

Magnetic Field Exposure to Clinical Isolates of *Acinetobacter baumannii*

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This paper presents the isolation of *Acinetobacter baumannii* from clinical sources such as wounds, burns and urinary tract infection. A total of 15 isolates of the studied bacteria were collected and identified by using macroscopic features, biochemical tests and the Vitek2 technique. The diagnosis was then confirmed at the species level. Antibiotics susceptibility test was performed following Kirby-Bauer procedure using 12 antibiotics before and after exposing the bacteria to a static magnetic field, to notice changes related to resistance or sensitivity of the antibiotic, in addition to experimenting with the bacterial viable count before and after exposure to the field as well. The results showed that the isolates had a high resistance to antibiotics, so that all the isolates were 100% resistant to both the third generation cephalosporin and ampicillin, while the most effective antibiotic against the isolates was Imipenem giving only 50% susceptibility. When the isolates were exposed to a magnetic field of 0.3 Tesla and for a period of 24 hours incubation at a temperature of 37 ° C, it was observed a decrease in the number of colony forming unit. Concerning with antibiotic testing after exposure, results indicated that Doxycycline was the most variable in the inhibition zone readings, as it increased significantly. We conclude from our study that the magnetic field can change the vital activity of bacteria by reducing its resistance to antibiotics, which is considered a health problem for the life of humans and their animals.

Keywords: *Acinetobacter baumannii*; Antibiotic Susceptibility; Static Magnetic field.

Acinetobacter baumannii is one of the most widely distributed bacterium in nature, and there are at least 25 different species of it. It covers both Iraq and Afghanistan, which is why it is called the “Bacteria of Iraq”¹. This is due to its high ability to acquire resistance against all currently used antibiotics, as well as its ability to stay for a long time in the hospital environment, which

led to its rapid spread and outbreak of infection². *Acinetobacter baumannii* is also responsible for many hospital-acquired infections, including: Pneumonia, Meningitis, Bacteremia, Soft tissue infection, Peritonitis, Endocarditis, and Urinary tract infection³. These bacteria possess many virulence factors that contribute to their pathogenicity. The increasing incidence of antibiotics resistance had

prompted intensification of research efforts to find new strategies that enhance the role of other antibiotics in therapeutic applications and less likely to develop resistance unlike traditional antibiotics⁴. Magnetism sciences have developed and become more complex since the discovery that their properties are related to all physical status: *solid*, *liquid*, and even *gaseous* as well as living things. It deals with transformations, chemical reactions and transport processes between gaseous, liquid, and solid matter. The metabolism of most living organisms can be regarded as multiphase processes involving gases like oxygen and carbon dioxide; liquids like water, blood, lymph, and plant sap; and solid or semisolid substances like bone, tissue, skin, and cellular membranes. Even primitive forms of life and metabolic activity under anaerobic conditions generally involve multiple liquid and solid or semisolid phases structured by cells and membranes. The current diversity of magnetic fields that are generated and used as applications and electronic devices cannot be completely avoided in contrast to the Earth's magnetic field which is weak, almost uniform, and can be ignored. Therefore, the effect of Magnetic field (MF) exposure depending on field type and strength has become a relevant topic of academic research and appropriate choice of inhibiting or encouraging the growth and reproduction of living cells⁵.

Depending on type and intensity of the magnetic field, and the living organism and its nucleic material, the biological effects of electromagnetic fields are explained by two main manners: The first is based on the possible effects on the permeability of ion channels in the membrane. This can affect the transport of ions into cells and can lead to biological changes in organisms due to existence of moving ions and charged groups within the living cells. The second manner is the formation of free radicals due to exposure to the magnetic field. The growth rate depends on the strength of the field and the nature of the magnetic field⁶. This work aimed to isolate and identify *Acinitobacter baumani* bacteria from different clinical sources and monitoring phenotypic differences before and after exposure to a static magnetic field related to total colony counts and susceptibility to the antibiotic.

MATERIALS AND METHOD

Sample Collection and Isolation

This bacterium were recovered from different sporadic clinical sources in a surveillance, however it was obtained from infected wounds, burns and urine cultures. Samples of wounds and burns injuries were collected by a sterile cotton carrier after sterilization and lifting the bandage under the supervision of the physician. As for urine samples, specimens were taken from patients with urinary tract infection, using sterilized containers designated for this purpose, after directing the patient to sterilize the area and hands with soap and water. Urine was taken from the middle of the stream and neglected the first drops to prevent contamination with the flora present on the skin. Samples were streaked on Maconkey and blood agar at a rate of two replicates per sample and incubated at a temperature of 37°C for 24 hours to obtain single colonies⁷.

Identification

Bacterial isolates were diagnosed based on the cultural characteristics of the colonies in terms of shape, size, color and type of growth in liquid media at 37°C, in addition to microscopic examination and biochemical tests. Whereas, it appeared light pink color on the Maconkey agar while on the blood agar in a light gray color and is not hemolytic, due to its inability to produce hemolysin, knowing that all isolates of *A. baumannii* bacteria have the ability to grow at a temperature of 44 °C which is a physiological characteristic that distinguishes *A.baumannii* from other species of the genus *Acinetobacter*⁸. Diagnosis was verified using automated Vitek2 technique (Biomeriucs, Marcyl Ettoile, France).

Antibiotics susceptibility testing

The sensitivity of the isolates to the antibiotics used in the study was carried out using the Kirby-Bauer disc diffusion method. Muller Hinton agar medium was inoculated with 0.1 ml of bacterial suspension, antibiotic discs were placed on the surface of the medium, spaced apart and incubated for 18-24 hours at 37°C. The areas of inhibition around each disc were read in mm and the results recorded⁹.

Viable count

Total counting of bacterial colonies was performed using Colony Meter by applying the formula:

$$\text{Total number of bacteria (cfu/ ml)} = \text{number of colonies} \times \text{inverted dilution factor.}$$

Decimal serial dilution was followed and growing colonies on the plates were visually counted. The viable colony counting was conducted pre and post exposure to the magnetic field. Knowing that dilution 10^{-6} was used for pre and post colony number measurement. The bacterial suspension prepared by comparing turbidity to McFarland tube that represents 1.5×10^8 cells / ml measured at 600nm spectrophotometry with optical density $OD = 0.5^{10}$.

Magnetic Field Exposure testing

A permanent magnet measuring 0.3tesla (Tesla unit) power was provided after measuring the field strength using a Teslometer, where the tubes of bacterial broth were transported were connected between the poles of the magnet and incubated at 37° for 24 hours after which 100µl of bacterial suspension were transferred and cultured on Muller-Hinton medium to test their antibiotics susceptibility as mentioned previously. Considering the results of the sensitivity test for the same isolates before the field exposure as control for the purpose of comparison¹¹.

Statistical Analysis

The data obtained from susceptibility testing were analyzed using one-way ANOVA test and average means were compared depending on Duncan multiple range with a level of confidence at 0.95 (SPSS 19.0 software program.)¹².

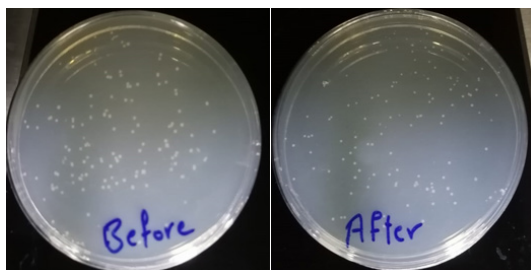


Fig. 1. Viable Count of Colony Isolates Before and After Magnetic Field Exposure

RESULTS AND DISCUSSION

Isolates were collected from patients' wounds, burns and urinary tract infection, fifteen *Acinetobacter baumannii* were identified out of 100 isolates. Regardless of the total number, the species isolates were obtained more from wounds, followed by urinary tract infection and then burns. The isolates were diagnosed based on routine examinations including microscopic examination, cultural characteristics and biochemical tests, where the genus was diagnosed initially, and then the species was confirmed using Vietek2 technology.

This study determined the differences in antibiotic resistance. The results indicated high resistance to the used antibiotics at different rates, as they totally (100%) resisted the antibiotics Ampicilin and Cefotaxime, followed by Amikacin and Ciprofloxacin by 83%, then the Gentamicin antibody by 58%, and finally the Imipenem by 50%. These results are in agreement with what was stated by Sohail¹⁴, where their study showed that the isolates of this species gave the least resistance to Imipenem by 5-19%.

Reasons behind the resistance of this bacterium to penicillins may be due to the widespread and indiscriminate use of these antibiotics, as well as the transmission of the resistance through plasmids or transposons. *Acinetobacter baumannii* strains have the potential to rapidly acquire antimicrobial resistance as newer antimicrobial resistance has been reported worldwide. In general, the resistance to the group of

Table 1. Results of Biochemical Tests

Test	Result
Catalase test	Positive
Oxidase test	Negative
Indole production	Negative
Methyl red	Negative
Vogus proskauer	Negative
Citrate utilization	Positive
Sugars utilization in TSI Agar	K/K
H ₂ S gas production	Negative
Motility test	Negative (non- flagellated)
Urease test	Negative
Nitrate reduction	Negative

cephalosporins is due to bacterium's possession of multiple resistance mechanisms and its production of β -lactamase enzymes and that it has the ability to change outer membrane proteins¹⁵. Bacteria resistance to third-generation cephalosporins (Cefotaxime, Cefixime, Ceftriaxone) is related to their indiscriminate use, which leads to the development of their resistance. Another cause is their production of broad-spectrum beta-lactamase enzymes as well as production of Ampc-type beta-lactam enzymes that inhibit the action of third and fourth generation cephalosporins. Resistance toward aminoglycosides came up from possession of plasmids that encode of aminoglycosides modifying enzymes represented by the small ribosomal unit 30s, thus preventing them from binding to ribosomes. The antibiotic imipenem was the optimal and effective treatment for this bacterium. It inhibits the activity of the enzyme transpeptidase and stimulates cell disruption. During the past few years from 2010-2018 the resistance of the bacteria under study to this antibiotic has increased, and the reason for this is due to its possession of several mechanisms of resistance, perhaps the most common are Carbapenem-hydrolyzing class D beta lactamase (CHDLs) enzymes that break down carbapenems also break down a protein in the outer membrane known as the core that is related to the resistance against these antibiotics¹⁶.

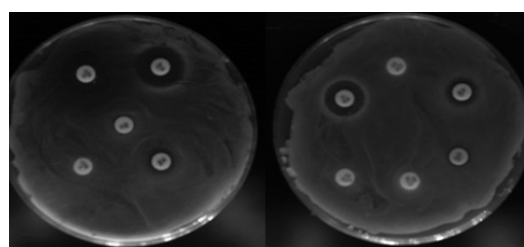
Magnetic Field Exposure

The growth rate for a culture subjected to static magnetic fields was significantly reduced in comparison with that of the control cultures fig.1 reveals the difference. In an average, number of colonies before exposure was approximately 128 colony forming units while it was 110 after being exposed to same strength of static magnetic field at same incubation's circumstances. This result was consistent in all fifteen bacterial isolates. This result is in line with Li *et al.*,¹⁷ who stated that the drop number of viable colonies is due to 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 cytoplasmic contents, retraction of the cytoplasmic membrane. This result is also consistent with Wenjin¹⁸ who had investigated the morphology of the bacterial strain using scanning and transmission electron microscope and found that the cell surface was damaged. Overall, magnetic fields inhibit the growth of this species.

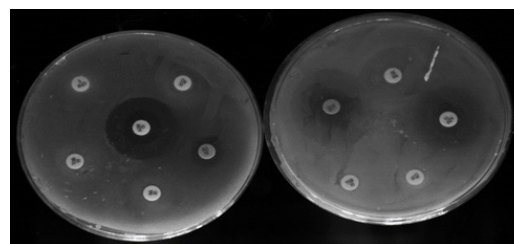
There are controversial explanations for the growth of microbes. For example, results from experiments with other bacterial genera suggest that the effects of magnetic fields may be on

Table 2. Antibiotic Susceptibility Before and After Exposure to Magnetic Field

Antibiotic	Susceptibility percentage %	
	Pre exposure	Post exposure
Ampicilin	0%	33.30%
Cefotaxime	6%	11%
Cefixime	0%	20%
Cefradine	0%	0%
Azithromycine	25%	50%
Ceftriaxone	8%	11%
Amikacin	18%	55.50%
Nalidixic acid	23.70%	33.30%
Ciprofloxacin	25%	55.50%
Gentamicin	40%	45%
Doxycycline	33%	88.80%
Imipenem	41.60%	52%



A) Before Exposure



B) After exposure

Fig. 2. Antibiotics Susceptibility Results Before and After Exposure to Static Magnetic Field

Table 3. Statistical Comparison of Microbial Susceptibility Before and After Exposure to the Magnetic Field

Isolates no. Antibiotics	1	2	3	4	5	6	7	8	9	10	11	12	
AZM	8Ca 0Db	13Bb 0Ea	27Aa 0Ea	16Aa 0Ba	15Da 0Ea	11Bb 0Da	10Ba 0Ca	18Aa 0Da	20Da 0Fa	16Ab 0Ca	26Ba 0Ea	17Aa 0Ca	0Ca 0Da
CTR	0Db 13Ca	0Ea 0Ea	0Ba 0Ba	0Ea 0Ea	0Ba 0Ba	0Da 0Da	0Ca 0Ca	0Da 0Da	0Fa 0Fa	0Ca 0Ca	0Ea 0Ea	0Ca 0Ca	0Da 0Da
CH	0Da 0Da	0Ea 0Ea	0Ba 0Ba	0Ea 0Ea	0Ba 0Ba	0Da 0Da	0Ca 0Ca	0Da 0Da	0Fa 0Fa	0Ca 0Ca	0Ea 0Ea	0Ca 0Ca	0Da 0Da
AM	0Da 0Da	10Cb 0Cb	22Ba 0Ba	0Ba 0Ba	15Ba 0Ba	0Da 0Da	0Cb 0Cb	20Da 0Cb	20Da 0Cb	0Cb 0Cb	26Ba 0Ca	0Ca 0Ca	0Da 0Da
Ipm	8cb 27Ba	5Cb 20Ba	16Aa 0Ba	0Eb 0Eb	16Ab 0Ba	12Ca 0Cb	11Ba 0Cb	13Ba 0Cb	0Fb 0Fb	9Ba 0Cb	0Eb 0Cb	15Aa 0Cb	19Ab 0Cb
CN	0Db 14Ca	0Ea 0Ea	0Ba 0Ba	0Ea 0Ea	0Ba 0Ba	0Da 0Da	0Ca 0Ca	0Da 0Da	0Fa 0Fa	0Cb 0Cb	10Da 0Cb	0Ca 0Ca	0Da 0Da
CIP	0Da 0Da	0Eb 16Ca	0Ba 0Ba	0Ea 0Ea	0Ba 0Ba	0Db 0Db	20Ba 0Ca	16Ab 0Cb	27Ca 15Ab	0Cb 0Cb	36Aa 9Ba	10Ba 0Cb	14Ca 0Cb
DO	23Ab 33Aa	0Ea 0Ea	17Ab 0Ba	25Ba 0Ba	15Ab 0Ba	22Ab 0Ba	25Aa 0Ca	10Cb 35Aa	27Ab 35Aa	7Bb 0Cb	35Aa 9Bb	12Ba 0Cb	22Ba 0Cb
NA	0Da 0Da	0Ea 0Ea	0Ba 0Ba	0Ea 0Ea	0Ba 0Ba	15Bb 24Aa	0Ca 0Ca	30Ba 0Ca	30Ba 0Ca	0Ca 0Ca	0Ea 0Ea	0Ca 0Ca	0Da 0Da
CFM	0Da 0Da	0Ea 0Ea	0Ba 0Ba	0Ea 0Ea	0Ba 0Ba	0Da 0Da	0Ca 0Ca	15Ea 0Ca	15Ea 0Ca	0Ca 0Ca	0Ea 0Ea	0Ca 0Ca	0Da 0Da
CTX	0Da 0Da	0Ea 0Ea	0Ba 0Ba	0Ea 0Ea	0Ba 0Ba	0Da 0Da	0Cb 0Cb	13Da 0Cb	0Fa 0Cb	0Cb 0Cb	12Da 0Cb	0Ca 0Ca	0Da 0Da
AK	15Ba 0Db	17Ab 20Ba	0Ba 0Ba	0Ea 0Ea	0Bb 10Ca	10Cb 22ABa	10Cb 10Ba	27Ba 0Cb	25Ca 0Cb	15Ca 0Cb	15Ca 0Cb	0Ca 0Ca	0Da 0Da

*: Before exposure

**: After exposure

quorum sensing mediated by the quorum sensing molecule AI-2. The presence of a strong magnetic field in the bacterial medium affects the life of bacteria, as this magnetic field works to create a large physical electrical voltage in the environment of bacteria, so that this effort will overwhelm the natural efforts that exist mainly in the middle of these small cells, which affects the process of ion flow through the cell wall and its loss of control¹⁹.

The results of our current study showed clear differences in the sensitivity of isolates to antibiotics compared to the results before exposure to the magnetic field, knowing that the isolates that were originally sensitive to most antibiotics were not counted when treated with the magnetic field, so the total isolates exposed to the field became 12 isolates. Table 2 shows the difference in percentages for each antibiotics before and after exposure to the field. It was found that the inhibition reading of Doxycycline was most variable giving 50 percent susceptibility, unlike the antibiotic Imipenem, whose readings differed only by a small difference.

The disc diffusion reading of susceptibility in figure2 shows the phenotypic differences in the diameter of the antibiotic inhibition before and after their exposure to the magnetic field, Table No.3 shows the details of these readings. The result of our research agrees with what was presented by Alkhazan and Saddiq⁵, who showed that when *Escherichia coli* bacteria are exposed to a magnetic field at a frequency of 50Hz and electromagnetic waves 2iT for different periods of time, it caused a significant inhibition in the growth of bacteria after 6 hours and became more sensitive toward antibiotics, with implications for the phenotypic characteristics of reduced bacterial cell length. The magnetic field significantly affects the bacteria wall through the separation of ions and changing the permeability of the cytoplasmic membrane containing porins or protein holes responsible for transporting ions and some other molecules, and one these ions is the proton H+. The flow of this proton through the cell wall is what maintains the pH balance function inside Cell solution. Also, the inhibitory effect may be due to a decrease in the activity of the bacterial cell and its metabolism and a decrease in the rate of replication of its genetic material²⁰. Li et al.,²¹ mentioned that static magnetic field had altered bacterial metabolism

by decreasing in glycolate oxidase activity and accumulating of long-chain fatty acids (LCFA, with more than 18 carbons) suggesting that free radicals generated by LCFA degradation are the primary target of static magnetic field action, which triggers the bacterial oxidative stress response and ultimately leads to growth inhibition.

From the point of view of statistics using ANOVA test, results depicted as lower-case letters assessing the difference before and after exposure to magnetic field in terms of number isolates (similar lowercase letters horizontally mean that there are no significant differences between them). Capital letters differences refer to antibiotics involved before and after exposure (similar capital letters vertically mean that there are no significant differences between them). It is clear that isolates number 2,4,7 and 9 had showed significant differences to Azithromycin (AZM) at $p < 0.05$ where: $a > b$, while the rest of isolates showed no differences.

Ceftriaxone (CTR) showed less differences except for isolate number 1. Same results with Cefradine (CH) since they belong to same group of cephalosporins, approximate interpretation to Woroszy³o²² despite their use of a rotating field test against a specifically different bacterial genus.

Imipenem (IPM) showed differences in isolates number 1,2,4,5,6,8 and 10. Amikacin (AK) had statistical differences of the isolates before and after exposure to the magnetic field, as the readings changed in the isolates 1,2,5,6,7,8,9 and 12. Anyway Doxycycline (DO) had the largest share in the statistical difference since all isolates gave dramatic changes after exposure according to the alphabetical differences in an ascending order. Differences in inhibition zones diameter around antibiotic discs pre and post exposure illustrated in table3. The current results are compatible with Kamel *et al.*,²³ who posted that moderate intensity static fields induced dramatic changes in the susceptibility to antibiotics and referred to the possibility that magnetic field could interferes with the surface charges of the bacterial membrane or the distribution of the charged antibiotic molecule eventually modifying the rate of antibiotic penetration.

CONCLUSION

In conclusion the results of this study shed light on the efficiency of the magnetic field in inhibiting the growth of *Acinitobacter baumannii* and increasing their sensitivity to antibiotics. It can change the life activity of bacteria and may open the way for scientific applications. We propose to monitor the bacterial growth and physiological characteristics of bacteria after exposure to different types of magnetic fields over longer periods of time by measuring optical density or bacterial counting, in addition to highlighting the gene expression of these species.

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