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Assessment of RF Radiation on E. Coli Antibacterial Resistance

Question: What is the effect of different radio frequencies, intensities, and exposure times emitted by Wi-Fi routers on E. Coli antibiotic resistance and normal growth?

Hypothesis: Increased radiation frequency, intensity, and exposure time will increase antibiotic resistance and normal growth due to added energy; thus, beneficial effects such as higher rates of metabolic activity may ensue.

Materials List:

1. E. Coli Bacteria K-12 Strain Broth Cultures, 5
2. Petri Dishes, 54
3. Agar, 500 mL
4. Inoculating Loops, 2
5. Blow Torch/Bunsen Burner
6. 2.4 GHz Wi-Fi Router, 2
7. 5.0 GHz Wi-Fi Router, 2
8. Disinfectant/Bleach
9. RF Power Meter
10. Aluminum Foil
11. Penicillin Diffusion Discs, 50
12. Millimeter Calliper/Ruler

Independent Variables (Isolated): Frequency: 2.4 GHz and 5GHz, Intensity: -40 dBm and -80dBm, and Exposure Time: 12 and 24 Hours

Dependent Variables: Normal Growth: Number of Colonies Per Petri Dish and Antibiotic Resistance: Inhibition Zone Radius in mm

Control: E. Coli exposed without any radiation

Constants

- Type of Petri Dishes
- Type of Agar
- Amount of E.Coli per Petri Dish
- Temperature of E. Coli Environments
- Aluminum Foil Thickness

Lab Procedure

1. Create a sterile environment for experiment.
    a. Clean all surfaces with bleach.
    b. Wash hands thoroughly with soap and wear sterile gloves.
2. Prepare petri dishes.
    a. Heat agar to a boil.
    b. Remove petri dishes from sterile packaging.
    c. Remove lid from petri dish.
    d. Pour approximately 8-12 mL of agar into dish.
    e. Swirl dish until bottom is covered fully.
    f. Immediately place lid onto dish and set aside to cool.

    g. Repeat step 2c to 2f for remaining dishes.
    h. Wait approximately 1-2 hours for agar to cool.
3. Transfer bacteria.
    a. Carefully open tube.
    b. Use blowtorch to head mouth of tube.
    c. Use blowtorch to heat inoculating loop until glowing.
    d. Wait 3-5 seconds to cool.
    e. Dip loop into broth culture and transfer liquid to petri dish.
    f. Repeat step 3c to 3e 3 times per petri dish.
4. Place penicillin discs in half of petri dishes.
5. Wash hands and cleanup inoculation space.
6. Prepare WiFi.
    a. Setup first router and place three normal growth and three penicillin dishes around router.
    b. Wrap three normal growth and three antibiotic test dishes in one layer of aluminum foil (for attenuation) and place around router.
    c. Use dBm meter to find location where first router strength dies.
    d. Prepare next router at this location.
    e. Repeat step 3c to 3d for remaining routers. Make sure that each location has no interference from any of the other routers.
    f. Find final location with no signal from any router. Place control here.
7. Run tests and collect data.

    a. Run one 2.4 GHz and one 5.0 GHz router for 12 hours per day and one each for 24 hours per day.
    b. Count number of visible colonies everyday for ten days.
    c. Measure inhibition zone everyday for ten days.
8. Analyze data and draw conclusions.
9. Dispose of bacteria.
    a. Put on gloves.
    b. Flush bacteria down lab sink with bleach.
    c. Wash hands afterwards.

Safety Precautions

- Make sure Agar is at a temperature able to hold.
- Wears gloves to keep everything sterile.
- Use blow torch carefully and point fire away from body.
- Do not inhale the chemicals used.

<u>**Quantitative Data**</u>

Control (No WiFi Exposure)						
DV:	**Inhibition Radius**			**Normal Growth**		
Day 1:	I	I	I	0	0	0
Day 2:	I	I	I	0	0	0
Day 3:	I	I	I	0	0	0
Day 4:	I	I	I	0	2	1
Day 5:	I	I	I	3	5	2
Day 6:	I	I	I	5	6	2
Day 7:	I	I	I	12	14	8
Day 8:	I	I	I	21	16	19
Day 9:	I	I	I	71	39	62
Day 10:	4	5	4	159	104	121

I= Indeterminate

Number of Colonies at 2.4 GHz												
Frequency:	**2.4 GHz**											
Exposure Time:	**12 Hours**						**24 Hours**					
Intensity:	**-40 dBm**			**-80 dBm**			**-40 dBm**			**-80 dBm**		
Day 1:	0	0	0	0	0	0	0	0	0	0	0	0
Day 2:	0	0	0	0	0	0	0	0	0	0	0	0
Day 3:	0	0	0	0	0	0	0	0	0	0	0	0
Day 4:	0	1	0	1	0	0	0	0	0	0	0	2
Day 5:	2	3	1	1	0	0	1	2	0	0	3	4
Day 6:	2	3	2	1	0	0	1	3	0	0	4	4

Day 7:	2	5	5	4	1	0	3	4	0	5	7	8
Day 8:	2	7	8	12	1	1	3	5	0	15	27	32
Day 9:	12	17	32	67	31	29	4	7	0	33	48	57
Day 10:	41	56	69	102	58	84	5	15	3	145	106	207

Antibiotic Resistance (Inhibition Zone Radius in mm) at 2.4 GHz												
Frequency:	**2.4 GHz**											
Exposure Time:	**12 Hours**						**24 Hours**					
Intensity:	**-40 dBm**			**-80 dBm**			**-40 dBm**			**-80 dBm**		
Day 1:	I	I	I	I	I	I	I	I	I	I	I	I
Day 2:	I	I	I	I	I	I	I	I	I	I	I	I
Day 3:	I	I	I	I	I	I	I	I	I	I	I	I
Day 4:	I	I	I	I	I	I	I	I	I	I	I	I
Day 5:	I	I	I	I	I	I	I	I	I	I	I	I
Day 6:	I	I	I	I	I	I	I	I	I	I	I	I
Day 7:	I	I	I	I	I	I	I	I	I	I	I	I
Day 8:	I	I	I	I	I	I	I	I	I	I	I	I
Day 9:	I	I	I	I	I	I	I	I	I	I	I	I
Day 10:	3	1	1	1	3	4	3	4	5	4	1	3

Number of Colonies at 5 GHz		
Frequency:	**5.0 GHz**	
Exposure Time:	**12 Hours**	**24 Hours**

Intensity:	**-40 dBm**			**-80 dBm**			**-40 dBm**			**-80 dBm**		
Day 1:	0	0	0	0	0	0	0	0	0	0	0	0
Day 2:	0	0	0	0	0	0	0	0	0	0	0	0
Day 3:	0	0	0	0	0	0	0	0	0	0	0	0
Day 4:	0	0	0	0	0	0	0	0	0	0	0	0
Day 5:	2	1	1	4	0	2	1	3	1	2	0	5
Day 6:	2	3	1	10	8	9	2	5	1	3	1	6
Day 7:	4	12	1	24	17	21	2	13	1	4	5	6
Day 8:	13	41	2	47	63	76	11	22	1	15	16	25
Day 9:	14	52	6	66	82	99	13	38	2	24	32	51
Day 10:	17	124	7	179	213	243	71	111	18	105	134	228

Number of Colonies at 5 GHz												
Frequency:	**5.0 GHz**											
Exposure Time:	**12 Hours**						**24 Hours**					
Intensity:	**-40 dBm**			**-80 dBm**			**-40 dBm**			**-80 dBm**		
Day 1:	I	I	I	I	I	I	I	I	I	I	I	I
Day 2:	I	I	I	I	I	I	I	I	I	I	I	I
Day 3:	I	I	I	I	I	I	I	I	I	I	I	I
Day 4:	I	I	I	I	I	I	I	I	I	I	I	I
Day 5:	I	I	I	I	I	I	I	I	I	I	I	I
Day 6:	I	I	I	I	I	I	I	I	I	I	I	I
Day 7:	I	I	I	I	I	I	I	I	I	I	I	I
Day 8:	I	I	I	I	I	I	I	I	I	I	I	I

Day 9:	I	I	I	I	I	I	I	I	I	I	I	I
Day 10:	I	I	I	1	1	1	1	1	3	4	2	1

Averages:

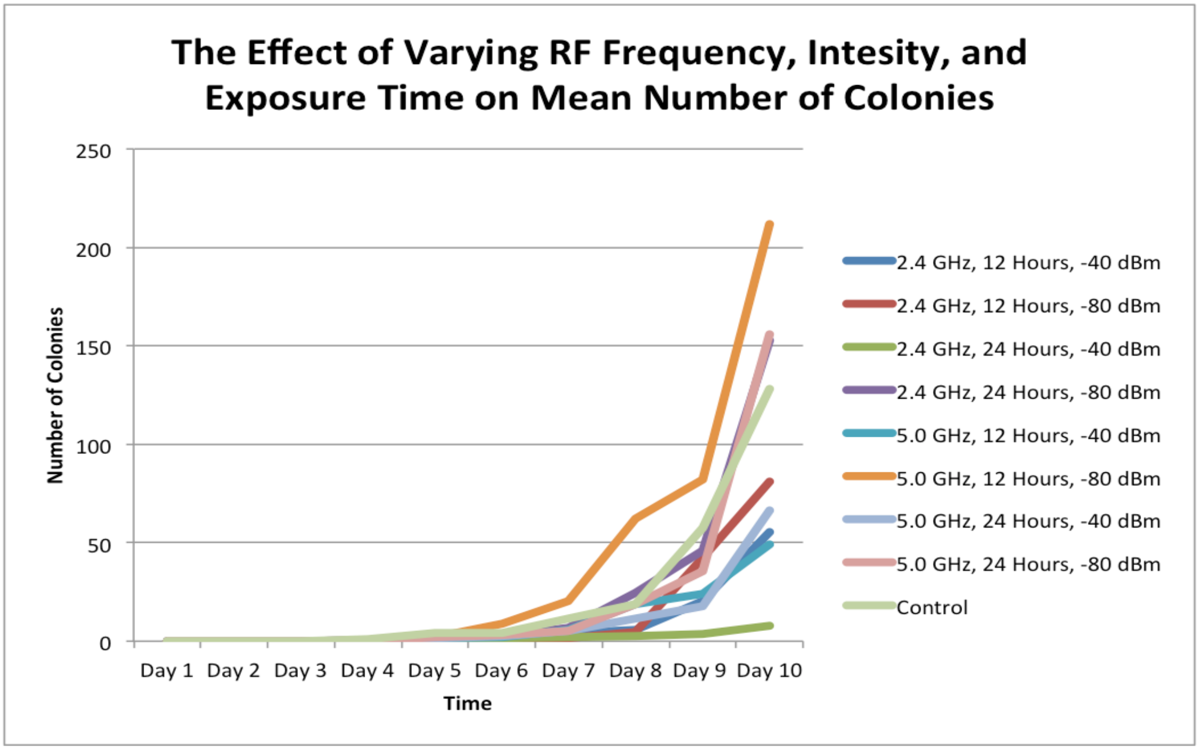
Number of Colonies:

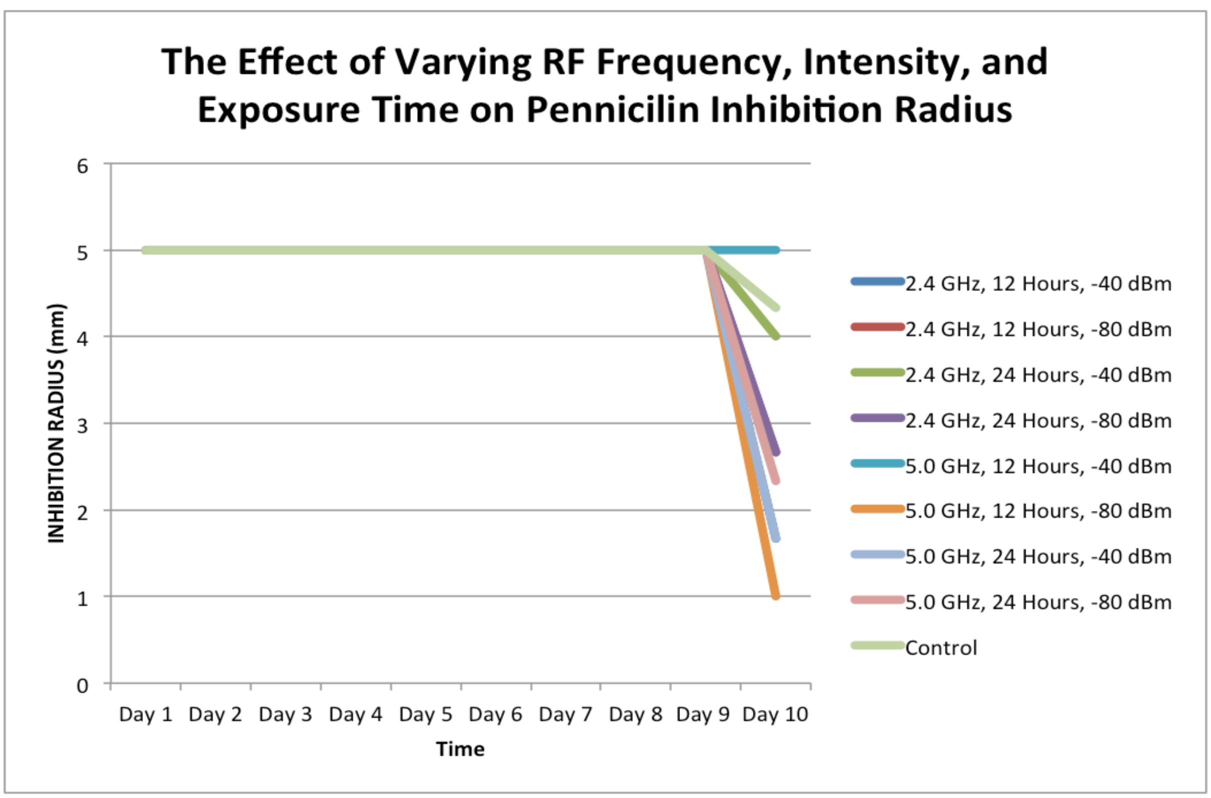
	2.4 GHz, 12 Hours, -40 dBm	**2.4 GHz, 12 Hours, -80 dBm**	**2.4 GHz, 24 Hours, -40 dBm**	**2.4 GHz, 24 Hours, -80 dBm**	**5.0 GHz, 12 Hours, -40 dBm**	**5.0 GHz, 12 Hours, -80 dBm**	**5.0 GHz, 24 Hours, -40 dBm**	**5.0 GHz, 24 Hours, -80 dBm**	**Control**
Day 1:	0	0	0	0	0	0	0	0	0
Day 2:	0	0	0	0	0	0	0	0	0
Day 3:	0	0	0	0	0	0	0	0	0
Day 4:	0.333333333	0.3333333333	0	0.6666666667	0	0	0	0	1
Day 5:	2	0.3333333333	1	2.333333333	1.333333333	2	1.666666667	2.333333333	4.333333333
Day 6:	2.333333333	0.3333333333	1.333333333	2.666666667	2	9	2.666666667	3.333333333	4.333333333
Day 7:	4	1.666666667	2.333333333	6.666666667	5.666666667	20.66666667	5.333333333	5	11.33333333
Day 8:	5.666666676	4.666666667	2.666666667	24.66666667	18.66666667	62	11.33333333	18.66666667	18.66666667
Day 9:	20.33333333	42.33333333	3.666666667	46	24	82.33333333	17.66666667	35.66666667	57.33333333
Day 10:	55.33333333	81.33333333	7.666666667	152.6666667	49.33333333	211.6666667	66.66666667	155.6666667	128

Inhibition Radius (mm):

	2.4 GHz, 12 Hours, -40 dBm	2.4 GHz, 12 Hours, -80 dBm	2.4 GHz, 24 Hours, -40 dBm	2.4 GHz, 24 Hours, -80 dBm	5.0 GHz, 12 Hours, -40 dBm	5.0 GHz, 12 Hours, -80 dBm	5.0 GHz, 24 Hours, -40 dBm	5.0 GHz, 24 Hours, -80 dBm	Control
Day 1:	I	I	I	I	I	I	I	I	I
Day 2:	I	I	I	I	I	I	I	I	I
Day 3:	I	I	I	I	I	I	I	I	I
Day 4:	I	I	I	I	I	I	I	I	I
Day 5:	I	I	I	I	I	I	I	I	I
Day 6:	I	I	I	I	I	I	I	I	I
Day 7:	I	I	I	I	I	I	I	I	I
Day 8:	I	I	I	I	I	I	I	I	I
Day 9:	I	I	I	I	I	I	I	I	I
Day 10:	1.666666667	2.666666667	4	2.666666667	I	1	1.666666667	2.333333333	4.3333333333

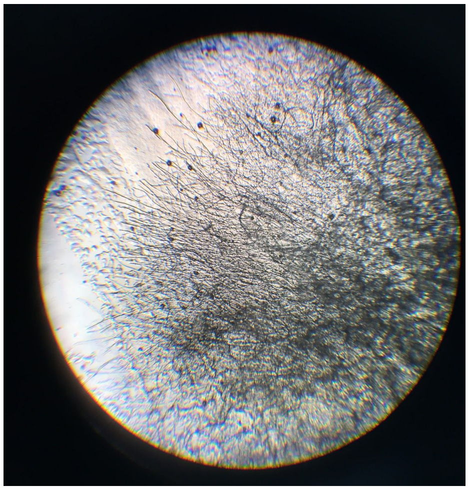
Graphs (Quantitative):



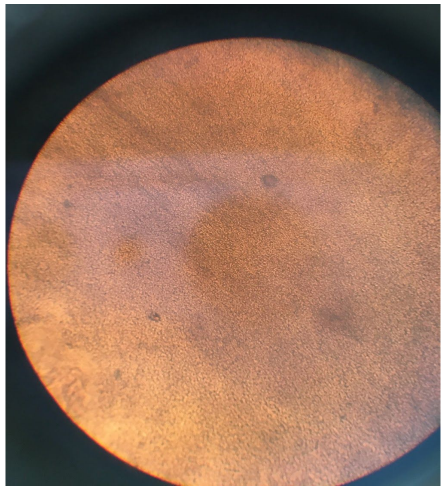


Qualitative Data:

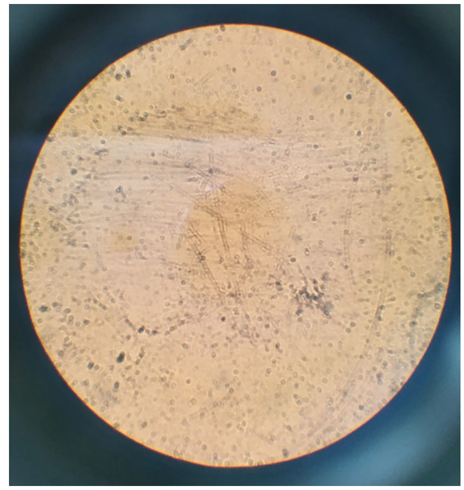
40x Fungus (Contaminant)



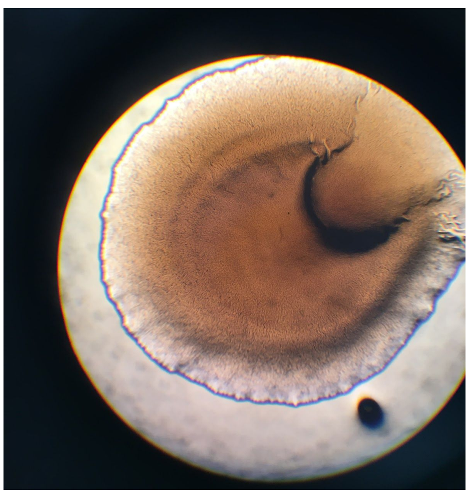
100x E. Coli



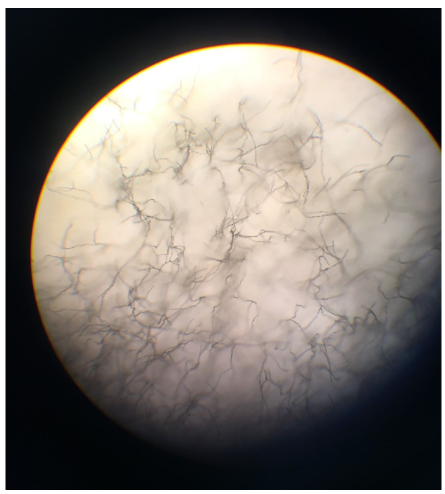
1000x E. Coli



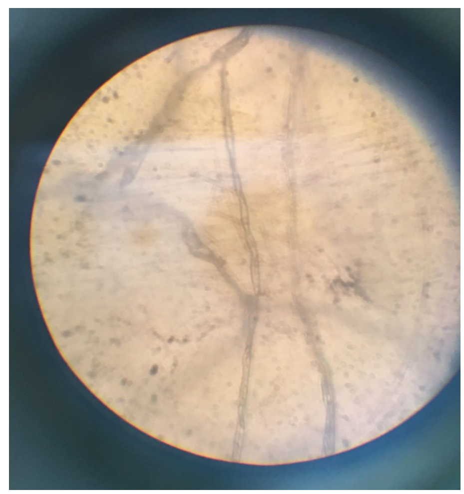
40x E. Coli



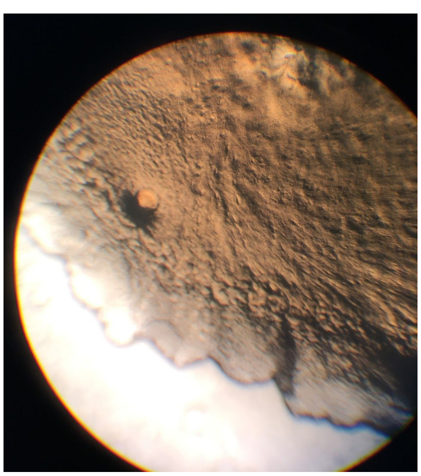
100x Fungus



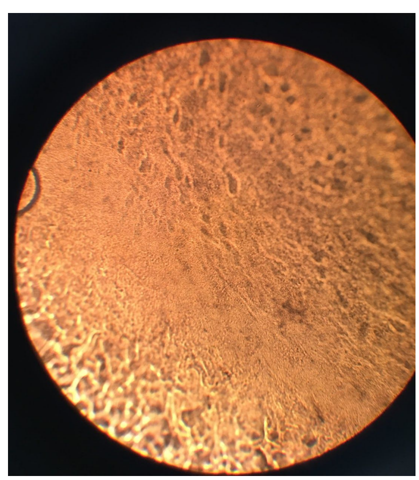
1000x Fungus



40x E. Coli



100x E. Coli



Observations (quantitative):

- The combination that resulted in most growth was the highest frequency (5.0 GHz) with lower intensity (-80 dBm) and less exposure time (12 hours).
- The radiation only favored growth over the control with -80 dBm.
- The E. coli grew exponentially, typically not growing until day 5.
- All the variables but one have a radius lower than the control.

Observations (qualitative):

- Contaminant fungal spore growing in one of the antibiotic resistance testing dishes in the 2.4 GHz, 24 hour, -80 dBm group.
- Contaminant fungus consists of long, threadlike cells. Appears to be hairy.
- E. Coli colonies grow circular, often connected along a single line.
- E. Coli cells are small, tubelike, and densely packed.
- E. Coli smell develops at Day 7. Resembles fecal matter.
- Bubbles appear on petri dish lid. Amount of bubbles appears to have relation with amount of bacterial growth (expected).

Analysis with Conclusion

The purpose of the above investigation was to better understand the connection between RF radiation and prokaryotic cell activity. The experiment assessed the impact of different frequencies, intensities, and exposure times, including 2.4 and 5.0 GHz, -40 and -80 dBm, and 12 and 24 hours, on the growth, measured in number of colonies per petri dish, and antibiotic resistance, measured by the inhibition radius in mm. The data proved to be interesting, showing numerous unexpected correlations. The growth exceeded the control in all of the lower intensity

-80 dBm groups, except 1 (2.4 GHz, 12 hours). Also, all experimental groups showed some antibiotic resistance over the control except 5.0 GHz, 12 hours, -40 dBm, the most (1 mm radius) being in the 5 GHz, 12 hour, -80 dBm group. This group also showed the highest mean growth, with an average of approximately 212 colonies per dish. Overall, the data suggests that high intensity radiation is detrimental to bacterial growth, as none of the -40 dBm groups grew more than the control. Also, some qualitative correlations were noted. And one fully developed contaminant fungus colony was found in the 5.0 GHz, 24 hour, -40 dBm group. Overall, this investigation demonstrated many interesting the trends that will no doubt be built upon by greater experimentation into the cellular mechanisms for prokaryotic adaptation to RF radiation.

Research summary (see works cited for references)

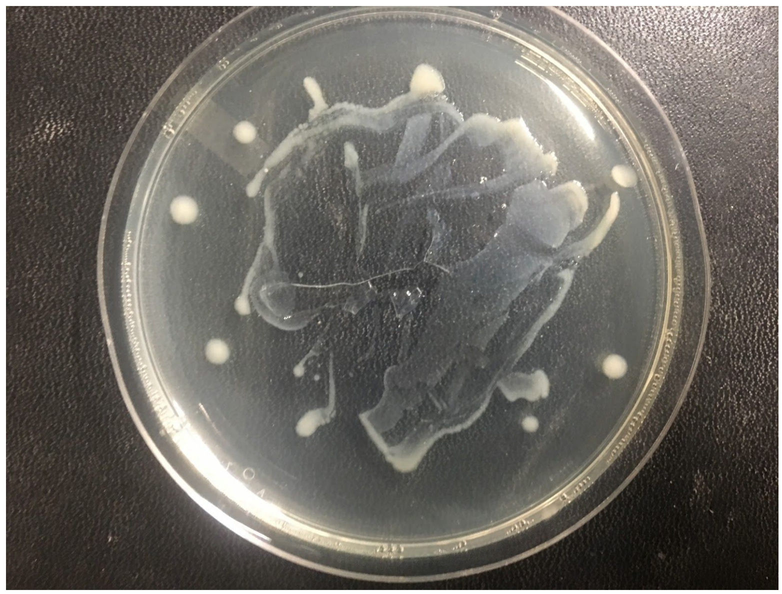
Electromagnetic energy constitutes the main source of radiation exposure experienced by the general population. The electromagnetic spectrum consists of seven distinct ranges of frequencies, and the energy of each range is dependent on the frequency.[3] These energies can be divided into two classes, ionizing and non-ionizing.[2,3] The term “radiation” is most commonly associated with the ionizing end of the spectrum, including gamma and x-rays, and the term carries a negative connotation, conveying the idea of damage. However, the non-ionizing form of radiation impacts organism differently.[3] For example, many organisms have adapted visible light receptors to better sense their environment. Other highly present forms of radiation in the modern age are radio and microwave radiation. Particularly present in the 21st century, radio and microwaves emitted from cellular phones and wifi routers (collectively called RF radiation) are poorly studied in terms of cellular effects.[6] In high quantities, RF can cause heating, particularly when absorbed by objects constituted of large amounts of water.[2] High energy RF exposure in relation to human body cells has been closely studied, linking the ensuing internal heat to numerous neurological complications and cataract development.[2] Even low energy RF radiation has been studied in relation to the human body, showing little change in function due to exposure.[2,3] A less commonly studied effect, however, is the impact of RF radiation on prokaryotic cells. Recent research suggests that RF exposure in pathogenic E. Coli strains, which are already extremely resilient to antimicrobial treatment, increases antibiotic resistance.[1,6] However, these studies are limited, and they fail to understand the root cause for the cellular adaptation.[4,5,6] Also, these studies fail to capture the effect of RF on other bacterial strains, so an experiment was designed to understand the impact on other prokaryotes. This experiment used the K-12 strain of E. Coli and assessed the effect of wireless network RF on the antibiotic resistance and normal growth.

Confirmation, modification, or rejection of hypothesis

Contradictory to the hypothesis: if radiation frequency, intensity, and exposure time increase, then the normal growth will increase; the normal growth was most affected by a higher frequency, lower intensity and lower exposure. Continued, for antibiotic resistance, all of the data showed resistance compared to the control, except one outlier. This could be due to an error as described in the section below. So, for the most part, the hypothesis pertaining to antibiotic resistance supported the data found with the inhibition radius.

Sources of Error

This experiment was carried out manually. Some sources of error in this experiment could include imprecise agar filling; therefore, creating an unstable environment for the E. coli. Further, the agar may have been punctured on the bottom of the dishes by the process of depositing culture with inoculating loop.



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