Exploring the Impact of Air Fresheners on the Lung Microbiome

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Introduction

People frequently use air fresheners to make their houses smell nice. In fact, air fresheners are commonly used by 72.8% of American households**.** However, air fresheners can be dangerous. They emit more than a hundred chemicals, including phthalates, formaldehyde, and toluene. Some of these chemicals are linked to cancer, reproductive issues, and other health problems. Moreover, these chemicals could cause unknown health impacts when mixed together because of their synergistic effects. In previous studies, researchers have shown the impact of these chemicals on multiple body systems, such as the nervous system, cardiovascular system, and the gut microbiome.

The gut microbiome is a bacterial community in your gut. charlieIt is responsible for maintaining digestion, the immune response, and overall health. Yet, no studies have looked at the lung microbiome, which is similar to the gut microbiome. The lung microbiome is the group of bacteria that is in your lungs. Since air fresheners are breathed in, they could potentially cause more damage to the lungs than the gut. If this were the case, then the lung microbiome's role in preventing infections, supporting respiration, and balancing cardiac function would be impacted. Thus, it is important to understand and prevent harm to the lung microbiome.

Therefore, the goal of this project is to determine the effect of chemicals in air fresheners on the lung microbiome. In order to do this, this study is examining one of the most common air freshener brands, Glade. Because these air fresheners are used in so many households, they are a good model for most of the air fresheners on the market. The general purpose of this project is to determine the effect of air fresheners on the lung microbiome. Specifically, it examined 1) the

effect of air fresheners on bacterial growth, 2) air fresheners' effect on antibiotic resistance, and 3) the air fresheners' impact on bacterial motility.

It is hypothesized that the lung microbiome will be harmed because of the potentially toxic compounds inside air fresheners. The 1) hypothesis is that the bacterial growth will be slowed. This decrease in growth would be caused by chemicals killing healthy bacteria and stifling growth. The 2) hypothesis is that air fresheners will harm the bacteria's antibiotic resistance. This can be attributed to compounds such as Styrene, Benzene, and Dichlorobenzene, which have been shown to hurt bacteria. Finally, the 3) hypothesis is that the 10% fragrance solution will have an increase in growth, and the 100% percent will have a decrease in growth. This is believed because the ten percent solution would not be enough to severely damage the bacteria. However, with the 100% solution, early damage will occur, affecting the bacterial motility.

Background Research

A **microbiome** is a group of bacteria. They exist in different parts of the body, including the lung and the gut. When these groups are in balance, they play a significant role in supporting bodily functions like respiration, digestion, and the immune response. Two of the most well known microbiomes are in the lung and the gut. Each microbiome performs different functions. This project focuses on the **lung microbiome**. The lung microbiome is in your lung and helps to keep the lung healthy by preventing diseases and supporting normal functions. **Bacteria** make up the microbiome and can support or harm the body systems they inhabit by replicating and protecting areas from other deadly bacteria..

Bacteria come in different shapes, including cocci, bacillus, and spirillum. **Cocci bacteria** are round and spherical. A **bacillus** is a rod-like shape. Finally, **spirillum** is a spiral

shape. An example of a spirillum bacterium is the bacteria that causes lyme disease. **E.coli and staph** are the bacteria that are tested in this experiment. E.coli are gram positive bacteria with a bacillus shape. **Flagella** are what bacteria such as E.coli use to move. They are like propellers. They are made of 9 pairs of a protein called **flagellin**. Staph are gram negative bacteria that have cocci shape. While these bacteria are only present in low amounts in the human body, they are a good model for the two different types of bacteria: gram positive and gram negative. Because of this, they allow the researcher to more accurately predict what would occur in an actual human lung.

It is important to understand the biology behind staph and e.coli. These bacteria are made of a single cell, making them **prokaryotic** or single-celled organisms. Unlike **eukaryotic** cells, prokaryotic cells do not have membrane bound organelles, instead DNA and other essential material is within one chamber. Because of this, prokaryotic cells are smaller than human cells and have **plasmid**s, which are circular molecules of DNA not bound by a nucleus. These plasmids regulate the bacteria's functions, growth, and characteristics. There is a **copy number,** such as 1, for each **chromosome**, and each chromosome codes for a specific trait. A very common trait is **antibiotic resistance**, the bacteria's ability to fight off medications and other lethal chemicals.

In order to survive, bacteria must reproduce and have a consistent fuel source. To find food, some bacteria, called **aerobic bacteria**, use oxygen as nutrients. They can sometimes swap between anaerobic and aerobic but sometimes they cannot. An **anaerobic bacteria** doesn't get nutrients from oxygen. Bacterial growth can be graphed using a **bacterial growth curve**. Bacteria start in the **lag phase**, where they are sensing what is around and if it is suitable for growing. Next is the **log phase**, when growth skyrockets. If the conditions are correct, the

bacteria grow to big sizes. When food runs out, growth slows down and slowly they all die. The growth curve directly impacts the health of the lung microbiome.

Additionally, bacteria can be sorted into two groups. They can be **gram negative** or **positive**, which is determined by their pedioglacan layers thickness. This shows in gram staining with their different coloration. Gram positive stains indicate a thick peptidoglycan layer and stain purple. Gram negative stains are typically pink and suggest a thin peptidoglycan layer.

When the lung microbiome is not balanced because of bacterial growth and death, it can cause bacterial infections and health problems. Therefore, it is important to examine how different chemicals and environments impact the lung microbiome. In this project, air fresheners were tested. **Air fresheners** are used in most American homes, schools, and institutions. They contain chemicals such as phthalates, voc's(volatile organic compounds), carcinogens, and madhye. These chemicals have been shown to cause various types of cancer, nerve damage, and harm to the gut microbiome.

To test the impact of air fresheners, various microbiology techniques were used. The **Kirby-Bauer infection test** is used to determine the sensitivity and resistance to antimicrobial compounds. This can be used to determine what antibiotics to use. Additionally, the MIC (**Minimal Inhibitory Contraction test**) is a way to test what amount of an antibiotic is needed to inhibit a bacteria. These tests allow people to measure antibiotic resistance. Moreover, they help demonstrate the true impact of air fresheners on lung health. Finally, the **motility test** is a way to test how much the bacteria is moving. Changes in movement can reflect threats or other dangers to healthy bacteria. If motility test results are too high or too low, it indicates an unhealthy environment.

Methods

Crate Aerosol Water

1. Step one is to put two holes in the lid of the coffee can
2. Cut the ends off of a pipet tube.
3. Put the pipet tube inside one of the holes.
4. Take the lid off and tape the air pump to the side.
5. Put the cord through the other hole.
6. Hot glue the aquarium tubing to the other end where the air comes out of the pump.
7. Hot glue the tube to the pump.
8. Hot glue the things into the holes.
9. Spray for one minute air freshener onto a paper towel.
10. Place the paper inside the aerosol chamber.
11. Close the lid.
12. Fill a flask with distilled water.
13. Put the tube in the water.
14. Cover the top with tin foil.
15. Plug in and run for 6 hours.

Spectrometer Test

1. Add 2 grams of tryptic soy broth powder.
2. Use a hot plate mixer for two minutes to create the plain media.
3. Autoclave the media.
4. Sterilize and inoculate the loop with fire then let cool.
5. Scrape bacteria off of an agar plate.
6. Put it inside the media.
7. Sterilize the inoculating loop.
8. Let it grow in an incubator for twenty four hours.
9. Take 100 micro liters and add it to another tube of media.
10. Let it grow for twenty four hours.
11. Put some into a spectrometer tube then.
12. Zero the spectrometer on an empty tube.
13. Put the spectrometer tube in the spectrometer to record the number it displays.
14. Repeat with control and arsol media, repeat with E.coli and Staph.

Antibiotic Resistance Test

1. Put 100 micro liters in a tryptic soy agar plate.
2. Sterilize bacteria spreader.
3. Use spread to make sure bacteria is on the whole agar plate.

4. Sterilize bacteria spreader.
5. Put two antibiotic discs on each side of the biofilm.
6. Let it grow for 24 hours then measure the diameter of each circle.
7. Average the diameter.
8. Repeat with control and arsol media and repeat with E.coli and Staph.

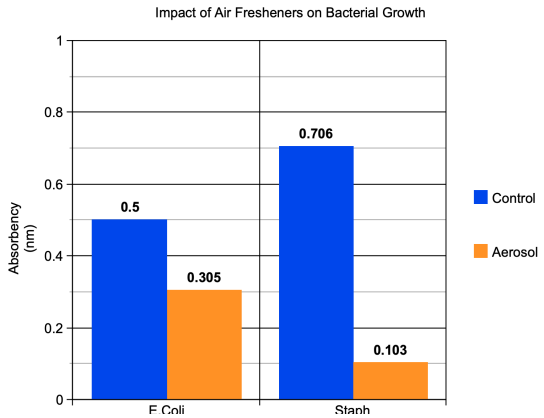
Motility Test

1. Sterilize the inoculating needle then cover in media.
2. Put in a straight line down the middle. Do not touch the bottom of the motitly tube.
3. Take out and sterilize the inoculating needle.
4. Repeat with control and arsol media for E.coli only.
5. Put in an incubator and let it grow for 24 hours.

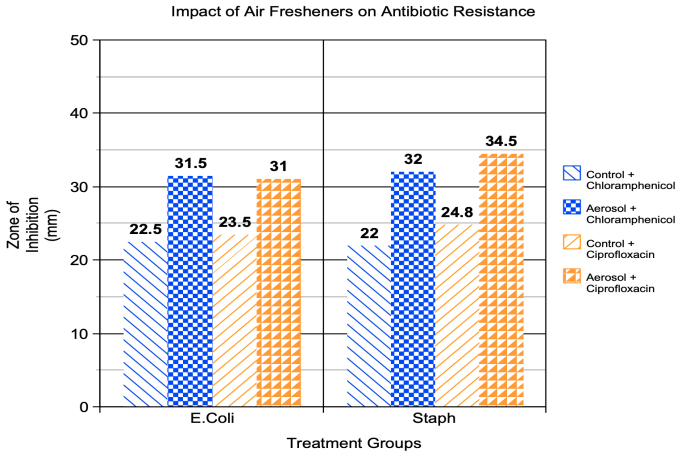
Biofilm Test

1. Put 2ml of media in the tube.
2. Put 100 microliters of bacterial media.
3. Let it grow for 24 hours.
4. Separate the biofilm and the media.
5. Use the spectrometer to zero on empty media.
6. Put the liquid media with bacteria in the spectrometer.
7. Record the number.
8. Put in 100 microliter of a 15mm is isopropyl alcohol and 5ml acetone solution.
9. Put in 1 milliner of crystal violet dye.
10. Let it set for thirty minutes.
11. Wash out the dye.
12. Put in an empty spectrometer tube and zero it.
13. Put in the tube and record the number.
14. Divide the larger number into the smaller number to get the biofilm index.
15. Repeat for staph and E.coli, control and arsol

Results

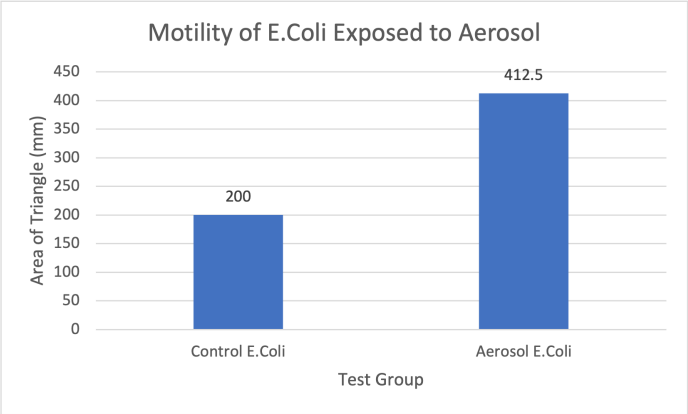


Graph 1. This graph shows how the bacteria growth was affected by the air fresheners in increasing the bacterial growth. There was an 85% decrease in how much light got through in Staph with fragrance media compared to the staph in normal media. Similarly, there was 39% less absorbency in the E.Coli in fragrance media compared to the E.Coli in normal media.

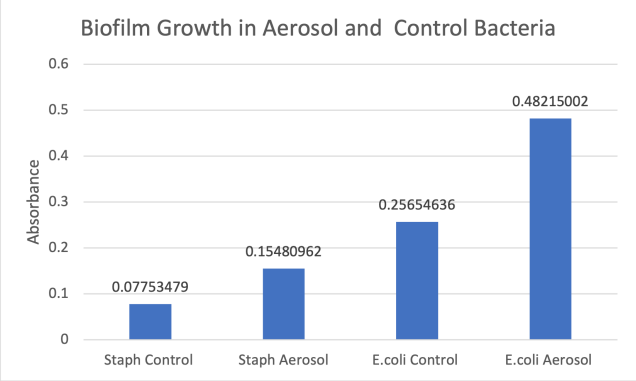


Graph 2. This graph displays the change in antibiotic resistance between Staph and E.Coli with the antibodies ciprofloxacin and chloramphenicol. The graph shows the bacteria antibiotic resistance was lowered by air fresheners and E.Coli was overall less affected. The results showed that the diameter was increased by 45.45% in chloramphenicol staph with fragrance media,

41.57% in chloramphenicol E.coli with fragrance media, 31.91% in E.Coli cipro's diameter with fragrance media, and 39.9% in Staph cipro's diameter with fragrance media.



Graph 3. There is a 69.4% increase in motility for the aerosol media compared to the normal media. This indicates that the motility is usually higher for bacteria that have been exposed to the aerosol media, which is a factor for virulence.



Graph 4. There is a 66.5% increase in the growth of the biofilm for staph aerosol compared to the staph control. There is a 61.1% increase in the biofilm for the E.Coli aerosol when compared to the E.Coli Control. This indicates that the biofilm is typically thicker for bacteria that have been exposed to the aerosol media, which is a factor for virulence.

Conclusion

It was hypothesized that the bacterial growth with air fresheners would be lowered because of the harmful compounds such as carcinogens and phthalates. However, the results disproved this hypothesis. There was an 85% decrease in how much light got through in Staph with fragrance media compared to the staph in normal media. Similarly, there was 39% less absorbency in the E.Coli in fragrance media compared to the E.Coli in normal media. While the E.coli was affected much less than the staphylococcus, there was still a significant decrease in the amount of light that passed through the media.

In a spectrometer test, lower percentages represent lower light absorbency. This means the media is thicker in test tubes with higher percentages. This change in color and thickness is due to new bacterial growth. So, based on the data, bacterial growth was higher in the tubes with the fragrance media. This could be due to a variety of factors. For instance, the chemicals in the media could be causing mutations, forcing the bacteria to multiply faster and in an uncontrolled fashion. Additionally, the bacteria in the normal media could have reached its log phase earlier or later than 24 hours. This means that it could have already passed its peak in growth, or it had not reached its peak yet. All of this could impact the amount of bacterial growth detected by the spectrometer.

However, if the bacteria growth was increased in the febreeze media, this would have a negative impact on the lung microbiome. The bacteria growth being increased is harmful because it increases the chances of harmful bacterial growth. Moreover, uncontrolled growth disrupts the balance of the microbiome, harming it and the functions it supports.

Additionally, it was hypothesized that the antibiotic resistance in bacteria with fragrance media would be lowered. This is because of the harmful compounds inside of air fresheners,

such as carcinogens and neurotoxins. My hypothesis was proven correct by my data. The results showed that the diameter was increased by 45.45% in chloramphenicol staph with fragrance media, 41.57% in chloramphenicol E.coli with fragrance media, 31.91% in E.Coli cipro's diameter with fragrance media, and 39.9% in Staph cipro's diameter with fragrance media.

In all of the plates with fragrance media, there was an increase in the diameter of the zone of inhibition. This suggests that the bacteria in these plates were more affected by the antibiotic discs than the bacteria in the normal media plates. Overall, these results indicate that antibiotic resistance was lowered in bacteria grown in fragrance media. As a result, air fresheners could be lowering the antibiotic resistance of bacteria in human lung microbiomes. This would make people more prone to respiratory infections and other lung conditions.

While all of the plates with fragrance media had larger zones of inhibition, different antibiotics affected the zones of inhibition on separate scales. Ciprofloxacin, a common treatment for pneumonia, impacted the zones of inhibition less than chloramphenicol. In fact, there was a 10% difference in the zones of inhibition between these two drugs for E.Coli. This indicates that different drugs impact antibiotic resistance in seperate ways. This could be due to the synergistic effect of the antibiotic and chemicals in the fragrance media. Overall, the data shows that bacterial growth is increased by air fresheners and antibiotic resistance is lowered. This has interesting implications for the lung microbiome.

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