Natural Antibiotic Effects of Honey, Garlic, Lemon and Ginger against Escherichia Coli

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Abstract

Antibiotic resistance is a serious issue with the overuse of synthetic antibiotics, which have been known to kill good bacteria and kill with one method, allowing bacteria to evolve and mutate, unlike natural antibiotics. The purpose of this research was to find out which natural antibiotics- Manuka Honey, Garlic, Lemon, and Ginger, is the most effective against inhibiting E.Coli growth. My hypothesis was that Garlic will be best with the biggest zone of inhibition. In this experiment, 20 Luria Broth Agar plates were swabbed with K-12 strain E.Coli bacteria and split into six parts each, consisting of: Negative control with nothing, Positive control with Ampicillin, and 4 different concentrations of the natural antibiotic on a disk (4000mg/ml, 2000mg/ml, 1000mg/ml, 500mg/ml), using a serial dilution with water. 5 samples per antibiotic (Honey, Garlic, Lemon and Ginger) were prepared, and the 20 plates were incubated for 24 hours at 37 degreesC. The zone of inhibition's diameter in millimeters for each disk was measured. 3 trials were performed. Based on the average zone of inhibition diameter for 15 samples in 3 trials, Garlic exhibited the largest zone at all concentrations (23.9mm at 4000mg/ml) and was better than Ampicillin. Manuka Honey (12.8mm at 4000mg/ml) and Lemon (11.4mm at 4000mg/ml) inhibited E.Coli but were not very effective. Ginger results are poor (8.6mm at 4000mg/ml), and dropped rapidly at lower concentrations. My hypothesis was correct, Garlic inhibits E.Coli best even at lowest concentrations of 500mg/ml (10.7mm). Garlic is effective due to its 100+ active compounds from Allicin, and targets enzymes that are needed for the production of energy for the bacteria. These results encourage people to take natural antibiotics instead of synthetic antibiotics, so that antibiotics resistance doesn't proliferate.

1 Introduction

Antibiotics are medicines that can inhibit and kill bacteria in many different ways. Antibiotics can inhibit cell wall production or the cell membrane surrounding bacteria. These membranes keep important substances inside that the bacteria need in order to survive. Another type of antibiotic can cause protein synthesis to be disrupted, which are needed for bacteria enzymes, cellular structures, in order for bacteria to multiply, and for a bacteria's overall survival. These antibiotics bind to parts of the bacteria's ribosomes, which produce proteins, and they make the proteins form incorrectly, causing bacteria death. Other antibiotics work by inhibiting or binding the molecules that are used to coil DNA or make RNA, or disrupt the process or enzymes necessary for cell functions.

Antibiotic resistance is fast becoming a problem in the world, with the overuse of synthetic antibiotics. Synthetic antibiotics are naturally produced by soil bacteria, mold, or fungi but are grown synthetically in a lab or combined with natural substances. However, synthetic antibiotics have been known to kill good bacteria in your body and even more, harboring antibiotic resistance. This is because they have been over-prescribed and overused, causing the bacteria to mutate and become resistant over time. Synthetic antibiotics also only kill target bacteria with one method only, allowing bacteria to evolve and mutate very easily and become resistant.

Natural antibiotics on the other hand, are hard to become resistant to due to the many compounds that can counteract against bacteria. They are not fungi or mold, and are mainly made from natural substances like plants or minerals from nature. Natural antibiotics do not destroy useful bacteria, which makes them a better antibiotic choice since they are healthy for the human body and our immune system. Natural antibiotics have many different ways of killing bacteria, making it very difficult for bacteria to become resistant to it, and hence is a necessary weapon against fast evolving antibiotic resistant bacteria, which synthetic antibiotic cannot keep up with. Some natural antibiotics that have shown to produce effective results are Manuka Honey, Garlic, Lemon, and Ginger.

Manuka Honey uses its antibacterial compounds Methylglyoxal and Bee Defensin 1, its high concentration of sugar, and more to fight off bacteria. When diluted with water, a honey enzyme glucose oxidase makes hydrogen peroxide which kills bacteria. Manuka has so much sugar that it creates an osmotic effect, such that there is no water for bacteria so they dehydrate and die.

Garlic uses its powerful enzyme Allicin, and its 100+ compounds to stop bacteria's production of energy. Garlic contains two key compounds called Allin, an amino acid and Alliinase, a protein-based enzyme. When the garlic is damaged, the Alliinase converts Allin into a third compound called Allicin which when oxidized, breaks down into 100+ antibacterial compounds. These compounds in turn can make many other effective defensive combination. Allicin in garlic targets enzymes that are needed for the production of energy and food for the bacteria cell. Hence bacteria are inhibited from functioning.

Lemons contain alkaloids, which are a group of compounds that are found in many antibacterial and medicinal drugs, and act by stopping DNA from being produced in a bacteria cell, which causes it to die. Essentially, lemon is a common and refreshing natural antibiotic that is easily accessible, and can inhibit bacteria by targeting bacterial DNA.

Finally, Ginger contains special compounds called Gingerols, which are responsible for ginger's spicy flavor as well as its antibacterial properties. These are very effective against breaking down a bacteria's cell wall which it needed to protect themselves, and interferes with cell membrane functions. Thus, preventing the cell from absorbing nutrients and protein, nucleic acid synthesis, and enzyme activity.

The goal of this research is to investigate in depth about natural antibiotics and how well they inhibit *Escherichia coli* (E. coli) bacteria growth. In my experiment, I will be using the natural antibiotics Manuka Honey, Garlic, Lemon, and Ginger, which I have researched to be effective against various bacteria, and can also be consumed. I will also be using the synthetic antibiotic, Ampicillin, as a positive control. With these results, I hope to discover which natural antibiotic inhibits E. coli growth the best, so that people may learn how effective natural antibiotics are. This is becoming extremely important nowadays because synthetic antibiotics have shown to harbor antibiotic resistance in bacteria, and we may be left with natural antibiotics as our ultimate weapon against these resistant bacteria.

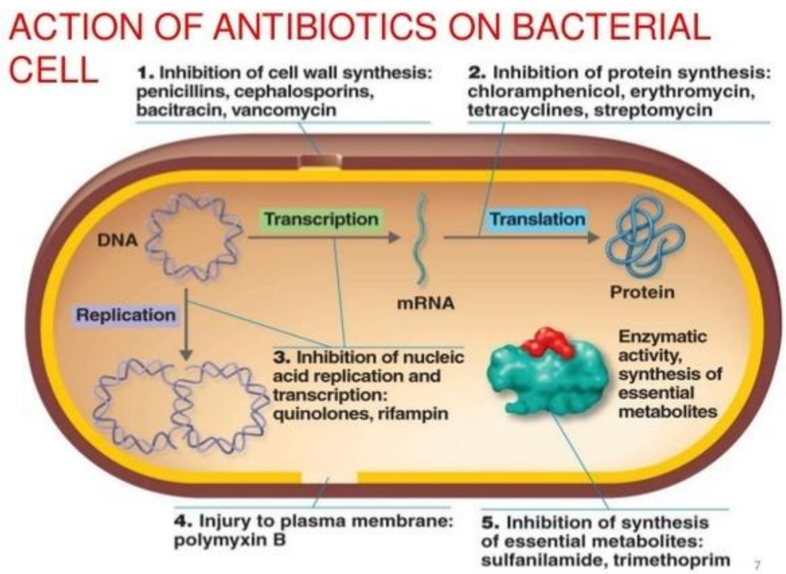


Fig. 1 Antibiotics Mechanisms of Actions (Keni)

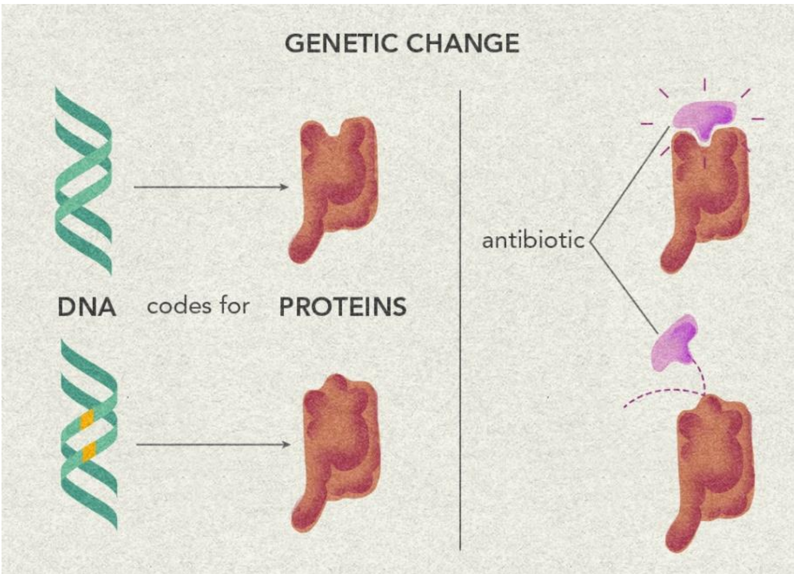


Fig. 2 Antibiotics Resistance caused by Bacteria Mutations

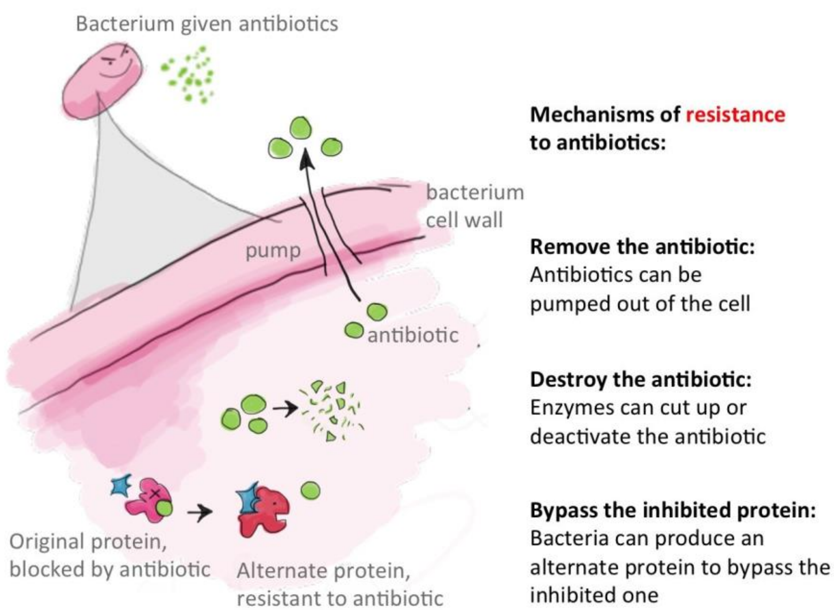


Fig. 3 Antibiotics Resistance caused by Bacteria Mutations (Cantley)

2 Background

Bacteria have existed since the beginning of early life on Earth. Although many of them are part of the environment and help our world be what it is today, there are some that have caused problems to the human body. Naturally, in our very modern and scientifically advanced society today, cures have been made and found. These medicines are called antibiotics and have saved countless lives. However, many synthetic or lab-made antibiotics, have been countered with a much stronger force: antibiotic resistance. In the need of survival, bacteria have learned to mutate and evolve to prevent successful antibiotic treatment. On the other hand, natural antibiotics, which are from natural substances that are mainly plant or mineral-based, have been shown and tested to be almost equal, or even better, than synthetic antibiotics. Although synthetic antibiotics have been proven to be useful in inhibiting bacterial growth, bacteria have started to become resistant to them, so using natural antibiotics has been shown to be a better solution for helping human health due to their broad range of natural compounds. My goal for the research I have conducted, was to learn about four well-known natural antibiotics: Manuka Honey, Garlic, Lemon, and Ginger, and to study in depth about bacteria and antibiotics mechanisms of actions.

2.1 The Biology of Bacteria

Bacteria are single-celled organisms. These microbes, microscopic organisms, have very simple structure. They have no nucleus, unlike a eukaryotic cell which contains a nucleus with special structures inside a membrane that have all of the cell's genetic information. Bacteria doesn't have some organelles, which are special structures that help cells survive by doing different tasks, such as chloroplasts and mitochondria. Bacteria have a cell wall and cell membrane that together, are referred to as the cell envelope.

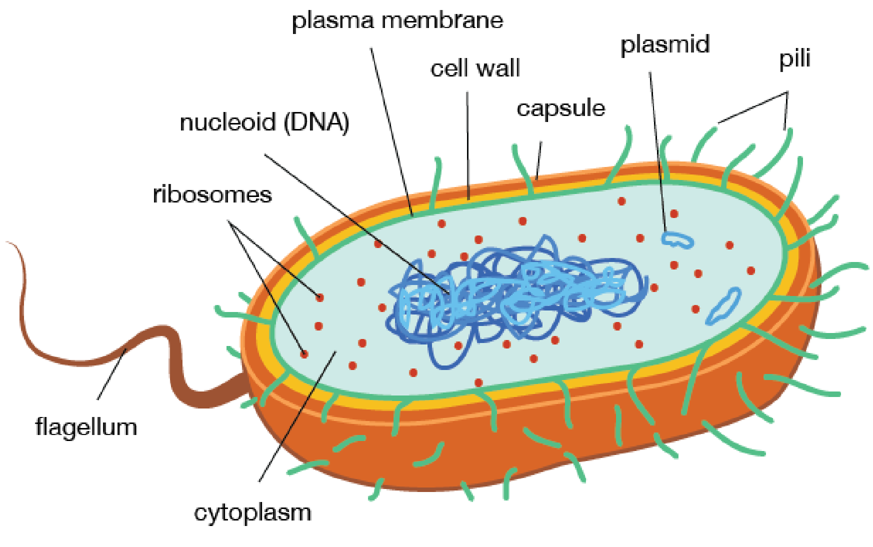


Fig. 4 Structure of Bacteria

2.1.1 Bacteria Genes

A bacteria's genetic information is contained in one single loop of DNA, unlike other organisms which have multiple strands. However, some bacteria and some eukaryotic cells have plasmid, which are small, DNA molecules that comprise extra genetic material that gives bacteria an extra trait, such as making a bacteria resistant to an antibiotic or genes that can make proteins to kill other bacteria. Some bacteria need plasmids if they find themselves in danger to something that will kill them, like an antibiotic. They can make plasmids to give them antibiotic resistance traits in order to survive. Not all bacteria have plasmid and they are technically not needed. Scientists have used plasmids to experiment with and have used them to clone and change genes. These experimental plasmids are called vectors. Plasmids can be passed onto offspring bacteria and can be transferred to other bacteria through conjugation. Conjugation is a process where a donor bacteria transfers genetic material, such as plasmid, to a recipient bacteria. The donor can turn the plasmid into a thin tube called a pilus and gives it to the recipient. Conjugation usually occurs in order to spread an antibiotic resistance trait to other bacteria, so that more can survive and spread it. In essence, the bacteria's plasmid is essential for providing genetic advantages such as antibiotic resistance traits.

2.1.2 Bacteria Survival

Bacteria can be found anywhere and in any habitat. They are both inside and outside of living organisms. They thrive in many food we eat and things we touch. They are about 10 times more bacterial cells than human cells in the human body and are both good and bad for the human body. Many of them live inside of us to help our digestive system, like the ones in our small intestine and stomach. Bacteria have very high tolerance for their living conditions. However, each species has their own environment that they thrive best in. For example, helicobacter pylori is an acidophilic, which are bacteria that live in acidic conditions, and thrives in our stomach. Bacteria need a food source and an environment in which they can live and tolerate. A lot of the food humans eat have many nutrients needed for bacterial survival, causing them to be prone to bacteria growth easily. The less common species of bacteria don't need food, but can gain nutrients through photosynthesis. In addition, bacteria need moisture because it helps bacteria dissolve food they need for energy and allows food to enter the bacteria. All in all, bacteria thrive in different food and environment conditions.

2.1.3 Bacteria Reproduction

Bacteria can reproduce in 3 ways. Binary fission is a type of asexual reproduction and is very common among bacteria. This is done when one parent cell splits into two to create child cells. These child cells are given the exact genetic information from the parent cells, which doubled its genetic material before splitting. Budding is another type of asexual reproduction. It happens when a mother cell grows a small nub on one end and makes a nucleus for it through a process called mitosis. When the bud grows the same size as the mother, it separates from it and can be a different organism by having different traits or it can be an exact clone of the parent cell. Bacteria can also sexually reproduce although it is rare in bacteria. When an antibiotic threatens it, asexual reproduction will have all the same genetic information, so the antibiotic can kill them very easily. On the contrary, when bacteria sexually reproduce, their offspring may have genetic traits that are resistant to antibiotics.

In summary, although asexual reproduction of Binary Fission and Budding are more susceptible to antibiotics than sexual reproduction which are rare in bacteria, genetic recombination through conjugation as described earlier, allows bacteria to survive antibiotic attacks and become resistant to them.

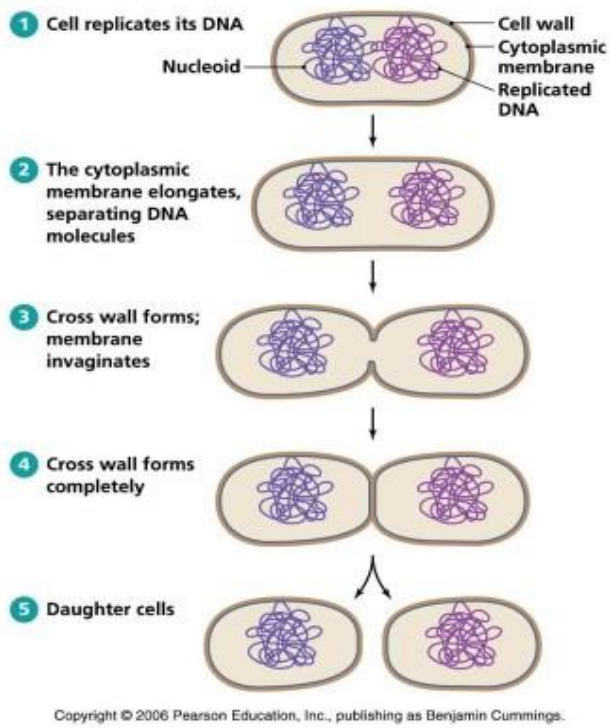


Fig. 5 Bacteria Reproduction Mechanisms

2.1.4 Bacteria Classifications

Bacteria can be classified and identified in many different ways. Some of the most common ways bacteria are classified are by shape and by cell wall (gram). There are many shapes and sizes of bacteria. The three main ones are coccus, spiral, and bacillus. Cocci are bacteria that are ovoid or spherical. After being made, they sometimes still stick together, staying as clusters, pairs, or chains. Spiral bacteria are spiral-shaped and can be thin, thick, or even comma shaped. Bacilli are rod-shaped and can also be in pairs, clumps, or chains.

Another way bacteria can be classified is into two different groups based on their cell wall: gram positive and gram negative. Gram positive bacteria only has one cell wall made of peptidoglycan, while gram negative bacteria contain a thinner layer of peptidoglycan and a thicker outside membrane. A process called gram staining uses a die called Crystal Violet to color the cell wall and then an alcohol rinse lets the color show. Peptidoglycan absorbs the Crystal Violet die. Gram positive bacteria contain 50-90% of this in their cell wall, causing it to stain completely violet. Gram negative bacteria contains a thin layer of this, 10%, and loses the violet color when it goes through the alcohol rinse, so it will appear reddish or pink. The outside membrane also prevents it from being colored. This process not only helps the bacteria cell wall to be classified, it helps bacteria be identified. Under a light microscope, bacteria is invisible, so the die allows the bacteria to be observed.

There is a variety of species of bacteria that can be both good and bad for our health. Some bacteria that aren't dangerous for humans are used to ferment foods such as sauerkraut and cheese. There are many bacteria that live in the human digestive system to help break down food and absorb nutrients. There are also bacteria that can cause harm through diseases. They can usually be killed by antibiotics, but some bacteria have grown resistant to them. In summary, bacteria can be classified through several methods, including by shape, cell-wall type, and whether they are harmful to our health (pathogenic) or not.

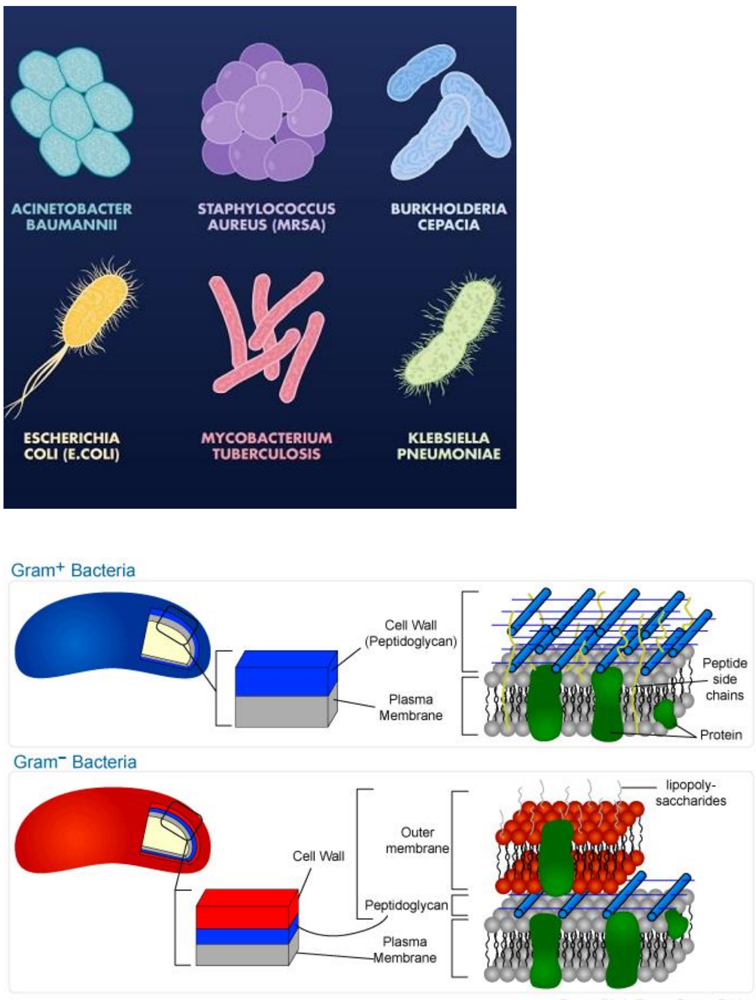


Fig. 6 Bacteria Classifications

2.2 Antibiotics

Antibiotics are medicines that help inhibit and fight off bacterial infection in humans and animals. There are different compounds that are effective against certain microorganisms. Antimicrobials have all medicines that work on general microorganisms, such as bacteria, fungi and viruses. Antibacterials only act against bacteria. Antibiotics can be naturally produced by microorganisms or synthetically made to inhibit the growth or kill other microorganisms. Antibiotics mainly work against bacteria, but not viruses.

There are many different types of antibiotics that kill or inhibit bacteria growth in different ways. A Bactericidal kills bacteria but a bacteriostatic stops bacteria from growing and does not kill the cells, leaving the immune system to clear the rest out. There are several classes of antibiotics that target different function in bacteria.

2.2.1 Antibiotics Mechanisms of Actions

Antibiotics can inhibit and kill bacteria in many different ways. Antibiotics can inhibit cell wall production. Cell walls are extremely important for bacterial survival and are constantly being made and broken down. Certain antibiotics can stop the synthesis of the cell wall, but do not affect the enzymes that break them down. Therefore, the cell wall will get destroyed along with the bacteria itself. Other antibiotics destroy the cell membrane surrounding bacteria. These membranes keep important substances inside that the bacteria need in order to survive. However, both bacteria and cells in our body have cell membranes, so medicines like these can be dangerous to the human body if not carefully selected and used.

Another type of antibiotic can cause protein synthesis to be disrupted, which are needed for bacteria enzymes, cellular structures, in order for bacteria to multiply, and for a bacteria's overall survival. These antibiotics bind to parts of the bacteria's ribosomes, which produce proteins, and they make the proteins form incorrectly, causing bacteria death.

Other antibiotics work by inhibiting or binding the molecules that are used to coil DNA or make RNA. There are also antibiotics that can disrupt the process of producing nucleic acids and other processes that are important for bacteria functions. Overall, antibiotic mechanisms of actions include destroying bacteria cell walls or membranes, disrupting protein synthesis, or attacking DNA processes.

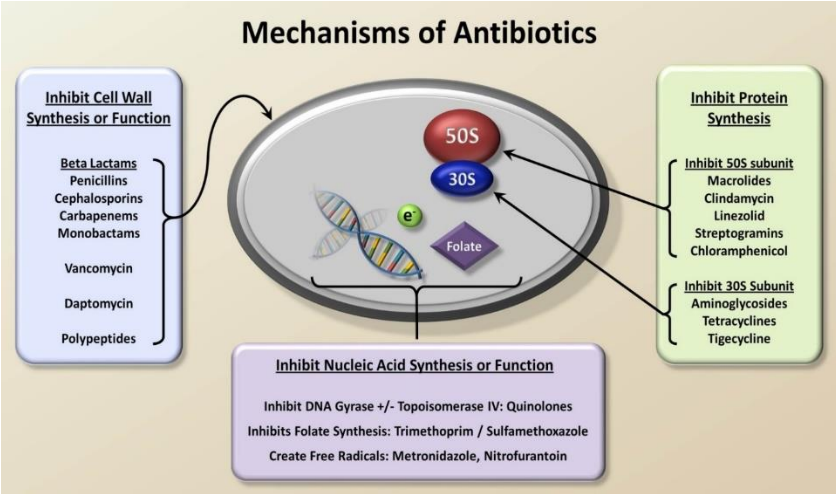


Fig. 7 Mechanisms of Antibiotics

2.2.2 Types of Antibiotics

Antibiotics can be classified into narrow or broad spectrum. Narrow range antibiotics work only against a certain group of bacteria and wide-spectrum antibiotics work against a wider range of bacteria. Narrow-spectrum antibiotics are more preferred over broad-spectrum antibiotics because broad-spectrum antibiotics can have side effects and kill non-disease causing bacteria that are good for the body. However, doctors usually use broad-spectrum antibiotics because of their convenience; since it is hard for doctors to find out what bacteria is causing an infection in time and they sometimes don't know how to correctly treat it effectively. In general, antibiotics fall into either narrow range or broad spectrum types, but are generally effective against only specified bacteria species for each antibiotic.

2.2.3 Synthetic and Natural Antibiotics

Synthetic antibiotic is a medicine that helps kill off bacteria and inhibit bacterial growth. Antibiotics are naturally produced by soil bacteria, mold, or fungi but can be grown synthetically in a lab or combined with natural substances.

Natural antibiotics are not fungi or mold, and are mainly made from natural substances like plants or minerals from nature. Any natural antibiotics are plant-based and have similar or the same qualities synthetic antibiotics have. However, synthetic antibiotics sometimes affect or kill off good bacteria that are needed in the human body. Natural antibiotics do not destroy useful bacteria, which makes them a better antibiotic choice since they are healthy for the human body and our immune system to begin with.

Another reason why natural antibiotics have a better effect for bacterial infections is because some bacteria have been growing resistant to synthetic antibiotic treatment. When a synthetic antibiotic has been over-prescribed when not needed, used for a long time, or have been overused, it contributes to the bacteria mutating process to not get affected by that antibiotic. This has caused many problems in antibiotic treatment. Synthetic antibiotics also only kill target bacteria with one method only, allowing bacteria to evolve and mutate very easily, and become antibiotic resistant. Natural antibiotics have many different ways of killing bacteria, making it very difficult for bacteria to become resistant to it. In addition, natural antibiotics have a much broader spectrum than synthetic antibiotics. However, natural antibiotics sometimes don't work fast enough or take a longer time to work, so synthetic antibiotics may be somewhat more convenient. Nevertheless, natural antibiotics are evidently becoming a necessary weapon against fast evolving antibiotic resistant bacteria, which synthetic antibiotic cannot keep up with.

2.3 Natural Antibiotics Effects on Bacteria

In this research, 5 types of natural antibiotics are investigated: Manuka Honey, Garlic, Lemon, Ginger and Oregano Oil, which have been found to be effective against many bacteria in several studies.

2.3.1 Manuka Honey

Manuka Honey is a well-known and effective natural antibiotic which is frequently used for medicinal drinks. Manuka Honey has been used as a medicine since ancient time to heal wounds and is found in New Zealand from the Manuka bush. It contains five main compounds that when combined together, make it a very effective natural antibiotic. When diluted with water, a honey enzyme called glucose oxidase makes hydrogen peroxide, a type of bleach that is effective at killing bacteria. Manuka has so much sugar that it creates the osmotic effect. The osmotic effect comes about because Manuka Honey has such a high sugar concentration, that there is no water for bacteria to absorb, so they get dehydrated and die. The nectar of Manuka flowers also contain a compound called dihydroxyacetone, which is converted into an antibacterial compound called Methylglyoxal. Other honeys contain this as well, but not as much as Manuka Honey. Another antibacterial protein it contains is called Bee Defensin 1. It is found in royal jelly, which is food that is eaten by queen bee larva. Lastly, honey is very acidic, resulting in bacteria being inhibited or their growth being slowed down. In essence, Manuka Honey's main mechanism of action is its high sugar content to dehydrate bacteria, as well as its several antibacterial compounds.

2.3.2 Garlic

Garlic is another natural antibiotic that is known to be an extremely effective natural antibiotic which is also used widely in preparing food. Garlic has been able to treat many bacterial infections and viruses. It is known to be one of the best infection fighters and doesn't make any resistant bacteria strains. There are many ingredients in raw garlic that makes it able to kill bacteria so well, but they don't become active until you "damage" the garlic cloves by slicing, chewing, or cooking them. Garlic contains two key compounds called Allin, an amino acid and Alliinase, and protein-based enzyme. When the garlic is damaged, the Alliinase converts Allin into a third compound called Allicin. When Allicin is oxidized, it breaks down into more than 100 other antibacterial compounds. These compounds in turn can make many other effective defensive combination.

Allicin in garlic targets enzymes that are needed for the production of energy and food for the bacteria cell. Hence bacteria are inhibited from functioning.
In addition to Allicin, garlic also contains many natural sugars, like glucose and insulin, trace minerals, like iron and calcium, enzymes, amino acids, flavonoids and many more bacteria-fighting compounds. Garlic has also been proved to balance blood sugar, help with digestion, and boost your immune system with its many medicinal factors. Garlic is not only antibacterial, but antifungal and antiviral, as well. It can be used as food in everyday dishes, medicine, and can sometimes be applied to wounds. It is one-fifteenth stronger than penicillin and is more effective than other antibiotics. All in all, Garlic is one of the best natural antibiotic that is very versatile as food and medicine, and destroys bacteria by targeting essential enzymes.

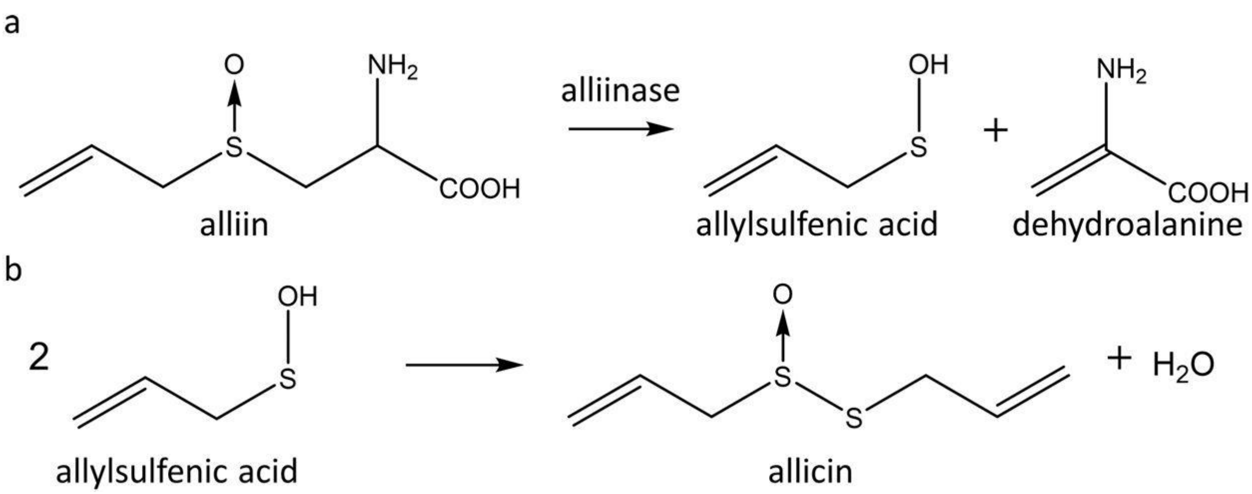


Fig. 8 Formation of Allicin from Alliin, when Garlic is crushed (Leontiev)

2.3.3 Lemon

Lemon is also another potent natural antibiotic that is used in many food and drinks. Lemons are very acidic and can help restore the body's pH. They also help dissolve poisons and uric acid in your liver, liquefies the bile in it, and helps with bowel movements. It has been proven to help with brain disorders, improve eye disorders, help with scurvy, and strengthen blood vessels, helping with high blood pressure.
Lemons contain alkaloids, which are a group of compounds that are found in many antibacterial and medicinal drugs. These alkaloids in lemons act by stopping DNA from being produced in a bacteria cell, which causes it to die.
Essentially, lemon is a common and refreshing natural antibiotic that is easily accessible, and can inhibit bacteria by targeting bacterial DNA.

2.3.4 Ginger

Ginger contains special compounds called Gingerols, which is responsible for ginger's spicy flavor as well as its antibacterial properties. These are very effective against breaking down a bacteria's

cell wall which it needed to protect themselves, and interferes with cell membrane functions. Thus, removing the cell from absorbing nutrients, and protein, nucleic acid synthesis, and enzyme activity.
Ginger has been known in cooking as an often used ingredient to boost flavor. It has many antibacterial compounds and have been known to do "health wonders." Its anti-inflammatory effects, gut problem and cancer cell killing are already famous. A study in which antibiotics and ginger extract were used to treat Staphylococcus aureus and S. pyreus infections, may have shown that the ginger was better.

2.3.5 Oregano Oil

Oregano oil, or Origanum vulgare, which is used in food and also as an essential oil, has also been shown to be a very potent natural antibiotic. Oregano oil is derived from oregano, an herb, and is a very concentrated version of oregano's active antimicrobial compounds. This essential oil is unlikely to cause many dangerous side effects that synthetic antibiotics caused, such as antibiotic resistance from using it too much, unhealthy gut from all of the good bacteria and cells being killed, and low nutritional value. Oregano oil basically contains antibacterial, antifungal, and antiviral factors. Its two main powerful compounds are Carvacrol, a liquid used in many essential oils and as a disinfectant, and Thymol. Both of them are very antibacterial and antifungal. Carvacrol and Thymol inhibit bacteria growth by destroying bacteria cell membranes.

Oregano's leaves also contain many antioxidant compounds such as phenols, triterpenes, and many acids. Oregano oil has also been known to be able to help with common health problems such as indigestion, bacterial and fungal infections, allergies, parasites, inflammation, and more. It has also been shown that it can reduce and help with side effects of medicines and drugs. In general, Oregano oil is not only a dressing in food and an essential oil, it has also strong medicinal properties, and acts by targeting bacteria cell membranes.

3 Purpose

The purpose of this research is to see which natural antibiotic out of manuka honey, garlic, lemon, and ginger is the most effective against E. Coli. I hope to discover which one inhibits antibiotic growth the best by seeing which one produces the biggest zone of inhibition. This is important because today, synthetic antibiotics have been often been overused and not used correctly, so bacteria have become resistant to them. This is very dangerous considering antibiotics are very important as medicines and used in hospitals. So if they don't work, many people will suffer. If we continue to use antibiotics too much and not in the right way, more bacteria will become resistant and antibiotics won't be able to be used anymore and won't be effective. However, bacteria cannot become resistant to natural antibiotics, so using them will help many and are also generally healthy for us. Some synthetic antibiotics can have side effects that are very dangerous for the human body. The goal is to figure out which natural antibiotic can help inhibit the growth of E. Coli, which is one of the bacteria known for causing food poisoning and others. I am specifically using natural antibiotics that can be eaten so they can be easily taken.

4 Hypothesis

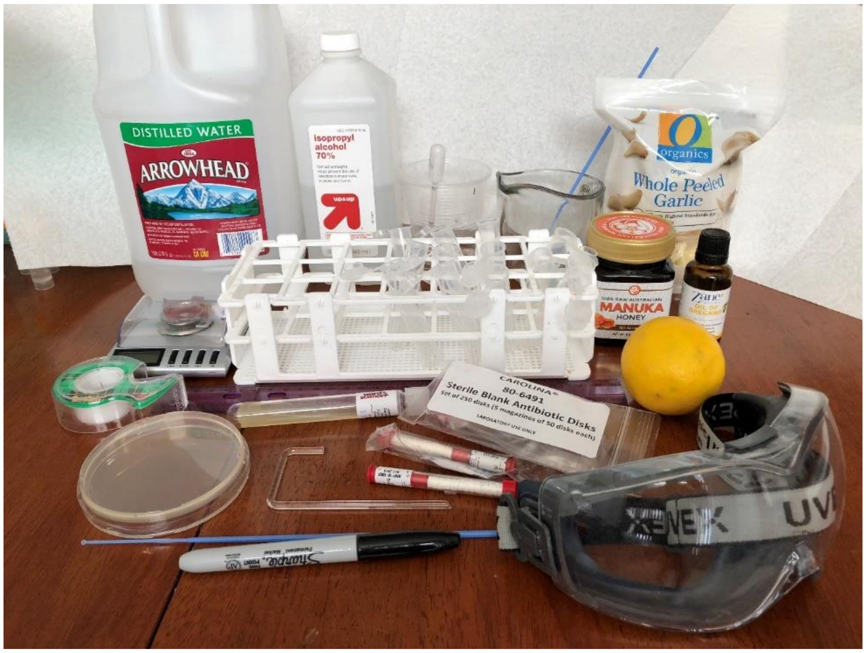
My hypothesis is that if garlic is added to E. Coli, the zone of inhibition will be the greatest against Manuka Honey, Lemon, and Ginger by about 100% of the time, constantly, even when it is diluted. I developed this hypothesis because during my research of all four of these natural antibiotics, I learned that all of them have many antibacterial factors. However, I think that Garlic got the lead because it does not contain just 3 main compounds, alliinase which converts allin into allicin when garlic is oxidized, further breaks down into more than 100 compounds. And although garlic becomes less effective when cooked, it still contains many antibacterial factors.

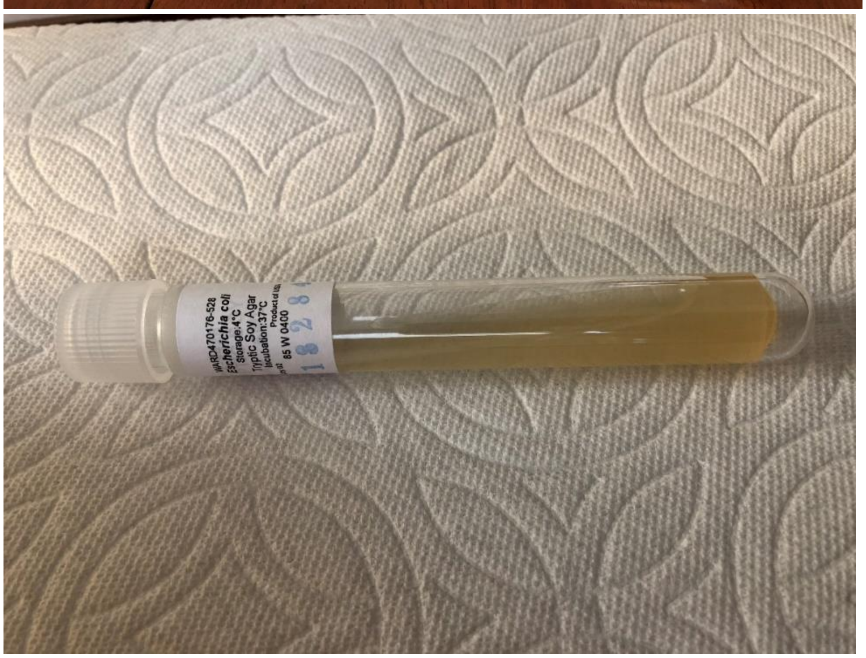
Garlic is also not only antibacterial, but antifungal and antiviral as well. It is even known to be more effective than penicillin, even though it is just a bit. Neither Manuka honey, lemon, nor Ginger contains more than 100 compounds. Allicin itself is very strong to begin with; it is sometimes used for medicinal purposes by itself. Allicin combined with alliinase, alliin, and many more effective compounds, makes garlic a very powerful natural antibiotic. Garlic is very useful for wounds, medicines, and as food. It contains many natural sugars, enzymes, amino acids, flavonoids, trace minerals, and much more. It also doesn’t have any side effects of overuse, specifically antibiotic resistance, which makes it able to be used multiple times without being ineffective and is known to be a very powerful infection fighter. I selected the percentage of my prediction, 100%, because I think that garlic will be the most effective against the other three natural antibiotics.

5 Materials

Black sharpie
K-12 Strain E.Coli (Ward Science)
60 Luria Broth agar plates
Manuka Honey (100% Raw Australian)
Garlic cloves
Lemon
Ginger roots
Pipette and 50 ul pipette tips
Distilled water
Goggles
Gloves
Inoculating loop
Glass spreader
Homemade incubator
6mm blank disks
6mm Ampicillin disks

High Precision digital Weighing scale
Heat lamp
Tin foil
Digital Thermometer
Ruler
Tape





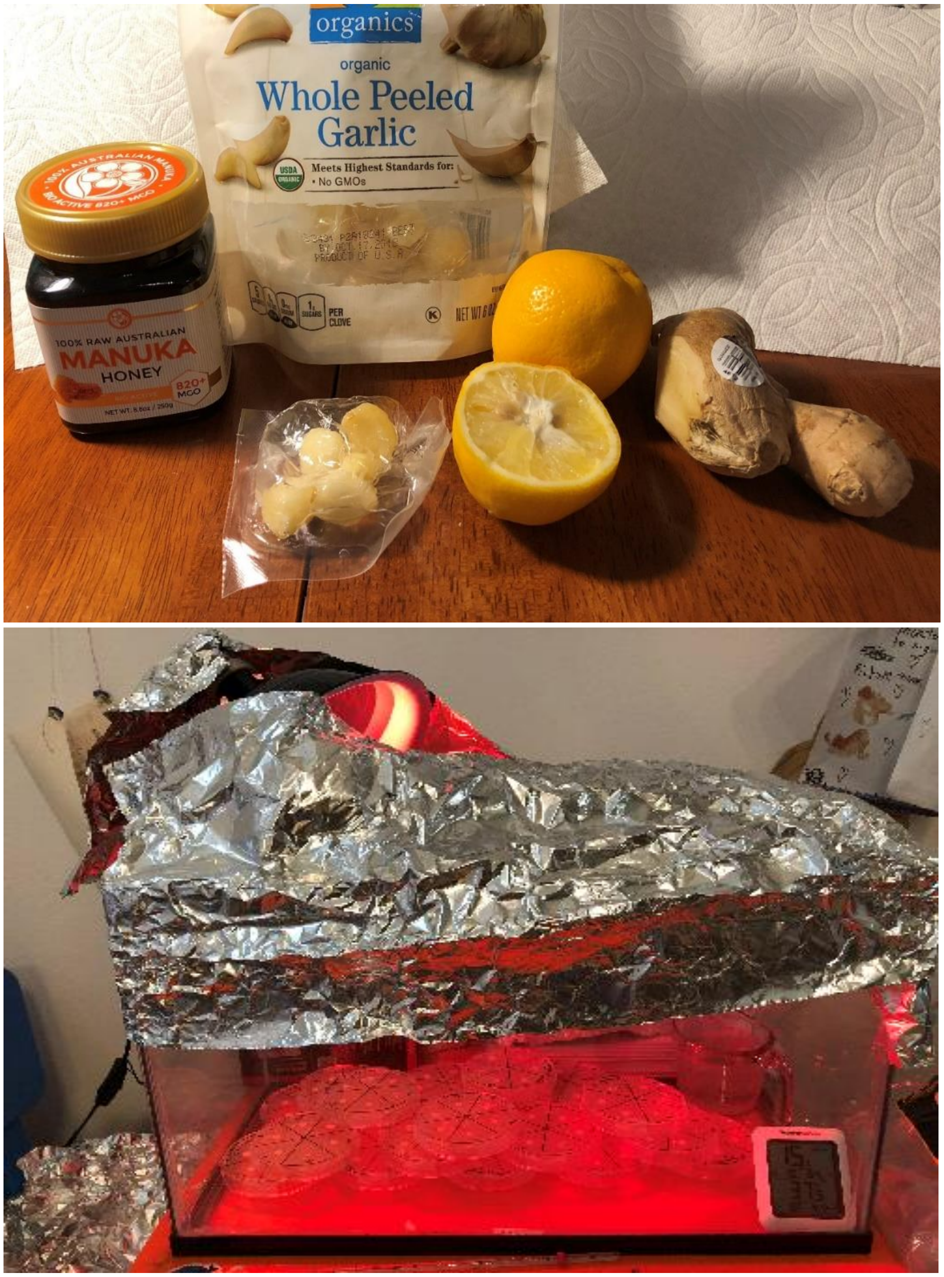


Fig. 9 Materials and Methods

6 Method

1) Put on goggles, gloves, and any other protective clothing.
2) Weigh with digital weighing scale and put 4000 mg of Manuka honey and 1 ml of water in a test tube. Shake well and label C1 (concentration 1).
3) Pipette 0.5 ml of the first tube and put it in the second test tube with 0.5 ml of water. Shake well and label C2 (concentration 2).

4) Repeat step 3 for the third and fourth test tube and label C3 (concentration 3) and C4 (concentration 4) correspondingly.
5) Open agar plate and swab E. Coli with inoculating loop and glass spreader to even it out.
6) Close agar plate, flip over, and divide it into six sections with a sharpie. Label each section - (negative control), + (positive control), C1 (concentration 1), C2 (concentration 2), C3 (concentration 3), and C4 (concentration 4).
7) Flip over agar plate and open. Place an ampicillin disk in the + section, one blank disk in the – section and each concentration section. Pipette 20 microliters of concentration solution to each corresponding c1, c2, c3 and c4 disk and nothing in the - or + disks.
8) Close agar plate and tape it around. Repeat steps 2-7 for 4 more agar plates.
9) Repeat steps 2-8 for garlic, lemon, and oregano oil.
10) For garlic, crush garlic clove into fine pieces. For lemon, cut in half and squeeze juice. For Ginger, cut slices of ginger roots and crush them.
11) Place all 20 agar plates in the incubator with sharpie/agar side up. Turn on heat lamp and adjust tin foil for a heat of 37 degrees Celsius. Leave for 24 hours.
12) After 24 hours, take out agar plates. Measure and record diameter of zone of inhibition with ruler in mm.
13) Repeat steps 1-12 for 2 more trials.

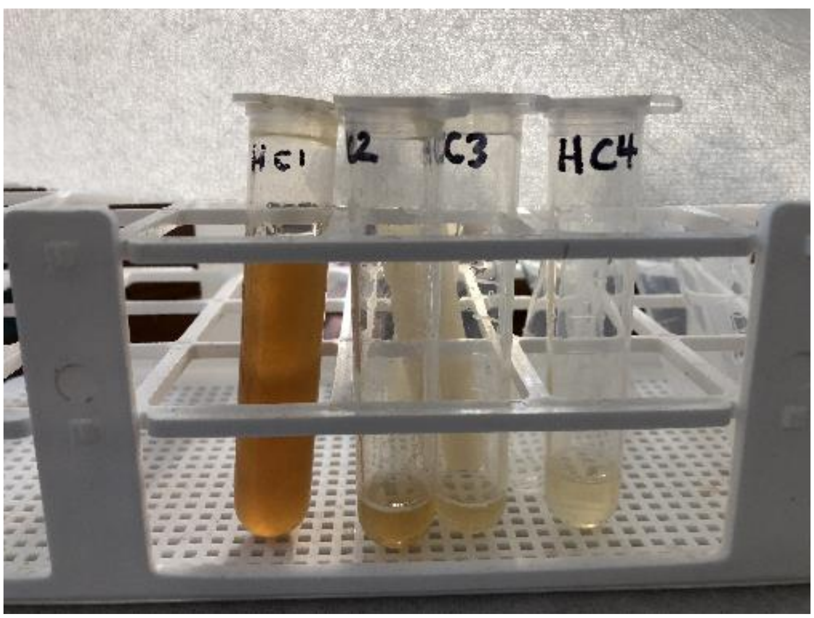


Fig 10. Honey Samples – C1, C2, C3, C4

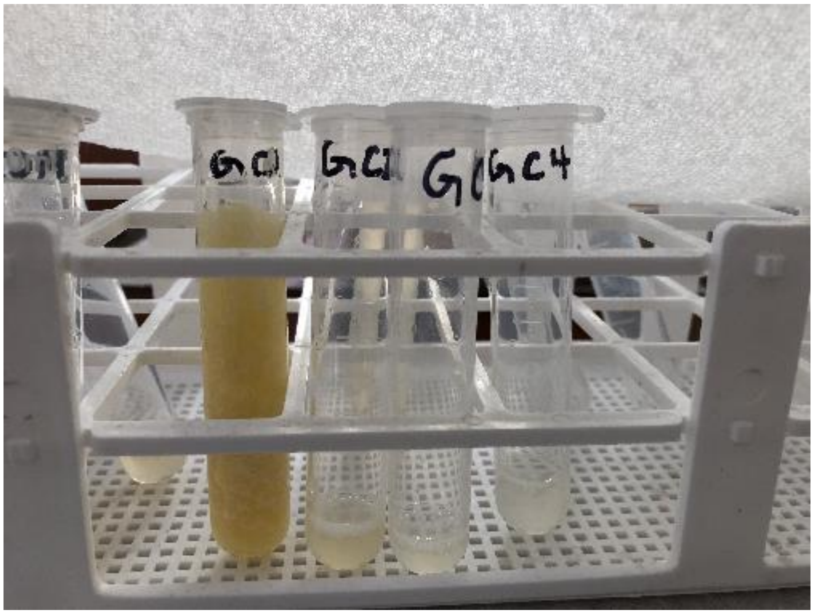


Fig. 11 Garlic Samples – C1, C2, C3, C4

Fig.12 Lemon Samples – C1, C2, C3, C4

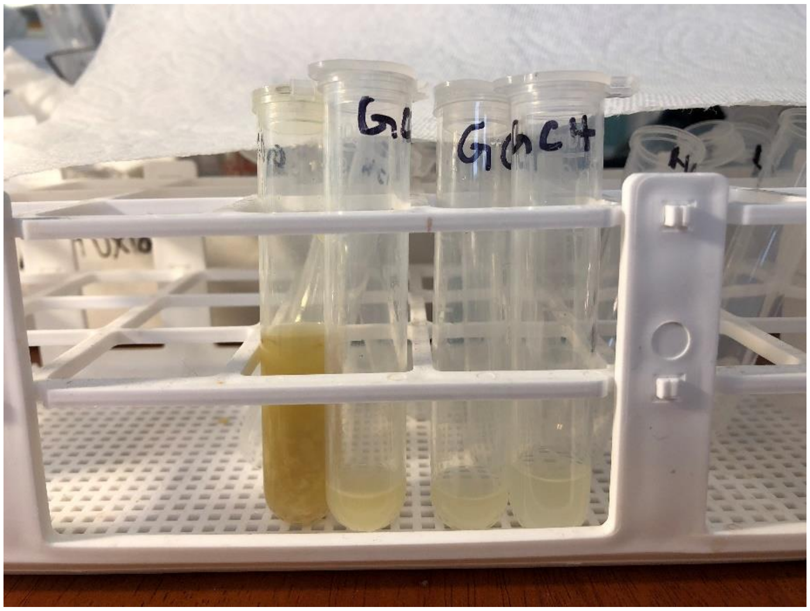


Fig.13 Ginger Samples – C1, C2, C3, C4

7 Results

7.1 Zone of Inhibition Data

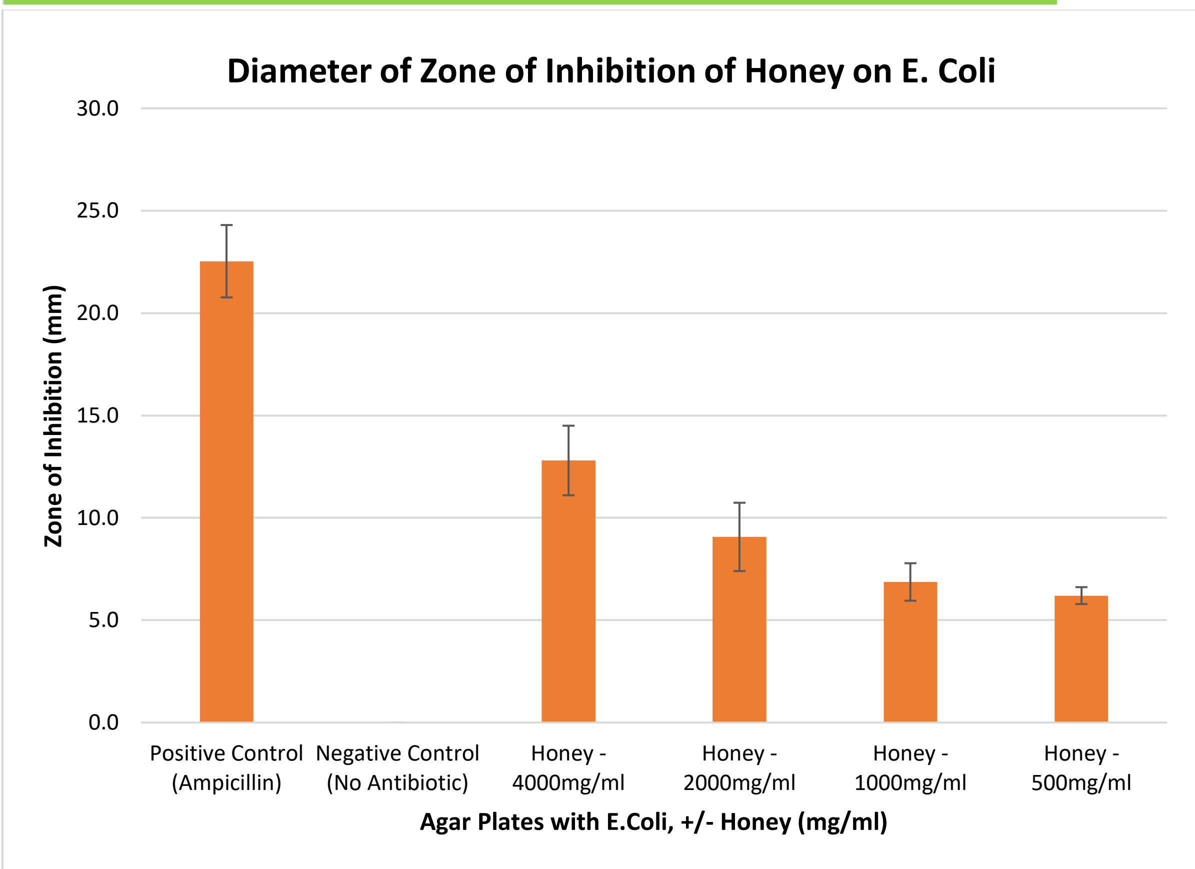
This section presents the results of the experiments, which consists of 3 trials of each Antibiotic (Honey, Garlic, Lemon, Ginger).
In each trial, the diameter of the zone of inhibition is measured for 5 samples of the following 6 plates:

(1) Positive Control (Ampicillin)
(2) Negative Control (No antibiotic)
(3) Concentration 1 (4000mg/ml of antibiotic solution)
(4) Concentration 2 (2000mg/ml of antibiotic solution)
(5) Concentration 3 (1000mg/ml of antibiotic solution)
(6) Concentration 4 (500mg/ml of antibiotic solution)

For each antibiotic, the averages of the 15 samples (over 3 trials, 5 samples per trial) were calculated, and the standard deviation was derived as well.
Finally, all the 4 antibiotic data were plotted against each other to compare their efficacies against each other at the same concentration levels.

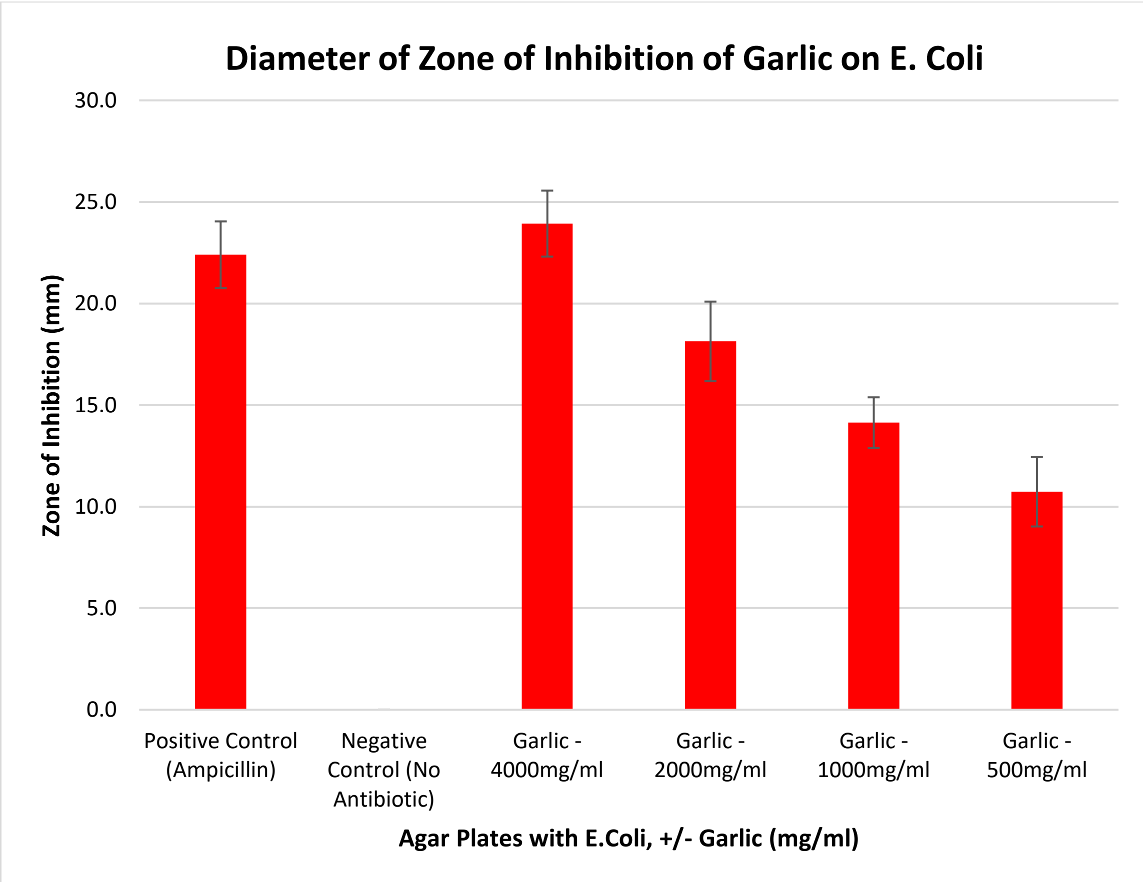
7.1.1 Honey

Honey (Diameter of Zone of Inhibition - mm)							
		Positive Control (Ampicillin)	Negative Control (No Antibiotic)	Honey - 4000mg/ml	Honey - 2000mg/ml	Honey - 1000mg/ml	Honey - 500mg/ml
Trial 1	Sample 1	20	0	11	9	8	6
	Sample 2	19	0	14	10	7	6
	Sample 3	22	0	14	12	6	6
	Sample 4	23	0	10	8	7	6
	Sample 5	20	0	15	12	9	7
Trial 2	Sample 1	25	0	14	7	6	6
	Sample 2	23	0	13	8	7	6
	Sample 3	24	0	10	7	6	6
	Sample 4	24	0	13	7	6	6
	Sample 5	21	0	11	8	6	6
Trial 3	Sample 1	23	0	14	9	8	7
	Sample 2	24	0	14	11	6	6
	Sample 3	23	0	11	9	7	6
	Sample 4	23	0	14	10	7	7
	Sample 5	24	0	14	9	7	6
	Average	**22.5**	**0.0**	**12.8**	**9.1**	**6.9**	**6.2**
Standard Deviation		1.77	0.00	1.70	1.67	0.92	0.41



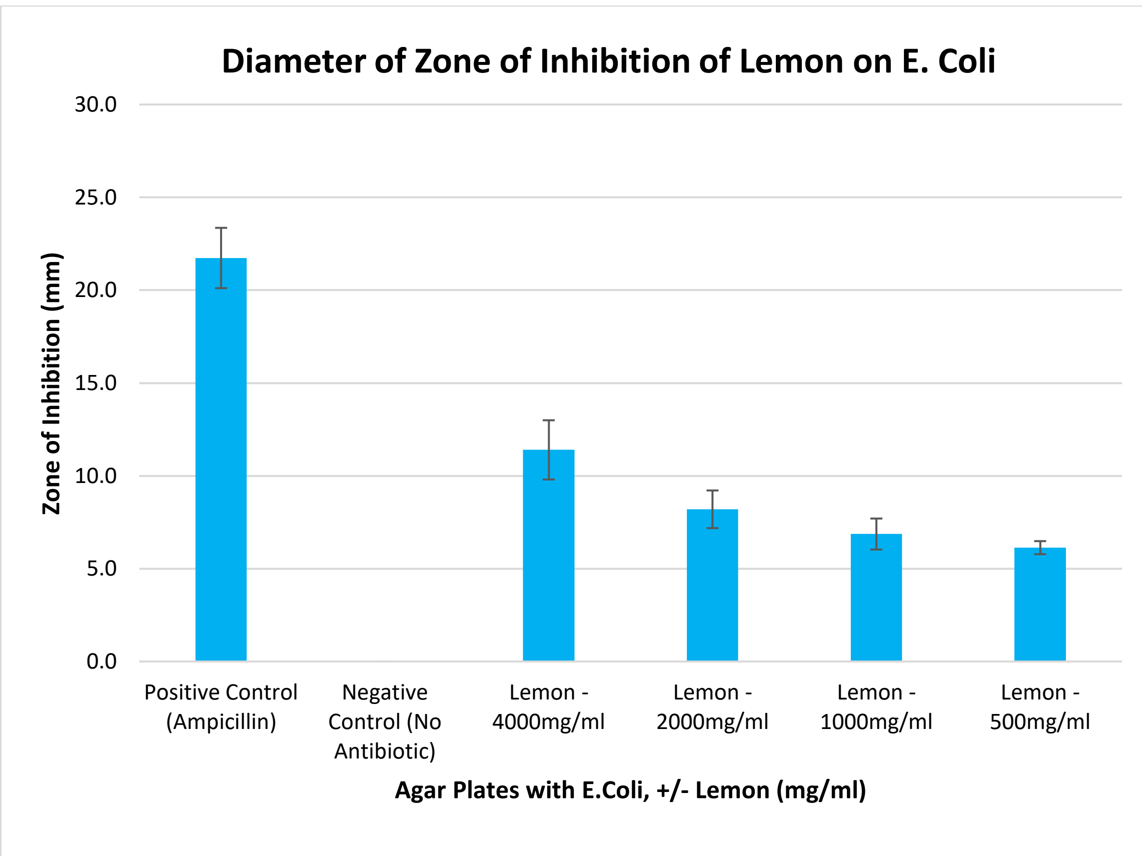
7.1.2 Garlic

Garlic (Diameter of Zone of Inhibition -mm)							
		Positive Control (Ampicillin)	Negative Control (No Antibiotic)	Garlic - 4000mg/ml	Garlic - 2000mg/ml	Garlic - 1000mg/ml	Garlic - 500mg/ml
Trial 1	Sample 1	21	0	23	19	13	11
	Sample 2	20	0	23	16	13	13
	Sample 3	22	0	21	17	13	11
	Sample 4	21	0	26	17	14	11
	Sample 5	22	0	22	14	15	11
Trial 2	Sample 1	21	0	25	21	12	8
	Sample 2	25	0	22	16	14	11
	Sample 3	22	0	22	17	14	8
	Sample 4	22	0	24	18	14	11
	Sample 5	21	0	25	19	14	8
Trial 3	Sample 1	24	0	25	20	17	14
	Sample 2	22	0	25	21	16	11
	Sample 3	25	0	26	19	14	12
	Sample 4	25	0	25	19	15	10
	Sample 5	23	0	25	19	14	11
	Average	**22.4**	**0.0**	**23.9**	**18.1**	**14.1**	**10.7**
Standard Deviation		1.64	0.00	1.62	1.96	1.25	1.71



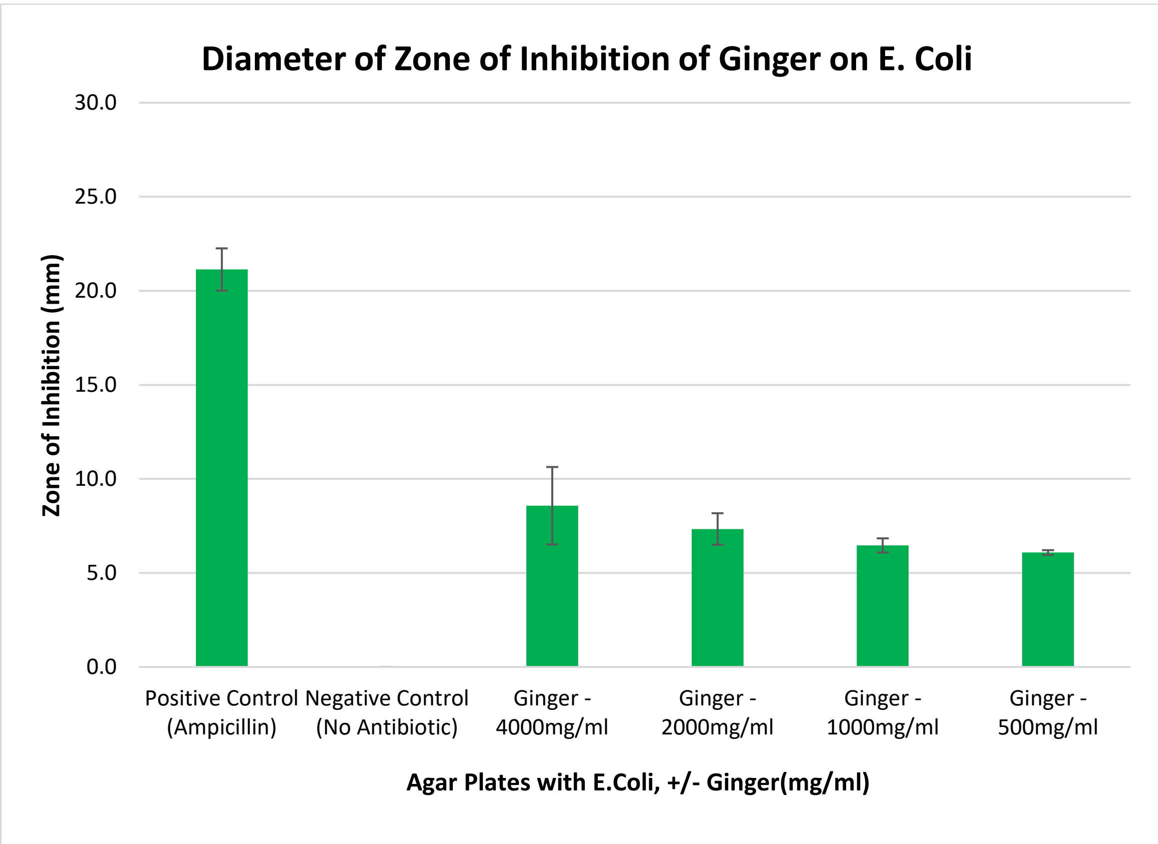
7.1.3 Lemon

Lemon (Diameter of Zone of Inhibition -mm)							
		Positive Control (Ampicillin)	Negative Control (No Antibiotic)	Lemon - 4000mg/ml	Lemon - 2000mg/ml	Lemon - 1000mg/ml	Lemon - 500mg/ml
Trial 1	Sample 1	23	0	10	8	6	6
	Sample 2	20	0	9	7	6	6
	Sample 3	19	0	9	8	6	6
	Sample 4	21	0	11	11	9	6
	Sample 5	21	0	10	8	7	6
Trial 2	Sample 1	22	0	11	8	7	6
	Sample 2	22	0	10	8	7	6
	Sample 3	21	0	12	8	7	6
	Sample 4	24	0	11	8	7	6
	Sample 5	20	0	13	9	6	6
Trial 3	Sample 1	24	0	13	9	8	7
	Sample 2	20	0	13	9	7	6
	Sample 3	22	0	13	7	6	6
	Sample 4	24	0	12	7	7	6
	Sample 5	23	0	14	8	7	7
	Average	21.7	0.0	11.4	8.2	6.9	6.1
Standard Deviation		1.62	0.00	1.59	1.01	0.83	0.35



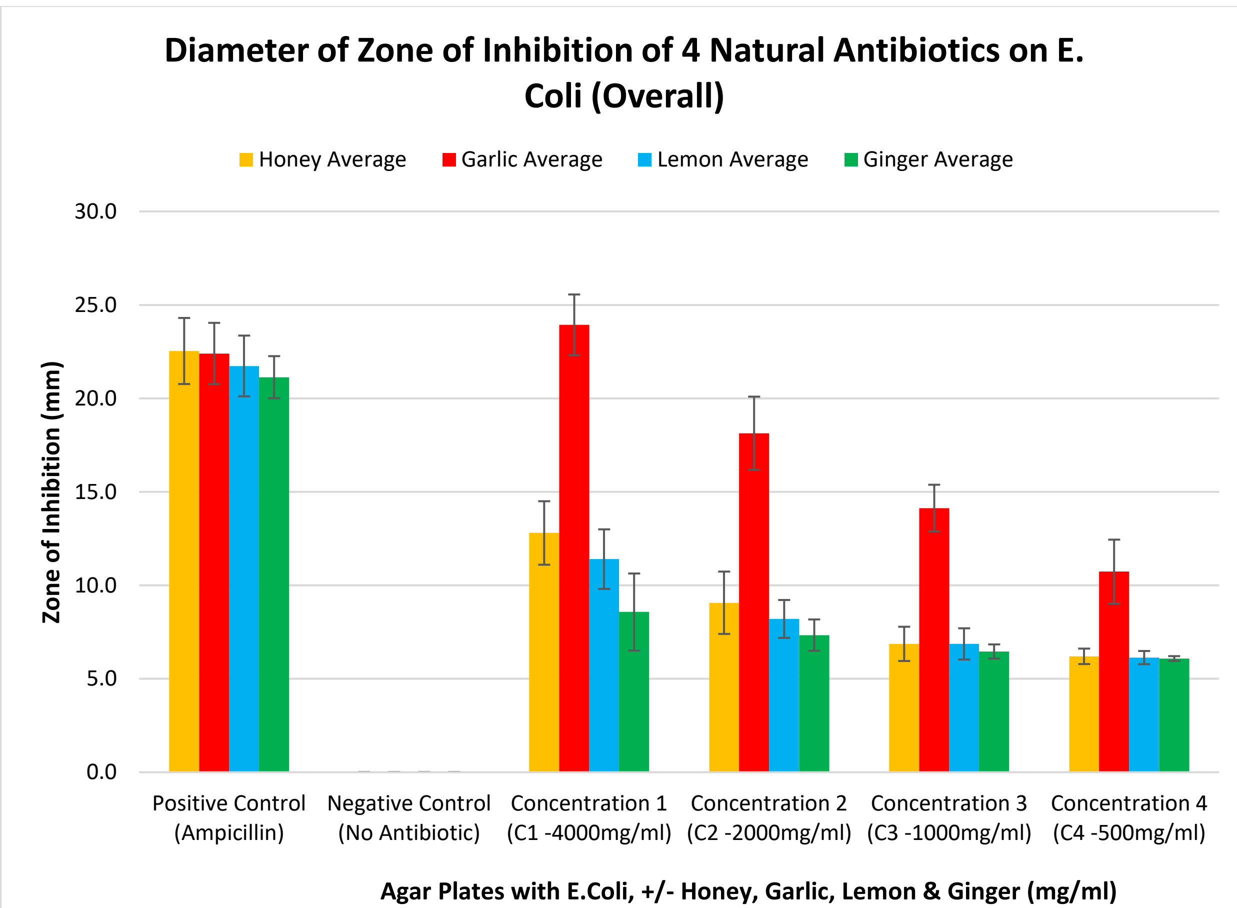
7.1.4 Ginger

Ginger (Diameter of Zone of Inhibition-mm)							
		Positive Control (Ampicillin)	Negative Control (No Antibiotic)	Ginger - 4000mg/ml	Ginger - 2000mg/ml	Ginger - 1000mg/ml	Ginger - 500mg/ml
Trial 1	Sample 1	21	0	8.2	7	6	6
	Sample 2	21	0	8	7	6.5	6.05
	Sample 3	20	0	8.2	7	6.2	6
	Sample 4	20	0	9	8	6.5	6.1
	Sample 5	20	0	8.5	7.5	7	6
Trial 2	Sample 1	21	0	6.5	6.45	6.1	6.05
	Sample 2	19	0	6.2	6.1	6.05	6.05
	Sample 3	21	0	15	9	6.2	6.1
	Sample 4	22	0	8	6.8	6.3	6.05
	Sample 5	22	0	9	8	6.5	6.05
Trial 3	Sample 1	21	0	8.5	7	6.5	6
	Sample 2	21	0	9	8.5	7	6.1
	Sample 3	23	0	6.5	6.2	6.1	6
	Sample 4	22	0	8	7.5	7	6.2
	Sample 5	23	0	10	8	7	6.5
	Average	**21.1**	**0.0**	**8.6**	**7.3**	**6.5**	**6.1**
Standard Deviation		1.13	0.00	2.06	0.84	0.38	0.13



7.1.5 Summary

Natural Antibiotics (Average Diameter of Zone of Inhibition - mm)						
	Positive Control (Ampicillin)	Negative Control (No Antibiotic)	Concentration 1 (C1 - 4000mg/ml)	Concentration 2 (C2 -2000mg/ml)	Concentration 3 (C3 - 1000mg/ml)	Concentration 4 (C4 -500mg/ml)
Honey	**22.5**	**0.0**	**12.8**	**9.1**	**6.9**	**6.2**
Std Dev	1.77	0.00	1.70	1.67	0.92	0.41
Garlic	**22.4**	**0.0**	**23.9**	**18.1**	**14.1**	**10.7**
Std Dev	1.64	0.00	1.62	1.96	1.25	1.71
Lemon	**21.7**	**0.0**	**11.4**	**8.2**	**6.9**	**6.1**
Std Dev	1.62	0.00	1.59	1.01	0.83	0.35
Ginger	**21.1**	**0.0**	**8.6**	**7.3**	**6.5**	**6.1**
Std Dev	1.13	0.00	2.06	0.84	0.38	0.13



7.1.6 Sample Agar Plate Results

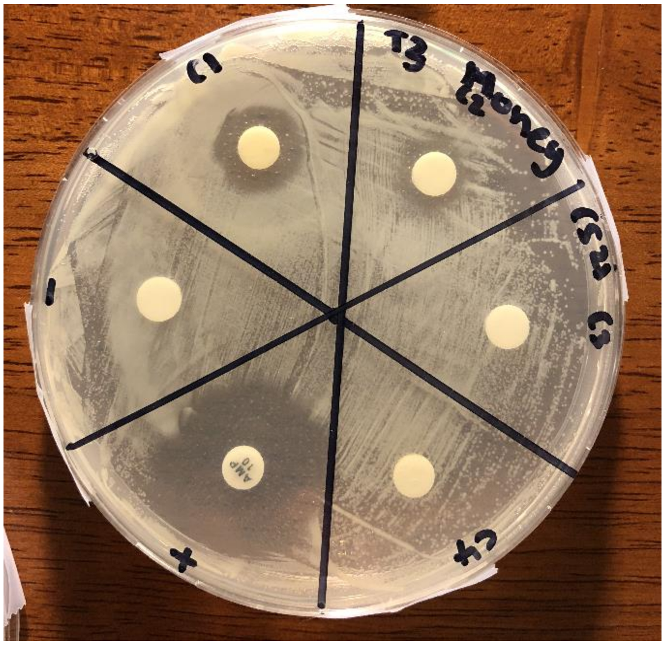


Fig.14 Sample Honey Plate

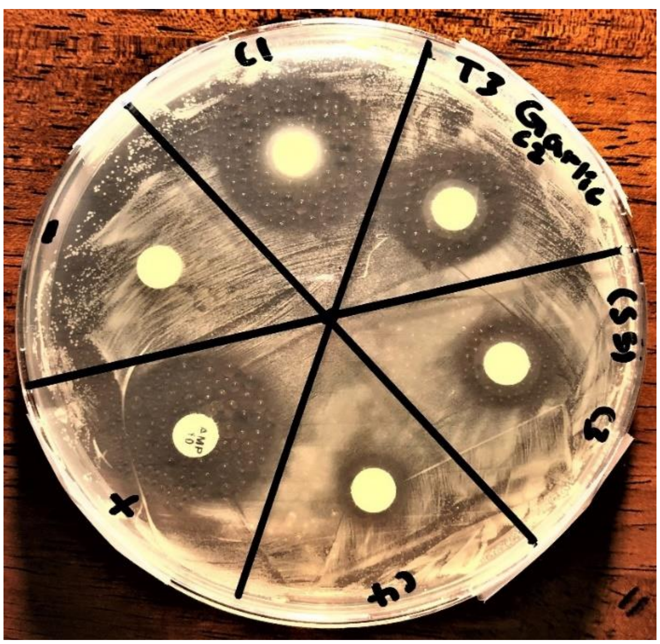


Fig.15 Sample Garlic Plate

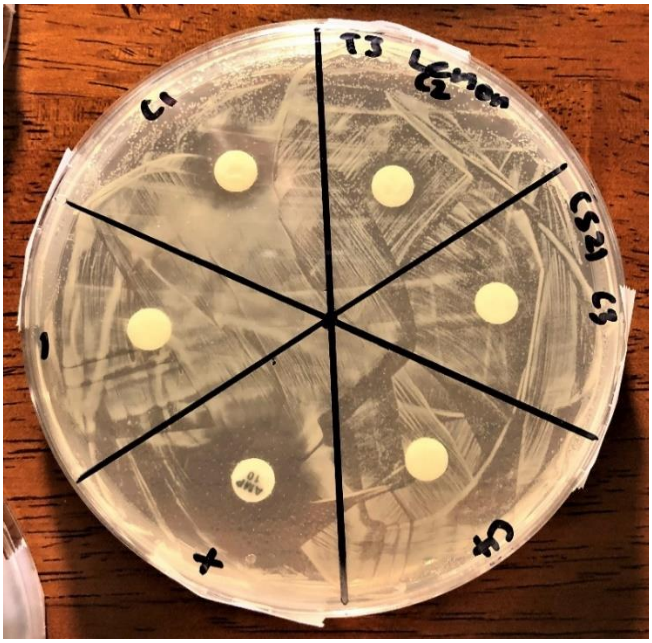


Fig.16 Sample Lemon Plate

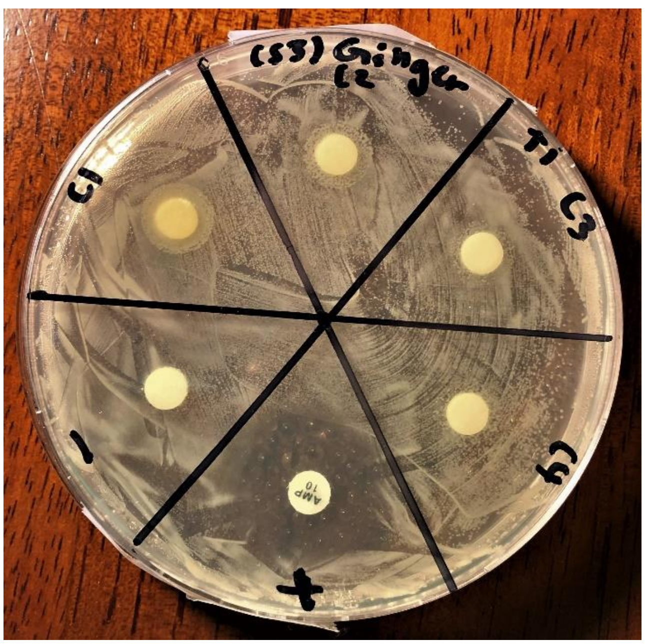


Fig.17 Sample Ginger Plate

7.2 Honey Plates

7.2.1 Honey -Trial 1

Fig.18 Honey – Trial 1

7.2.2 Honey -Trial 2

Fig.19 Honey – Trial 2

7.2.3 Honey - Trial 3

Fig.20 Honey – Trial 3

7.3 Garlic Plates

7.3.1 Garlic -Trial 1

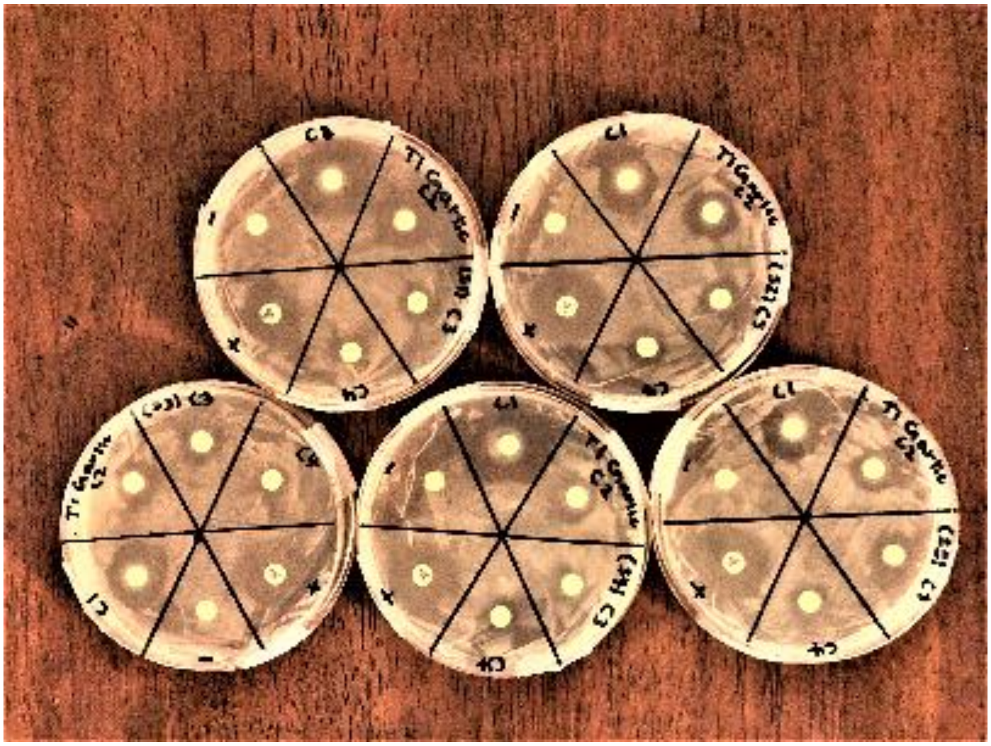


Fig.21 Garlic – Trial 1

7.3.2 Garlic - Trial 2

Fig.22 Garlic – Trial 2

7.3.3 Garlic - Trial 3

Fig.23 Garlic – Trial 3

7.4 Lemon Plates

7.4.1 Lemon - Trial 1

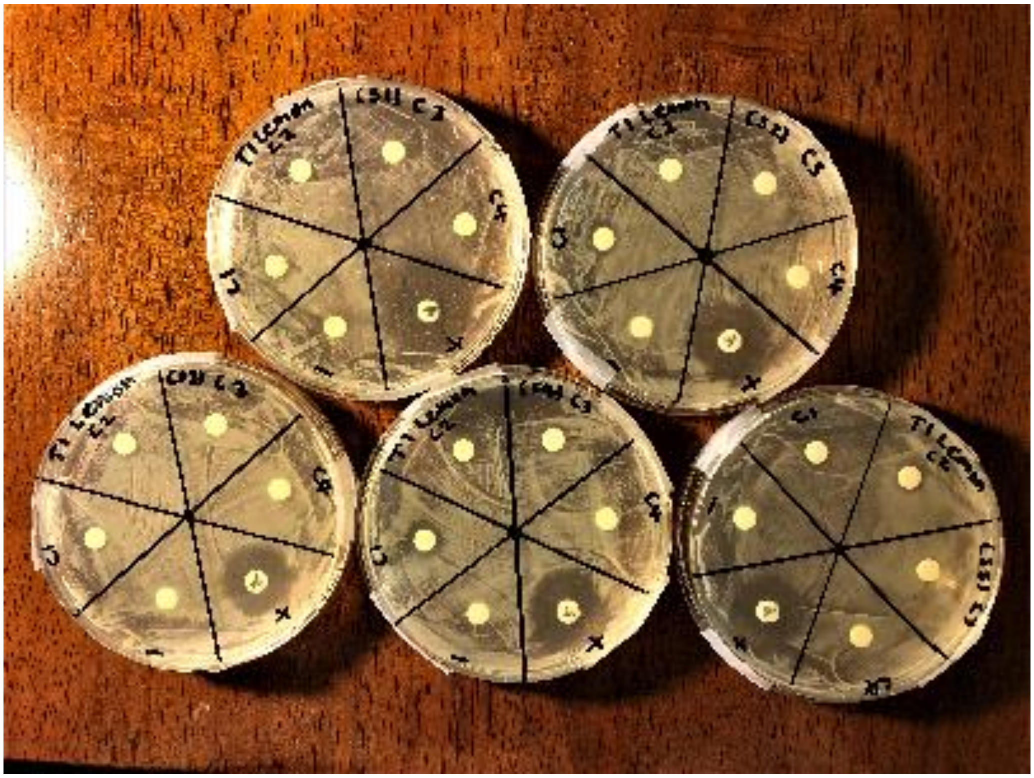


Fig.24 Lemon – Trial 1

7.4.2 Lemon - Trial 2

Fig.25 Lemon – Trial 2

7.4.3 Lemon - Trial 3

Fig.26 Lemon – Trial 3

7.5 Ginger Plates

7.5.1 Ginger - Trial 1

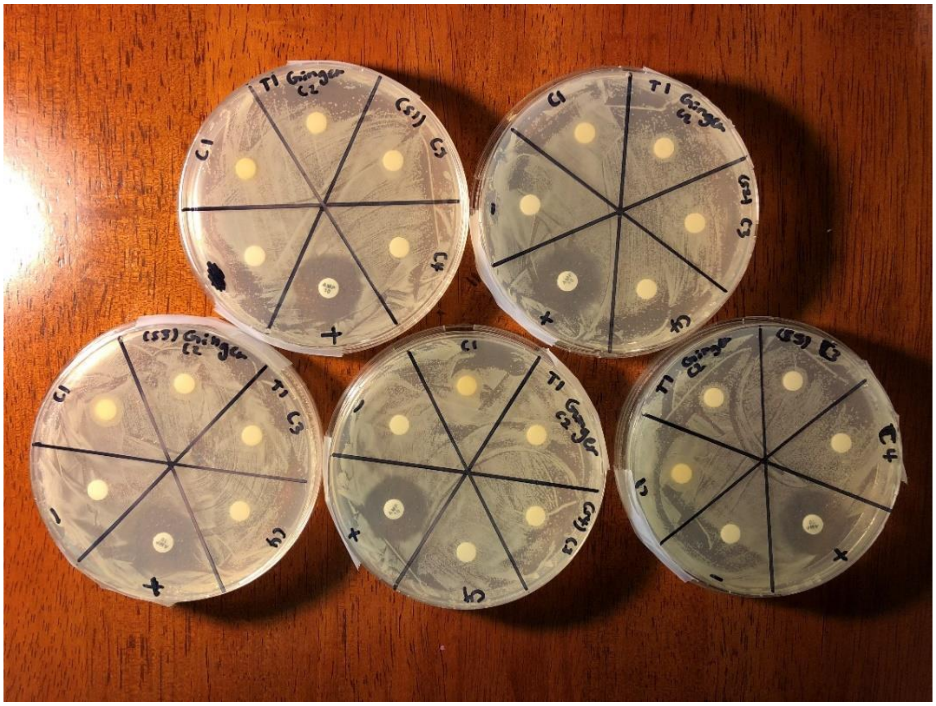


Fig.27 Ginger – Trial 1

7.5.2 Ginger - Trial 2

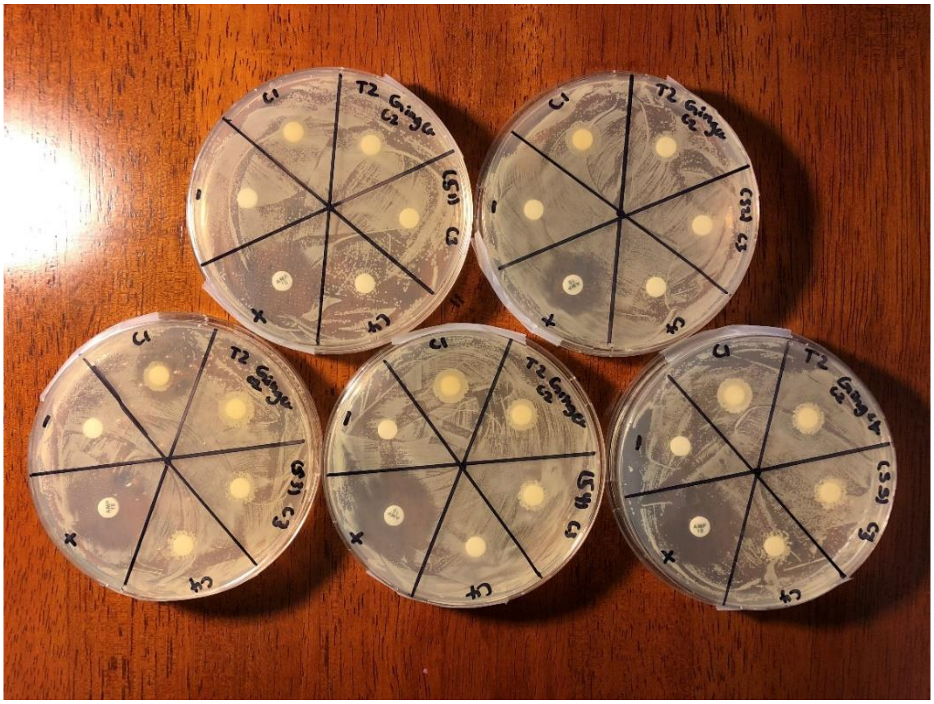


Fig.28 Ginger – Trial 2

7.5.3 Ginger - Trial 3

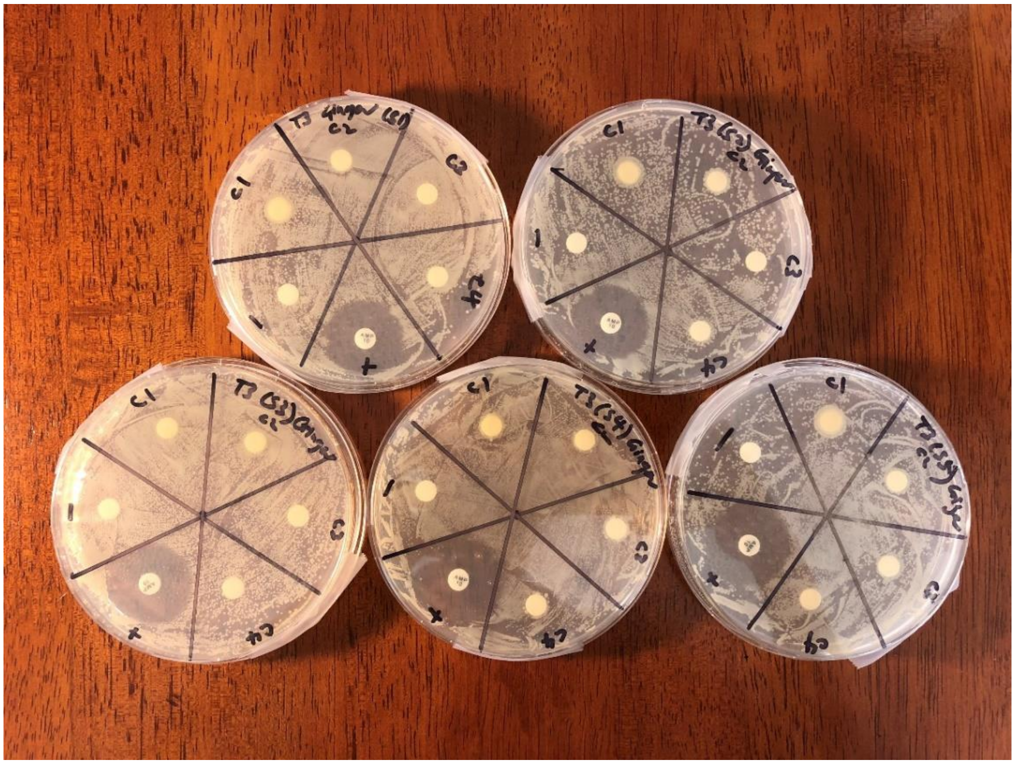


Fig.29 Ginger – Trial 3

8 Conclusions

This experiment tested out four natural antibiotics (Manuka Honey, Garlic, Lemon, and Ginger) and with one synthetic antibiotic, Ampicillin, as a positive control. My hypothesis was that Garlic would best inhibit E. coli growth compared to the other three natural antibiotics. I thought this because many articles have explained that garlic may be even better than the well-known antibiotic Penicillin. Many studies have also said that Garlic is very effective against bacteria because it doesn't only have a couple of compounds, like other natural antibiotics, but have hundreds of them, which when combined together, make a very strong force. One of its compounds is Allicin, which is Garlic's main reason of being so effective. I was pleasantly surprised by the result, which was that my hypothesis was correct and the zone of inhibition of **Garlic was the greatest – at 23.9 mm average diameter of zone of inhibition, at the highest concentration of 4000mg/ml, 18.1mm at 2000mg/ml, 14.1mm at 1000mg/ml, and 10.7mm at 500mg/ml.**

Manuka Honey (12.8mm at 4000mg/ml) and Lemon (11.4mm at 4000mg/ml) were almost the same amount of effectiveness, but the results were pretty low overall, with Manuka Honey in the slight lead.

Ginger was not as effective as I thought it would be and was in fact, the worst natural antibiotic (8.6mm at 4000mg/ml, to about 6.1mm at 500mg/ml). I had done research on Ginger, saying that it was a very health beneficial and strong natural antibiotic. However, this may be for certain and different types of bacteria, and may not be effective on E.Coli.

All of the natural antibiotics were very consistent in that they became less effective the more they were diluted. **Garlic was in the lead in every concentration level – being still quite effective (at 10.7mm zone of inhibition diameter) even at the lowest concentration of 500mg/ml**, even beating the others at higher concentrations of 2000mg/ml. This illustrates the superior efficacy of Garlic, followed by Manuka Honey, Lemon and Ginger.

I hope to use these results to encourage people to use more natural antibiotics, such as Garlic, to prevent antibiotic resistance, which is a big issue all around the world today and have caused many people to suffer. Many people see synthetic antibiotics as their first choice mainly because it is fast to get and they trust it because it is made in a lab. Not many people know that natural antibiotics, such as Garlic, can be just as effective.

9 Recommendations

Overall, I think my experiment went pretty well and there were only a couple of unexpected results. Three of the antibiotics, Manuka Honey, Lemon, and Ginger, were a lot less effective than I deemed and researched them to be, but it may just be because these 3 natural antibiotics are less effective against E.Coli bacteria.

Hence one thing I could extend on this experiment is to try different types of bacteria for these 4 natural antibiotics, because I discovered that different natural antibiotics may be more effective for different types of bacteria. Garlic may be the best against E. coli out of the four I tested, but Lemon could be better at inhibiting a different bacteria’s growth.

Another thing I would also definitely do is to try different natural antibiotics, including some essential oils (Oregano Oil, Tea Tree Oil. Eucalyptus oil, Lavender oil), and compare with synthetic antibiotics, against different types of bacteria.

10 Acknowledgements

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