

# Natural Antibiotics: Effects of Honey, Garlic, Lemon, and Ginger on Escherichia Coli

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## Introduction

Antibiotic resistance is fast becoming a problem in the world, with the overuse of synthetic antibiotics. Synthetic antibiotics are medicines that 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 in addition to 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 usually 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. Some natural antibiotics that have shown to produce effective results are Manuka Honey, Garlic, Lemon, and Ginger.

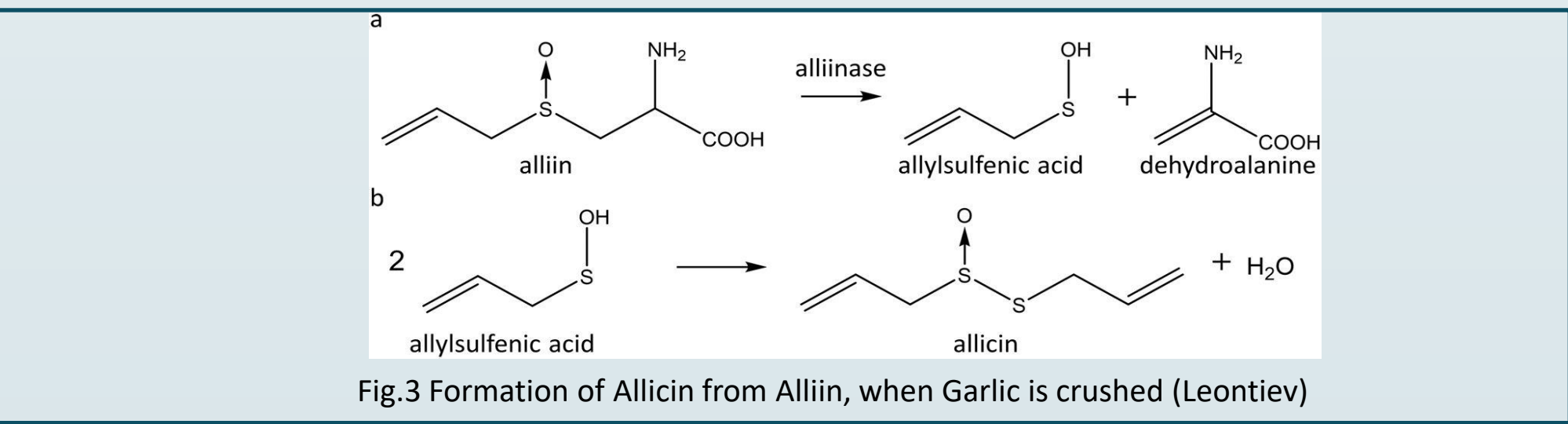
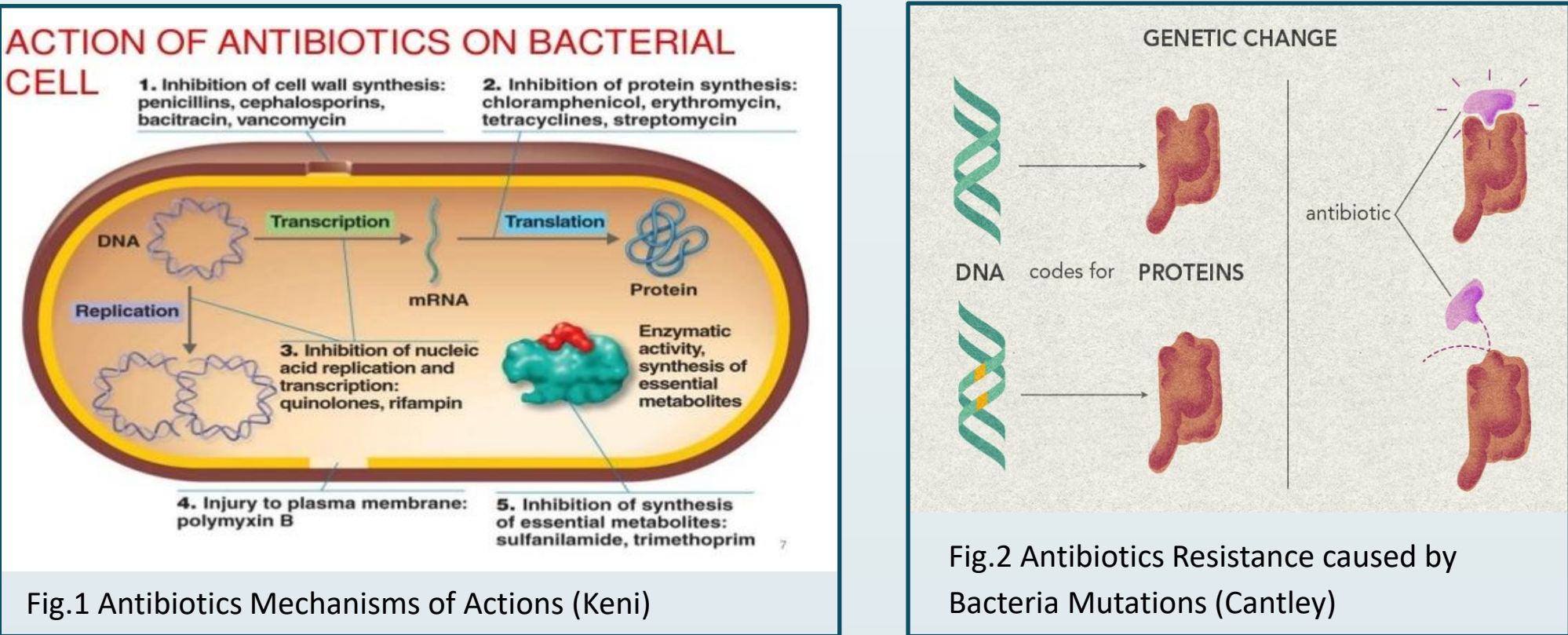
Antibiotics inhibit and kill bacteria in many different methods. Antibiotics can inhibit cell wall production or the cell membrane surrounding bacteria. These membranes keep important substances inside that the bacteria need in order to survive. Another type of antibiotic can cause protein synthesis 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.

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 also 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 Alliin, and its 100+ compounds to stop bacteria's production of energy. Garlic contains two key compounds called Alliin, an amino acid and Alliinase, a protein-based enzyme. When the garlic is damaged, the Alliinase converts Alliin 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, causing it to die. Essentially, lemon is a 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.



## Purpose

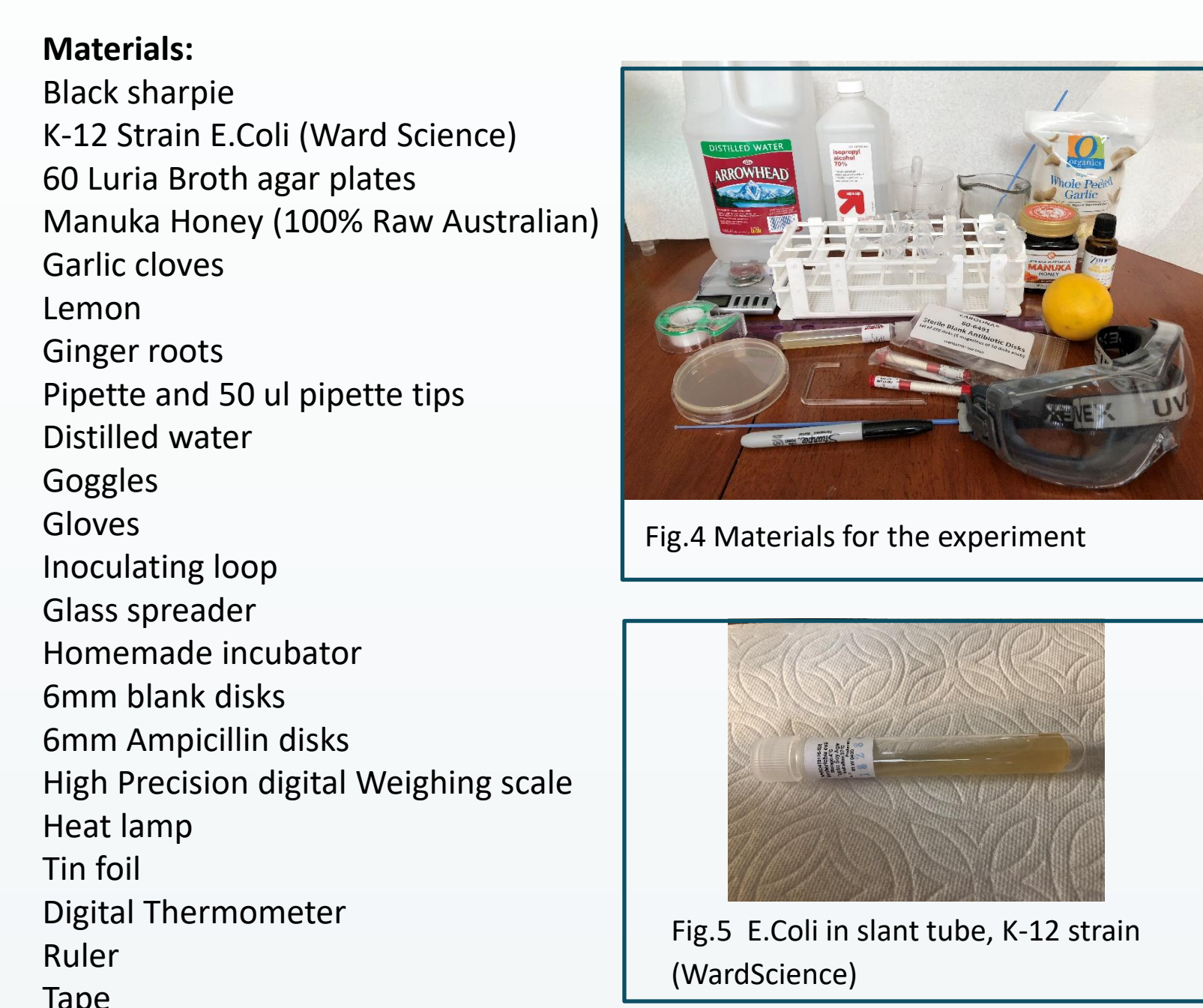
The goal of my project is to investigate natural antibiotics and how well they inhibit *Escherichia coli* (E. coli) bacteria, which is one of the bacteria known for causing food poisoning and others. In my experiment, I will be using Manuka Honey, Garlic, Lemon, and Ginger, which are researched to be effective against various bacteria. I will also be using the synthetic antibiotic, Ampicillin, as a positive control. With these results, I hope to discover which of the 4 natural antibiotics inhibits E. coli growth the best with the largest zone of inhibition, by measuring the zone's diameter, so that people may learn how effective natural antibiotics are. This is becoming extremely important nowadays because synthetic antibiotics have been overused, and have shown to harbor antibiotic resistance in bacteria, hence we may be left with natural antibiotics as our ultimate weapon against them. I hope to help people with their health and let people know how useful natural antibiotics are so they can eat them when they are sick or even include them in their diets to grow stronger immune systems.

## Hypothesis

My hypothesis is that if natural antibiotics are added to a disk placed in an agar plate with E. Coli, its growth will be inhibited within a circular zone of inhibition around the disk. I hypothesized 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 believe Garlic got the lead because it not only contains 3 main compounds, alliinase which converts alliin into allicin when garlic is oxidized, it further breaks down into more than 100 compounds. Neither Manuka honey, lemon, nor ginger contains more than 100 compounds.

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. 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. I selected 100% percentage of my prediction, since I think that garlic will be the most effective among the other 4 natural antibiotics.

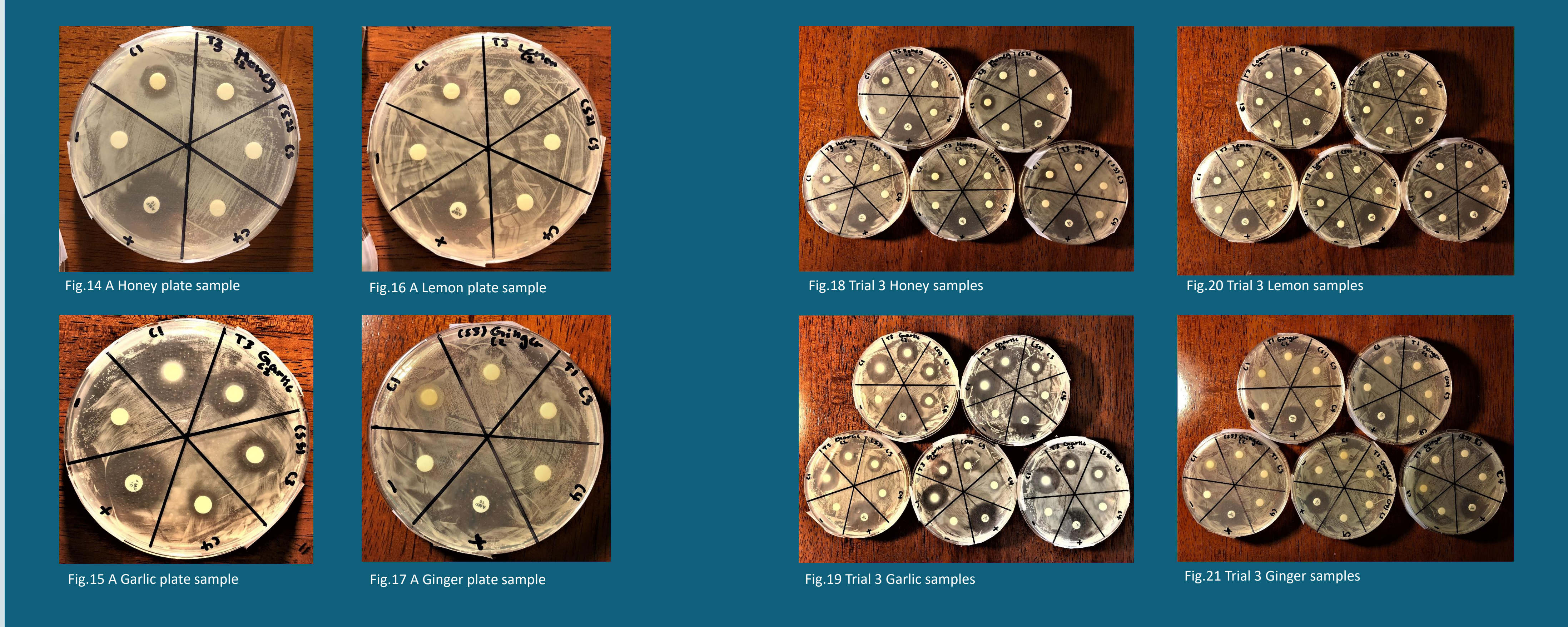
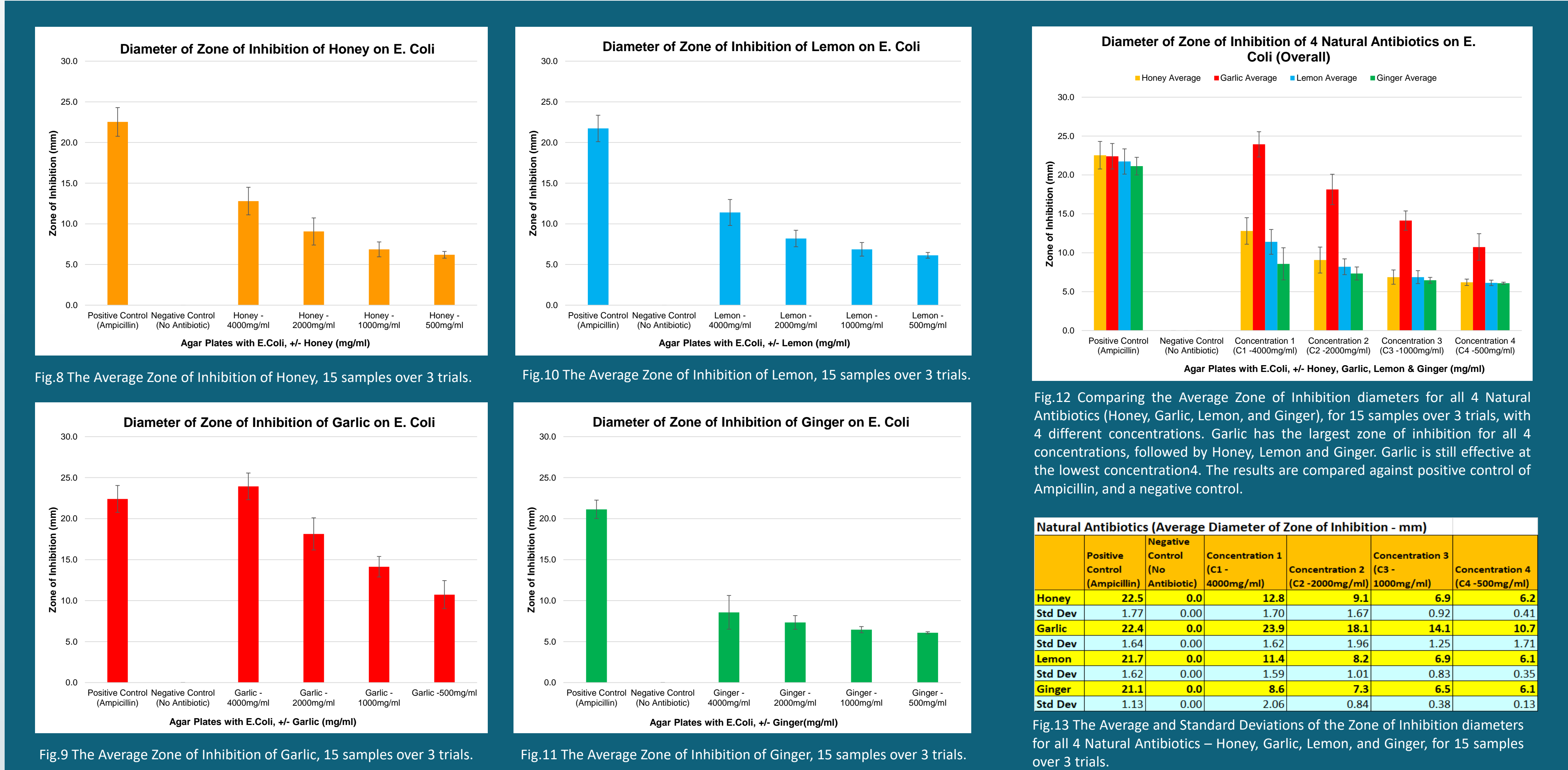
## Materials and Procedures



- Procedures:**
- 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 ginger.
  - 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.



## Results



## Conclusion

My 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 when I researched about it, 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. I was surprised that Manuka Honey wasn't very effective because doctors and other people I know recommend it when I am sick and some of my research has written about its effectiveness.

I was disappointed and very surprised indeed that 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 am very glad that I ended up answering my initial question and that my hypothesis was correct. 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.

## 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.

## Acknowledgements

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