Magnetic Field Effect on Growth and Antibiotic Susceptibility of Staphylococcus aureus

Fouad Houssein Kamel¹, Chimam Hameed Saeed² and Saleem Saad Qader²
¹Erbil Medical Technical Institute, Hawler Polytechnic University, Erbil-Iraq.
²Medical Research Centre, Hawler Medical University, Erbil-Iraq.
¹E-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 10⁶) 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 ±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 °C 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 °C 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 instructions. 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.

<table>
<thead>
<tr>
<th>Time of exposure to magnetic field in hour</th>
<th>Optical Density (O.D.) at 600 nm</th>
<th>Bacterial cells count(McFarland)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>400G</td>
</tr>
<tr>
<td></td>
<td>O.D. x 10^6</td>
<td>O.D. x 10^6</td>
</tr>
<tr>
<td>0</td>
<td>0.034</td>
<td>10.2</td>
</tr>
<tr>
<td>2</td>
<td>0.139</td>
<td>41.7</td>
</tr>
<tr>
<td>4</td>
<td>0.138</td>
<td>41.4</td>
</tr>
<tr>
<td>6</td>
<td>0.110</td>
<td>33.0</td>
</tr>
<tr>
<td>8</td>
<td>0.098</td>
<td>29.4</td>
</tr>
<tr>
<td>20</td>
<td>0.055</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Fig. (1) Absorbance at 600 nm of Staph.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.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Mode of action</th>
<th>Inhibition antibiotics zone diameter in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Un exposed to M.F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>Inhibition of protein synthesis (30 S-R)</td>
<td>25</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Inhibition of protein synthesis (30 S-R)</td>
<td>25</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Inhibition of protein synthesis (50S-R)</td>
<td>18</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>Inhibition of cell wall</td>
<td>25</td>
</tr>
<tr>
<td>Ceftazidium</td>
<td>Inhibition of cell wall</td>
<td>17</td>
</tr>
<tr>
<td>Rifampin</td>
<td>Inhibition of nucleic acid</td>
<td>32</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>Inhibition of nucleic acid</td>
<td>R</td>
</tr>
</tbody>
</table>


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.
Conclusions

It is concluded that the growth rate of *Staphy. 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


الخلاصة

هدف الدراسة الحالية في تقصي تأثير الحقل المغناطيسي بقوى مختلفة (0011، 011، 11، 0011) على معدل النمو والحساسية للمضادات الحيوية إضافة إلى الخواص الكيميائية لبكتريا Escherichia coli. اجريت الدراسة في مركز هولير للبحوث الطبية على عزلات بكتيريا تم عزلها من إصابات مرضية في مستشفى رزكاري. لفحص حساسيتها للمضادات الحيوية، أظهرت النتائج انخفاضاً كبيراً في معدل نمو البكتريا العنقودية في الطور اللوغارثمي نتيجة التعرض للحقل المغناطيسي عالي التردد مقارنة بنموذج السيطرة السالب للمعرضة للحقل المغناطيسي، كما لوحظ زيادة في حساسية البكتريا للمضادات الحيوية خلال فترة قصيرة (0-2 ساعة) من التعرض للمجال المغناطيسي وزيادة مقاومتها للمضادات الحيوية في نفس الظروف عند التعرض لفترة زمنية طويلة (18-32 ساعة)، أوضح نتائج التحليل الكيميائي باستخدام API وجود تأثير إيجابي 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).

كما وعملت من التغييرات الحاصلة في معدل النمو الخلايا، حساسيتها للمضادات الحيوية وفي فعالية الإنزيمات البكتريا حدوث التغير في البنية الداخلية لخلايا البكتريا.