Original Article

Bacteriological and molecular study of the effect of magnetic field on Methicillin Resistance Staphylococcus aureus (MRSA)

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Abstract

A group of staphylococcal bacteria strains were exposed to the magnetic field and the characteristics of these bacteria were studied and diagnosed through phenotypic and biochemical characteristics. In addition to studying the sensitivity of these isolates to antibiotics, which included (AK 30, Lev 5, Tm 5, VA 5, Met 10, CEC 5, SAM 20, GM 10, OX 5, NIT 100, CIP 5) and noted the differences between these antibiotics in terms of its effect on the bacterial isolates under study, where the isolates resistant to methicillin (Methicillin Resistance Staphylococcus aureus) were identified (3 thee isolates B1,B2,B3) and then exposed to the magnetic field with one strength and one dimension, but at different times (30 sec, 45 sec, 60 sec), where it was observed that the number of bacterial cells decreased in lower concentrations, but by the influence of the magnetic field. Molecular markers: Most of treatments were distinguished with unique bands and absent bands the all mutant bands (28). unique bands (21) and absent bands (7), As for the B3 obtained mutant bands which reached (11) bands of which (8) unique bands and (3) Absent bands. As for the B2 obtained which reached (7) bands of which (6) unique bands and (1) Absent band. As for the B1 obtained which reached (6) bands of which (4) unique bands and (2) Absent bands. The molecular differences between the strains were determined from the molecular side at the stage before and after exposure to the magnetic field through random primers used in the polymerase chain reaction technique. There is a difference in the results between the three bacterial because of the difference in the genome of them due to different sources. The magnetic field creates mutations in the genome of bacterial . and the mutation increases with the period of exposure of the plant to the magnetic field. The efficiency of the RAPD markers was high in detecting the producing mutation in the genome of bacterial.

Keywords: Staphylococcus aureus, magnetic field, antibiotic, polymerase chain reaction.

INTRODUCTION

Staphylococcus aureus, a member of the family Micrococcaceae, is a Gram –positive coccus whose cells tends to occur either singly or if dividing cells do not separate, form pairs, tetrads and distinctive irregular (grape like) structures. Humans are commonly colonised by S.aureus on external skin and upper respiratory tract, particularly the nasal passages .Healthy individuals are usually unaware of staphylococcal carriage but they may suffer from minor skin infections such as boils and abscesses (1).

However, S. aureus is an opportunist pathogen; and given the right circumstances can cause more serious infection. Burns and surgical wound infections are commonly invaded by S. aureus, where the production of toxins by S. aureus can e.g. give rise to toxic shock syndrome leading to fever, sickness and in some cases death. Infections caused by S. aureus include, pneumonia, (inflammation of lungs), mastitis (infection of the mammary glands), infections of skin (impetigo, cellulites and staphylococcal scalded skin syndrome) .osteomyelitis (infection of bone), endocarditis (infection of the endothelial lining of the heart and valves) and bacteremia (bacteria present in blood). S. aureus can also cause food poisoning, the result of enterotoxin

production (2).

Treatment of S. aureus infections before the 1950 involved the administration of benzyl penicillin (penicillin G), a beta- lactam antibiotic, but by the late 1950 S. aureus strain resistance to benzyl penicillin were causing increasing concern (3). Resistant strains typically produced an enzyme, called a beta- lactamases, which inactivated the beta-lactam. Efforts were made to synthesise penicillin derivatives that were resistant to beta-lactamases hydrolysis. This was achieved in 1959 with the synthesis of methicillin, which had the phenol groups of benzyl penicillin disubstituted with methoxy groups .The methoxy groups produced steric hindrance around the amide bond reducing its affinity for staphylococcal beta- lactamases. Unfortunately, as

soon as methicillin was used clinically, methicillin- resistant S. aureus (MRSA) strains were isolated (4).

Resistance was not due to beta- lactamases production but due to the expression of additional penicillin- binding protin (PBP2a), acquired from another species, which was resistance to the action of the antibiotic .

The use of different types of antibiotics over the years has led to the emergence of multiresistant MRSA strains, the result of mutations in genes coding for target proteins and through the acquisition and accumulation of antibiotics resistance- conferring genes (5)

The evolution of the molecular markers are distinguished by the speed, accuracy and shorten time and effort to select the best of genetic performance (6). The discovery of PCR- technique in the last more than two decades has helped many researchers to develop various markers to study the genetic diversity, fingerprinting and gene detection which are responsible for many important qualities in plant breeding, among these markers is RAPD (7).

Material and methods

Bacterial isolates:

Fifteen of clinical bacterial isolates from wound were used in this study, all these isolates were submitted to identification tests, which in clude: Gram stain, Catalase, Citrate, Coagulase, IMVC, Urease, hemolysis. In addition to detection the ability of fermentation sugars, which are lactose, glucose and Mannitol using different bacterial media these, Mannitol salt agar, Nutrient agar and Kligler Iron agar (8).

Antibiotic Suscebtibility(disc diffusion test):

This test was performed according to (9).

1-3 to 5 of bacterial colonies were transfer to a tube of saline.

2-The turbidity of tube was compared to 0.5 McFarland turbidity standard using salin.

3-Mueller- Hinton plate agar was inoculated by sterile swab into the inoculums.

4- Streaking the plates by the swab over the surface of the medium many times, allowed to dry,

then fixed antibiotics using sterile forceps at 4 discs per dish.

5-All Petri dishes were incubated at 370C for 24 hours.

6-The inhibition zones measured using ruler, then recorded.

Exposure method:

This test was performed according to (10).

1- 5 ml of bacteria under study were cultured in Brain Heart Infusion Broth (BHIB) medium, and incubated at 37oc for 24 hours.

2- Samples are placed in test tube and centrifuged for 10 minute at a speed 6000 cycle/ min.

3- Removal the suspension and keep the deposit down the tube.

4- Add 5 ml of saline solution and wash (by centrifuge) twice and incubated the tube (sample wash) next day at 370c.

5- Take 5 tubes and put in each tube 9 ml saline solution.

6- Add (1) ml of the original sample to one tube for dilution (0.1), then add 1ml to the second

tube (0.01) , thus all tubes to get dilutions (0.001-0.0001).

7- Taken from each dilution (0.1) ml and placed in sterile Petri dish , then added culture media ,

that to calculate the number of bacteria before exposure to the magnetic field .

8- taken (0.1) ml from each Epinedroff tube in Petri dish and pour selected culture media , then count bacteria after exposure to the magnetic field.

9- If bacterial growth is obtained after exposure to the magnetic field , examined antibiotic sensitive test again to observe the difference.

2- Sample collection:

The three bacterial species were cultured on broth medium with four groups for each bacteria, three treatments were exposed

to the influence of the static magnetic field with the same intensity and at different times (30, 45, 60) seconds and one was designated as control, and after the treatment all samples were incubated in the incubator at 37°C for 24 hours. to transfer to the laboratory to isolate DNA from them.

DNA Extraction Analysis

Genomic DNA was extracted for The three bacterial species under study before and after exposure to the static magnetic field as reported in Method (11,12). After modulation, DNA was confirmed by deportation on the Agarose Gel electrophoresis device(13,14).

RAPD-PCR reactions

RAPD-PCR reactions were performed based on (15) on the control sample and the three coefficients for The three bacterial species using (5) primers prepared by Operon Technologies, U.S.A. As described in Table (1), Mixed solution (Master Reaction) was prepared by mixing the reaction components into sterile Eppendorf tube (2ml), then centrifuged in Microfuge for 3-5 sec. This work was done inside a sterilized hood. The solutions and materials used are shown in table (2).

Table (1) primers used in the RAPD-PCR study.

Primer name	Primer Relay -	Primer name	Primer Relay -
P-1	GTGTGCCCCA	P-3	GTTGCGATCC
P-2	GTCGCCGTCA	P-4	AACGGTGACC
P5	GATGACCGCC		

Table (2) solutions used in the RAPD markers

C	Components	Volume
1	Green Master mix	12.5 µ l
2	Primer	2 µ l
3	Nuclease free water	8.5µ l
4	DNA template	2µ l
5	Total Volume	25µ l

RAPD-PCR program performed by the following: first denaturation 1 cycle at 95 ºC for 5 min, 40 cycle (denaturation 94 ºC for 30 sec; annealing 36 ºC for 40 sec; extension 72 ºC for 1 min) and final extension 1 cycle at 72 ºC for 7 min. After the PCR amplifications program was finished, 4µL of PCR products were separated using gel electrophoresis in a concentration of 1.5% with DNA marker, after migration the gel stained by ethidium bromide for 60 min and visualized under UV- transilluminator.

Diagnosis of mutations:

The variation in genetic material DNA which can obtained from RAPD-PCR markers can be adopted to identify mutations in treatments compared with control, and that is done by converting the bands which appeared in the gel to description table, by putting 1 when there is unique band and 0 at the absence of the band, that is, the band which appears in the treatment and dew varieties.

Results and Discussion

Staphylococcus aurous was diagnosed based on the phenotypic characteristics, as it was found to be Gram-positive cocci arranged in a grape cluster shape. It was also diagnosed by biochemical tests to determine the enzymatic activities of the bacteria. These tests included (Mannitol fermentation, Oxidase, Catalase, Coagulase and coagulability test). H2s production, Hemolysin as in the diagnosis table (3), as well as the diagnosis was made by Vitek 2 compact device agreement with (16)

Table (3) results of biochemical tests for diagnosis

Test	Result
Coagulase	+
Oxidase	-
Catalase	+
Motility	-

Bacitracin		+
Mannitol fermentation		+
H2s production		-
Hemolysin		+
Dnase		+
Proteinase		+
Urease		+
Lipase		+
Lecithinase		+
Novobiocin		+
Sugar	Glucose	+
fermentation	Lactose	+
	Sucrose	+
	sorbitol	+
	Arabinose	-
	xylose	-

As for the results of antibiotics (AK30, Lev 5, Tm 5, VA 5, Met 10, CEC 5, SAM 20, GM 10, OX 5, NIT 100, CIP 5), it was noted that the results varied, and most of these antibiotics were sensitive and some of the isolates I resisted vancomycin and that the reasons for resistance to this antibody may be due to changes in the manufacturing pathway of the cell wall sensitive to vancomycin anti-vancomycin, which leads to a decrease in cross-links or an increase in their thickness or an increase in the ends of D-Ala..D-Ala. and then a change in this antagonist. In general, resistance is achieved, and the effect of the antigen is neutralized by producing enzymes that lead to enzyme failure or a modification of the target position on which the antibody is working, or a change in the permeability of bacteria to prevent cross-over of the antibody, or by the bacteria's possession of the flow systems, Efflux pump systems (17). Resistance to oxacillin occurs as a result of the secretion of broad-spectrum beta-lactamases enzymes, which neutralize the activity of the beta-lactam ring by breaking it, and another group was moderate. The extrusion of the cytoplasmic contents and the shrinkage of the cytoplasmic membrane (18) according to Table (4)

Table (4) Antibiotic Sensitivity Tests

	AK 30	**VA 5**	**TM 5**	**LEV 5**	**MET 10**	**CEC(30l 10)**	**SAM 20**	**GM 10**	**OX 5**	**NIT 100**	**CIP 5**
1	**R**	**S**	**S**	**S**	**---**	**R**	**I**	**S**	**---**	**S**	
2	**S**	**S**	**S**	**S**	**---**	**---**	**I**	**S**	**S**	**S**	**S**
3	**R**	**S**	**I**	**S**	**---**	**R**	**I**	**---**	**---**	**S**	**S**
4	**R**	**S**	**S**	**S**	**---**	**R**	**I**	**S**	**---**	**S**	**S**
5	**I**	**S**	**S**	**S**	**---**	**S**	**R**	**S**	**I**	**S**	**S**
6	**R**	**S**	**S**	**S**	**I**	**R**	**----**	**S**	**---**	**S**	**S**
7	**R**	**S**	**R**	**R**	**S**	**I**	**I**	**S**	**R**	**S**	**S**
8	**R**	**S**	**S**	**S**	**I**	**R**	**I**	**S**	**---**	**S**	**---**
9	**R**	**S**	**S**	**S**	**I**	**R**	**I**	**S**	**---**	**S**	**S**
11	**R**	**S**	**S**	**S**	**I**	**R**	**R**	**S**	**---**	**I**	**S**
11	**I**	**I**	**I**	**R**	**I**	**R**	**I**	**S**	**S**	**I**	**S**
12	**I**	**S**	**S**	**S**	**R**	**I**	**R**	**S**	**R**	**I**	**I**
13	**I**	**S**	**I**	**R**	**S**	**R**	**R**	**I**	**I**	**I**	**S**
14	**I**	**I**	**S**	**S**	**S**	**R**	**I**	**I**	**S**	**R**	**S**

15	**I**	**S**	**S**	**R**	**R**	**I**	**I**	**S**	**---**	**R**	**I**

The resistant isolates, Methicillin Resistance Staph aureus (MRSA) were selected in order to determine them and expose them to the magnetic field and to investigate the ability of the magnetic field to inhibit or reduce growth with variations in time (30 s, 45 s & 60 s) and gradually with the stabilization of the field strength. The bacteria have begun to diminish..... and the reason for this is that the magnetic field creates a large physical electric voltage in the environment of bacteria, causing the growth of bacteria to decline, and that this effort dominates the natural efforts that are mainly found in the middle of these small cells, which affects the process of ion flow through The cell wall and consequently the loss of control over it (19) and this is consistent with (9).

In this study , 5 primers were used as shown in Table (1) of the studied models using RAPD-PCR markers. All the primers found binding sites on the genome and produced different bands detected on the glucose gel in the presence of DNA ladder 100bp DNA Marker. These bands in the production of general bands Main Bands / Monomorphic bands and differentiated Polymorphic band.

Most of the coefficients as shown in Tables (5) marked by the shares in the pictures (1-1) were characterized by distinctive bands (unique band, absent band). Characteristic compared to the control sample, of which (21) unique band and (7) absent band, the primer OPA- 06 produced the highest number of band reached (8) bands while the bands produced the primer OPB- 14 the less number of bands (3) As for the B3 obtained a percentage of the characteristic mutant bands which reached (11) bands of which (8) unique bands and (3) Absent bands. As for the B2 obtained a percentage of the characteristic mutant bands which reached (7) bands of which (6) unique bands and (1) Absent band. As for the B1 obtained a percentage of the characteristic mutant bands which reached (6) bands of which (4) unique bands and (2) Absent bands. These bands are discriminatory and their diagnosis of these treatments indicates that the wavelengths affect the genetic material of DNA because the emergence of different bands between treatments is caused by the different type of isolation and its genetic structure and its ability to repair damage in the genetic material and that the increase of mutant bands is caused by an increase in The effect of the static magnetic field, which may reach the degree of total killing of bacteria and inferred from the occurrence of a mutation in a particular site led to the identification of the initiator and the emergence of the unique band, but for the absent bands as well as a mutation in the site only know the Primers in that treatment, which led to the elimination of this identification and concealed War package that is compatible with many former researchers, the results of (20).

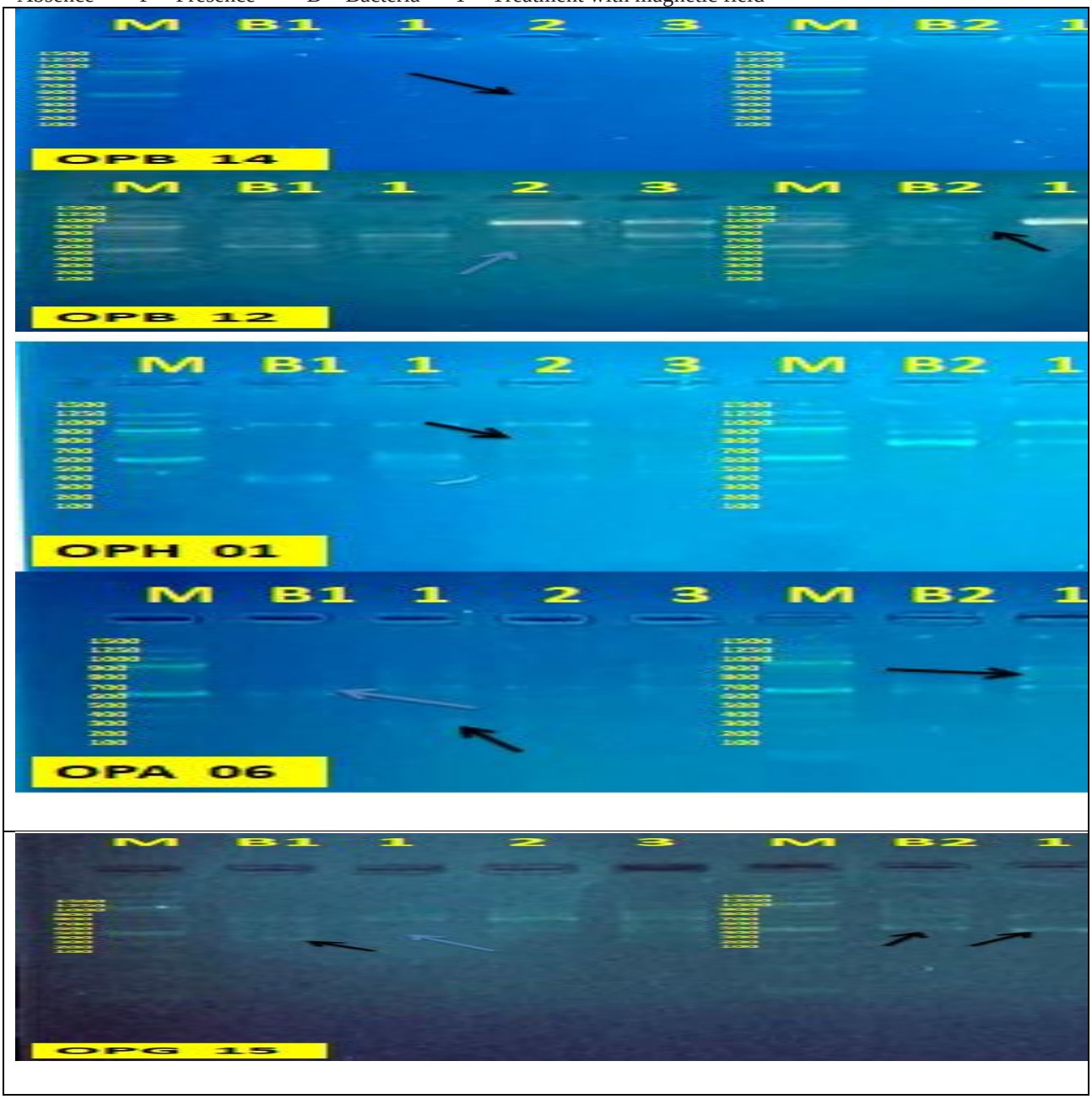
Through the molecular results, the effect of the waves on the bacteral was very high in all treatments. The results of the RAPD-PCR all distances had a significant effect on the genome of bacteria because the average distance of 5 meters had a greater effect and the reason for the incompatibility of sensitivity with molecular that most mutations that occurred in the genome of bacteria in non-coding genes and therefore all these mutations have no effect on the traits The bacterial phenotype of the bacterium does not have a gene expression, and the reason is that the proportion of the non-coding genome of the genes in the bacteria is much higher than the genome encoded and this is consistent with the results of most researchers (21).

Table (5): Shown Transaction- characteristic mutation in S.aureus isolates using 5 random primer

Primer name	**Molecular size Bp**	**Mutation in bacteria 1**								**Mutation in bacteria 2**								**Mutation in bacteria 3**							
		B1		**1 T**		**2 T**		**3T**		**B2**		**1 T**		**2 T**		**3T**		**B3**		**1 T**		**2 T**		**3T**	
		P	**A**	**P**	**A**	**P**	**A**	**P**	**A**	**P**	**A**	**P**	**A**	**P**	**A**	**P**	**A**	**P**	**A**	**P**	**A**	**P**	**A**	**P**	**A**
OPG-15	**450-1500**	**1**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**1**	**-**	**1**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**-**
OPB-14	**450-1500**	**-**	**-**	**-**	**-**	**1**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**
OPB-	**200-1250**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**1**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**1**	**2**		**-**	**-**	**-**

12																								
OPH-01	**350-1250**	**-**	**-**	**-**	**-**	**2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**-**	**-**	**1**	**1**		**-**	**-**	**-**
OPA-06	**300-1150**	**-**	**1**	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**2**	**-**	**2**	**-**	**-**	**-**
المجموع	**28**	**1**	**1**	**1**	**1**	**3**	**1**	**0**	**0**	**2**	**0**	**3**	**0**	**1**	**0**	**3**	**0**	0	**0**	**5**	**3**	**3**	**0**	**0**	**0**

A = Absence P = Presence B = Bacteria T = Treatment with magnetic field



These results agreed with many studies that showed that the magnetic field has an effect on bacterial growth (22) and reduce their numbers (23). Studies have assumed that the Arctic has the ability to disrupt and suppress the sources of ATP energy

production necessary for the growth and reproduction of microbes (24), and the field energy has an effect in causing major changes in the metabolic processes of living organisms. These changes lie in the exchange of ions through the cell membrane and in the transfer of ions and enzymes, including The process of transporting calcium ions in the system through the plasma membrane, where it acts as a physical stimulus to increase calcium ions (25,26), The results of this study also agreed with (27), which confirmed that magnetically treated water affects cells and living organisms, and this effect varies between stimulatory and inhibitory, as the effect depends on the strength of the magnetic field and the type of bacteria as well as on the total number of microbial content, which indicates the great effect of the magnetic field in inhibiting the growth of microbiology.

The magnetic field also has an effect on gene expression and thus different metabolic responses related to carbohydrate metabolism (28). The results differed with the findings of (29) that the magnetic field had no significant effect on S. aurous bacteria and no significant differences were observed in the number of colonies compared with the control treatment. (30) reported in 2016 that gene expression of S. aureus enterotoxins was down-regulated when exposed to magnetic fields of 34 mT. They also shown that the treatment affected the growth rate of the microbe(31,32). Our point of view regarding the variation in gene expression of the genes under study is mainly due to strain variation, as different strains of S. aureus might react differently to the treatments under study and thus led to the variations. On the other hand, the effect of each treatment on the up or down regulation of the target genes is unclear, as no study has explored such type of treatments on those genes. However, those point mutations are the most likely cause of the variance, as different strains produced varied expression of the genes. Experiment technical error and primers' specificity might have contributed to the variations. Mechanical effects of treatments might have affected the gene expression by mechanical disruption of the proteins produced during cell division, as the bacterial population was exposed to the treatments for 24 hrs. Finally, due to the net charge of ions in the cytoplasm, the net charge produced by magnetic field may have an effect on gene expression, influencing the activity of DNA or RNA polymerase, and consequently the transcription/translation process as a whole.

Conclusions:

The effects of high frequency magnetic fields to inhibit or reduce growth with variations in time(30 s, 45 s & 60 s) and gradually with the stabilization of the field strength. The bacteria have begun to diminish and the reason for this is that the magnetic field creates a large physical electric voltage in the environment of bacteria, causing the growth of on selected bacteria were studied to understand the impact of radiation stress in environment on biological systems. Bacteria were selected for the study since they grow quickly and have relatively short generation time when compared to other organisms. There is a difference in the results between the tree bacterial because of the difference in the genome of them due to different sources. The magnetic field creates mutations in the genome of bacterial . and the mutation increases with the period of exposure of the bacteria to the magnetic field. The efficiency of the RAPD markers was high in detecting the producing mutation in the genome of bacterial.

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