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CYTOTOXICITY TEST ON THE EFFECT OF MODULATED ELECTROMAGNETIC WAVE ON NON-PATHOGENIC *E.COLI* CULTURE

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Abstract: We investigated the effects of modulated electromagnetic wave on non-pathogenic *E. coli* HB101 bacteria culture by performing cytotoxicity test with *Alamar* blue dye. The *E. coli* samples were prepared and grouped (*i.e.* control group and treated group) in a 96-culture well plate. The electromagnetic wave with a ***3.3 MHz*** carrier frequency is modulated at several audio frequencies to find the appropriate resonant frequency that can debilitate *E. coli's* viability. Cytotoxicity test showed that from ***12,342.60 Hz*** to ***12,413.72 Hz*** modulating frequency, there is a significant reduction of *E. coli* bacterial growth under *37.5 °C* incubation temperature. Other modulating frequencies showed a significant increase in *E. coli* bacterial growth under the same cytotoxicity test. With proper frequency control program settings, the highly selective resonant frequency for killing the *E. coli* bacteria and widely available frequencies for bacterial growth can be an alternate non-invasive treatment tool in managing non-pathogenic *E.coli's* population under prevailing laboratory conditions. Under these resonant frequencies, it is possible to indirectly determine the *E.coli's DNA* base pair size.

Keywords: *Escherichia coli*; Resonance; Cytotoxicity; Electromagnetic Wave; *DNA* Base Pair Size; Medical Instrumentation

1. INTRODUCTION

Diarrhea related diseases are one of the major causes of mortality under the age of 5. It is responsible for the death of 5 million children every year. A statistical study conducted by the Department of Health in the Philippines, diarrhea related disease is the second leading cause of infant deaths and is the sixth cause of mortality in all age group. According to the Institute for Tropical Medicine Rotavirus and E. coli strains are the primary cause of diarrhea in Metro Manila. The ingestion of *Escherichia coli (E. coli)* contaminated food or water can cause gastrointestinal infection and diarrhea which can lead complications and ultimately death. However, due to budget constraints the government is unable to regulate the water present for the consumption for the public.

E. coli bacteria are fast producing bacteria that are mostly present in the intestines of warm blooded animals. Although adult patients infected with pathogenic *E. coli* bacteria do not need treatment, but in extreme cases the treatment only includes the replacement of fluids and electrolytes to only prevent dehydration and not the bacteria itself. Studying alternative remedy for *E. coli* bacteria could provide a cost-effective approach in treating *E. coli* related diseases. Developed in the 20th century, the Rife machine is a non-invasive and non-conventional treatment created by Dr. Royal Rife. It is said that the Rife machine eliminate a specific virus bacteria based on its bio frequency using the concept of resonance. The potential use of the Rife machine as an alternative treatment device could possibly reduce the cost of the treatment procedure and it might ease the preparation protocol since its mode of treatment is non-invasive in practice.

Since the Rife machine is an electromagnetic wave generator, a well balanced scientific study has to be developed on how radio waves can affect the viability of the *E. coli* bacteria. The researchers investigated the

effects of the modulated electromagnetic wave signal on non-pathogenic *E. coli* HB101 bacteria culture by performing cytotoxicity test using *Alamar* blue assay. The statistical "t" tests will be conducted in studying the effect of ultrasound on the E. coli bacteria to determine how significant is the effect of the ultrasound irradiation on the E. coli bacteria using the Rife machine. A control group is considered to determine if the exposed group would have a significant decrease or increase in the population size of the E. coli bacteria.

2. METHODOLOGY

2.1 Calculation of Rife Frequency Bands

Different audio frequencies modulated by the ***3.3 MHz*** carrier frequency of the beam ray were first calculated and determined. The frequencies were calculated by correlating the total length the DNA structure into wavelength. The speed of the radio wave is then divided by the calculated wavelength. The quotient will be the fundamental Rife frequency and is divided down by orders of two to access the side bands of the ***3.3 MHz*** carrier frequency from the audio frequency range. However, the speed of the radio wave differs significantly for each medium. In order to compute for the speed of the radio waves, the permittivity and the permeability of each medium were considered.

The four different medium which the radio wave will travel were air, water, biological tissue and cell membrane and the calculated frequencies were ***250.05Hz***, ***223.28Hz***, ***176.60Hz***, and ***166.70Hz*** respectively. In this study we include the highest octave before reaching the ultrasound range of the calculated frequencies mainly ***16002.94 Hz***, ***14277.41 Hz***, ***11302.54 Hz*** and ***10668.65 Hz*** respectively. In this study, the researchers also included the frequencies provided by the *Beam Ray, Inc.* in treating *E. coli* infection.

The researchers also included the calculations of Dr. Philip Hoyland's sideband frequencies of ***3.3MHz*** carrier. Using Dr. Philip Hoyland's sideband frequencies of ***3.3MHz*** carrier frequency calculated from the ***8,020 Hz*** and ***17,220 Hz*** audio frequency with a 10-minute exposure time. By integrating both the idea of the frequencies at different speed of radio wave at different medium and Dr. Philip Hoyland's 3.3MHz sweep the researchers have formulated a different set of frequencies. The frequencies representing Air ***194.52 Hz*** and ***12,449.28 Hz***, for water ***176.74 Hz*** and ***11,311.36 Hz*** and for biological membrane are ***198.51 Hz*** and ***12,704.64 Hz*** each with a 10-minute exposure time.

2.2 Preparation of *E. Coli Hb101*

The *E. coli* were provided by the Molecular Biology Laboratory. The *E. coli* were cultivated in a culture media like Luria-Bertani medium (an Erlenmeyer flask that contains all the nutrients the bacteria needed in order to grow). From a culture broth of *E. coli*, it was inoculated in several media for different testing.

2.3 Distribution of Sample

The *E. coli* were placed in a 96 culture well plates using the micropipette. Three wells will serve as a replicate for each specific frequency and time. Each well contains 100µl of the bacterial sample.

2.4 Exposure of *E. Coli* in Specific Rife Frequency Bands

The Rife machine was employed during the experiment to be able to determine its effect on *E. coli.* The proponents will prepare two (2) different set-ups in this undertaking. The first set of sample consists of *E. coli*

unexposed to the Rife machine, which were the control group. Second set of sample is the experimental group in which the *E. coli* were exposed to the commercially available rife machine.

2.5 Distribution of Dye/Alamar Blue

After exposure of the *E. coli* to the Rife machines, the wells with the bacteria were added with 5.0 µl alamar blue.

2.6 Cytotoxicity test of the Samples

After the treatment, the *E. coli* were incubated for 15 minutes at 37ºC and the treated wells were scanned individually with a visible spectrophotometer. The spectrophotometer was used to determine the spectroscopic profile of the treated samples. The spectroscopic profile can directly indicate the cytotoxicity level of *E. coli* at the absorbance wavelength of *570 nm*.

2.7 Statistical Analysis Using *t*-Test

Results of the cytotoxicity of the control group and the exposed groups of E. coli will undergo statistical test (t-test). If the "p" value is less than the alpha level there is a statistical difference between the control and exposed group however if "p" value is greater than the alpha level there is no statistical difference between the control and exposed group. The t-test was implemented using Microsoft EXCEL application software installed in a desktop computer.

3. RESULTS AND DISCUSSION

3.1 Initial Resonant Frequencies for Different Media

The frequencies were calculated by converting the length of the DNA into frequencies with respect to the speed of the radio wave at different medium and each sample were exposed for 10 minutes. ***250.05 Hz*** and ***16,002.94 Hz*** were used to represent the speed of radio waves in air, ***176.6 Hz*** and ***11302.54 Hz*** for the biological membrane, ***223.28 Hz*** and ***14277.41 Hz*** at water and ***166.7 Hz*** and ***10668.65Hz*** for the cell membrane. *Alamar* blue assay was used to measure the cytotoxicity of the samples and then later compare the control group against the exposed group. The cytotoxicity measurement is the measure of quantities of biological chemicals in a sample, in return one can measure the metabolic function of the *E. coli* thus it also identifies if the cell growth of the sample increases or decreases.

In table 1 the researchers found out that the cytotoxicity of the exposed cell increased approximately by 5-24% compared to the control group. Also by using the statistical analysis *"t" test* the researcher found out that there is a significant growth in almost all of the exposed cells with respect to the control group because the "p" value is less than the alpha level. Furthermore, the values on the table do not show any linear relationship between the Rife frequencies and the mean cytotoxicity percentage.

Table 1 Comparison of Cytotoxicity in Different Frequency

Medium	Rife Freq.Hz	1	2	3	$Mean_a$-$Mean_b$	*t*	P one-tailed
Air	**250.05**	1.749	1.775	1.771	0.2603	4.83	0.004
	16,002.94	1.554	1.607	1.611	0.086	1.53	0.100
Biological Membrane	**176.6**	1.785	1.821	1.805	0.299	5.51	0.003
	11,302.54	1.677	1.619	1.647	0.143	2.56	0.03
Water	**223.28**	1.811	1.805	1.850	0.3173	5.76	0.002
	14,277.41	1.699	1.681	1.650	0.172	3.12	0.018
Cell Membrane	**166.7**	1.700	1.852	1.885	0.308	3.94	0.008
	10,668.65	1.701	1.739	1.738	0.2213	4.04	0.009
	Control	1.535	1.401	1.578	Δt= 10 minutes		

3.2 Clinically Prescribed Frequencies

The researchers also include the thirty-four frequencies that are used in treating the *E. coli bacteria* that were provided by the beam ray company. The thirty-four frequencies range from ***282-7849 Hz***. Each frequency has duration of 2 minutes.In table 2, with the same statistical parameters for the clinically prescribed frequency set; the computed *t* value is 4.53 which gave a *p* one-tailed value of 0.005. Since the *p* value < α, it shows a significant increase in the population size.

Table 2 Comparison of Cytotoxicity in Clinic Frequency

Hertz	1	2	3	$Mean_a$-$Mean_b$	t	P one-tailed
Clinic Frequency	0.905	1.078	1.079	0.3153	4.53	0.005
Control	0.739	0.749	0.628	Δt= 66 minutes		

3.3 The Rife Frequency Side Bands for Different Media

Using Dr. Philip Hoyland's sideband frequencies of ***3.3MHz*** carrier frequency calculated from the ***8,020 Hz*** and ***17,220 Hz*** audio frequency with a 10-minute exposure time. In table 3, the results show a 4-20% increase in the cytotoxicity level. This is indicative of the test organism's viability and multiplicity. Using the concept of the frequencies at different speed of radio wave at different medium and Dr. Philip Hoyland's 3.3MHz sweep the researchers have formulated a different set of frequencies. The frequencies representing Air ***194.52 Hz*** and ***12,449.28 Hz***, for water ***176.74 Hz*** and ***11,311.36 Hz*** and for biological membrane are ***198.51 Hz*** and ***12,704.64 Hz*** each with a 10-minute exposure time. In this study, the exposed samples shows a 4%-16% increase in cytotoxicity level and using the statistical analysis *t*-test the researchers conclude that almost all of the exposed cells have no significant increase or decrease of cytotoxicity with respect to the control group but at frequencies ***194.52 Hz***, ***12,704.64 Hz*** and ***11,311.36 Hz*** the exposed groups shows a 1%, 3% and 5% decrease of cyototoxicity respectively. However, using statistical analysis *"t" test* the researchers

found out that there is still no significant decrease in the cytotoxicity of the exposed cells with respect to the control group.

Table 3 Cytotoxicity Level of Samples Exposed to the Original Rife Frequencies

Medium	Rife Freq.Hz	1	2	3	$Mean_a$-$Mean_b$	*t*	P one-tailed
Air	194.52	1.474	1.602	1.561	-0.016	-0.36	0.369
	12449.28	1.451	1.527	1.566	-0.046	-1.18	0.152
Biological Membrane	176.74	1.68	1.784	1.822	0.201	4.26	0.007
	11311.36	1.372	1.577	1.500	-0.078	-1.24	0.141
Water	198.51	1.548	1.634	1.61 0	0.036	1.1	0.167
	12704.64	1.773	1.674	1.759	0.174	4.69	0.005
	Control	1.545	1.602	1.537	Δt= 10 minutes		

3.4 Searching Scheme to Determine the Resonant Frequency from *11,311.36 Hz* to *12,229.28 Hz*

Based from the cytotoxicity results from the original frequency side bands of Dr. Raymond Royal Rife, we managed to identify two prominent frequencies in Table 3 indicative of decrease in cytotoxicity level. Although the decrease of the cytotoxicity level is still insignificant, these frequencies could guide us in finding the resonant frequency that can debilitate the *E. coli* samples. For frequencies from ***11,311.36 Hz*** to ***12,229.28 Hz***, it shows no significant decrease in the cytotoxicity level between the exposed *E. coli* group and the control *E. coli* group. It is important that we developed a searching scheme from ***11,311.36 Hz*** to ***12,229.28 Hz*** on how to find the right resonant frequency that will debilitate a non-pathogenic type of *E. coli* bacteria. The developed searching scheme used the sweep function of the beam ray which has a 50 Hz less range. In total there are 25 frequency range and have an average of 3.37 seconds per frequency.

Among the list of frequencies discussed two major effects that made a significant change in the cytotoxicity level of *E. coli* bacteria. In table 4, first effect can be characterized as those frequencies that have significant *E. coli* bacterial growth which can be attributed to the significant increase in the cytotoxicity level of the treated *E. coli* bacteria while in table 5, the second effect that can be regarded as those frequencies that have significant *E. coli* reduction or debilitation.

Table 4 Cytotoxicity Level of Samples Exposed to the Original Rife Frequencies

Frequency, Hz	***p* one-tailed**	**Significant**	**Frequency, Hz**	***p* one-tailed**	**Significant**
11,408.08-11,453.60	0.003	Yes	11,915.99-11,951.44	0.036	Yes
11,809.20-11,844.76	0.026	Yes	11,951.44-11,987.00	0.043	Yes
11,844.76-11,880.32	0.034	Yes	12,307.04-12,342.60	0.048	Yes

Table 4 is the list of frequencies that showed significant increase in the cytotoxicity level of the treated *E. coli* bacteria. All these data has *t* value greater than **2.13** and *p* one-tailed value less than the alpha level (**0.05**) which is the threshold value for significant change. These statistical parameters facilitated the alternate hypothesis in the statistical *t*-test that there is a significant increase in the bacterial growth manifested in the cytotoxicity level of *E. coli* bacteria.

Table 5 Cytotoxicity Level of Samples Exposed to the Original Rife Frequencies

Frequency, Hz	*p* one-tailed	Significant
12,342.60-12,378.16	0.028	Yes
12,378.16-12,413.72	0.033	Yes

Table 5 is the list of frequencies that showed significant decrease in the cytotoxicity level of the treated *E. coli* bacteria. All these data has *t* value greater than **2.13** and *p* one-tailed value less than the alpha level (**0.05**) which is the threshold value for significant change. These statistical parameters facilitated the alternate hypothesis in the statistical *t*- test that there is a significant *E. coli* bacterial mortality. We can now state that one of the several resonant or Rife frequencies of non-pathogenic type of *E. coli* (HB101) bacteria is possibly between ***12,342.60 Hz*** to ***12,413.72 Hz*** with an average exposure time of ***3.37*** seconds per frequency.

The increase temperature can contribute to the increase of bacterial growth. At higher temperature, bacterial cell growth increases as shown by B. Rudolph et al. The continued usage of the plasma tube may contribute to the slight increase in temperature of the surrounding medium. Most especially if the increase in temperature is within the optimum temperature range of growth of *E. coli* which is around 37.5C. Another reason to the increase of bacterial growth is the activation of heat shock proteins during RF exposure of the bacteria that increases protein activation according to F. Jebai. Thus, if there is an increase inbacterial cell metabolism then it would eventually lead to bacterial cell growth.

On the other hand, the decreased growth of non-pathogenic *E. coli* is fairly limited with the available frequencies tested. This behaviour is a well-known wave phenomenon in *Physics* – resonance. For this case, resonance happens when the fundamental frequency of the incident *EM* or *RF* wave or its harmonics matches with the DNA base pair length of the non-pathogenic type of *E. coli* HB101 bacterium. In resonance, *E. coli* can constructively absorb the energy and momentum of the incident *EM* wave resulting into the amplification of the internal energy of *E. coli*. The increase in the internal energy of *E. coli* would consequently lead to the bacterial cell death due to internal heating. Under these resonant frequencies, it is possible to directly determine the non-pathogenic type of *E. coli's* HB101 DNA base pair size as listed in Table 6 below.

Table 6. DNA Base Pair Size of Non-Pathogenic *E. coli* HB101 Bacteria with Error Bars

Medium	Rife Frequency, Hz	Harmonic	DNA Base Pair Size	Error Bar
Water	12,413.72	11th	5,474,162	±273,708
	12,342.60	11th	5,505,705	±275,285

Since the *E. coli* culture is mostly surrounded by water in this case, we were able to compute the DNA base pair length of the *E. coli* bacterium taken from the resonance length of the incident *RF* or *EM* wave that can be derived from the Rife frequency. Below is the outcome of the calculations done for the non-pathogenic type of *E. coli* HB101 bacterium with error bar delimited by the statistical parameter alpha level set at **0.05** or set at 5% uncertainty.

4. CONCLUSION

Many studies has been done on the effect of electromagnetic wave on living cells. The quest to find a reliable and accessible treatment tool for most dreaded diseases has been motivated by the development of new medical techniques and instrumentation. *E. coli* bacteria have been one of the causative agents on the onset of diarrhea cases in the Philippines. In this study, radio waves emission from modulated frequency bands with carrier frequency centered at ***3.3 MHz*** was used to target the non-pathogenic type or of *E. coli* HB101 bacteria.

With proper frequency control settings in the Rife machine, it has the potential to facilitate a control system with a fail-safe mechanism for the elimination of good type of *E. coli* bacteria and an accessibility mechanism for a wide selection of frequency to increase the bacterial growth. This is an ideal treatment tool in managing good type of *E. coli* bacteria given these inherent mechanisms. Therefore, this particular Rife machine can be an alternate non-invasive treatment tool in managing the *E. coli* population under prevailing laboratory conditions. Under these resonant frequencies, it is possible to indirectly determine the *E.coli's DNA* base pair size.

5. REFERENCES

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