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Phenotypic and Genetic Effects of Wi-Fi Waves on Some Bacterial Species Isolated from Otitis Media Infection

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ABSTRACT

An increasing use of telecommunication technologies such as bluetooth, and Wi-Fi has led to an increase in exposure of living organisms to electromagnetic radiations. The aim of this study was to investigate the effects of Wi-Fi waves on some pathogenic bacteria including *Kocuria kristinae*, *Staphylococcus aureus* and *Proteus mirabilis* isolated from patients suffering from Otitis media infection. The organisms were identified using phenotypic and biochemical characteristics. Four cultures of each of the bacterial isolates were respectively exposed to a 2.4 GHz Wi-Fi waves at a distance of 0, 0.5, 5 or 10 m from the radiation source. Sensitivity of the bacterial isolates was tested against nalidixic acid, tetracycline and tobramycin before and after exposure. Also, DNA was extracted, purified and quantified from the test bacterial isolates before and after exposure. These extracts were used to perform a random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR), the products were separated on an agarose gel electrophoresis and data were analyzed. The results of the antibiotic sensitivity testing indicated a significant effect of the Wi-Fi waves on it. All the isolates were resistant to the antibiotics before exposure, but most of them became sensitive after exposure. RAPD-PCR markers showed that the Wi-Fi waves have serious effect on the bacterial genetic materials. Our finding revealed that Wi-Fi waves have significant effects on the phenotypic and genetic traits of test bacterial isolates. Therefore, it is recommended that cautions should be taken when handling electromagnetic-emitting devices as a result of the serious health risk associated with them.

Keywords: Bacteria, DNA, RAPD-PCR, Wi-Fi waves.

Introduction

In recent times, increase in the use of telecommunication technologies such GSM and local network (bluetooth, cordless phones and Wi-Fi) has led to an increase in exposure of living organisms to frequency waves of electromagnetic fields from different sources.[1,2] The frequency waves are in the forms of microwaves and radio-frequency radiation signals.[2] Typically, Wi-Fi waves are in operation when using 2.4 GHz radio frequencies and are part of non-ionizing radiation of the electromagnetic spectrum. Recently, a new field has emerged and it is concerned with the effect of radiations on vital functions of cells and organisms in relation to human health and the environment.[3] Studies have demonstrated that electromagnetic waves have an influence on the functions of cells of an organism.[4] Thermal or non-thermal effects are forms of non-ionizing electromagnetic radiation that has an effect on biological system.[5,6] Non-thermal electromagnetic fields cause different responses on bacteria, depending on the type of organism, frequency strength and exposure time.[7] In particular, it has been shown that electromagnetic fields (EMFs) have effects on bacteria and their functional parameters which include cell growth and antibiotic resistance.[8,9] Also, other studies have revealed that electromagnetic fields cause alteration in the phenotypic form of bacteria,[10] growth rate,[11] antibiotic sensitivity,[12] and DNA repair processes.[13] EMFs have the potential of inducing a severe or even chronic influence inside cells by increasing the levels of free radicals, leading to the potential damage of DNA, thereby causing harmful cellular responses.[14]

Growth of bacteria can be influenced by electromagnetic radiations by either promoting,[15] or inhibiting,[16] cell growth. Low-frequency of electromagnetic radiations cause an accumulation of mineral ions (such as Ca^{2+}) inside cells,[17] essential for the function of bacterial membrane by activating ATPase that provides energy for the flow pumps and transport ions through cell membrane.[18] Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique is one of the modern methods used in molecular diagnostics to determine mutations occurring in the genome of an organism. An advantage of this molecular method over other techniques is that it does not require a prior knowledge of the genome of the test bacterium.[19] The present study was aimed at investigating the effect of radio waves of electromagnetic radiation emitted by a Wi-Fi router on the phenotypic and genetic traits of some bacterial species isolated from Otitis Media infection.

Materials and Methods

Source and identification of bacterial isolates

Three bacteria, *Kocuria kristinae*, *Staphylococcus aureus* and *Proteus mirabilis* were isolated from patients with Otitis media infection attending the Tikrit Teaching Hospital, Tikrit, Iraq from a period of March to July, 2019. The bacterial isolates were identified phenotypically by employing the MacConkey agar and Mannitol salt agar culture media. Also, biochemical tests which include Catalase, Oxidase, Indole test, Methyl test, Voges–Proskauer test, Citrate test were performed. These phenotypic and biochemical tests were carried

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out according to the methods previously described,[20] and the identification of bacteria was confirmed by Vitek 2 compact system.

Antibiotic susceptibility testing of bacteria isolated from Otitis media infection

The Kirby-Bauer method,[21,22] was used for testing the sensitivity of bacterial isolates to three types of antibiotics: nalidixic acid (30 µg/mL), tetracycline (10 µg/mL) and tobramycin (10 µg/mL). Bacterial suspension was prepared by individually picking 3-5 bacterial colonies and placed in a sterile test tube containing 5 ml of nutrient broth. The cultures were incubated at 37°C for 24 h, after which the bacterial cultures were compared with McFarland No. 0.5 Standard. Inoculation of bacterial cultures on Muller Hinton Agar plate was carried out and antibacterial disks were layered on the cultures using sterilized forceps. All the cultures were incubated at 37°C for 24 h. At the end of the incubation period, inhibition zones around each antibiotic disc was measured in millimeter according to the Clinical and Laboratory Standards Institute guidelines for antibiotic susceptibility assay.[23] Data were recorded for bacterial isolates before and after exposure to the wireless router waves.

Wi-Fi radio wave exposure on test bacterial isolates

The effects of Wi-Fi radio wave exposure on bacterial resistance to antibiotics was investigated according to the procedure described by Taher,[24] with some modifications. Each of the three bacterial isolates was respectively inoculated into 5 mL of nutrient broth (4 tubes/bacterial type) and divided into four treatment groups: Group 1, without treatment (control); Group 2, cultures were placed at a distance of 0.5 meters (M1) from the Wi-Fi router device; Group 3, cultures placed at a distance of 5 meters (M2) from the radiation source and Group 4, cultures were placed at a distance of 10 meters (M3) from the wireless router. All the cultures were incubated at 37°C for 18 h. The radio frequency simulator used for the study was a 2.4 GHz Wi-Fi router.

DNA extraction from test bacterial isolates before and after Wi-Fi radio wave exposure

Genomic DNA was extracted from bacterial isolates before and after exposure to Wi-Fi radio waves as described by Onasanya,[25] and Hamzah *et al*.,[26] with slight modifications. One milliliter of the bacterial culture was transferred to 1.5 mL Eppendorf tube and centrifuged at 14,000 rpm for 3 min. The supernatant was discarded and the bacterial cells were washed with 200 µL TE buffer. In 200 µL of 2× CTAB buffer (0.7 mM NaCl, 50 mM Tris, pH 8.0, 10 mM EDTA, 2% hexadecyltrimethylammonium bromide, 0.1% 2-mercaptoethanol), the washed bacterial cells were suspended and mixed thoroughly by a vortex mixer. An aliquot of 100 µL of 20% sodium dodecyl sulfate (SDS) solution was added and incubated in a water bath at 65°C for 20 min. The resulting DNA was purified by three successive extraction processes with phenol:chloroform:isoamyl alcohol (25:24:1). The top aqueous layer was transferred to a new Eppendorf tube and a double volume of 100% absolute ethanol was added to precipitate the DNA. After precipitation, the tube was centrifuged at 10,000 rpm for 10 min, thereafter the supernatant was discarded. The DNA pellet was washed with 1 mL of 70% ethanol, dried and resuspended in 50 µL of sterile distilled water. Quality of the isolated DNA was confirmed on 1% agarose gel electrophoresis in 1xTAE (45 mM Tris-acetate, 1 mM EDTA, pH 8.0).[27,28] The DNA concentration and purity were determined by measuring absorbance values of diluted DNA solution at 260 and 280 nm using Nanodrop spectrophotometer (Thermo Scientific, Germany) and these values were used to determine DNA purity.

Random amplified polymorphic DNA-polymerase chain reaction analysis

RAPD-PCR was set up using the isolated bacterial DNAs from all the experimental groups as templates, employing the procedure described by Williams *et al*.[29] Eight RAPD primers were used in this study and the nucleotide sequences of the primers are presented in Table 1. The RAPD-PCR was performed using a Green Master mix (Promega Company, USA) in a total volume of 25 uL according to the company's instructions. The reaction condition involved: a pre-denaturation step at 94°C for 5 min, followed by 40 cycles of denaturation step at 93°C for 30 sec, annealing step at 36°C for 45 sec, extension step at 72°C for 1.5 min; and a final extension step at 72°C for 10 min. At the end of the amplification cycles, 4µL of the PCR products were separated on 1.5% agarose gel electrophoresis with the inclusion of a DNA marker (100 bp DNA ladder). When the electrophoresis was completed, the gel was stained by ethidium bromide for 60 min and visualized under UV- transilluminator.

Table 1: Primers used for RAPD-PCR analysis

Primer code	Primer sequence (5' – 3')
P-1	TGTGCCCCA
P-2	GTCGCCGTCA
P-3	GTTGCGATCC
P-4	AACGGTGACC
P-5	ATGACCGCC
P-6	ACAGGAGGT
P-7	CTGGGACTC
P-8	GAGGGTGTT

RAPD-PCR data scoring and analysis

In order to identify changes in genetic materials of the treatment groups in relation to the control group, banding patterns from the RAPD markers were scored. This was achieved by converting bands which appeared on the gel to values by recording 1 for the presence of a unique band and 0 for the absence of a band.

Results and Discussion

Effect of Wi-Fi radio wave exposure on bacterial resistance to antibiotics

In this study, bacterial sensitivity to three types of antibiotics was tested. The results showed that the bacterial isolates were resistance to the three test antibiotics: nalidixic acid (NA), tetracycline (TE) and tobramycin (TOB) as shown in Table 2. The bacterial isolates were exposed to electromagnetic waves emitted by a Wi-Fi router at a distance of 0, 0.5, 5 or 10 meters for 24 hours and the results (Table 3) indicated that the waves affected the sensitivity of the test bacteria to the antibiotics. *Proteus mirabilis* was sensitive to TOB at a distance of 0.5 and 5 meters only, while there was no change in the level of sensitivity to other antibiotics as shown in Figure 1. *S. aureus* which was resistant to NA, TE and TOB before exposure to Wi-Fi waves became sensitive to the three antibiotics after exposure (Figure 2). After exposure, *K. kristinae* (Figure 3) was sensitive to the three antibiotics at distances of 0.5 and 5 m, while only sensitive to TOB and NA at a distance of 10 m.

In modern times, our world is surrounded by various devices such as Wi-Fi router and computers that emit massive electromagnetic waves which have harmful effects or can cause several health problems on humans. Studies have indicated that these electromagnetic waves can lead to genetic modifications in bacteria leading to a change in antibiotic sensitivity or resistance.[2,30] Also, other studies have shown that high frequency non-thermal electromagnetic field such as Wi-Fi has different effects on strains of bacterial species.[31,32] It was found that the effect of electromagnetic waves on *E. coli* ATCC 25992 at a frequency of 50 Hz caused a change in the phenotypic shape and growth curve of bacterial cells, as well as sensitivity to antibiotics such as nalidixic acid, amoxicillin and erythromycin.[8,33] Taheri and coworkers,[12] also showed the effect of electromagnetic waves

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emanating from a Wi-Fi device at 2-4 GHz wavelength on *Klebsiella pneumonia* led to an increase in its sensitivity to the antibiotics Cefteriaxone, Imipenem, Cefotaxime, Piperacilline after an incubation period of 18 hours. Other reports have indicated that exposure of *Escherichia coli* and *Listeria monocytogenes* to radiations of Wi-Fi and GSM caused changes in diameters of growth rate and zone of inhibition of the bacteria. Taheri and his group,[24] found that after exposure of *E. coli* to electromagnetic waves emitted from Wi-Fi, increased its sensitivity to Cefotaxime and Ciprofloxacin antibiotics. In the same study, *Listeria* bacteria became sensitive to antibiotics Dox, while there was no change to Cefotaxime and Ciprofloxacin after 18 hours of incubation. These results agree with our current study. Salman,[34] found that the electromagnetic waves emitted from 2-4 GH Wi-Fi on *Staphylococcus epidermidis* increased their sensitivity to penicillin after exposure, but did not affect sensitivity to Chloramphenicol, Oflaxicin, Gentamycin, whereas, there was no change in sensitivity of these antibiotics to *Staphylococcus aureus*. Also, this finding is in agreement with our results in relation to the bacterial isolate, *Proteus mirabilis* which was sensitive to TOB, while there was no change in sensitivity to other antibiotics. Furthermore, a research conducted by Segatore *et al*.,[35] found that electromagnetic waves have an effect on the sensitivity of *Pseudomonas. aeruginosa* and *E. coli* to kanamycin and amikacin.

The effect of electromagnetic waves on bacterial resistance to antibiotics depends on the physical properties of the electromagnetic waves which include the wave frequency intensity, power emitted, exposure period to wave and types of bacteria species. Therefore, the effect of these waves on bacteria is not enough for detecting the effects of these waves on the environment, but also to detect the pattern of bacterial resistance to antibiotics in medical and environmental laboratories.[36-37]

Different mechanisms of bacterial sensitivity to antibiotics induced by electromagnetic fields have been described. Membrane potential changes and surface charge of bacterial cell membrane lead to an imbalance in respiratory chain system. Addition of the production of energy through proton motive force in the bacteria, weakens its ability to control the transport through the wall of the bacteria and thus facilitates the entry of antibiotics.[38,39] Increase in bacterial sensitivity to antibiotics could be linked to the structure of the bacterial cell wall and nature of the peptidoglycan.[40] It has been shown that electromagnetic waves can induce a change in the composition of fatty acids of bacterial cell membrane, as well as a change in the composition of the murein layer of the cell wall, consequently affecting the sensitivity to antibiotics.[12,41] Also, electromagnetic fields can change the sensitivity of efflux pumps or ion transfer channels by allowing molecules to enter the bacterial cell where the efflux pumps and ion transfer channels located in the cell membrane have an important role in the transport of antibiotics by the cell.[12,35,42] More so, composition, charge and size of antibiotics can affect sensitivity of bacteria when exposed to electromagnetic waves.[43]

Effect of Wi-Fi radio wave exposure on genetic material of bacterial isolates

A total of eight primers were used as RAPD markers and the primers initiated binding loci on the genome producing different bands that were detected on agarose gel electrophoresis. Some of the bands obtained were monomorphic, while others were polymorphic. The different forms of the bands obtained are presented in Table 4. The total number of loci identified by bands on sample genotype was 107, of which 5 were general for all the samples and 102 were differentiated. The highest number of productive loci reached 17 and the complete number of bands produced from these loci was 527 total bands, of which 60 were general bands (main bands) and 467 were differentiated polymorphic bands. A maximum number of 92 bands were obtained from Primer OP O -11. General variation ratio for the produced primers was 95 %. The sensation of the variance of the three constants associated with control samples is an indication of the influence on genome of bacterial isolates, whenever the high variation after management designates the effect of treatment.[30,31] Bands showed different sizes, ranging from 100-3350 bp; the smallest was 100 bp of Primer D -08, while the largest size was 3350 bp in the P-5 primer.

Table 4 and 5 showed the characteristics of the RAPD-PCR bands and Figures 4-11 showed distinctive bands (unique and absent bands). Distinguishing the association with the control sample, of which 100 were unique bands and 112 absent bands. The primer P-4 showed high number of 18 unique bands, while the absent bands formed the largest number of absent bundles.[31] As for the three bacterial isolates, it was obtained for isolate B1, a percentage of the characteristic mutant bundles which reached 103 bundles, of which 63 unique bands and 40 absent bands, followed by isolate B3, which received 69 distinctive bands disseminated into 21 unique bands and 48 absent bands. This was followed by isolate B2, which received 40 distinctive bands separated into 17 unique bands and 23 absent bands. These bands were discriminatory and the diagnosis of these treatments indicated that the wavelengths affected the genetic materials, substantially the DNA because of the emergence of different bands between isolates. This was caused by the different type of isolates, genetic structure and its ability to repair damaged DNA strands and that the upsurge of mutant bands was caused by an increase in the waves, which may influence the degree of bacteria death and inferred from the occurrence of a mutation in a particular site led to the documentation of the primers and the emergence of the unique band, but for the absent bands as well as a mutation in the site only know the initiator in that treatment, which led to the elimination of this identification and concealed war package that is compatible with many former researchers.[30, 32- 33]

To the best of our knowledge, this current study is the first report in the country on the effect of electromagnetic radiations on the genome of bacteria in all its forms. It was shown that the markers of RAPD-PCR have high efficiency in the diagnosis of mutation and a small number of primer shaped treatments presented a variance in the number and excellence of mutant band. The results presented revealed that the treatments have effects on the genetic substantial of the bacterial isolates used, but there are different ratios rendering to the different treatments where the third treatment presented M3 which was 10 m away from the source of the waves for the first isolate. *Proteus mirabilis* (B1) had the maximum result because it obtained the number of distinctive mutant bands, 44 divided into two types of bands which included 17 absent bands that was originally present in the control sample and disappeared after the treatment, while 27 unique bands were found after treatment. These were not originally present in the control sample. This was followed by the second treatment M2, which was away from the source of the router 5 meters, which had 36 distinctive bands that were separated into 13 absent bands and 23 unique bands. Followed by the first treatment M1, which is 0.5 meters from the source of the wave that had the lowest number of mutant beams amounted to 23 bands separated between 10 absent bands and 13 unique bands.

For the second isolate, *S. aureus* (B2), where the third treatment M3, which was 10 meters away from the source of the waves presented the highest effect because it got several distinctive mutant bands that amounted to 14 distinctive bands. These were divided into two types of bands, where 7 absent bands were originally contemporary in the control sample and disappeared after the treatment and 7 unique bands originated after the treatment that was not previously in the control sample. The second treatment, M2, which is away from the source of the router by 5 meters, had 13 distinctive bands, separated into 9 absent bands and 4 unique bands. This was followed by the first treatment M1, which was 0.5 meters from the source of the wave, had the lowest number of mutant bands that amounted to 13 bands that were divided between 7 absent bands and 6 unique bands. The second treatment, M2, which was 5 m away from the source, showed the third isolate, *K. kristina* (B3) had the highest effect for obtaining the number of distinctive mutant bands, 29 distinctive bands that were divided into two types of bands. There were 21 absent bands which stood originally contemporary in the control sample that disappeared after the treatment and 8 unique bands were found after the treatment which did not exist previously in the control sample. This was followed by the third treatment M3, which was 10 meters away from the source of the wave which had 22 distinctive bands, separated into 16 absent bands and 6 unique bands. This was followed by the first

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treatment M1, which was 0.5 meters from the wave source and had the lowest number of mutant bands (18 bands) separated between 11 absent bands and 7 unique bands. The results of the three coefficients for each isolate showed that an increase in the distance was directly proportional to the effect on DNA by cumulative the number of distinctive mutant packets was the highest number of mutant packets for treatment M3 in the first separation number of absent mutant beams was highest in the second treatment M2 for the third isolation B3. In the absence of mutant bands, it was found that all distances had a strong effect on the genome of bacteria and the effect increased with increasing distance, which controlled to an upsurge in the destruction of DNA sequences and indication of the disappearance of the induction site of the primers originally in the control sample.[13,44]
On the other hand, the electromagnetic waves and their frequencies have a high effect on the bacteria and the best evidence for this is the large number of absent bands mutant and this corresponds to previous studies.[2,45,46] Through the relationship between antimicrobial resistance and molecular results, the effect of the waves on the three bacterial isolates was very high in all the treatments. The results of the RAPD-PCR at all distances had a significant effect on the genome of test bacteria because the average distance of 5 meters had a greater effect. The reason for the incompatibility of sensitivity at the molecular level is that most mutations that occurred in the genome of bacteria occurred in the non-coding genes and therefore, all these mutations have no effect on the traits The bacterial phenotype 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 dependable on the results of most researchers.[27,35,47,48]

Table 2: Antibiotic sensitivity testing of bacterial isolates from Otitis media infection

Bacterial isolates	**Antibiotics**		
	TOB	**NA**	**TE**
Staphylococcus aureus	R	R	R
Kocuria kristinae	R	R	R
Proteus mirabilis	R	R	R

TOB: Tobramycin; NA: Nalidixic acid; TE: Tetracycline; R: Resistance to antibiotics

Table 3: Antibiotic sensitivity of test bacterial isolates before and after exposure to Wi-Fi radio waves

Antibiotics code	**Bacterial isolates**											
	Staphylococcus aureus				***Kocuria kristinae***				***Proteus mirabilis***			
	Control Before treatment	**Wi-Fi Exposure After treatment**			**Control Before treatment**	**Wi-Fi Exposure After treatment**			**Control Before treatment**	**Wi-Fi Exposure After treatment**		
		0.5*m	**5*m**	**10*m**		**0.5*m**	**5*m**	**10*m**		**0.5*m**	**5*m**	**10*m**
TE	R	18 mm	16 mm	14 mm	R	14 mm	10 mm	R	R	R	R	**R**
NA	R	25 mm	21 mm	17 mm	R	11 mm	9 mm	9 mm	R	R	R	**R**
TOB	R	18 mm	17 mm	15 mm	R	21 mm	20 mm	19 mm	R	12	9	**R**

R: Resistance

Table 4: Characteristics of RAPD-PCR bands for detecting genetic variation in test bacterial isolates exposed to Wi-Fi waves

SN	**Primer Number**	**Loci number**	**Monomorphic loci number**	**Polymorphic loci number**	**Bands number**	**Monomorphic bands number**	**Polymorphic band number**	**Unique bands**	**Absent bands**	**Variation Ratio**
1	P-1	17	-	17	66	-	66	8	24	100
2	P-2	15	2	13	90	24	66	14	8	86
3	P-3	13	1	12	62	12	50	9	9	92
4	P-4	15	1	14	65	12	53	18	14	93
5	P-5	10	-	10	26	-	26	14	12	100
6	P-6	12	1	11	92	12	80	12	5	91
7	P-7	12	-	12	59	-	59	9	9	100
8	P-8	13	-	13	67	-	67	16	31	100
Total		**107**	**5**	**102**	**527**	**60**	**467**	**100**	**112**	**95**

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Table 5: Characteristic mutations of bacterial isolates exposed to Wi-Fi waves

Primer	Molecular weight (bp)	(B1) *Proteus mirabilis*						(B2) *S. aureus*						(B3) *K. kristinae*					
		M1		M2		M3		M1		M2		M3		M1		M2		M3	
		Unique	Absent	Unique	Absent	Unique	Absent	Unique	Absent	Unique	Absent	Unique	Absent	Unique	Absent	Unique	Absent	Unique	Absent
P1	100-3000	-	1	2	1	3	1	-	-	-	1	-	-	1	2	1	9	1	9
P2	150-1500	2	-	2	-	3	1	2	1	2	1	4	-	-	-	1	5	-	-
P3	450-2250	1	3	2	3	3	3	1	-	1	-	1	1	-	-	-	-	-	-
P4	200-3250	1	3	3	3	3	3	1	-	-	-	2	-	4	2	2	1	2	2
P5	350-2350	3	-	5	-	5	-	-	4	-	4	-	3	-	1	1	-	-	-
P6	300-2000	2	-	4	-	3	1	-	1	-	-	-	-	1	1	1	1	1	1
P7	150-1500	-	-	4	-	3	2	2	-	-	-	-	-	-	2	-	2	-	2
P8	200-1800	4	3	1	6	4	6	-	1	1	3	-	3	1	3	2	3	2	2
Absent and unique bands for each treatment		13	10	23	13	27	17	6	7	4	9	7	7	7	11	8	21	6	16
Total mutant bands per treatment		23		36		44		13		13		14		18		29		22	
Total mutant bands per bacteria		103						40						69					
Total mutant bands		212																	

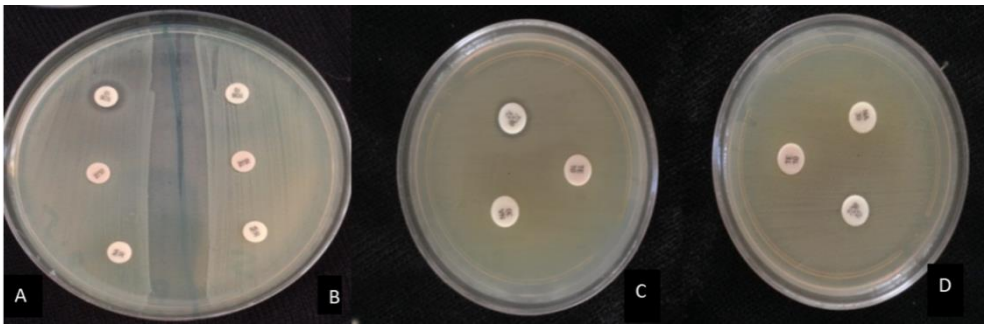

Figure 1: Effect of Wi-Fi waves on *Proteus mirabilis* bacterial sensitivity to test antibiotics.
A: Control; B: 0.5 m away from radiation source; C: 5 m away from radiation source; D: 10 m away from radiation source; Nalidixic acid (NA), Tetracycine (TE), Tobramycin (TOB) on different distance

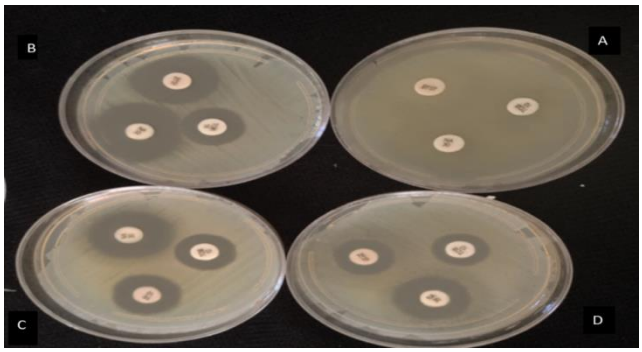

Figure 2: Effect of Wi-Fi waves on *Staphylococcus aureus* bacterial sensitivity to test antibiotics.
A: Control; B: 0.5 m away from radiation source; C: 5 m away from radiation source; D: 10 m away from radiation source; Nalidixic acid (NA), Tetracycine (TE), Tobramycin (TOB) on different distance

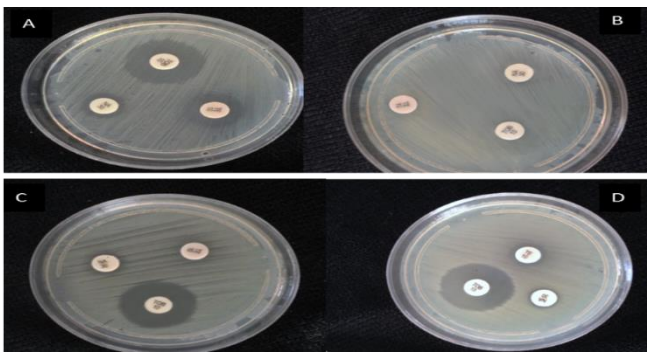

Figure 3: Effect of Wi-Fi waves on *Kocuria kristinae* bacterial sensitivity to test antibiotics.
A: Control; B: 0.5 m away from radiation source; C: 5 m away from radiation source; D: 10 m away from radiation source; Nalidixic acid (NA), Tetracycine (TE), Tobramycin (TOB) on different distance

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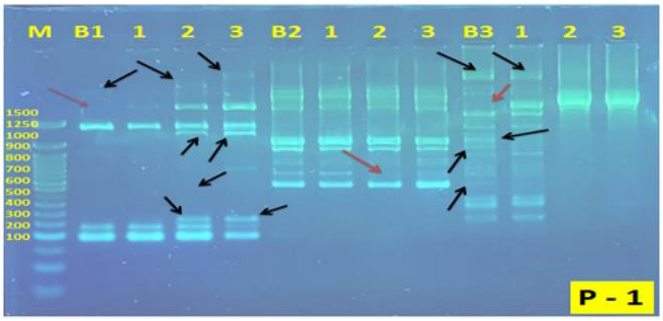

Plate 1: RAPD-PCR assay on test bacterial isolates using P-1 primer.
B1: *Proteus mirabilis*; B2: *Staphylococcus aureus*; B3: *Kocuria kristinae*; Black arrows: Unique bands; Red arrows: Absence of bands.

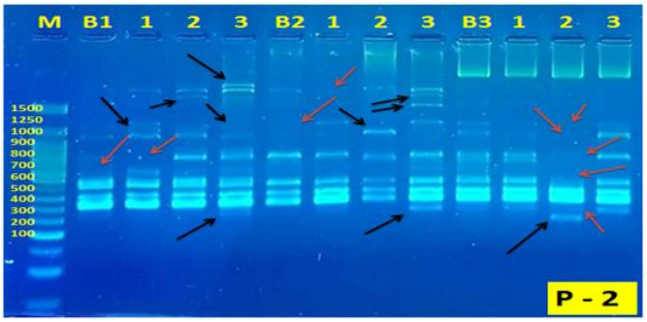

Plate 2: RAPD-PCR assay on test bacterial isolates using P-2 primer.
B1: *Proteus mirabilis*; B2: *Staphylococcus aureus*; B3: *Kocuria kristinae*; Black arrows: Unique bands; Red arrows: Absence of bands.

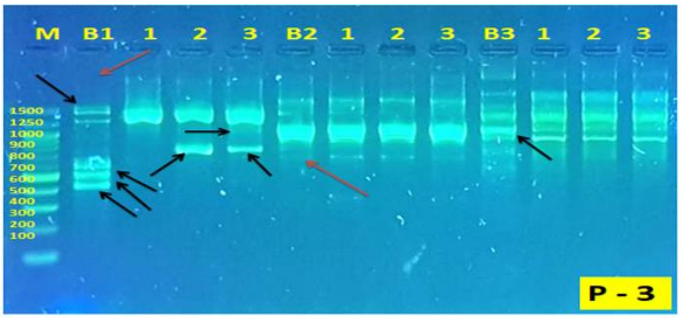

Plate 3: RAPD-PCR assay on test bacterial isolates using P-3 primer.
B1: *Proteus mirabilis*; B2: *Staphylococcus aureus*; B3: *Kocuria kristinae*; Black arrows: Unique bands; Red arrows: Absence of bands.

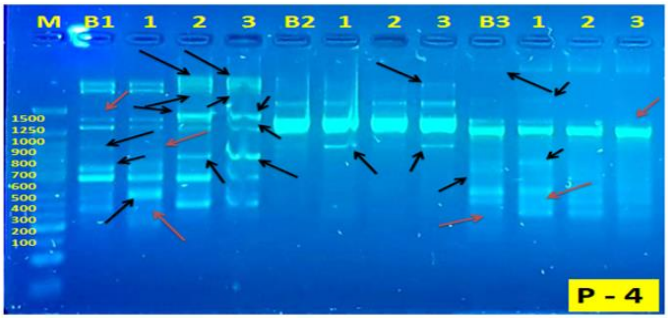

Plate 4: RAPD-PCR assay on test bacterial isolates using P-4 primer.
B1: *Proteus mirabilis*; B2: *Staphylococcus aureus*; B3: *Kocuria kristinae*; Black arrows: Unique bands; Red arrows: Absence of bands.

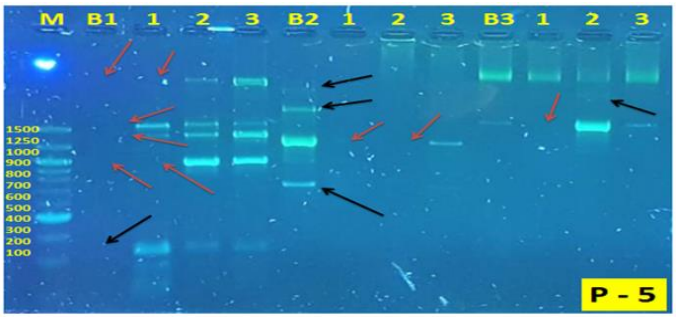

Plate 5: RAPD-PCR assay on test bacterial isolates using P-5 primer.
B1: *Proteus mirabilis*; B2: *Staphylococcus aureus*; B3: *Kocuria kristinae*; Black arrows: Unique bands; Red arrows: Absence of bands.

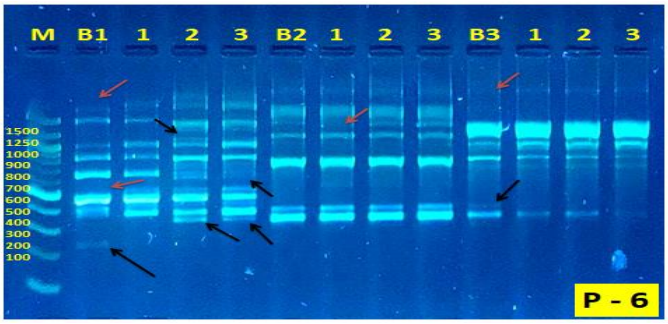

Plate 6: RAPD-PCR assay on test bacterial isolates using P-6 primer.
B1: *Proteus mirabilis*; B2: *Staphylococcus aureus*; B3: *Kocuria kristinae*; Black arrows: Unique bands; Red arrows: Absence of bands.

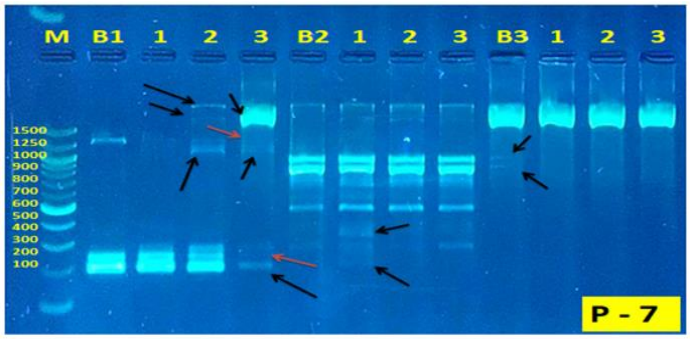

Plate 7: RAPD-PCR assay on test bacterial isolates using P-7 primer.
B1: *Proteus mirabilis*; B2: *Staphylococcus aureus*; B3: *Kocuria kristinae*; Black arrows: Unique bands: Red arrows: Absence of bands.

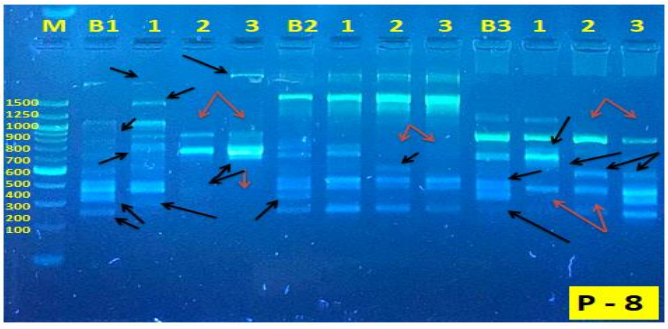

Plate 8: RAPD-PCR assay on test bacterial isolates using P-8 primer.
B1: *Proteus mirabilis*; B2: *Staphylococcus aureus*; B3: *Kocuria kristinae*; Black arrows: Unique bands; Red arrows: Absence of bands.

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Conclusion

The results from this study have shown that Wi-Fi radio waves have significant effects on antibiotic sensitivity of the test pathogenic bacteria, *Kocuria kristinae*, *Staphylococcus aureus and Proteus mirabilis*. Before exposure, the bacterial isolates were resistant to the test antibiotics, but they became sensitive after exposure to the Wi-Fi waves at various distances from the radiation source. Also, the Wi-Fi waves altered the genetic materials of the test bacteria in relation to the control. It is therefore, recommended that serious precautionary measures should be taken when handling electromagnetic-emitting devices as a result of the health hazard associated with them.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Hardell L and Sage C. Biological effects from electromagnetic field exposure and public exposure standards. J Biomed Pharma. 2008; 62(2):104-109.
2. Balmori A. Radiotelemetry and wildlife: highlighting a gap in the knowledge on radiofrequency radiation effects. J Sci Environ. 2016; 9:543-662.
3. Foletti A, Lisi A, Ledda M, de Carlo F, Grimaldi S. Cellular ELF signals as a possible tool in informative medicine. Elec Bio Med. 2009; 28(1):9-71.
4. Oncul S, Cuce EM, Aksu B, Inhan Garip A. Effect of extremely low frequency electromagnetic fields on bacterial membrane. Int J Rad Bio. 2016; 92(1):9-42.
5. Ng KH. Non-ionizing radiations–sources, biological effects, emissions and exposures. Int Con Rad. 2003; 20:1-16.
6. Belyaev I. Non-thermal biological effects of microwaves. Micro Rev. 2005; 11(2):13-29.
7. Salmen SH. Non-thermal biological effects of electromagnetic field on bacteria-A review. Am J Res Commun. 2016; 4:16-28.
8. Gaafar ES, Hanafy MS, Tohamy EY, Ibrahim MH. Stimulation and control of E. coli by using an extremely low frequency magnetic field. Rom J Bioph. 2006; 16(4):96-283.
9. Ibraheim MH and El-Din Darwish D. Hz frequency magnetic field effects on *Pseudomonas aeruginosa* and *Bacillus subtilis* bacteria. IOSR J Appl Phys. 2013; 5(3):49-56.
10. Inhan-Garip A, Aksu B, Akan Z, Akakin D, Ozaydin AN, San T. Effect of extremely low frequency electromagnetic fields on growth rate and morphology of bacteria. Int J Rad Bio. 2011; 87(12):61-1155.
11. Tessaro LW, Murugan NJ, Persinger MA. Bacterial growth rates are influenced by cellular characteristics of individual species when immersed in electromagnetic fields. Microbiol Res. 2015; 172:26-33.
12. Taheri M, Mortazavi SM, Moradi M, Mansouri S, Nouri F, Mortazavi SA, Bahmanzadegan F. Klebsiella pneumonia, a microorganism that approves the non-linear responses to antibiotics and window theory after exposure to Wi-Fi 2.4 GHz electromagnetic radiofrequency radiation. J Biomed Phys Eng. 2015; 5(3):1-115.
13. Phillips JL, Singh NP, Lai H. Electromagnetic fields and DNA damage. Pathophys J. 2009; 16(2-3):79-88.
14. Montagnier L, Aissa J, Ferris S, Montagnier JL, Lavalléee C. Electromagnetic signals are produced by aqueous nanostructures derived from bacterial DNA sequences. Interdisciplinary Sciences: Compu L Sci. 2009; 1(2):81-90.
15. Torgomyan H, Kalantaryan V, Trchounian A. Low intensity electromagnetic irradiation with 70.6 and 73 GHz frequencies affects Escherichia coli growth and changes water properties. Cell Biochem Biophys. 2011; 60(3):275-281.
16. Tadevosyan H, Kalantaryan V, Trchounian A. Extremely high frequency electromagnetic radiation enforces bacterial effects of inhibitors and antibiotics. Cell Biochem Biophys. 2008; 51(3):97-103.
17. Ming-Yan L, Kun S, Xu Z, Imshik L. Mechanism for alternating electric fields induced-effects on cytosolic calcium. Chin Phys Lett. 2009; 26(1):12-17.
18. Martins A, Machado L, Costa S, Cerca P, Spengler G, Viveiros M, Amaral L. Role of calcium in the efflux system of *Escherichia coli*. Int J Antimicrob Agent. 2011; 37(5):410-414.
19. Psifidi A, Dovas CI, Bramis G, Lazou T, Russel CL, Arsenos G, Banos G. Comparison of eleven methods for genomic DNA extraction suitable for large-scale whole-genome genotyping and long-term DNA banking using blood samples. PLoS One 2015; 10(1):e0115-960.
20. Forbes BA, Sahm DF, Weissfeld AS. Baily and Scott's Diagnostic Microbiology. 12th ed. Mosby (Elsevier). USA. 2007. 171-178 p.
21. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Natur Prot. 2008; 3(2):163.
22. Cockerill FR, Wikler MA, Alder J, Dudley MN, Eliopoulos GM, Ferraro MJ, Hardy DY, Hecht DW, Hindler JA, Patel JB, Powell M. Performance standards for antimicrobial susceptibility testing: twenty-second informational supplement. Clin Lab Standards Inst. 2012; 32(3):22-100.
23. Varaldo PE. Antimicrobial resistance and susceptibility testing: an evergreen topic. J Antimicrob Chemother. 2002; 50(1):1-4.
24. Taheri M, Mortazavi SM, Moradi M, Mansouri S, Hatam GR, Nouri F. Evaluation of the effect of radiofrequency radiation emitted from Wi-Fi router and mobile phone simulator on the antibacterial susceptibility of pathogenic bacteria *Listeria monocytogenes* and *Escherichia coli*. Dose-Resp. 2017; 15(1):155-581.
25. Onasanya A, Mignouna HD, Thottappilly G. Genetic fingerprinting and phylogenetic diversity of Staphylococcus aureus isolates from Nigeria. Afr J Biotech. 2003; 2(8):246-250.
26. Hamzah HM, Salah RF, Maroof MN. Fusarium mangiferae as New Cell Factories for Producing Silver Nanoparticles. J Micro Biotech. 2018; 28(10):1654-1663.
27. Sambrook JF and Russell DW. Molecular Cloning: A Laboratory Manual. 3rd ed. New York: Cold Spri Har Lab Press. 2012; 4:1-1879 p.
28. Mukhlif AS. The Genetic Diversity of a Number of Beansplant (FabaVicia) Genetic Compositions and their Individual Hybrids are Loaded using RAPD PCR Technology. Tikrit, Iraq: J Tikrit University for Agric Sci-Special Issue of the Sixth Sci Conf of Agric Sci. 2017; (17):579-595.
29. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers

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are useful as genetic markers. Nucl Acid Res. 1990; 18(22):6531-6535.
30. Belyaev I. Toxicity and SOS-response to ELF magnetic fields and nalidixic acid in *E. coli* cells. Mutation Research. Gen Toxi Environ Mutagen. 2011; 722(1):56-61.
31. Cranfield C, Wieser HG, Madan JA, Dobson J. Preliminary evaluation of nanoscale biogenic magnetite-based ferromagnetic transduction mechanisms for mobile phone bioeffects. IEEE Trans Nanobio. 2003; 2(1):3-40.
32. Nakouti I, Hobbs G, Teethaisong Y, Phipps D. A demonstration of a thermal effects of continuous microwave irradiation on the growth and antibiotic sensitivity of *Pseudomonas aeruginosa* PAO1. Biotech Pro. 2017; 33(1):37-44.
33. Gaafar ES, Hanafy MS, Tohamy EY, Ibrahim MH. The effect of electromagnetic field on protein molecular structure of *E. coli* and its pathogenesis. Rom J Biophys. 2008; 18(2):145-169.
34. Said-Salman IH, Jebaii FA, Yusef HH, Moustafa ME. Evaluation of Wi-Fi Radiation Effects on Antibiotic Susceptibility, Metabolic Activity and Biofilm Formation by Escherichia Coli O157H7, *Staphylococcus aureus* and *Staphylococcus epidermis*. J Biomed Phys Eng. 2019; 9(5):579-576.
35. Segatore B, Setacci D, Bennato F, Cardigno R, Amicosante G, Iorio R. Evaluations of the effects of extremely low-frequency electromagnetic fields on growth and antibiotic susceptibility of *Escherichia coli* and *Pseudomonas aeruginosa*. Int J Microb. 2012; 2:12-20.
36. Torgomyan H and Trchounian A. Escherichia coli membrane-associated energy-dependent processes and sensitivity toward antibiotics changes as responses to low-intensity electromagnetic irradiation of 70.6 and 73 GHz frequencies. Cell Biochem Biophys. 2012; 62(3):451-461.
37. Torgomyan H, Tadevosyan H, Trchounian A. Extremely high frequency electromagnetic irradiation in combination with antibiotics enhances antibacterial effects on *Escherichia coli*. Curr Microb. 2011; 62(3):962-967.
38. Polk C. Electric fields and surface charges induced by ELF magnetic fields Bioelectromagnetics. J Bioelectromagnetics Society. 1990; 11(2):189-201.
39. Fadel MA, Mohamed SA, Abdelbacki AM, El-Sharkawy AH. Inhibition of S almonella typhi growth using extremely low frequency electromagnetic (ELF-EM) waves at resonance frequency. J Appl Micro. 2014; 117(2):358-365.
40. Lee S, Hinz A, Bauerle E, Angermeyer A, Juhaszova K, Kaneko Y, Singh PK, Manoil C. Targeting a bacterial stress response to enhance antibiotic action. Proc Nat Acad Sci. 2009; 106(34):14570-4575.
41. Ayari S, Dussault D, Millette M, Hamdi M, Lacroix M. Changes in membrane fatty acids and murein composition of Bacillus cereus and Salmonella Typhi induced by gamma irradiation treatment. Internat J Food Microbiol. 2009; 135(1):1-6.
42. Blair JM, Richmond GE, Piddock LJ. Multidrug efflux pumps in Gram-negative bacteria and their role in antibiotic resistance. Fut Micro. 2014; 9(10):1165-1177.
43. Ke YL, Chang FY, Chen MK, Li SL, Jang LS. Influence of electromagnetic signal of antibiotics excited by low-frequency pulsed electromagnetic fields on growth of Escherichia coli. Cell Biochem Biophys. 2013; 67(3):1229-1237.
44. Cowell BA, Twining SS, Hobden JA, Kwong MS, Fleiszig SM. Mutation of lasA and lasB reduces Pseudomonas aeruginosa invasion of epithelial cells. J Microbio. 2003; 149(8):2291-2299.
45. Fregel R, Rodriguez V, Cabrera VM. Microwave improved Escherichia coli transformation. Lett Appl Microbio. 2008; 46(4):498-499.
46. Strašák L, Vetterl V, Fojt L. Effects of 50 Hz magnetic fields on the viability of different bacterial strains. Electro Bio Medic. 2005; 24(3):293-300.
47. Oncul S, Cuce EM, Aksu B, Inhan Garip A. Effect of extremely low frequency electromagnetic fields on bacterial membrane. Int J Radi Bio. 2016; 92(1):9-42.
48. Blair MW, Díaz LM, Buendía HF, Duque MC. Genetic diversity, seed size associations and population structure of a core collection of common beans (Phaseolus vulgaris L.). Theo Appl Gene. 2009; 119(6):955-972.

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