Elecromagnetic radiation effect on microbes in urine contaminated soil

By

Ajayi A.O[1*] and Ajayi N.O.[2]

1. Department of Microbiology, Adekunle Ajasin University, P.M.B 01, Akungba-Akoko, Ondo State, Nigeria
2. Department of Physics, Adekunle Ajasin University, P.M.B 01, Akungba-Akoko, Ondo State, Nigeria

*Corresponding author: E-mail:jidet02@yahoo.com,

Abstract

This study shows the effect of low frequency (Lf) magnetic field on microorganisms obtained from urine contaminated soil sources in Akungba-Akoko community, Nigeria. Thirteen (13) microorganisms isolated from these sources include ten (10) bacteria isolates and three (3) fungi isolates. They were identified as Bacillus subtilis, Bacillus cereus, Clostridium tetani, Clostridium sporoene, Enterobacter cloacae, Micrococcus spp., Staphyloccocus aureus, Proteus mirabilis and Pseudomonas aeriginosa while the fungi isolates were Saccharomyces cerevisae and Rhizopus spp. The microorganisms were treated with Lf magnetic field (50mG, 100mG, 250mG, 500mG, and 1000mG) at constant time (30minutes). Increase in the intensity of the field during the study show a decrease in microbial population some isolates determined by their coliform forming unit while there is increase in some instances. This may mean an initiation of growth abilities in some microorganisms and elimination in the pathogenic ones. The antibiotic susceptibility was determined by using the Kirby-bauer disc diffusion technique. The isolates that are resistant to antibiotics are also exposed to electromagnetic field for 45 minutes and were tested again for their susceptibility pattern. They thereafter show tangible susceptibility responses to the antibiotics used. Therefore resistant bacteria could be susceptible to antibiotic by exposure of the patients or source of environmental contaminants to the Lf magnetic field. Similarly, some of the strains killed or attenuated during the process can be used for vaccine formulation.

Keywords: Lf magnetic field, Environment, Microbes, Radiation.

INTRODUCTION

There are many environmental determinants that influence the survival of variety of microbes in the environment. This includes antimicrobial agents and electromagnetic field (EMF). Microbes have been known to cause urinary tract infection (UTI) (also known as acute cystitis or bladder infection) [1]. This infection affects part of the urinary tract. When it affects the lower urinary tract it is known as a simple cystitis (a bladder infection) and when it affects the upper urinary tract it is known as pyelonephritis (a kidney infection). Symptoms from a lower urinary tract include painful urination and either frequent urination or urge to urinate (or both), while those of pyelonephritis include fever and flank pain in addition to the symptoms of a lower UTI. In the elderly and the very young, symptoms may be vague or non specific.

In this study the effects of Lf magnetic field on some environmental microbes were determined. For biological materials, the values of B and H are related by the constant μ_0. Two idealizations of wave propagation are commonly used: spherical waves and plane waves [2; 3].

In the last few decades, the use of devices that emit electromagnetic fields has increased considerably. This proliferation has increased the concern of the damaging effect of the electromagnetic fields and the possible health effects of exposure to the fields [3; 4; 5, 6]. As a result, many organizations, both governmental and nongovernmental throughout the world,, have established safety standards or guidelines for radiation exposure

The antimicrobial effect of oscillating magnetic field pulses is not due to the temperature effect, but rather to the ability to cause damage **[7].** In this study, the relative effect of Lf magnetic field on some environmental samples of, specifically urine contaminated soil was determined with the aim of using it for monitoring and controlling some ecologic factors of epidemiologic significance in our environment.

MATERIALS AND METHODS

Urine contaminated soil samples were taken from the following five (5) spots in Akungba-Akoko: behind Akungba town hall, beside Microbiology Laboratory AAUA, behind Intercontinental hall, behind Access bank ATM machine and beside Health Center.

The test was carried out at Microbiology Laboratory in Adekunle Ajasin University Akungba-Akoko, Nigeria. All the glassware used for the study were adequately washed and sterilized in the oven at 160^0C for One hour. Liquid media used were sterilized in the autoclave at 121^0C for 15minutes, and then cooled to 45^0C before pouring into plates. Serial dilutions of the samples were made, 1mL of appropriate diluents incubated at 37^0C for 18 to 24 hours and 25^0C for 3 to 5days for bacteria and fungi respectively. Specimens showing no growth or lower colony counts were incubated for another period (24 hrs) of time. At the end of which the final colony forming units (CFU) and spore counts were taken.

The media routinely used for culturing the isolates were Nutrient agar and Peptone water. Carbohydrate sources such as mannitol, sucrose, lactose, maltose and fructose were also used for biochemical test. This was coupled with endospore staining and Gram's staining used in classifying the gram positive and the gram negative for standard identification of bacterial isolates. Physical characteristics and microscopy using Lacto phenol-in-cotton blue stain were used to identify the fungi. The experiment was carried out under stable ambient conditions to exclude possible effect of the environment.

ANTIBIOTIC SENSITIVITY TESTING

After cultivation of specimens and isolation; organisms isolated were subjected to sensitivity testing using disc diffusion method. The antibiotics employed for Gram-

positive were: Ampicillin (Amp) 10 µg; Chloramphenicol (Chl) 10µ; Cloxacillin (Cxc) 5µ; Erythromycin (Ery) 5µg; Gentamycin (Gen) 10µg; Tetracycline (Tet) 10µg; Streptomycin (Str) 10µg. those employed for Gram-negative isolates were: streptomycin (Str) 25µg; Tetracycline (Tet) 25µg; Ampicilin (Amp) 25µg; Cotrimoxazole (Cot) 25µg; gentamicin (Gen) 10µg; Nalidic acid (Nal) 30µg; and Nitrofurantoin (Nit) 200µg.

The minimum inhibitory zone was determined by disc diffusion method on Diagnostic sensitivity agar. A standardized inoculum of 100,000 colony forming units (CFU) was prepared, and the plates were then inoculated by pouring method, and then incubated at 37^0C for 48 hours. Sensitivity was determined by the extent of zone of inhibition of the organisms' growth by the coated discs.

Treatment of isolates with electromagnetic fields

This test was conducted in the Department of Physics, Adekunle Ajasin University, Akungba-Akoko where a solenoid coil to produce an Lf magnetic field existed. Isolates from the stock culture are transferred unto freshly prepared plates and afterwards multiplied in liquid nutrient medium (broth). From every breed, 1ml inoculums were introduced into 9ml freshly prepared broth in test tubes. The test tubes were treated with magnetic field of 0, 50,100,250,500 and 1000mG for 30minutes. While, the resistance breeds were then treated will the same magnetic field for 10minutes, 20minutes and 45minutes.the untreated isolates were taken as control.

After the treatment with magnetic field the isolates were seeded on a Petri dish containing selected medium, incubated at 37^0C for 24 hours and 25^0C for 3-5days for both bacteria and fungi respectively. The same process was done applied for the untreated ones, the coliform count was done for bacteria and the spore count was done for the fungi to determine the effect of magnetic field using colony counter.

RESULTS

Thirteen (13) microbial isolates were obtained from urine contaminated soil, out of which ten (10) were bacteria isolates and three fungi species. The bacterial isolates coded as MCB_1, MCB_2, ATM_1, ATM_2, ATM_3 ,ICH_1 ,ICH_2, LAW_1, LAW_2, and HTC_1 were identified to be *Bacillus subtilis, Clostridium tetani, Enterobacter cloacae, Bacillus cereus, Clostridium sporoene, Micrococcus spp., Staphyloccocus aureus, Proteus mirabilis and Pseudomonas aeruginosa* while the fungi species coded $FMCB_1$, $FATM_1$, $FICH_1$ were identified as *Saccharomyces cerevisae and Rhizopus spp.*

Identification of the isolates was done based on their morphological characteristics (fungi) and biochemical characteristics (bacteria). These characteristics were correlated with compendium for fungi identification and Bergey's Manual of Systematic bacteriology respectively.

Table 1: Biochemical Characteristics of some urine contaminated soil microflora

Isolates	Shape	Gram's Reaction	Glucose	Lactose	Fructose	Mannitol	Indole	Motility	Ornithine	Catalyses	endospore	H_2S	peptone	Methyl red	Starch hydrolysis	Probable organism
MCB$_1$	Rod	+	A	-	A	A	+	-	-	+	+	-	+	-	+	*Bacillus subtilis*
MCB$_2$	Rod	+	-	-	A	G -	+	+	-	-	+	-	+	+	+	*Clostridium tetani*
ATM$_1$	Rod	-	A	A	A	A G	-	+	-	+	+	-	+	+	+	*Enterobacter cloacae*
ATM$_2$	Rod	+	A	-	A	-	+	-	-	+	+	-	+	-	+	*Bacillus cereus*
ATM$_3$	Rod	+	A	-	A	A	+	-	-	+	+	-	+	-	+	*Bacillus subtilis*
ICH$_1$	Rod	+	A G	-	A	G -	+	+	-	-	+	-	+	+	+	*Clostridium sporoene*
ICH$_2$	Cocci	+	-	-	A	G A	-	-	+	+	-	-	-	+	-	*Micrococcus spp.*
LAW$_2$	Cocci	+	A	A	A	A	-	-	-	+	-	-	-	+	-	*Staphyloccocus aureus*
LAW$_2$	Rod	-	A G	-	A	G -	+	+	+	-	-	+	-	-	-	*Proteus mirabilis*
HTC$_1$	Rod	+	-	-	A	-	-	-	-	-	-	-	-	-	-	*Pseudomonas aeruginosa*

KEYS:
MCB$_1$ and MCB$_2$=Isolates from sample collected from microbiology laboratory, AAUA.
ATM$_1$, ATM$_2$ and ATM$_3$=Isolates from sample collected behind the ATM machine beside OBJ HALL
ICH$_1$ and ICH$_2$= Isolates from sample collected behind intercontinental hall
LAW$_1$ and LAW$_2$= Isolates from sample collected behind faculty of law
HTC$_1$=Isolate from sample collected beside health centre
(+) = POSITIVE **(-) = NEGATIVE** **(A/G) = ACID/GAS PRODUCTION**

Table 2: Antibiotics sensitivity pattern of the isolates

Isolates	CN	PEF	E	SXT	STR	CPX	R	AM	Z	APX	SP
MCB$_1$	-	-	-	-	-	-	-	-	-	-	-
MCB$_2$	-	-	-	-	-	-	-	-	-	-	-
ATM$_1$	17S	-	-	-	-	20S	-	-	-	-	-
ATM$_2$	15S	20S	20S	20S	18S	12R	15S	9R	-	-	-
ATM$_3$	-	15S	-	-	-	12R	13R	-	-	-	-
ICH$_1$	20S	10R	-	-	-	15S	-	-	-	-	-
ICH$_2$	-	-	-	-	-	-	-	-	-	-	-
LAW$_1$	-	-	-	-	-	-	-	-	-	-	-
LAW$_2$	-	-	-	-	-	-	-	-	-	-	27S
HTC$_1$	-	20S	-	-	22S	23S	19S	-	-	-	-

KEYS S= Sensitive, R= Resistant,
Z = Zinnacef, PEF= Pefloxacin, AM = Amoxacillin, CN = Gentamycin
CPX = Ciprofloxacin, R = Rocephin STR = Streptomycin, APX= Ampiclox
SXT = Septrin, E = Erythromycin

Isolate	Appearance	Shape	Elevation	Surface	Hyphae	Septa	Spore	Spread feactures	Probable organisms
F MCB	Cotton candy like, whitish colonies which later turns gray	Circular	raised	rough	branched	aseptate	Sporangiophore pale to dark brown	Sporangiophores that terminate with black sporangigium containing a collumella rhizoids penetrates the m	*Rhizopusspecies*
F ATM	White to cream color	Elongated	Flat	smooth	Absent	Non septate	Blastocondia are unicellular, Globose and ellips	Large globose to ellipsoidal budding yeast-like cells or blastocondia	*Saccharomyces cerevisae*
F ICH	White to cream color	Elongated	Flat	smooth	Absent	Non septate	Blastocondia are unicellular, Globose and ellips	Large globose to ellipsoidal budding yeast-like cells or blastocondia	*Saccharomyces cerevisae*

KEYS: F MCB= Isolate from sample collected from microbiology laboratory, AAUA.
F ATM= Isolate from sample collected behind the ATM machine beside OBJ HALL
F ICH= Isolate from sample collected behind intercontinental hall

TABLE 4: Results of the coliform forming unit count made after the exposure to EMF

Isolates	Control 0mG	50mG	100mG	250mG	500mG	1000mG
MCB$_1$	188	52	47	88	53	86
MCB$_2$	164	168	36	52	48	42
ATM$_1$	TNTC	40	47	27	152	48
ATM$_2$	162	72	22	84	94	96
ATM$_3$	106	89	52	86	22	106
ICH$_1$	154	92	92	40	52	22
ICH$_2$	96	53	48	28	38	124
LAW$_1$	218	52	53	36	56	42
LAW$_2$	112	TNTC	94	84	47	54
HTC$_1$	86	48	32	48	35	32
FMCB	25	12	9	12	17	3
FATM	54	84	22	47	42	35
FICH	63	68	40	48	28	21

KEY:
TNTC = Too numerous to count

TABLE 5: Results of the antibiotics sensitivity test for resistant isolates exposed to EMF at 1000mG for 45minutes

Isolates	CN	PEF	E	SXT	S	CPX	R	AM	Z	APX	SP
MCB$_1$	1.9	23mm	-	-	-	24mm	16mm	17mm	-	-	-
MCB$_2$	-	25mm	12mm	-	-	18mm	-	-	-	-	-
ICH$_2$	15mm	20mm	20mm	20mm	18mm	12mm	15mm	9mm	-	-	-
LAW$_1$	19mm	27mm	18mm	20mm	22mm	25mm	18mm	-	-	-	-

KEYS

Z = Zinnacef PEF= Pefloxacin AM = Amoxacillin
CN = Gentamycin CPX = Ciprofloxacin R = Rocephin
S = Streptomycin, APX= Ampiclox SXT = Septrin
E = Erythromycin

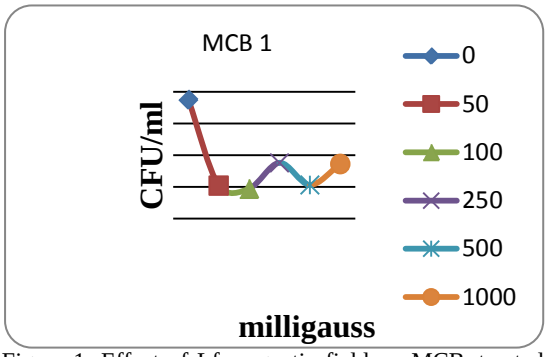

Figure 1: Effect of Lf magnetic field on MCB$_1$ treated for 30 minutes with different electromagnetic field

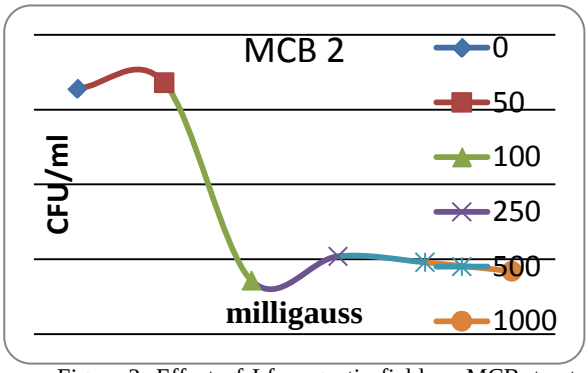

Figure 2: Effect of Lf magnetic field on MCB$_2$ treated for 30 minutes with different electromagnetic field.

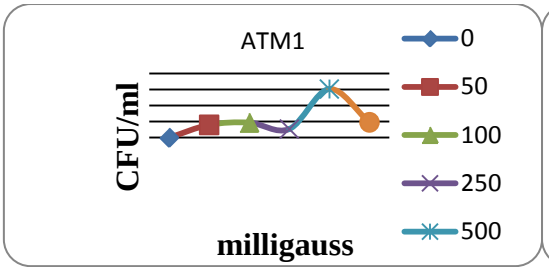

Figure 3: Effect of Lf magnetic field on ATM$_1$ treated for 30 minutes with different electromagnetic field.

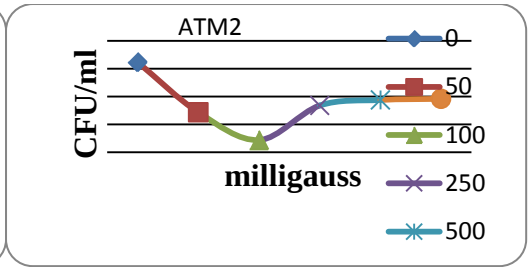

Figure 4: Effect of Lf magnetic field on ATM$_2$ treated for 30 minutes with different electromagnetic field.

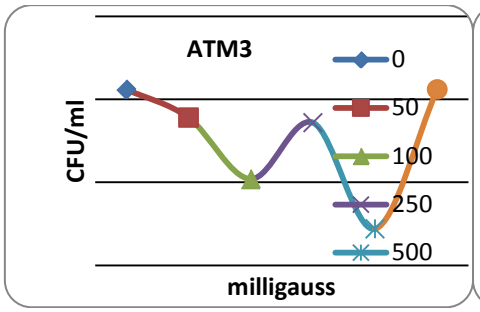

Figure 5: Effect of Lf magnetic field on ATM_3 treated for 30 minutes with different electromagnetic field.

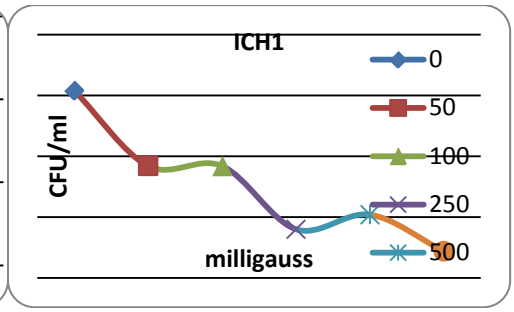

Figure 6: Effect of Lf magnetic field on ICH_1 treated for 30 minutes with electromagnetic field of varying frequency.

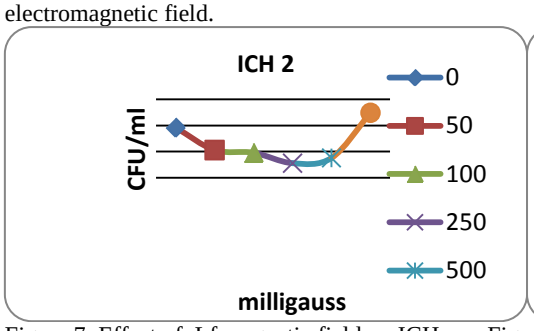

Figure 7: Effect of Lf magnetic field on ICH_2 treated for 30 minutes with electromagnetic field of varying frequency.

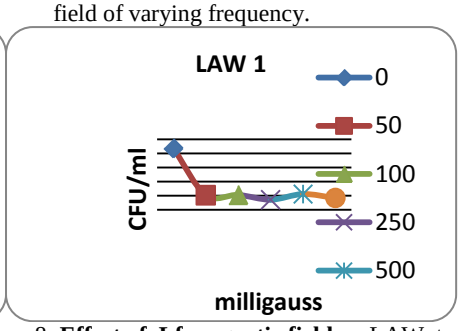

Figure 8: **Effect of Lf magnetic field** on LAW_1 treated for 30 minutes with electromagnetic field of varying frequency.

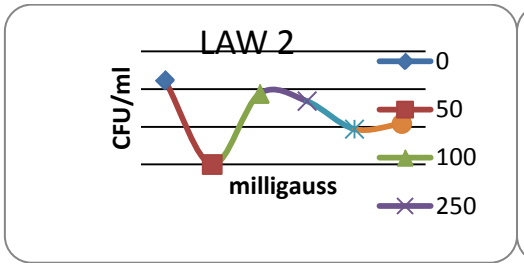

Figure 9: Effect of Lf magnetic field on LAW_2 treated for 30 minutes with electromagnetic field of varying frequency.

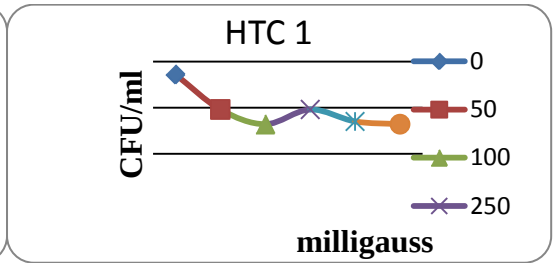

Figure 10: Effect of Lf magnetic field on HTC_1 treated for 30 minutes with electromagnetic field of varying frequency.

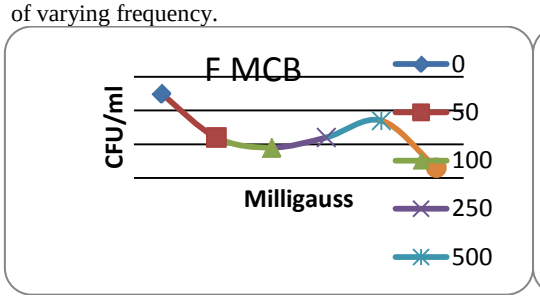

Figure 11: Effect of Lf magnetic field on FMCB treated for 30 minutes with electromagnetic field of varying frequency.

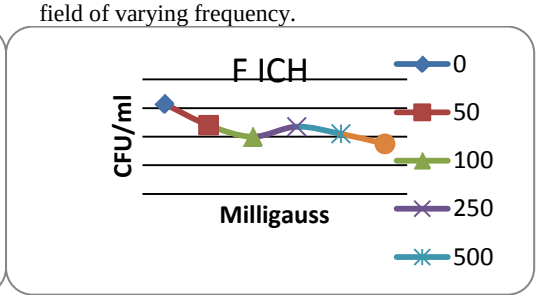

Figure 12: Effect of Lf magnetic field on FICH treated for 30 minutes with electromagnetic field of varying frequency.

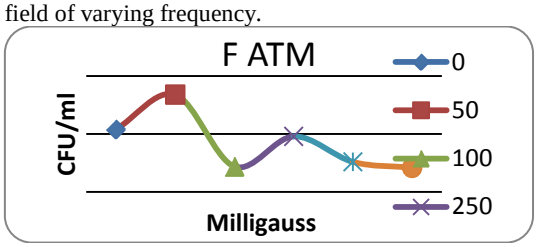

Figure 13: Effect of Lf magnetic field on FATM treated for 30 minutes with electromagnetic field of varying frequency.

DISCUSSION AND CONCLUSION

This study shows the effect of Lf magnetic field at various intensities on microorganism. It was found that an increase in the intensity of the field shows a decrease of microbial population of some isolates and an increase in others. This study reveal an initiation of some growth potentials in some microorganisms by this magnetic field and a kill off of some essential particles in others. It can be seen that the Lf magnetic field causes the decrease of colony forming units (CFU) in all exposed samples at certain level of exposure.

The thirteen microorganisms isolated and identified during the study include, ten (10) bacteria and three (3) were fungi. Eight (8) of the bacterial species with their respective codes were Gram positive. This were MCB1 – *Bacillus subtilis*, MCB2 – *Clostridium tetani*, ATM2 - *Bacillus cereus*, ATM3 - *Bacillus subtilis*, ICH1 - *Clostridium sporoene*, ICH2 - *Micrococcus spp.*, LAW1 - *Staphyloccocus aureus*, HTC - *Pseudomonas aeriginosa.* The Gram negative bacterial isolates were ATM1 - *Enterobacter cloacae* and LAW2 - *Proteus mirabilis.* The fungi isolates identified were FMCB – *Rhizopus species*, FICH – *Saccharomyces cerevisae* and FATM - *Saccharomyces cerevisae.*

In this study, the exposure of the isolates to Lf magnetic field of 50mG, 100mG, 250mG, 500mG and 1000mG show a decrease in microbial growth rate. This finding correspond with the work of Lipiec et al. [8] who investigated the effect of extremely low frequency (<300 Hz) magnetic fields (EMF) on the growth rate of Gram-positive and Gram-negative bacteria.

The results showed a decrease in the growth rate of exposed samples with respect to control.. MCB_1, ATM2, ATM3 and ICH_2 recorded an increase in growth when they were exposed at a field of magnitude of 1000mG. This shows that a modification has occurred in the cell composition of the microorganisms. MCB_2, ATM_1, ICH_1, LAW_1, LAW_2 and HTC_1 recorded a decrease in the coliform count due to the inhibitory effect of electromagnetic field. ATM_1 show an increase at an electromagnetic field of 500mG. This study corroborates with the work of Samarbaf-Zadeh et al., [9] who found that the exposure of *P. aeruginosa* to extremely low-frequency electromagnetic fields (2mT; 50Hz) at 4, 6, and 8 h of incubation the number of cells was significantly decreased in bacteria exposed to electromagnetic field when compared with the control. Additionally, at 24 h of incubation, the percentage of cells increased (*P. aeruginosa* ~ 42%; E. coli ~ 5%) in treated groups with respect to control groups suggesting a progressive adaptive response.

The isolates that are resistant to antibiotics are also exposed to Lf magnetic field for 45 minutes and were tested again for their sensitivity which show a tangible susceptibility to the antibiotics used. Therefore resistant bacteria could be susceptible to antibiotic by exposure of the patients to the EMF. Samarbaf-Zadeh et al., [9] found out that the antibiotic sensitivity test of microorganism cells indicated that the use of electromagnetic field could cause either a decrease or an increase in the sensitivity of exposed cells resulted in either inhibition or stimulation case for the bacteria depending on the drug mode of action on the bacterial cell.

CONCLUSION

This study shows that each organism responds to the exposure of electromagnetic field in a specific manner depending on the adaptation mechanism of each organism. Similarly, the exposure to electromagnetic field may have beneficial effects as well as harmful effects that the safety limits for exposure to these fields are strength and frequency dependent.

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