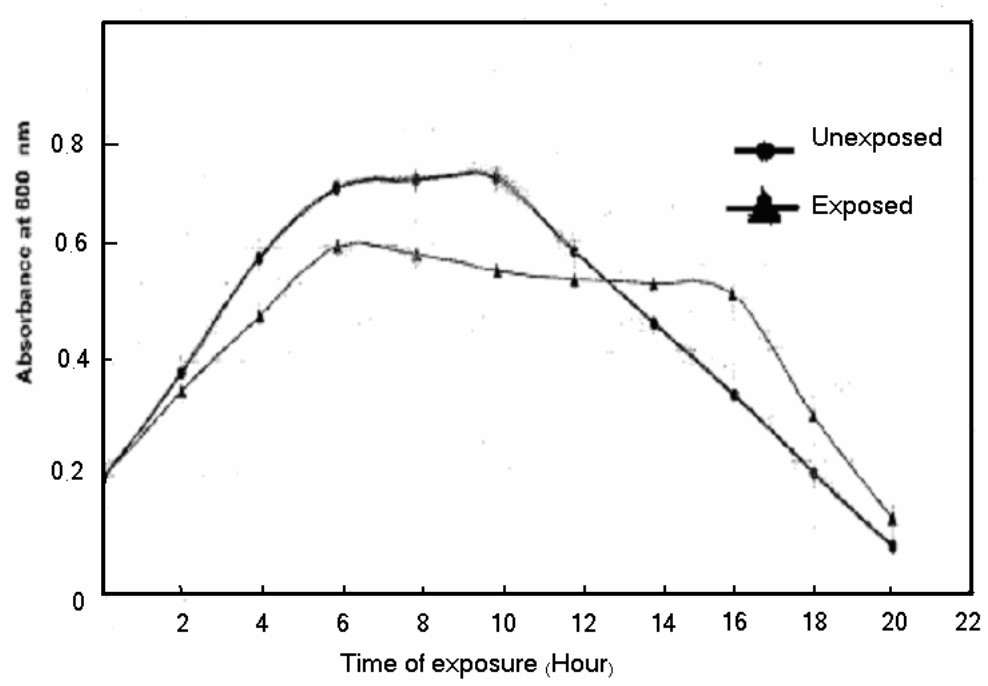

Fig. 3. Absorbance at 600 nm of *E. coli* cells at a different exposure periods.

EFFECT OF MAGNETIC FIELD ON BACTERIAL GROWTH RATE

Fig. 4 shows the growth rate of volumes A and B. It is clear from the figure that there is a decrease in the growth rate of the *E. coli* cells exposed to 6 h relative to its unexposed ones.

Fig. 5 explains the growth rate of volumes C and D. It is clear from the figure that there was a slight increase in the growth rate of the exposed *E. coli* cells relative to its unexposed.

The results in Figs. 4–5 and the calculated data from these curves in Table 1 indicate considerable changes in the growth curve characteristics for the two exposure periods 6 and 16 hrs. For the exposure period of 6 h (volume B) the maximum growth occurred at 16 h while for the unexposed cells at 18 h; also, the

maximum number of microorganisms decreased to be 2×10^7 cells/ml as compared with the unexposed cells 8×10^9 cell/ml. These results are in a good agreement with M. Li *et al*. [12] who used magnetic field for 4 h (0.2 kWh/m^2) to decrease the survivability of *E. coli* to reach 0.01%.

However, for the exposure period of 16 h (volume D) the maximum growth occurred at 14 h with increasing the maximum number of the microorganism to be 2×10^{10} cells/ml as shown in the table. Moreover, from these results one sees how the period of the active growth (log phase) decreased for the two volumes B and D, which became 12 and 10 h, respectively, while it was for the unexposed cell 14 h and also the lag phase was short. In spite of these facts, the exposure period of 16 h increased the cell division rate in a good agreement with Nascimento *et al* [19] who concluded that the electromagnetic field (8 h, 5 G, 60 Hz) had a positive effect in the consume of glucose and growth of *E. coli.* They attributed the increase in the growth to the shortening of lag phase and excitement of log phase.

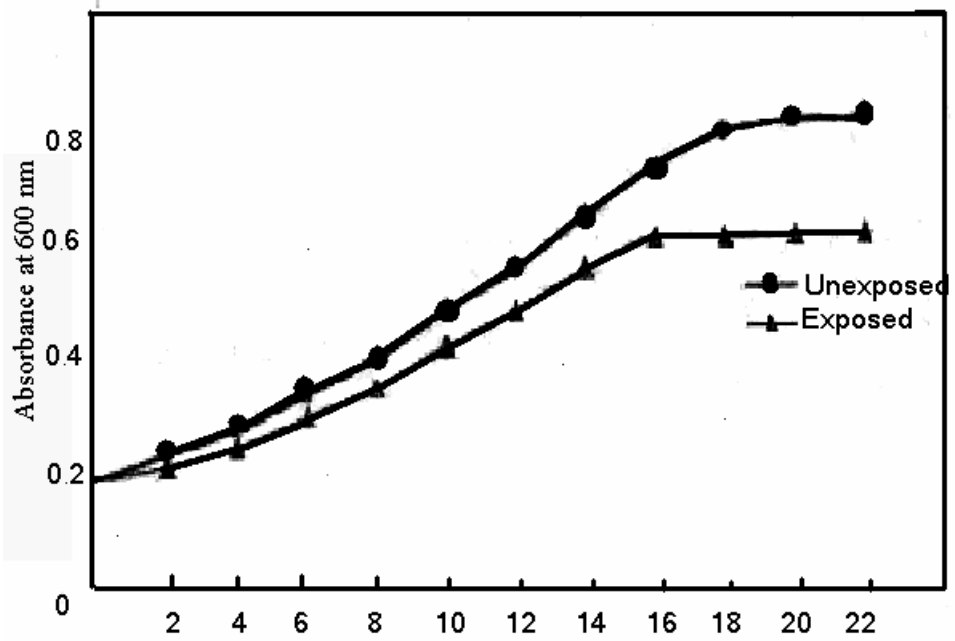

Fig. 4. Growth rate of *E. coli* before (unexposed) and after exposure period of 6 h.

Potenza [24] suggested that exposing *E. coli* cultures to 300 mT static magnetic field may stimulate transposition activity.

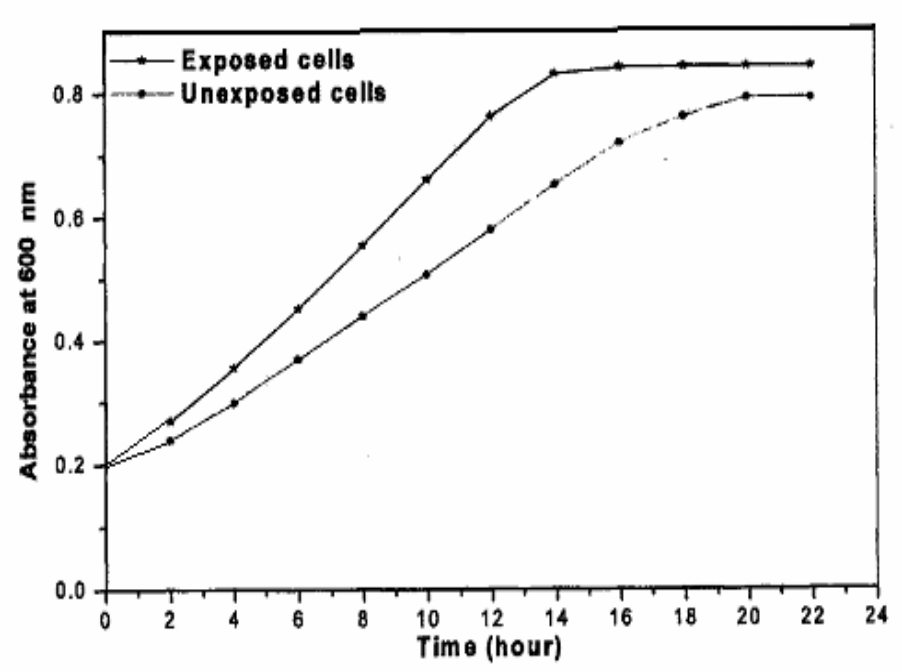

Fig. 5. Growth rate of *E. coli* before (unexposed) and after (exposed) exposure period of 16 h.

Table 1

Growth characterization of *E. coli* before and after exposing to the magnetic field

Samples	Log phase (h)	Stationary phase (h)	No. of cells/ml at stationary phase
Volume A	14	18	8×10^9
Volume B	12	16	2×10^7
Volume C	14	18	7×10^9
Volume D	10	14	2×10^{10}

The inhibitory effect of EMF after an exposure period of 6 h on the growth of bacteria may be due to the interaction between electric charges induced by EMF and that of the cytoplasmic membrane resulting in partial abolishment of electric potential of the cytoplasmic membrane with a subsequent decrease in the macromolecular biosynthesis. Also EMF may cause damage of bacterial DNA and inhibition of its replication [9, 14, 27].

Since the present data proved the cellular membrane of the microorganism had been affected by the external magnetic field, then one expects a disturbance in their metabolic activity and, consequently, a change in their cell division in a good agreement with Mohammed *et al.* [18] who reported that exposing *S. tyhi* to a 20 G magnetic field increased their cell division and cell number.

EFFECT OF MAGNETIC FIELD ON BACTERIA ANTIBIOTIC SENSITIVITY

Table 2 and Fig. 6 illustrate an increase in the sensitivity of volume B to the antibiotics used especially erythromycin, nalidixic acid and amoxicillin as revealed in the increase of the zone diameter of the microorganism of that volume. These results indicated that the exposed cells became more resistant to the field. Fig. 6 and Table 2 illustrate a decrease in the sensitivity of the exposed cells, volume D, where the diameter of its zones decreased for some antibiotics such as chloramphenicol, amoxicillin and nalidixic acid. These results indicate that the viability of cells exposed to 16 h increased as compared with the unexposed cells (stimulation case).

All these results indicate that there are effects of the used electromagnetic field to drug mode of action on bacterial cell through inhibition of, cell wall synthesis, protein synthesis, nucleic acids, essential enzymes and change in membrane permeability [11].

Moreover, Stansell *et al*. [29] stated that exposing the bacteria to medium strength magnetic field could significantly alter antibiotic sensitivity. He, also, found that exposing *E. coli* to the magnetic fields considerably increased antibiotic resistance.

Table 2

The antibiotic test of exposed and unexposed *E. coli*

Antibiotics	*E. coli*			
	Inhibition zone diameter in cm			
	Mode of action	Unexposed (A)	Exposed (6 h) (B)	Exposed (16 h) (D)
Chloramphenicol	Inhibition of protein synthesis	1.8	1.7	1.1
Garamycin	Inhibition of bacterial enzymes, prevents formation of PAB (para amino benzoic acid)	0.9	1.1	1.1
Cefaroxil	Inhibition of respiration of RNA, DNA	1.5	1.9	1.8
Amoxicillin	Inhibition of cell wall synthesis of bacteria	2	2.4	1.5
Nalidixic acid	Inhibition of bacterial protein synthesis and act on ribosome	1.2	1.8	1.1
Erythromycin	Inhibition of protein synthesis and binding of ribosome	2.5	2.8	2.4

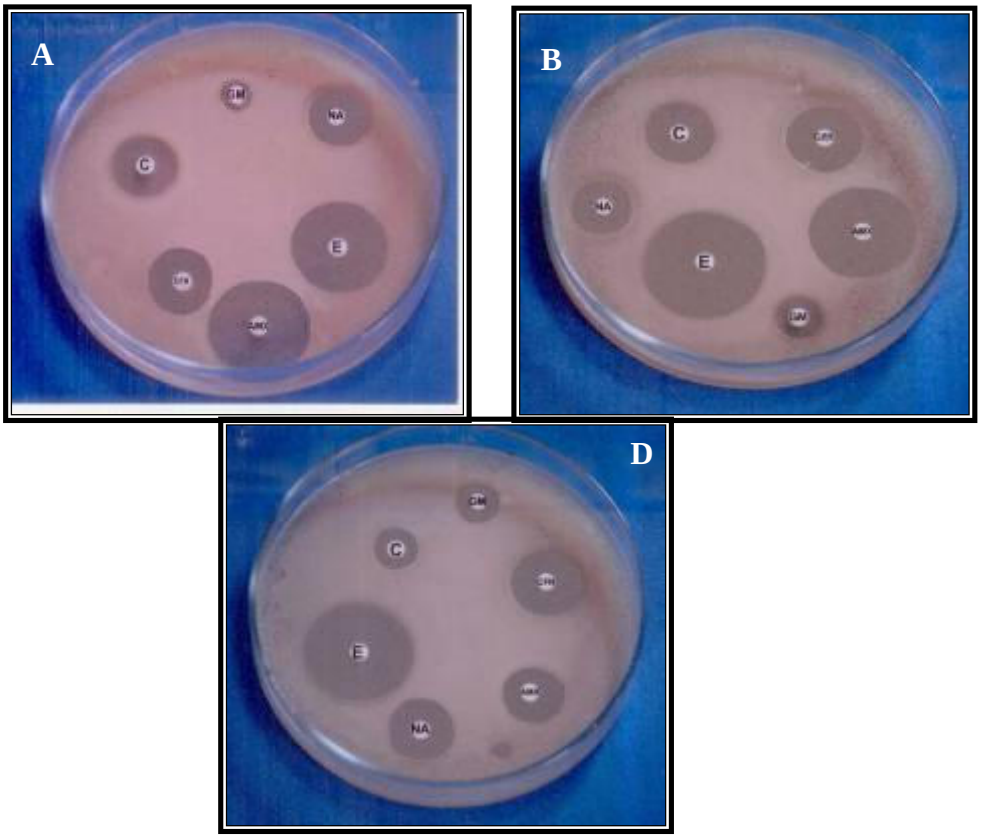

Fig. 6. The antibiotic zones for the unexposed and exposed bacteria (6 h and 16 h, respectively).

EFFECT OF MAGNETIC FIELD ON ULTRA STRUCTURE OF BACTERIA CELLS

Fig. 7 shows the ultra structure of *E. coli* cells for the three volumes, A, B and D. The figure illustrates a complete lyses of the cell wall without destruction of cytoplasmic membrane, granular ribosomal distribution and no vacuoles appear in the cytoplasm for volume B. But for volume D, an elongation of the cells was observed with an increase in the wall thickness of cell and the majority of the cytoplasmic component disappeared.

Strasak *et al.* [30] found that magnetic field effects depend on the cells shape.

To get better understanding of the interaction mechanism of the magnetic field with biological systems an understanding of the bioelectrical signals resulting from the biological system during metabolic activity is required.

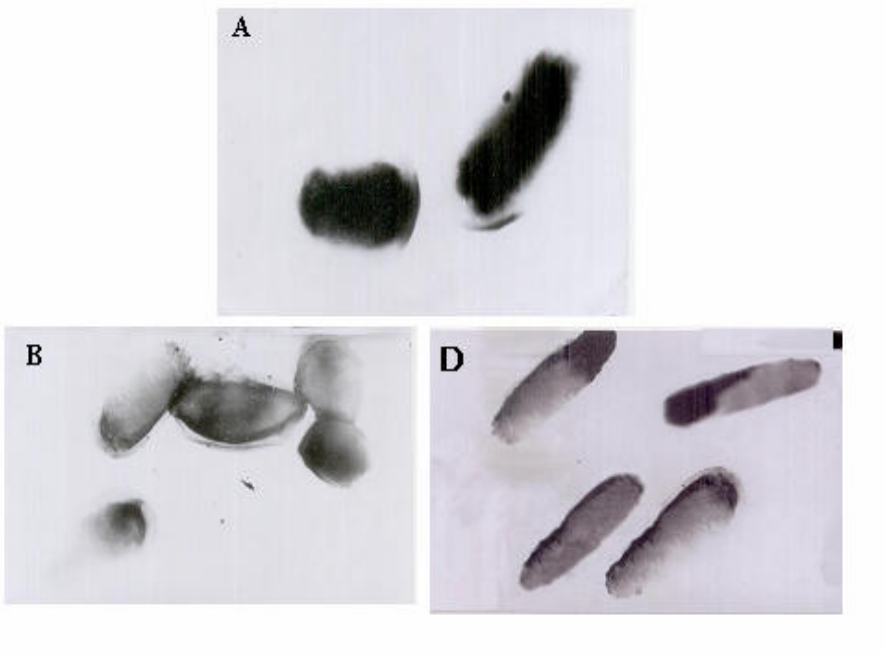

Fig. 7. Ultrastructure of the unexposed and exposed bacteria cells (magnification 20×10^3).

Mohamed *et al*. [18] reported that the bioelectrical signals from the microorganism normally were carried out through bending of their cellular membranes, which generate an electric impulse through a phenomenon known as flexoelectricity. The amplitude and the frequency of these impulses depend on the amount and frequency of bending. These impulses travel through the medium separating the microorganisms and were received by the signal receptors at the surface and that impeded in the cell membrane. Therefore the flexibility of the membrane is the most important parameters for generation of these signals. Also mentioned is that the biomagnetic field from the biological system associating to the bioelectrical signals from the membrane of the cells through its metabolic function is very weak, in nanogauss range (20×10^{-8} G). When the biological systems exposed to an external magnetic field whose strength is very large relative to the biomagnetic field of the cells a disturbance in their metabolic function will be expected and lead to death of the cells or to the increase of their cell division [7, 26]. Del-Re *et al*. [6] found that *E. coli* bacteria that had been exposed for a long time to a 50 Hz, low intensity (0.1–1 mT) magnetic field gave colonies with significantly lower transposition activity compared to sham-exposed bacteria. Such reduction in transposition activity was positively correlated to the intensity of the

EMF, in a dose effect manner also Zhang *et al*. [32] concluded that strong SMF induce mutations through elevated production of intracellular super oxide radicals in *E. coli* cells.

From the present data it is easily deduced that the cellular membrane of the microorganism had been affected by the external magnetic field in a good agreement with Fadel *et al*. [7]. Then we can expect the disturbance of cell division and hence, a change in the number of the cells per ml or the measured change in the membrane sensitivity to antibiotic demonstrated also the change in the internal structure of the cells.

CONCLUSION

From this work, it is concluded that the electromagnetic field (20 G) affected considerably the virulence of *E. coil* cells. 6 h exposure time was found to cause an inhibition case whereas 16 h exposure time enhanced the virulence.

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