Magnetic Field Effect on Growth and Antibiotic Susceptibility of *Staphylococcus aureus*

Fouad Houssein Kamel[*1], Chiman Hameed Saeed[**] and Saleem Saaed Qader[**]
[*] Erbil Medical Technical Institute, Hawler Polytechnic University, Erbil-Iraq.
[**] Medical Research Centre, Hawler Medical University, Erbil-Iraq.
[1]E-mail: fhkamel2013@yahoo.com.

Abstract

It is aimed to investigate the effect of exposure of different magnetic fields (400, 800.1200 and 1600 Gauss for 2 to 24 hours) on the growth rate and antibiotic sensitivity of *Staphylococcus aureus*. The experiments were done in Hawler Medical Research Centre Erbil, Iraq. The bacteria were isolated from medical case in Rizgary hospital and identified using API STAPH system. The antibiotic susceptibility of *Staphylococcus aureus* measured according to Kirby-Bauer disc diffusion technique. Results showed a significant decrease in the logarithm in the number of *Staph. aureus* (41.4 to 27 X 10^6) treated with high frequency magnetic field. Sensitivity of *Staph.aureus* to antibiotic increase during a short period (4-6 hours) and increase its resistance to same antibiotic at log term of exposure (18-24 hours). Some results of biochemical tests also showed positive effects of magnetic fields on the biochemical properties according to API STAPH results. The bacterial enzymes MAL (Maltose), LAC (Lactose), TRE (D-Trehalose), MAN (D-Mannitol), SAC (Sucrose) and NAG (N-acetyl-glucosamine) were affected by magnetic field at 24 hours of incubation.

It is concluded that the cellular membrane of the microorganism had been affected by the magnetic field, also the response increased when the field intensity increased. So the magnetic field effects on bacteria are considered bactericidal, 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.

Keywords*:* Magnetic field**,** *Staphylococcus aureus***,** Antibiotics susceptibility test, API Staph.

Introduction

For the first time, in 1976, the biological effects of electromagnetic fields usage were considered. With the growing development of technology in various fields and waves, greater use of technologies leads to increasing exposure to electromagnetic fields (EMFs), such as power lines and ordinary devices used inside house and work places. As consequence, organisms and especially the human who has affected today [1].

Ma *et al*. [2] 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.

The study of effects of ELF-EMF on bacteria is essential not only for investigation of environmental stress influences on biological systems, but also to explore the possibility of controlling the sensitivity of bacteria toward antibiotics in the environment or in clinical laboratories [3].Investigations sponsored by Bio-Magnetics Systems, Inc. have shown that unidirectional magnetic fields inhibited or increased the growth of cancer cells, depending on the field polarity, as disclosed by Trappier [4].

So the effects of magnetic fields were studied in different areas such as drug delivery, cancer therapy, sterilization, and water treatment [5]**.**

Magnetic field affects DNA synthesis and transcription [6] as well as ion transcription through all membrane [7]. Piatti *et al*.[8] found that the exposure of the bacteria *Serratia marcescens* to a static magnetic field 80 $\pm$20 Gauss resulted in inhibition of growth. The effect of magnetic field was variable depending on the type of the microorganism and field. Novak *et al*. [9] clarify that

magnetic field has significant effect on bacterial cell as well as on its life and they added that the effect of magnetic field was enclosed in cell membrane.

The aim of our objectives were to study the effects of different exposure periods (400, 800, 1200 and 1600 G locally prepared static magnetic field) on the cell activity. The effects of such magnetic fields on the growth rate and antibiotic sensitivity were explored, too.

Material and Methods

The bacterium *Staphy. aureus* was isolated and identified on culture medium of patient samples in Rizgary hospital and suspend into 10 ml of nutrient broth, incubated at 37 ºC for 24 hours as a stock culture.

Dipolar magnetic field was prepared locally with different forces including 400, 800, 1200, 1600 Gausses and measured by Teslometer in Physical Department, College of Science, University of Salahddin, Erbil, Iraq. Later 0.1 ml of stock bacterial suspension was inoculated into five groups of tubes contained 5ml of nutrient broth. Four groups of tubes were subjected to magnetic field (400, 800, 1200, 1600 Gauss) respectively. While the fifth group was subjected to magnetic field as a negative control, later all tubes were incubate at 37 ºC for 2 to 24 hours.

The effects of different forces of magnetic fields on growth rate were evaluated by measurement of the optical density using McFarland Turbidity Standards (0.5). The API STAPH kits were prepared by BioMerieux Company and used due to BioMerieux Company instrctions. Inoculation of API Staph kit with bacteria from each group done separately. Antibiotic susceptibility test was carried out using Muller-Hinton Agar medium depending Kirby-Bauer Disk Diffusion technique. Gentamycin (30 mcg), Tetracycline (10mcg), Chloramphenicol (30µg), Rifampcin (5mcg), Ceftazidium 30mcg), Ceftriaxone (30mcg), Metronidazol (5 µg) disks were placed over the medium. The antibiotics used in this study were chosen to be with different modes of action. The diameters of the inhibition zone were measured after 24 hours from the exposure process.

Results and Discussion

Results indicated that magnetic fields (400, 800, 1200, and 1600 G.) increased the logarithmic phase of *Staphyl. aureus* growth (within 4 hrs of treatment, but decreased growth curve after a period of 8 hrs (Fig.(1)). A considerable change in the growth rate of *Staphy. aureus* (Table (1)). A decrease in the colony forming units (CFU) started immediately after the magnetic field was switched on and that magnetic field effect on bacteria could be considered as bactericidal.

These results are in agreed with others [5, 10-12] who reported the exposure of *E. coli*, *Staph. aureus* and *Salmonella typhi* to the magnetic field has similar effects. Fojt *et al*. [13] found that *E. coli*, bacteria decarboxylation and *Staphy. aureus* viability was affected with the magnetic field (10 mT, 50 Hz). Nasher and Hussein [11] concluded that magnetic field effect on bacteria could be considered as bactericidal. Babushkina *et al*. [14-17] demonstrated that ELF-EMF positively affect functional parameters (cell growth and viability) and bacteria antibiotic sensitivity depending on physical parameters of the electromagnetic field (frequency and magnetic flux density) applied, the time of the exposure, and/or the type of bacteria cells used.

Table (1)
Growth rate of Staph.aureus for each group.

Time of exposure to magnetic field in hour	Optical Density (O.D.) at 600 nm and bacterial cells count(McFarland)									
	Control		400G		800G		1200G		1600G	
	O.D.	Bac. count x 10^6	O.D.	Bac. count x 10^6	O.D.	Bac. count x1 0^6	O.D.	Bac. count x10^6	O.D.	Bac. count x 10^6
0	0.034	10.2	0.034	10.2	0.034	10.2	0.034	10.2	0.034	10.2
2	0.139	41.7	0.123	36.9	0.115	34.5	0.113	16.95	0.102	30.6
4	0.138	41.4	0.120	36	0.113	33.9	0.110	33	0.090	27
6	0.110	33	0.108	32.4	0.106	31.8	0.099	29.7	0.075	22.5
8	0.098	29.4	0.085	25.5	0.080	24	0.060	18	0.050	15
20	0.055	16.5	0.040	12	0.036	10.8	0.030	9	0.015	4.5

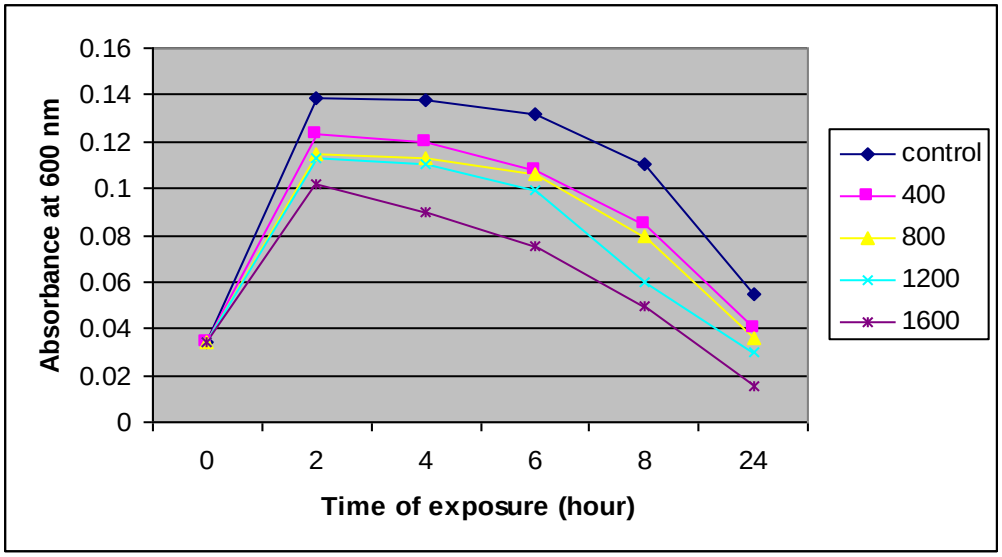

Fig. (1) Absorbance at 600 nm of Stap.aureus cells with different exposure periods.

Table (2) showed antibiotics susceptibility test at different periods of exposure (2, 4, 6, 8, 24 hours) which evaluated according to the mode of action, the results concluded that *Staph.aureus* were sensitive for Gentamycin, Ceftazidium, Tetracycline, Chloramphenicol, Rifampcin, Ceftriaxone whereas resistant to Metronidazol. Also the results indicated that magnetic field alter antibiotic sensitivity and found that exposing *Staphy.aureus* to magnetic field increased antibiotic resistance absolutely in Chloramphenicol, Rifampcin and Ceftriaxone. The diameters of the inhibition or stimulation zone of the different magnetic forces were measured after 24 hours from the exposure process compared with unexposed samples.

These results were in agreement with the work of Stansell and colleagues [18] who found that moderate intensity static fields were able to cause a decrease in the antibiotic sensitivity and resistance of *E. coli*. [16] found that electromagnetic field also induced transcriptional changes and the acquisition of resistance to Cephalosporins (Cefuroxime and Ceftazidime). Therefore, the possibility that magnetic field could interferes with the surface charges of the membrane or the charge distribution on the antibiotic molecule modifying the rate of antibiotic penetration may exist.

Table (2)
Antibiotic test of exposed and unexposed Staph.aureus to magnetic filed.

Antibiotics	Mode of action	Inhibition antibiotics zone diameter in mm							
		Un exposed to M.F	M.F exposure (G) time						
			2 hour				20 hour		
			400	800	1200	1600	400	800	1200	1600
Gentamycin	Inhibition of protein synthesis (30 S-R)	25	35	25	30	30	22	17	16	16
Tetracycline	Inhibition of protein synthesis (30 S-R)	25	36	38	35	30	17	16	16	16
Chloramph-enicol	Inhibition of protein synthesis (50S-R)	18	12	10	10	10	10	10	R	R
Ceftriaxone	Inhibition of cell wall	25	30	R	R	R	R	R	R	R
Ceftazidium	Inhibition of cell wall	17	30	25	23	23	16	15	13	10
Rifampin	Inhibition of nucleic acid	32	18	R	R	R	R	R	R	R
Metronidazole	Inhibition of nucleic acid	R	R	R	R	R	R	R	R	R

R: Resistance, M.F: Magnetic Field, S- R: Subunit- Ribosome.

According to API STAPH, the bacterial enzymes MAL, LAC, TRE, MAN, SAC and ARA affected by magnetic field at 24 hours of incubation. These results suggest that the biological effects of magnetic fields may critically depend on the physical characteristics of the magnetic signal, in particular the wave forces. So treating enzyme with different magnetic fields can inhibit or promote enzyme activity according to API STAPH. *Staphy. Aureus* may be identified by this test (Fig.(2)). Results agree with results of [16, 19, and 20] which demonstrated that short-term exposure (20–120 min.) to an ELF-EMF with a sinusoidal waveform of amplitude ranging from 0.1 to 1mT and frequency of 50 Hz affected both cell viability and morphology of cultured *E. coli* ATCC 700926. Results also exhibited that magnetic field can affect membrane functions; however the magnetic field could interact with other specific processes that help the adaptation of bacteria to the new environment. In this regard, bacteria are able to respond to environmental stresses by activating suitable inducible systems, such as the DNA repair system, and exploit processes which increase the genetic variability.

API STAPH System of Stap. aureus before exposure to magnetic field

API Staph system of Staph.aureus after 24 hours of exposure to magnetic filed

Fig. (2) API Staph System of exposed and unexposed of Staphy. aureus to magnetic field.

Conclusions

It is concluded that the growth rate of *Staph.aureus* cells was affected by exposure to magnetic forces (400, 800, 1200 and 1600). The magnetic field decreased the logarithmic phase within 4 -6 hours of treatment compared with the control. Furthermore, the bacterial sensitivity to antibiotics increased after exposure period of 6 hours to certain antibiotics, but become resistant after 16 hours. The bacterial enzymes MAL (Maltose), LAC (Lactose), TRE (D-Trehalose), MAN (D-Mannitol), SAC (Sucrose) and NAG (N-acetyl-glucosamine) were affected by magnetic field. Treating of enzymes with different magnetic fields forces could inhibit or promote enzyme activity according to API STAPH tests.

References

[1] Faychiting M., Ahlbom A., Kheifets L. EMF and health, Annu. Rev. Public Health, 26: 165- 189, 2005.

[2] MA, H., DENG Y., CHU J., MA H., DENG Y., CHU Y. Sterilization of watermelon juice with high voltage pulse magnetic field and its mechanism analysis, Transactions of the Chinese Society of Agricultural Engineering, 19(2), 163–166, 2003.

[3] Segatore B., Setacci D., Bennato F., Cardigno R., Amicosante G., Iorio R. Evaluations Effects of Extremely Low-Frequency Electromagnetic Fields on Growth of Antibiotic Susceptibility of the Escherichia coli and *Pseudomonas aeruginosa*. International Journal of Microbiology: 1-7, 2012.

[4] Trappier A. Evolving Perspectives on the exposure risks from magnetic Fields, Journal of Nat. Med. Assoc., 82:621-24, 1990.

[5] Haghi M., Maghsoodi MJ., Janipor MB, Seyedgholizadeh S. Effect of static magnetic field on E. coli growth. International Journal of Advanced Biotechnology and Research; 3(4):777–81, 2012.

[6] Phillips J. L., Haggren W., Thomas W. J, Ishida-Jones T., Adey W. R. Magnetic field induced changes in specific gene transcription. Biochim Biophys. Acta 112, 140- 44, 1992.

[7] Liburdy R P, Callahan D E, Harland J., Dunham E., Sloma T.R., Yaswen P. Experimental evidence for 60 Hz magnetic fields operating through the signal transduction cascade: effects on calcium influx and c- MYC mRNA induction., FEBS. Lett 334: 301-08, 1993.

[8] Piatti E, Albertini MC, Baffone W, Fraternale D, Citterio B, Piacentini MP, *et al*. Antibacterial effect of a magnetic field on *Serratia marcescens* and related virulence to *Hordeum vulgare* and *Rubs fruticosus* callus cells, Comparative Biochemistry and Physiology, B, Biochemistry and oleculerBiology132(2), 359–65, 2002.

[9] Novak J, Strasak L, Fojt L, Slaninova I, Vetterl V. Effects of low-frequency magnetic fields on the viability of yeast *Saccharomyces cerevisiae*. Bioelectrochemistry 70(1): 115-21, 2007.

[10] EL-Sayed A G, Magda S. H, Eman YT, Mona HI. The effect of electromagnetic field on protein molecular structure of *E. coli* and its pathogenesis. Romanian J Biophys. 18 (2): 145–69, 2008.

[11] Nasher SH and Hussein AA. Effect of Magnetic field on the growth of *Escherichia coli* and *Staphylococcus aureus*. Eng. & Tech; 26(4), 2008.

[12] Mohamed AA, Ali FM, Gaafar EA, Magda HR. Effects of magnetic field on the biophysical, biochemical properties and biological activity of *Salmonella typhi*, Master thesis submitted for Biophysics department, Faculty of science, Cairo University, Egypt, 1997.

[13] Fojt L., Strav L., Vetterl V. Comparison of the low-frequency magnetic field effects on bacteria *Escherichia coli*, *Felecia adecarboxylata* and *Staphylococcus aureus*, Bioelectrochemistry; 63: 337–39, 2004.

[14] BabushkinaV., Borodulin. B., Shmetkova V.A., *et al.* The influence of alternating magnetic field on *Escherichia coli* bacterial cells, Pharmaceutical Chemistry Journal, ٣٩(8):398-400, 2005.

[15] Justo O. R., Perez V. H., Alvarez D. C., Alegre R. M. Growth of *Escherichia coli* under extremely low-frequency electromagnetic fields, Applied Biochemistry and Biotechnology, 134(2): 155–163, 2006.

[16] Cellini L., Grande R., Di Campli E. *et al.* Bacterial response to the exposure of 50Hz *electromagneticfields, Bioelectromagnetics*, *29(*4): 302–311. 2011.

[17] Belyaev. Toxicity and SOS-response to ELF magnetic fields and nalidixic acid in *E. coli* cells, Mutation Research 722 (1): 56–61, 2008.

[18] Stansell M.J., Winters WD., Doe RH., Dart BK. Increased antibiotic resistance of *E. coil* exposed to static magnetic field, and Bioelectromagnetics, 22(2), 129–37, 2001.

[19] Kohno MF, Yama ZA, Kimura I, Wafa MC. Effect of static magnetic field on bacteria *Streptococcus mutans* and *Escheriachia coli*. J Am Pathophysiology; l7 (2): 143- 148, 2000.

[20] Ceon R and Martin JT. Low- level, magnetic field induced growth modification of *Bacillus subtilis*. J. electromagnetic; 8 (3):275-82, 2005.

الخلاصة

هدفت الدراسة الحالية في تقصي تأثير الحقل المغناطيسي بقوى مختلفة (١٢٠٠،٨٠٠،٤٠٠،١٦٠٠ كاوس وخلال ٢-٢٤ ساعة) على معدل النمو والحساسية للمضادات الحيوية إضافة إلى الخواص الكيموحيوية لبكتريا المكورات العنقودية الذهبية (*Staphylococcus aureus*) اجريت الدراسة في مركز هولير للبحوث الطبية على عزلات بكتيريا تم عزلها من إصابات مرضية في مستشفى رزكاري باستعمال عدة API Staph system وطريقة Kirby-Bauer لفحص حساسيتها للمضادات الحيوية.

أظهرت النتائج انخفاضا كبيرا في معدل نمو البكتريا العنقودية في الطور اللوغارثمي نتيجة التعرض للحقل المغناطيسي عالي التردد مقارنة بنموذج السيطرة السالبة غير المعرضة للحقل المغناطيسي، كما لوحظ زيادة في حساسية البكتريا للمضادات الحيوية خلال فترة قصيرة (٤-٦ ساعة) من التعرض للمجال المغناطيسي وزيادة مقاومتها للمضادات الحيوية في نفس الظروف عند التعرض لفترة زمنية طويلة (١٨-٢٣ ساعة). أوضحت نتائج التحليل الكيموحيوي باستخدام API وجود تأثير ايجابي للمغناطيسية على البكتريا، حيث تأثرت فعالية بعض الإنزيمات بذلك مثل MAL (Maltose), LAC (Lactose), TRE (D-Trehalose), MAN (D-Mannitol), SAC (Sucrose) and NAG (N-acetyl-glucosamine) بعد التحضين لفترة ٢٤ ساعة على 37 درجة مئوية. يستخلص من النتائج تأثر البكتريا بالحقل المغناطيسي وزيادته مع زيادة قوة الحقل المغناطيسي. كما ويستدل من التغييرات الحاصلة في معدل النمو الخلايا، حساسيتها للمضادات الحيوية وفي فعالية الإنزيمات البكتريا حدوث التغيير في البنية الداخلية لخلية البكتريا.