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(RESEARCH ARTICLE)

Effects of extremely low-frequency electromagnetic fields on the susceptibility and resistance mechanisms of multi-drug resistant *Pseudomonas aeruginosa*

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Abstract

The purpose of this study is to assess the impact of low-frequency electromagnetic fields (LF-EMFs = 0.5 MHz) on the phenotypic and genotypic levels concerning the antibiotic resistance, antimicrobial-resistant (AMR) genes, and resistance mechanisms of *Pseudomonas aeruginosa (P. aeruginosa)*. In this study, the bacterial isolates were exposed to 0.5 MHz and then inoculated to a new medium for the assessment of their antibiotic sensitivity. The results showed that there were statistically significant changes in the antibiotic sensitivity upon exposure to extremely low-frequency waves along with significant changes in the antimicrobial genes and resistance mechanisms. We conclude that extremely LF-EMFs appeared to be effective in changing the antibiotic sensitivity and could be considered a future promising method for controlling bacterial resistance.

Keywords: Electromagnetic Fields; Antibacterial Effects; Resistance; Pseudomonas; NGS

1. Introduction

The management of chronic wounds, which further exacerbates into infected wounds, is becoming a difficult and costly issue[1]. *Pseudomonas aeruginosa* (*P. aeruginosa*) is one of the most common bacteria that infect wounds. It is considered a troublesome microbe because of its ability to form resistant biofilms [2-4]. Nowadays, the extraordinary use of technologies increases the chances of exposure to non-ionizing, extremely low-frequency electromagnetic fields (ELF-EMFs) created by devices that are used inside houses, and workplaces[5]. Extremely low-frequency electromagnetic waves (ELF-EMWs) exert several effects on the biological functions of life forms such as the induction of genetic damage, cellular changes, and an increased risk of cancer[6]. These observations necessitate the study of the effects of ELF-EMF on bacteria for the investigation of environmental stress impact on the biological systems and extending the discussion of the possibility of controlling bacterial susceptibility toward antimicrobial agents *in-vitro and in-vivo.*

2. Patients, Materials, and Methodology

In total, 25 different resistant *Pseudomonas Aeruginosa*, according to inclusion (antimicrobial-resistant [AMR]* *P. aeruginosa*) and exclusion criteria (non-AMR *P. aeruginosa*), were collected from clinical samples (sputum, wound swabs, urine, and blood) of the patients in different clinical wards and intensive care units via aseptic techniques. These samples were transported immediately to the Medical Microbiology Department. Patients' data such as their personal

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history, clinical diagnosis, the number of hospitalization days, and antibiotic therapy used were also collected for this study.

As per the distribution of the 25 AMR *P. aeruginosa* isolates among the different clinical samples, the majority of the isolates were recovered from wound samples (11/25, 44%).

Table 1 Summarizes the distribution of *P. aeruginosa* isolates among the different clinical samples

	Sample type	**Count**	**%**
Pseudomonas aeruginosa	Wound	11	44%
	Sputum	6	24%
	Urine	5	20%
	Blood	3	12%
	Total	25	100

The collected samples were inoculated on nutrient agar, MacConkey agar, blood agar (Oxoid, England), and Chromogenic media (Biomerieux, France), then incubated aerobically for 48 hours at 37°C. *P. aeruginosa* was isolated and identified phenotypically according to the morphological and biochemical reactions. The final identification was confirmed by VITEK 2 Identification System (Biomerieux, France).

P. aeruginosa was identified by its colonial morphology on different culture media as greenish colonies on nutrient agar and non-lactose fermenting colonies on MacConkey agar, and beta-hemolytic on blood agar. Biochemical reactions such as sugar fermentation, indole, and oxidase, urease, and H2S production were performed. *In vitro*, antimicrobial susceptibility tests for various antimicrobial agents were conducted using VITEK 2 AST (N222) Cards according to the guidelines of the Clinical and Laboratory Standards Institute (2020)[7].

A 3 ml of 0.5 McFarland suspension of each isolate was prepared using sterile broth and was exposed to ELF-EMFs. Exposure of the isolates to extremely low-frequency electromagnetic waves (0.5 to 0.8Hz) for one hour in each session was done using the prototype apparatus for induction of the waves (figure 1).

Then, the isolates (before (control) and after exposure) were stored at −80°C till performing the genetic study by Illumina Next-Generation Sequencer (Illumina, U.S.A).

Figure 1 Electromagnetic wave prototype apparatus.

After the first exposure session, antimicrobial susceptibility tests using VITEK 2 were performed on the isolates and control. *Pseudomonas aeruginosa* ATCC 27853 was used as a control strain for susceptibility testing.

Both the isolates after exposure and control were sub-cultured on nutrient agar and incubated for 24 hours at 37ºC. The 0.5 McFarland solution was prepared from each subculture, which was then exposed again to the ELF-EMF session for one hour at the same resonance frequency and so on. These steps were repeated for five successive sessions.

2.1. Next-generation sequencing (NGS) using Illumina MiSeq machine

2.1.1. Sequence pre-processing and quality control

For each isolate, the read pairs were interleaved into a single FASTQ file using seqtk (v.1.3-r106) followed by the quality assessment of the interleaved files using FastQC (v. 0.11.8). The high-quality (mean per-base-sequence-quality ≥ 30) interleaved read pairs were merged using BBMerge (BBMap v. 38.94) into a single read to facilitate the alignment process. Using PRINSEQ (v. 0.20.4), the summary statistics of the reads were produced before and after the merging step. Thereafter, the format of the reads' files was converted from FASTQ to FASTA using seqtk. Next, after adding the name of each isolate to the header of its reads, all the isolates were concatenated together into a single FASTA file.

2.1.2. Alignment

The detection of AMR genes and drug classes took place by aligning the isolates against the CARD database (v.3.0.2) using Diamond (v.0.9.22)[8]. Then, the alignment results were saved in a tabular format.

2.1.3. Analysis and Visualization

The results of the alignments were imported to R studio for further analysis. Reads with less than 90 % identity or $1e^{-4}$ e value were filtered out. Firstly, the gene coverage was defined as the percentage of covered bases in each gene. Then, the gene copy number was calculated by dividing the number of reads aligned to each gene by its length. Another parameter, hits-per-thousand-reads, was calculated by dividing the number of aligned reads to each gene with the total number of aligned reads to all the genes. Consequently, the genes with less than 85 percent of coverage were filtered out. The resulting values were used to plot the heatmaps for both the genes and drug classes versus isolates, along with drug class's proportions graph and lollipop graph for the mean of coverage in all the isolates for each gene.

3. Results

In this study, antibiotic susceptibility was measured by using the VITEK 2 AST system before and after exposure to electromagnetic waves for five successive sessions for one hour each with an interval of 36 hours.

Before exposure, the 25 tested isolated were 100% resistant to Amikacin, Ampicillin, Ampicillin/sulbactam, Cefepime, Cefoxitin, Ceftazidime, Ciprofloxacin, Gentamycin, Levofloxacin, Meropenem, Nitrofurantoin, Piperacillin/tazobactam, Tobramycin. After the first session, there were minimal changes in the minimal inhibitory concentration (MIC) of some antibiotics, and resistance was still present in all the tested antibiotics. After the second session, the result revealed an increased susceptibility to the specific antibiotics group such as meropenem and ciprofloxacin. During the next three sessions, the MICs of different antibiotics were changed in different degrees of sensitivity (intermediate and highly sensitive). Additionally, there was no more improvement after the fourth session for all the isolates.

Table 2 Changes in the antibiotic's sensitivity pattern of the tested isolates before and after exposure to ELF-EMFs

Sensitivity pattern	Session 1	Session 2	Session 3	Sessions 4 and 5
Intermediate	None	Tobramycin Levofloxacin Cefepime	Tobramycin Levofloxacin Cefepime Ceftazidime	Levofloxacin Ceftazidime
Sensitive	None	Meropenem Ciprofloxacin	Meropenem Ciprofloxacin Gentamycin	Meropenem Ciprofloxacin Gentamycin Amikacin Tobramycin Cefepime Piperacillin/tazobactam

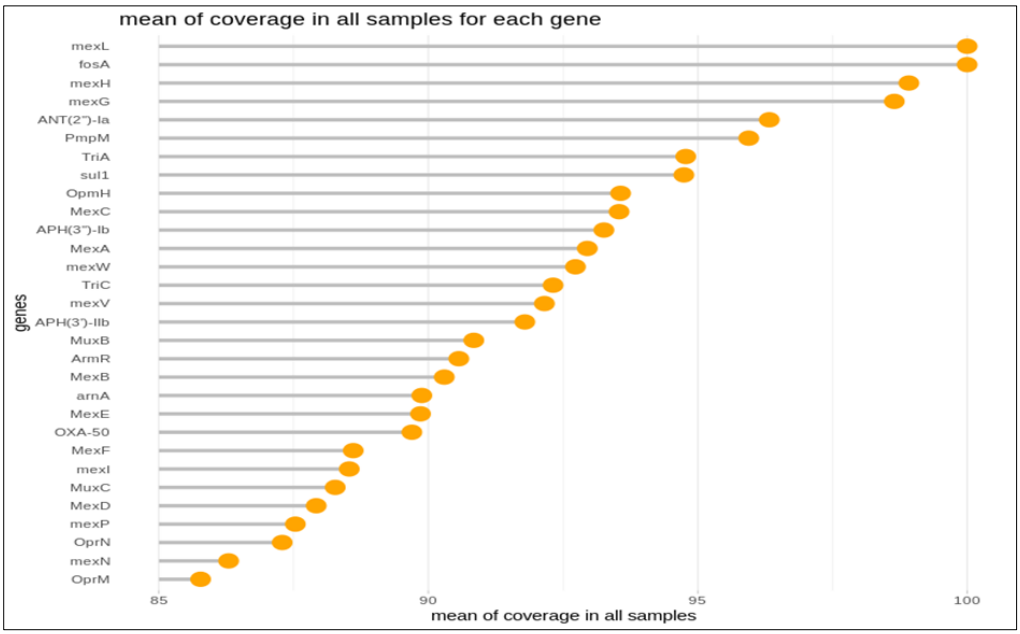

Figure 2 Lollipop plot of the mean coverage for each gene in all the samples. Each line represents the mean percent length of a single gene covered by sequencing reads. The mean was calculated from the read coverage in each sample. Genes included in further data analysis exceeded the cut-off coverage of 85%

Table 3 Clarifies the genetic profile and their correlation with the phenotypic susceptibility profile in the control sample that was not exposed to ELF-EMW

Sample name	protein.acc	Resistant gene	coverage	No. of reads	Gene copy num.
Control Sample (before exposure)	NP_248848.1	TriC	92.3152709	40	0.039409
	AAG07762.1	mexV	91.7553192	10	0.026596
	NP_252895.1	mexH	98.9189189	24	0.064865
	BAE06006.1	mexN	85.4247104	35	0.033784
	AAB41956.1	MexC	93.5400517	19	0.049096
	NP_251216.1	MuxC	86.1003861	36	0.034749
	NP_251184.1	MexF	89.2655367	37	0.03484
	NP_253661.1	OpmH	93.5684647	21	0.043568
	AAA74437.1	MexB	88.2409178	48	0.045889
	NP_251217.1	MuxB	88.9741131	39	0.037392
	NP_251183.1	MexE	89.8550725	19	0.045894
	NP_252368.1	mexL	100	12	0.056604
	NP_249116.1	MexA	87.4673629	14	0.036554
	NP_252896.1	mexI	88.5325559	29	0.028183
	AAG07763.1	mexW	92.7308448	43	0.04224
	NP_249820.1	fosA	100	7	0.051852
	AEJ33969.1	sul1	99.6415771	17	0.060932
	NP_250052.1	PmpM	96.6457023	32	0.067086

By using NGS, five randomly selected isolates (one for each session) and a randomly selected control sample underwent a genetic study. The genetic study showed 30 antimicrobial resistance genes in general. (Figure 2) shows the mean of coverage in all samples for each gene where we only included the genes that exceeded the cut-off coverage of 85%. The fourth session isolate was filtered out according to the filtration parameters because it did not reach the cut-off level.

In the control sample, 18 AMR genes conferring resistance to 11 drug classes were found (Table 8).

Table 4 clarifies the changes that occurred at the genotypic level and their correlation with that occurred at the phenotypic level after session one in the tested isolate. After session one, 19 genes were detected conferring resistance to 16 drug classes. (Table 8).

Table 4 AMR genes appearing after session one of exposure to ELF-EMW

Sample name	protein.acc	Resistant gene	coverage	No. of reads	Gene copy num.
1st session	NP_252244	arnA	89.8791541	35	0.05287
	AEJ33969.1	sul1	92.4731183	22	0.078853
	NP_252894.1	mexG	100	9	0.060811
	AAQ76277.1	OXA-50	89.6946565	13	0.049618
	NP_251216.1	MuxC	90.4440154	53	0.051158
	NP_251217.1	MuxB	92.7133269	52	0.049856
	AAA74437.1	MexB	92.3518164	67	0.064054
	NP_250052.1	PmpM	100	23	0.048218
	NP_251184.1	MexF	87.9472693	42	0.039548
	BAE06006.1	mexN	87.1621622	33	0.031853
	NP_251185.1	OprN	87.2881356	16	0.033898
	NP_252368.1	mexL	100	7	0.033019
	CAA62365.1	APH (3')-IIb	91.7910448	17	0.063433
	AAB41957.1	MexD	87.9194631	42	0.040268
	AAG07762.1	mexV	92.5531915	15	0.039894
	NP_248846.1	TriA	94.7780679	20	0.052219
	NP_249118.1	OprM	85.7731959	24	0.049485
	NP_249820.1	fosA	100	8	0.059259
	BAE06007.1	mexP	87.5324675	13	0.033766

After session two, only one gene (mexG) -as other genes were rolled out due to not exceeding the cut-off coverage of 85%- was detected and conferring to abroad spectrum of drug classes (Tables 5, 8).

Table 5 AMR genes appearing after session two of exposure to ELEMW

Sample name	protein.acc	Resistant gene	coverage	No. of reads	Gene copy num.
2nd session	NP_252894.1	mexG	97.2972973	5	0.033784

Total six genes in session three appeared conferring to four drug classes (Table 6, 8).

Table 6 AMR genes appearing Session 3 of exposure to ELF-EMW

Sample name	protein.acc	Resistant gene	coverage	No. of reads	Gene copy num.
3rd session	AEJ33969.1	sul1	92.1146953	12	0.043011
	NP_249116.1	MexA	98.4334204	14	0.036554
	NP_249820.1	fosA	100	5	0.037037
	NP_252408.1	ArmR	90.5660377	1	0.018868
	NP_250052.1	PmpM	91.1949686	16	0.033543
	AAC64365.1	ANT (2'')-Ia	99.4350283	6	0.033898

After session five, the two genes that appeared expressed aminoglycoside resistance (Table 7, 8).

Table 7 AMR genes appearing after session 5 of exposure to ELF-EMW

Sample name	protein.acc	Resistant gene	coverage	No. of reads	Gene copy num.
5th session	AAC64365.1	ANT (2'')-Ia	93.220339	6	0.033898
	ABK33456.1	APH (3'')-Ib	93.258427	7	0.026217

Table 8 The grouping of AMR genes according to the drug classes and resistance mechanisms

Gene	Drug Class	Resistance Mechanism
OXA-50	cephalosporin, penam	antibiotic inactivation
arnA	peptide antibiotic	antibiotic target alteration
ArmR, MexA, MexB, MexD, MexE, MexF, MexG, MexH, MexI, MexL, MexN, Mexp MexV, MexW, MuxB MuxC, OpmH, OprM, OprN, PmpM, TriA TriC	tetracycline antibiotic, acridine dye, triclosan, antibacterial free fatty acids, aminoglycoside antibiotic, phenicol antibiotic, diaminopyrimidine antibiotic, aminocoumarin antibiotic, monobactam, glycylcycline, macrolide antibiotic, penam, carbapenem, fluoroquinolone antibiotic	antibiotic efflux
ANT (2'')-Ia APH (3'')-Ib APH (3')-IIb	aminoglycoside antibiotic	antibiotic inactivation
Sul1	sulfonamide antibiotic, sulfone antibiotic	antibiotic target replacement
FosA	fosfomycin	antibiotic inactivation

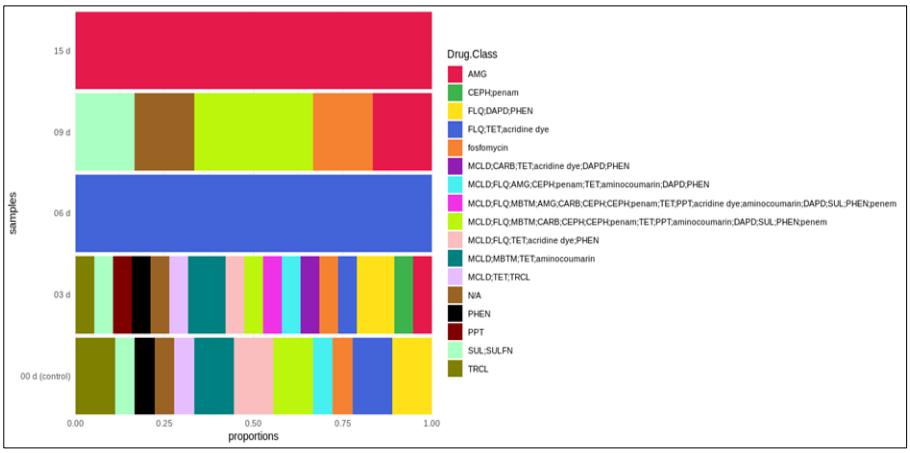

Figure 3 Stacked bar graph showing the drug classes represented in each isolate after the grouping of the AMR genes

Each gene of the previously mentioned 30 AMR genes represent a specific drug class that has a specific resistance mechanism. Some genes can share the same drug class, and some drug classes also can share the same resistance mechanism as shown in *Figures (3, 4) and Table 8*.

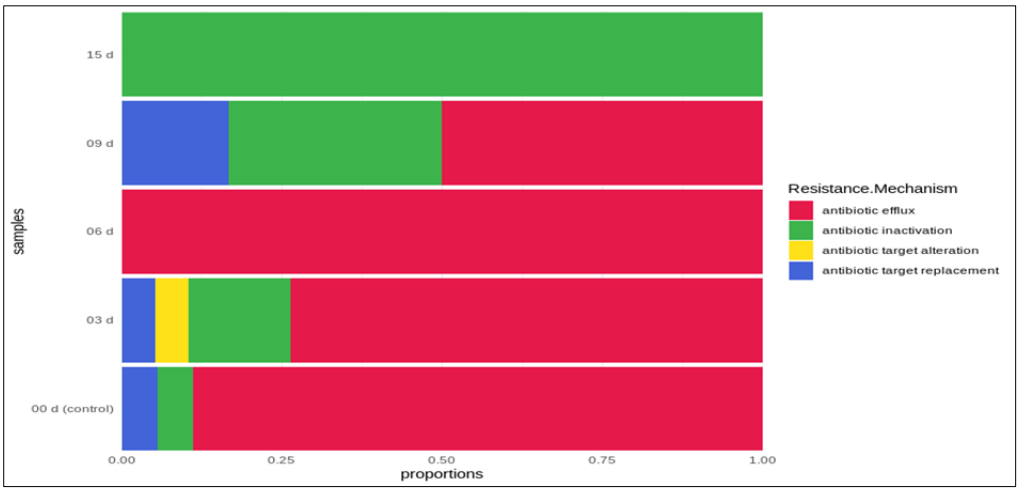

Figure 4 Stacked bar graph showing the resistance mechanisms represented in each isolate after the grouping of the AMR genes by different mechanisms. Each color represents resistance mechanism: blue, antibiotic target replacement; yellow, antibiotic target alteration; green, antibiotic inactivation, and red, antibiotic efflux.

4. Discussion

Pseudomonas are omnipresent and are found around the world. These organisms are known for their potency to develop an innate resistance to many antibiotics. *P. aeruginosa* is one of the most important species that can infect humans. It is the most common human saprophyte; however, it is seldom a cause of disease in a healthy person[9].

Pulsed electromagnetic field (PEMF) technologies have demonstrated its usefulness in the treatment of infected wounds. These relatively simple devices use an external, non-invasive PEMF to generate shorts bursts of electrical current in the injured tissue without producing heat or affecting nerve or muscle function[10]. With these devices, PEMF therapy has been broadened to include the treatment of postoperative pain and edema, thereby offering the physician a more adaptable tool for the management of patients[11].

NGS technology is a great revolutionary technology that delivers fast, inexpensive, and accurate genome information. The inexpensive production of large volumes of sequence data is its primary advantage over conventional methods[12].

Next-generation DNA sequencing can dramatically accelerate biomedical research by enabling the comprehensive analysis of genomes, transcriptomes, and interactomes to develop into a routine, widespread, and inexpensive method, rather than requiring significant production-scale efforts[13].

The objectives of this study were to apply and evaluate a new method to control the high resistance of *P. aeruginosa* through the exposure of these resistant isolates to the ELF-EMW and observe its effects at the phenotypic and genotypic levels concerning the isolates' antibiotic resistance, AMR genes, and resistance mechanisms.

After the first session of exposure to ELF-EMW at a significant resonance frequency, all the exposed isolates showed minor changes represented as reduced MICs of meropenem but without reaching the cut-off concentration of susceptibility. However, after the second session of exposure, three antibiotics (meropenem, ciprofloxacin, and tigecycline) showed significant susceptibility toward the isolates, and three other antibiotics (tobramycin, levofloxacin, and cefepime) had shown intermediate susceptibility. By continuing exposure, in the third session, one more antibiotic (gentamycin) showed a decrease in its MIC, thus exceeding the cut-off value of susceptibility, whereas ceftazidime showed intermediate susceptibility.

After the fourth session, three antibiotics altered their susceptibility pattern from being intermediately susceptible (tobramycin and cefepime) and completely resistant (piperacillin/tazobactam) to completely sensitive to all the isolates. All the isolates showed no further improvement after the fourth session.

After all the sessions, all the isolates showed a gradual improvement to the antimicrobial agent through the successful five sessions, thereby revealing the significant effects of ELF-EMF on antibiotics' susceptibility. The depressive effect of electromagnetic waves at different frequencies on the growth and viability of bacteria could be an alteration of membrane proteins conformation, changing cell morphology and sizes leading to change in bacterial sensitivity toward the chemicals, especially to antibiotics [14]. Our results were in concordance with Stansell et al., 2001 [15] who found that the static fields of moderate-intensity were able to cause a decrease in the antibiotic sensitivity and resistance of *E. coli* and *P. aeruginosa*.

Our finding was also matched with a study by Ibraheim & El-Din Darwish, 2013 [16] they demonstrated that there was a little decrease in the susceptibility of exposed pseudomonas isolates to the antibiotics Amikacin, ceftriaxone, norfloxacin, Rifampicin, and ciprofloxacin revealed by a decrease in the zone of inhibition by 1mm. While studying Bacillus subtilis after exposure to the electromagnetic field for 14 hours they found a decrease in the resistance to Amikacin, ceftriaxone, norfloxacin, Rifampicin revealed by an increase in the zone of the diameter of the microorganism to antibiotics.

Kamel et al., 2013 [17] detects the effect of electromagnetic waves on changing the antibiotic susceptibility on *P. aeruginosa* isolates revealed an increase in their zone of inhibition after 6 hours of exposure. While after 16 hrs. exposure, it became more resistant to the antibiotics. So, the duration of exposure to electromagnetic waves could have a different effect on bacterial resistance.

To investigate whether the changes that occurred at the phenotypic level had occurred at the genotypic level as well, six isolates including the control underwent genetic study by NGS to detect changes in AMR genes and the mechanisms of drug resistance. After applying bioinformatics analysis on the 6 isolates, the session 4 isolate results were filtered out according to the filtration parameters. In general, we obtained 30 anti-microbial resistance genes in the six studied isolates.

Before their exposure to ELF-EMW, "the control sample" bacteria exhibited a broad range of antibiotic resistance, which was based on three resistance mechanisms (antibiotic target replacement, antibiotic inactivation, and majorly unspecific antibiotic efflux mechanism) expressed by 18 AMR genes conferring to 11 drug classes.

Antibiotic target replacement is expressed by the (Sul1) gene, which is responsible for resistance toward sulfonamide and sulfone antibiotics. The antibiotic inactivation mechanism is expressed by the (FosA) gene, which is responsible for fosfomycin resistance. There are 16 (TriC, mexV, mexH, mexN, MexC, MuxC, MexF, OpmH, MexB, MuxB, MexE, mexL, MexA, MexI, mexW, and fosA) genes that expressed the same antibiotic efflux mechanism and shared resistance to a broad range of substances with antimicrobial effect as tetracycline antibiotic, acridine dye, triclosan, antibacterial free fatty acids, aminoglycoside antibiotic, phenicol antibiotic, diaminopyrimidine antibiotic, aminocoumarin antibiotic, monobactam, glycylcycline, macrolide antibiotic, penam, carbapenem, and fluoroquinolone antibiotic[8].

After the first session of exposure to ELF-EMW at a significant resonance frequency, the genetic study shows the introduction of a new mechanism (antibiotic target alteration) in addition to the previously mentioned three mechanisms as a strategy to overcome the stress effect of electromagnetic radiation expressed by the appearance of one more AMR gene (arnA).

After this session, this gene was responsible for peptide antibiotics resistance, namely, actinomycin, bacitracin, colistin, and polymyxin B.

However, this strategy failed to eliminate the stress effect of radiation; therefore, the bacteria switched totally to an alternative unspecific antibiotic efflux mechanism after the second session was expressed by the (MexG) gene with a broad unspecific range of drug classes. In a failure to overcome the damaging effect of electromagnetic waves, *P. aeruginosa* introduced two extra strategies (antibiotic inactivation and antibiotic target replacement), which were expressed by six AMR genes. However, this approach did not come up with lengthy exposure to ELF-EMW. This strategy explains the sharing of some genes between previous sessions with each other and with a control sample. After the last session, the bacteria were specially designed with genetic machinery to specifically inactivate aminoglycoside drug class expressed in two genes (ANT (2'')-Ia and APH (3'')-Ib), but these two genes seemed to be mutated and inactivated concerning phenotypic susceptibility result.

Overall, the approach of *P. aeruginosa* tends to overcome the stress effect of ELF-EMW by designing and shifting among the resistance mechanisms, but all the trials resulted in failure.

5. Conclusion

Finally, we conclude that the use of ELF-EMWs, the new non-invasive technique, could be considered as a future promising method for the control of bacterial resistance because of its effect on susceptibility to antibiotics. Additionally, it will be of considerable interest for use in medical and biotechnological applications.

Recommendation

Unfortunately, we e couldn't inspect all variations that occurred after exposure to extremely low frequency electromagnetic waves, A largescale study in the form of the project is recommended for better evaluation of all factors affecting the results including different durations of exposure, different wavelengths, different types of bacteria…… etc. The research should include the examination of transcriptomics as well as proteomics through different techniques (MALDI TOF and Electron Microscope) for documentation of electromagnetic wave effect. Proceeding to animal trials to evaluate the effect *in-vivo*.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Lu J, Cokcetin NN, Burke CM, Turnbull L, Liu M, Carter DA, et al. Honey can inhibit and eliminate biofilms produced by Pseudomonas aeruginosa. Scientific reports. 2019;9(1):1-13.

[2] Bjarnsholt T, Kirketerp-Møller K, Jensen PØ, Madsen KG, Phipps R, Krogfelt K, et al. Why chronic wounds will not heal: a novel hypothesis. Wound repair and regeneration. 2008;16(1):2-10.

[3] Ammons MC, Ward LS, Fisher ST, Wolcott RD, James GA. In vitro susceptibility of established biofilms composed of a clinical wound isolate of Pseudomonas aeruginosa treated with lactoferrin and xylitol. Int J Antimicrob Agents. 2009;33(3):230-6.

[4] Ratjen F, Saiman L, Mayer-Hamblett N, Lands LC, Kloster M, Thompson V, et al. Effect of azithromycin on systemic markers of inflammation in patients with cystic fibrosis uninfected with Pseudomonas aeruginosa. Chest. 2012;142(5):1259-66.

[5] Fadel M, Mohamed S, Abdelbacki A, El-Sharkawy A. Inhibition of S almonella typhi growth using extremely low frequency electromagnetic (ELF-EM) waves at resonance frequency. Journal of applied Microbiology. 2014;117(2):358-65.

[6] Su L, Yimaer A, Wei X, Xu Z, Chen G. The effects of 50 Hz magnetic field exposure on DNA damage and cellular functions in various neurogenic cells. Journal of radiation research. 2017;58(4):474-86.

[7] CLSI.Performance Standard for Antimicrobial Susceptibility Testing. 30th ed. CLSI suplement M100. Wayne, PA: Clinical and Laboratory Standard Inistitute; 2020 .

[8] Alcock BP, Raphenya AR, Lau TT, Tsang KK, Bouchard M, Edalatmand A, et al. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic acids research. 2020;48(D1):D517-D25.

[9] Yayan J, Ghebremedhin B, Rasche K. Antibiotic resistance of Pseudomonas aeruginosa in pneumonia at a single university hospital center in Germany over a 10-year period. Plos one. 2015;10(10):e0139836.

[10] Strauch B, Herman C, Dabb R, Ignarro LJ, Pilla AA. Evidence-based use of pulsed electromagnetic field therapy in clinical plastic surgery. Aesthetic Surgery Journal. 2009;29(2):135-43.

[11] Aaron RK, Boyan BD, Ciombor DM, Schwartz Z, Simon BJ. Stimulation of growth factor synthesis by electric and electromagnetic fields. Clin Orthop Relat Res. 2004;419(419):30-7.

[12] Metzker ML. Next generation technologies: basics and applications. Environ Mol Mutagen. 2010;51(691):691.

[13] Shendure J, Ji H. Next-generation DNA sequencing. Nat Biotechnol. 2008;26(10):1135-45.

[14] Soghomonyan D, Trchounian K, Trchounian A. Millimeter waves or extremely high frequency electromagnetic fields in the environment: what are their effects on bacteria? Applied microbiology and biotechnology. 2016;100(11):4761-71.

[15] Stansell MJ, Winters WD, Doe RH, Dart BK. Increased antibiotic resistance of E. coli exposed to static magnetic fields. Bioelectromagnetics. 2001;22(2):129-37.

[16] Ibraheim MH, El-Din Darwish D. Hz frequency magnetic field effects on Pseudomonas aeruginosa and Bacillus subtilis bacteria. IOSR J Appl Phys. 2013;5(3):2278-4861.

[17] Kamel FH, Saeed CH, Qader SS. The static magnetic field effect on Pseudomonas aeruginosa. Trends Biotechnol Res. 2013;40:M371-M6.