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The Inhibitory Effects of Static Magnetic Field on *Escherichia coli* from two Different Sources at Short Exposure Time

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Abstract

This study was intended to investigate the effectiveness of static magnetic field on the growth of *Escherichia coli* (*E. coli*) provided from two sources, the urine samples of patients with urinary tract infections and the reference strain *E. coli* ATCC 25922. Bacterial samples in Nutrient Broth were subjected to a range of magnetic intensities (2, 4, 6, 9, 14, 16, 18, and 20 mT) at various exposure times (0, 15, 30, 45, 60, 75, and 90 min). The survival rate was measured in the presence and absence of the magnetic field over time. The cell counts of uropathogenic *E. coli* did not statistically differed from those of the standard strain if exposed to the magnetic field. The fluctuation was observed in cell viabilities at different magnetic intensities below 18 mT. Both groups presented a significant decline in survival rate as exposed to 18 and 20 mT.

Keywords: *Escherichia coli*, Intensity, Magnetic field, Urinary tract infections

Introduction

In the postmodern era along with the globalization, particularly development of electronic gadgets, all known life forms have been subjected to much higher electromagnetic fields (1, 3). Legions of studies have addressed the impacts of the electromagnetic fields on animals, cells, tissues, enzymes and microorganisms (4- 8).

The healthcare or industrial implications of the effects of magnetic fields on prokaryotes have drawn much more attention to cell response to different situations. It has been well established that proliferation rates are on decline as exposed to magnetic fields, either pulsed or static. In this regard, Moore (1979) demonstrated the inhibitory potentials of magnetic fields on microorganisms' growth, which, in turn, varies depending on field strength and frequency (9). This is followed by Strasak et al.'s (1998) work, where 5–21 mT magnetic fields were applied to *E. coli* for 0–24 h (10). Likewise, they observed a decrease in cell viability. More specifically, Fojt et al. (2004) reported a reduction in colony forming units of some bacterial strains, namely *Escherichia coli* (*E. coli*), *Leclercia adecarboxylata* and *Staphylococcus* aureus at extremely low-frequency electromagnetic fields (6). Similarly, static magnetic fields were also associated with a decline in the growth of microorganisms (11,12); put it differently, it concludes that cell viability undergo changes in response to different exposure times and magnetic intensities.

Microorganisms are of utmost importance for human beings. They are widely utilized in numerous food processing (13). More to the point, they possess essential benefits for the function of gastrointestinal system (14-16). Nevertheless, there have been several reports of bacteria-induced epidemics per annum, on the ground of bacterial food-borne diseases and hospital-acquired illnesses in particular. To overcome such threatening conditions, antibiotics being developed by pharmaceutical breakthroughs come up with promising results (17). Yet, multiple resistant bacteria to antibiotics

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as well as the emergence of new pathogens are continuously being observed (18, 19).

Therefore, more efforts are required to explore new treatments or procedures for fighting against antibiotic-resistant pathogens. On the other hand, the use of any antibiotic additives to control over microorganisms is a controversial issue, especially for the food industry (18).

The objective of the present study was to the effectiveness of static magnetic field at a broad range of magnetic intensities and exposure times against the microbial growth of *E. coli* from two different sources, clinical specimens and standard ones. The latter served as the reference.

Materials and Methods

Electromagnetic Field Exposure System

The exposure system was composed of a pair of Helmholtz coils and a variable DC transformer (Iransanat Co., Iran), which allowed generation of an effective magnetic field in the range 0–25 mT. In this study, a pair of Helmholtz coils with 20 cm inner diameter and 1000-turn copper wire was employed to generate the magnetic field. The magnetic field at the center of coils was gauged using a gauss-meter (Gaussmeter HT201, Hengtong, China). The exposure system can provide various intensities of magnetic field by adjusting the rheostat. To monitor the temperature, a thermometric sensor was inserted in the Helmholtz coils system throughout the experiments. This temperature was manually maintained constant at around 25.0 ºC by means of airflow.

Bacterial strains and experimental design

Urinary samples with a positive culture for more than 105 colony forming units per milliliter (CFU/mL) of *E. coli* in urine were obtained from human subjects referred to Razi Pathobiology Laboratory, Rasht. The samples with more than one type of microorganism were ruled out. Eosin methylene blue (EMB) agar and MacConkey agar were utilized for urine culture. The isolates were identified according to their cultural and biochemical characteristics (20, 21). Culture of *E. coli* was re-cultivated on Nutrient Agar at 37 °C for 24 hrs. The initial cell population of 1×106 CFU/mL was achieved following serial dilution with Nutrient Broth (Merck, Germany).

The lyophilized ampoules containing *E. coli* ATCC 25922 were provided by Daarvaash Co. (Iran). They were grown in Nutrient Broth for 24 hours at 37 °C. Afterwards, the microorganisms were poured on Nutrient Agar (Merck, Germany). Bacteria were harvested from Nutrient Agar plates, suspended in Nutrient Broth, and then justified to McFarland 0.5; 2.5×10^8 CFU/mL.

Bacterial from both sources (uropathogenic *E. coli* and reference strain *E. coli* ATCC 25922, groups A and B in order) was subsequently exposed to magnetic intensities (2, 4, 6, 9, 14, 16, 18, and 20 mT) and time durations (0, 15, 30, 45, 60, 75, and 90 min) at 25 ºC. A total of 244 bacterial cell suspensions were utilized for experiments. The control groups were not treated with the magnetic field and incubated in the same conditions at 25 ºC. After the exposure period, two experimental samples and their corresponding control from groups A and B underwent another serial dilution with normal saline, placed on Nutrient Agar plates. Thereafter, they were incubated at 37 ºC for 24 h for subsequent colony counting.

Statistical Analysis.

All measurements were carried out in three replicates. Data was described as mean ± standard deviation (SD). Given normal distribution, independent t-test, one-way ANOVA and Duncan's multiple range tests were conducted to determine any significant difference at P-values <0.05 microbiological counts (SPSS 19.0 software Package, IBM Inc., Chicago IL, USA).

Results

In this study, *E. coli* from two various sources was subjected to a wide range of the magnetic field. Data collected regarding untreated and treated cells was summarized in Table 1. In general, it was apparent that the use of magnetic field remarkably impacted the bacterial growth as compared with the control ($p < 0.05$). Interestingly, the cell counts of uropathogenic *E. coli* (group A) did not statistically differed from those of the standard strain (group B) if treated with the magnetic field ($p > 0.05$). It was revealed that the cell viability at each magnetic field intensity (i.e. from 2 mT to 16 mT) fluctuated during exposure period ($p > 0.05$) (Table 1). Nevertheless, a tendency of growth inhibition was notably obvious by exposing bacterial cells (group A) to 18 mT and 20 mT over time ($p < 0.05$); put it differently, as indicated in Table 1 the population of uropathogenic *E. coli* at 15 min was 145 ± 18.3 and 121 ± 12.3 CFU/g, which approximately diminished to 106 ± 6.9 and 70 ± 3.3 CFU/g at 90

minutes when treated with 18 and 20 mT, respectively. The maximum rate of growth inhibition (51 CFU/g) occurred only when uropathogenic *E. coli* cells exposed to 20 mT for 90 min. Similar to uropathogenic bacteria, group B showed a considerable decline in survival rate when received the magnetic field at 18 and 20 mT *($p<0.05$)*. In other words, it was substantially reduced during 90-min exposure, reaching the lowest count of 71 ± 5.3 CFU/g at 20 mT-90 min.

Table 1. Total viable counts of *E. coli* exposed to different magnetic intensities (CFU/g).

Group	Magnetic intensity	Exposure time (min)						p-value
		15	30	45	60	75	90	
Uropathogenic *E. coli* (A)	0 mT (Ctrl)	82 ± 6.2	85 ± 5.3	90 ± 5.6	86 ± 6.6	86 ± 6.5	86 ± 6.5	0.121
	2 mT	97 ± 8.6	106 ± 6.5	91 ± 4.6	95 ± 7.2	96 ± 7.3	100 ± 9.2	0.230
	4 mT	95 ± 4.5	95 ± 6.9	97 ± 4.9	96 ± 6.6	90 ± 5.2	89 ± 6.6	0.061
	6 mT	89 ± 8.3	89 ± 9.5	99 ± 5.6	98 ± 8.3	95 ± 5.5	90 ± 7.6	0.111
	9 mT	102 ± 4.9	94 ± 10.6	90 ± 2.3	89 ± 9.3	89 ± 9.9	87 ± 4.4	0.801
	14 mT	155 ± 5.8	142 ± 10.5	160 ± 10.2	145 ± 9.1	145 ± 12.3	140 ± 4.3	0.813
	16 mT	140 ± 4.6	169 ± 15.3	166 ± 24.6	156 ± 20.3	155 ± 15.9	155 ± 17.9	0.074
	18 mT	145 ±8.3a	135 ± 15.7b	129 ± 14.3c	120 ± 15.5d	111 ± 11.1e	106 ± 6.9f	0.046
	20 mT	121 ± 12.3a	112 ± 10.6b	100 ± 9.9c	94 ± 14.6d	81 ± 7.6e	70 ± 3.3f	0.023
Reference strain E. coli (B)	0 mT (ctrl)	90 ± 4.9	86 ± 4.3	86 ± 4.9	95 ± 7.2	79 ± 5.6	86 ± 6.3	0.065
	2 mT	88 ± 4.9	91± 5.6	91 ± 6.0	84 ± 7.3	85 ± 3.6	96 ± 3.5	0.341
	4 mT	91 ± 5.5	96 ± 4.4	87 ± 6.6	89 ± 5.3	95 ± 5.3	94 ± 4.3	0.078
	6 mT	96 ± 6	95 ± 7.2	84 ± 4.8	89 ± 4.9	86 ± 6.3	85 ± 5.9	0.153
	9 mT	102 ± 7.5	94 ± 4.6	90 ± 4.9	96 ± 11.6	85 ± 5.5	89 ± 5.9	0.120
	14 mT	151 ± 15.9	144 ± 14	137 ± 15.6	151 ± 15.3	160 ± 15.3	151 ± 13.3	0.064
	16 mT	157 ± 10.7	165 ± 13.3	155 ± 14.2	145 ± 4.8	156 ± 17.2	150 ± 5.0	0.057
	18 mT	135 ± 12.9[a]	130 ± 11.2[b]	122 ± 23.6[c]	116 ± 20.3[d]	105 ± 5.9[e]	95 ± 17.9[f]	0.034
	20 mT	126 ± 8.6[a]	116 ± 11.5[b]	106 ± 10.6[c]	89 ± 8.3[d]	79 ± 7.5[e]	71 ± 5.3[f]	0.011

Different letters in each row indicating significant difference (P<0.05). Ctrl: control

Discussion

There has been a great breadth of conflicting evidence regarding the impact of the magnetic field on microorganism proliferation. This implies that such influence relies on the cell type, exposure time, magnetic intensity, frequency, and the like (2,11,22-24). Bayir et al. reported that treating *E. coli* with 4 mT-20 Hz magnetic field for 6 hours resulted in significant inhibition of bacteria (85%) (18). Our findings indicated that an exposure to 18 mT or 20 mT (90 min at the most) produced the inhibitory effects ranging from 39 to 55 CFU/g for the standard and uropathogenic bacteria. This drop in the cell survival was explained by the correlation between the induction of magnetic field and changes in cell physiology, metabolism, and morphology. It was proved that an exposure to the magnetic field was associated with an increase in the permeability of ion channels in the cytoplasmic membrane, formation of free radicals and active oxygen, disintegration of the cell wall, extrusion of the cytoplasmic contents, retraction of the cytoplasmic membrane, and blebbing (6, 7, 25-31). It was corroborated that the field effect was on the rise with an increase in time of exposure. The present

finding was in accordance with Inhan-Garip et al. (2011), El-Sayed et al. (2006), Fojt et al. (2004), and Strasak et al. (1998), whose studies showed an adaptive behavior in response to the magnetic field (6, 7, 10, 32). In other words, the fluctuation occurred in cell viabilities at the magnetic intensities below 18 mT might be the result of adaptation for the field stress. Nawrotek et al. (2013) and Dunca et al. (2005) also demonstrated that the inhibitory and stimulatory impact of the magnetic field on *E. coli* relies on the time of exposure (33, 34). The results of the present study shed light on the medical and industrial potency of the magnetic field against bacterial growth, which, in turn, comes up with promising outcome for its application in the process of microorganism decontamination and food preservation (7, 18, 35).

In this study, we attempted to address the effectiveness of the magnetic field on the growth of *E. coli* from two different sources when subjected to a range of magnetic intensities at certain exposure time. To the best of our knowledge, the present study was the first effort made to compare the impact of the magnetic field on the uropathogenic bacteria, meaning that this pathogenic microorganism was sensitive to the magnetic field as the reference strain. Moreover, the static magnetic field at 18 and 20 mT for 90 min can afford to retard bacterial growth. For further studies, it is proposed to appraise this reductive influence on other microbial strains in the presence or absence of antibiotics.

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