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Effect of Magnetic Field on Growth and Antibiotic Sensitivity of *Escherichia coli*

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ABSTRACT

The aim of this work was to study the effect of different magnetic intensities on the dry weights and the antibiotic sensitivity of *E. coli* bacteria. *E. coli* were exposed to three magnetic intensities (130, 260, and 390 T) for two-time intervals (24 and 72 hours). The results indicated that exposure of the bacteria to different magnetic fields caused changes in the dry weight after the exposure period of 24 hours, but there was a slight decrease after the exposure period of 72 hours. Further, changes in antibiotic sensitivity were observed after an exposure period of 24 h. *E. coli*, where bacteria became more sensitive to certain antibiotics such as amoxicillin, nalidixic acid, and erythromycin, while after a 72-hour exposure period, they became more resistant to the same antibiotics. In conclusion, the results of the current investigation show that exposing bacteria to static magnetic fields of varying strengths can considerably affect their susceptibility and resistance to antibiotics.

INTRODUCTION

Since the postmodern period, along with globalization, all known forms of life have been subjected to significantly greater electromagnetic fields Claassen et al. (2016). This is particularly true with regard to the spread of electronic devices. One of the most contentious topics of discussion in the world of biology is the subject of magnetic fields' influence on biological systems (Brocklehurst and McLauchlan 1996). The increased use of electric energy in industry, medicine, research, communication networks, and home electric appliances has resulted in an increase of orders of magnitude in the exposure of biological systems to electromagnetic fields across a broad frequency range stretching from 0 to 100 GHz (Hirata *et al.*, 2021). This increase in exposure is occurring across the entire frequency range. There are a number of natural environments in which *Escherichia coli* (*E. coli*) may be found, including the soil, water, and air (Tran *et al.*, 2020). The bacterium known as *Escherichia coli* belongs to the genus Enterobacter and the family Enterobacteriaceae. The bacteria is often found in the lower intestine of warm-blooded animals, including humans, and is released into the environment via feces or wastewater effluent (Leclerc *et al.*, 2001). It has long been believed that finding *E. coli* in water sources is a sign of recent fecal contamination.

However, several recent investigations have shown that certain strains of *E. coli* may live outside of the intestines for extended periods of time and even proliferate outside. This finding suggests that *E. coli* may coexist with naturally occurring microorganisms (Liao *et al.,* 2020).

Multiple studies show that magnetic fields can have negative effects on living organisms. Research into the effects of magnetic fields spans several sectors, including those of medicine delivery, cancer treatments, water purification, and others (Zadeh-Haghighi and Simon 2022). Moreover, medical equipment, medication delivery systems, biotechnology, and bioprocess optimization are just a few of the sectors that have benefited from the application of magnetic fields in the life sciences and healthcare industries (Schwaminger *et al.,* 2019). As a result of its mechanical and thermodynamic properties, the magnetic field is utilized in medical equipment, which may be broken down into two categories: permanent magnetically driven devices and magnetically driven devices. Permanent magnetically powered medical devices, as the name implies, employ permanent magnets as their driving force in the diagnosis, therapy, treatment, and cure of illnesses.

Microbial species' shape, vitality, and growth in response to magnetic fields have also been studied. The proliferation of nitrite-oxidizing bacteria and the survival rate of *P. aeruginosa* sp. are two examples (Martirosyan *et al.,* 2013, Segatore *et al.,* 2012). The development of bacteria has been shown to be affected by magnetic fields in a variety of experiments, with results indicating that the effect can range from inhibition to stimulation, depending on the intensity and frequency of the field (Bajpai *et al.,* 2012, Inhan-Garip *et al.,* 2011).

The aim of this work was to study the effect of exposure to different intensities of magnetic field (130, 260 and 390 µT) on the growth rate of pathogenic bacteria *Escherichia coli* and the antibiotic sensitivity of the exposed cells during two periods 24 and 72 hours.

MATERIALS AND METHODS

Bacterial Strain:

Escherichia coli were obtained from the Laboratory of Jeddah King Fahad Hospital, Saudi Arabia. It was cultured on Nutrient agar media (Oxioid CM 41) at 37°C.

Magnetic Field Exposure Facility:

The bacterial suspension was exposed to different magnetic intensities (Micro-tesla; µT). Treatments included 130, 260, and 390 µT in addition to control. The magnetic intensity was measured by Gaussmeter (Schecklmann *et al.,* 2020) in the Faculty of Engineering, University of Sudan for Science and Technology. Each treatment was replicated three times. The temperature during the exposure period was 37° C.

Bacteriological Treatment:

An overnight culture of *E. coli* was cultivated on Nutrient broth (Oxioid) at 37° C; each ml of bacterial suspension contained 1.3 x 106 CFU/ml. 50 ml of volumes of the bacterial strains were incubated for 18 h and then exposed to two exposure periods those were 24 and 72 hours. For each exposure volume, there was a corresponding control volume and then they were chosen for another investigation which was the antibiotic sensitivity. The dry weight of the tested microorganism was measured (The filter's pre- and post-use weights were subtracted to get the dry weight of the remaining solids); The biomass is expressed as mg dry weight /ml (Bratbak and Dundas 1984).

Antibiotic Susceptibility Test:

The *E. coli* cells were tested for their in vitro susceptibility to different types of antibiotics with a different mode of action by disk diffusion test (De Backer et al. 2006). Tested antibiotics include Nalidixic acid (NA) 30 µg, Norfloxacin (Nor) 25 µg, Nitrofurantoin (NI) 30 µg, Cefalothin (Kf) 30 µg, Ampicillin(AP) 10 µg, (Ts) mast Diagnostic Amiens, France. The diameters of the inhibition or stimulation zone (mm) were measured after 24 h and 72 from the exposure process.

Statistical Analyses:

With SPSS V.20.0 software, the one-way ANONA test was used to analyze all of the experimental data presented here (SPSS Institute Inc., Cary, NC). When p is less than 0.05, data is considered statistically significant. The 2- factor ANOVA with the type III sum-of-squares method by means of multivariant general linear models (GLM) were used to investigate the effect of exposure time to different magnetic strength and their effect on bacterial growth and antibiotic sensitivity.

RESULTS

Figure 1 shows the effects of the three magnetic intensities and the two exposure periods on the dry weight of *E. coli*. In general, dry weight decreased with increasing exposure periods and magnetic intensity. The changes in dry weight after an exposure period of 24 hours for all magnetic densities are clear; the lowest reading in dry weight was at 390 T, which was 10.0 mg/ml, while unexposed cells have a dry weight of 163.33. After 72 hours of exposure, the decrease was significant; it reached 39.17 mg/ml when comparing these results with the control. When we compared the decrease of 24 and 72 hours of exposure at a magnetic density of 390 T, we recognized the highest decrease recorded at 24 hours; this may be due to the death of bacterial cells after 24 hours.

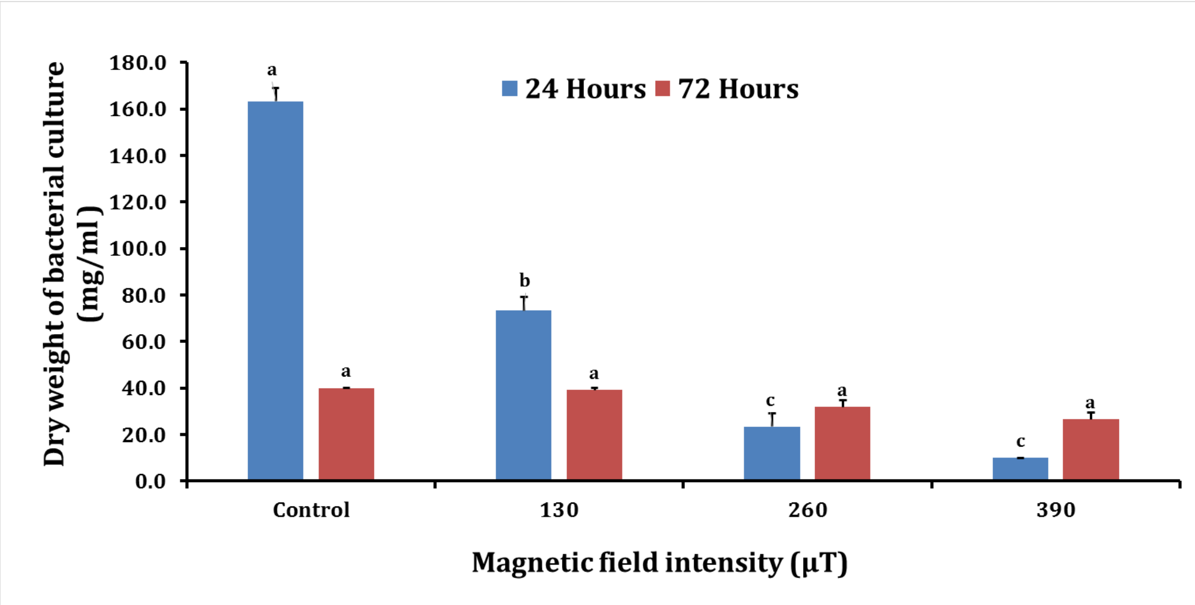

Fig. 1: Effect of different intensity magnetic fields on the dry weight of bacterial cultures. Data are expressed as mean±SD. For each time, the same letter is shown. There is no statistical difference between the means. Different letters, on the other hand, mean that there is a statistically significant difference between the different exposures ($p<0.05$ one-way ANOVA test).

For antibiotic susceptibility, Figure 2 illustrates a clear increase in the sensitivity of *E. coli* to different antibiotics examined, especially cefalothin, ampicillin, and norfloxacin, as indicated by the increase in the zone diameter of the microorganism growth. The result indicated that the cell treated with norfloxacin and exposed to the three magnetic intensities caused no changes in the inhibition zone through the period of 24 hours, while after the 72-hour exposure period, the inhibition zone increased at a magnetic intensity of 390 T. Treatment with Nitrofurantion antibiotic showed no significant changes in inhibition zone, on the other hand, cefalothin has the biggest sensitivity which indicated the by the large inhibition zone after 72 hours of exposure, especially for the magnetic intensity of 269 and 390 µT. the current findings indicate that *E. coli* has the strongest resistance to ampicillin, especially for the exposure period of 24 hours, but at 72 hours there was a slight change. The results showed that E. coli is more resistant to antibiotic TS than ampicillin, but after 72 hours there was no difference between the magnetic intensity of 260 and 390 µT in the diameter of the inhibition zone of the bacteria measured.

A two-way analysis of variance was conducted to determine whether magnetic intensities and time of exposure, influence how the dry weight of bacteria decreases. Results in Table (1) indicated a significant effect of magnetic intensities on antibiotic sensitivity against bacteria. Results also showed a main effect for time of exposure, as well as a significant magnetic intensities * time interaction for both dry weight of E coli as well as antibiotic sensitivity for all tested antibiotics except for Ampicillin.

Table 1: Effect of exposure of bacteria to 3 magnetic field strength at two-time intervals on bacterial growth and antibiotic sensitivity.

Dry weight	Time (T)	Magnetic field (M)	Time*Treatment (T*M)
Antibiotic sensitivity			
Norfloxacin	9.410*	7.597*	12.256*
Nalidixic acid	59.897*	96.862*	26.609*
Nitrofurantion	50.701*	45.038*	54.409*
Cefalothin	12.666*	26.299*	9.983*
Ampicillin	8.051*	12.293*	2.436
TS	58.006*	87.523*	39.171*

(*) Significantly at p-level <0.05 of the main effect of time (T), exposure to Magnetic field (M) and the interaction between both of them (T*M).

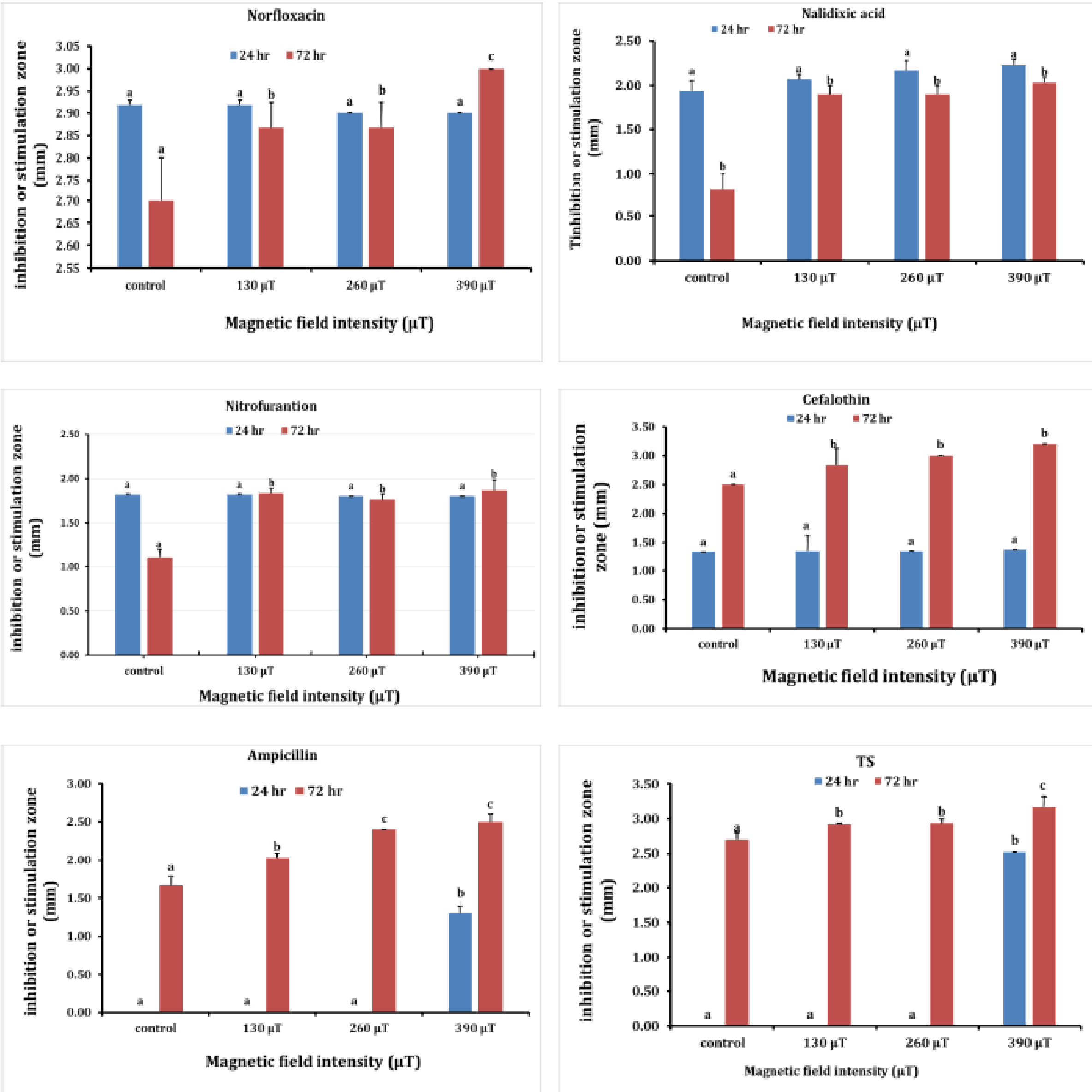

Fig. 2: Effect of different intensity magnetic fields on antibiotic susceptibility against E. coli. For each time, the same letter is shown. There is no statistical difference between the means. Different letters, on the other hand, mean that there is a statistically significant difference between the different exposures ($p<0.05$, one-way ANOVA test).

DISCUSSION

The results acquired in this work involve the induced modifications that occurred in *E. coli* as a direct result of being subjected to three different intensities of the magnetic field. These findings could be of tremendous significance for determining whether or not being subjected to a low-frequency and low-level magnetic field poses more risks than it does advantages. Additionally, the significance of this discovery rests in the observation that *E. coli*, although a bacterium housed within a unit cell, acts in the same manner as a fully functional functioning biological system.

In the present investigation, *E. coli* subjected to magnetic fields of different intensities revealed a decrease in dry weight after 24 hours but showed no significant effect after 72 hours. This is in accordance with that reported by previous studies in which researchers declared the ability of bacteria to decrease colonies with increasing magnetic field intensity and with increasing time of exposure (Bajpai *et al.,* 2012, Fojt *et al.,* 2004). According to the findings presented by Gaafar *et al.* (2006), the magnetic field was utilized to either impede or accelerate the development of the microbe depending on the environmental circumstances. When a biological system is exposed to an external magnetic field with a strength that is very large in comparison to the biomagnetic field of the cells, a disturbance in the metabolic function of the cells is expected to occur, which will either cause the cells to die or cause an increase in the rate at which they divide. Del Re *et al.* (2003) found that Long-term exposure of *E. coli* bacteria to a 50 Hz, low-intensity (0.1-1 mT) magnetic field resulted in colonies with dramatically reduced transposition activity compared to sham-exposed bacteria.

In contrast to our findings previous study revealed that after 24 hours in various types of the static magnetic field, there was no correlation between the growth of bacteria and the presence of the magnetic field for different species (*Bacillus circulans, Micrococcus luteus, Pseudomonas fluorescens* and *Salmonella Enteritidis*) (László and Kutasi 2010). Recently Woroszyło *et al.* (2021) applied 30, 50 and 80 mT MF on three microorganisms, *E. coli*, Staphylococcus aureus and B. subtilis. They reported a decrease in the growth rates of *E. coli* and S. aureus and an increase in the growth rate of B. subtilis. The growth rate of the latter microorganism increased with increasing the intensity of the magnetic field. In an attempt to explain these phenomena, it was reported that the cellular membrane of the microorganism is affected by the external magnetic field, also one expects a disturbance in their metabolic activity and, consequently, a change in their cell division (Gaafar *et al.*, 2008).

Regarding antibiotic sensitivity assay in the present investigation treatment with Nalidixic acid antibiotic showed no significant changes in the inhibition zone, on the other hand, Cefalothin has the biggest sensitivity on the other hand E. coli exhibited resistance to Ampicillin as well as TS, especially for the exposure period of 24 hours, but at 72 hours there were slight changes. All of these results indicated that there are effects of the use of magnetic intensity to drug sensitivity. Gaafar *et al.* (2008) found that electromagnetic fields affect the drug mode of action on bacterial cells through inhibition of, cell wall synthesis, protein synthesis, nucleic acids, essential enzymes and changes in membrane permeability. In addition, Elbourne *et al.* (2020) stated that exposing bacterial cells to the medium-strong magnetic field could significantly alter antibiotic sensitivity. The authors also reported that exposing *E. coli* to magnetic fields considerably increased antibiotic resistance.

CONCLUSION

The results of this study led the researchers to the conclusion that the magnetic intensity had a significant impact on the pathogenicity of E. coli cells. It was discovered that exposing the bacteria for a period of 24 hours led to a reduction in their dry weight. When it comes to the usage of antibiotics, inhibition might occur after an exposure time of either 24 or 72 hours. Additional research might be carried out making use of the various magnetic fields; this could lead to the discovery of more intriguing facts about our biological items. In order to understand the molecular processes at play in our findings, further research is necessary. Furthermore, future research will study trials incorporating other MF signals, utilizing strains from a variety of genetic backgrounds, with the hope of clarifying these discoveries.

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