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The Differences in Antibacterial Activities of Various Honey Produced by One Source of Pollen

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ABSTRACT

The objective of this study is to investigate the differences in antibacterial activities of various honey produced by one source of pollen against *Escherichia coli* and *Staphylococcus epidermidis.* Primary, quantitative data was obtained in this study using the Kirby Bauer Test. 8 different types of honey were tested in this experiment against both gram-negative and gram-positive bacteria on plates inoculated by both spread and pour method. Each honey plate was tested with three replicate discs in their quadrant to allow for the establishment of accurate standard deviations. Using the results of inhibition zones, conclusions were made regarding which type of honey was most effective than the others tested in the experiment.

Introduction

Bees are responsible for producing the delicious natural product: honey, which is regarded for its nutritional content and health benefits (Stagos et al. 2018). Honey has a rich history of use in Indian culture as an add-on to daily meals, treatment of wounds, and managing respiratory issues (Guruvu et al. 2020). Since the beginning of time, people have used customary medicine to treat infections. Honey is among one of the oldest traditional medicines still used today and is useful in the treatment of several human diseases (Mandal and Mandal 2011). Honey can be used to remove free radicals from burns or wounds, which also lessens contractures and scarring; Honey has anti-inflammatory and antibacterial properties that will keep the damaged region stay moist and, as a result, stop it from deteriorating and fibrosing (Almasaudi 2020). These findings led to a recent expansion of new studies to explore how different honeys influence the development of skin bacteria.

The Human System

Honey is valued for its nutritional content in addition to its antioxidant properties, which are thought to be crucial for human health (Stagos et al. 2018). Due to their favorable effects on human health, propolis and honey are both very well-liked (Rahman et al. 2010). The medical grade honeys exhibit strong in vitro bactericidal action against bacteria resistant to antibiotics that can cause a number of potentially fatal diseases in people (Mandal and Mandal 2011). *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus* have all been shown to be successfully eradicated by manuka honey, according to research (Mandal and Mandal 2011). Honey and plant extracts work together to combat contagious illnesses like hepatitis, TB, tetanus, influenza, and human immune deficiency syndrome (Khan et al. 2018). This has made it possible to create a natural medication that is both safe and extremely effective (Khan et al. 2018). Although many people are unaware of the benefits honey can offer, this is an aspect that should be taken into account when evaluating honey's possible future applications for treating inflammatory issues that are prone to certain variables.

Skin Microbiome

Infections of the skin and chronic wounds are serious and expanding global health issues (Morroni et al. 2018). The human skin is a sophisticated physiological barrier created to protect the host from opportunistic microorganisms and preserve internal equilibrium (Brown and Horswill 2020). Honey prevents skin maceration because of its high osmolarity and ability to keep the wound wet (Almasaudi 2020). Some investigations evaluated the potential of honey against certain infectious intestinal bacteria and pathogenic bacteria that frequently cause skin wound infections in both humans and animals (Almasaudi 2020). However, many people are not aware of the potential honey can provide for them, which is why it is a factor that ought to be taken into consideration while assessing its potential future application in treating skin conditions that are prone to certain variables.

Honey and its Properties

Not only does honey allow foods to taste sweeter, but it also has antioxidants and flavonoids, which allow it to acquire antimicrobial properties (Rahman et al. 2010). The color of honey is a reflection of the numerous ingredients it contains, including polyphenols, minerals, and pollen, with black honey containing more pigments like flavonoids (Albaridi 2019). Additionally, honey's phenolic components, organic acids, vitamins, and flavonoids enhance its antibacterial effect and have the potential to be antioxidants (Almasaudi 2020).

Bacterial Anatomy and Antibacterial Efficiency

There are certain types of bacteria with certain anatomy that allow them to be susceptible to certain antibiotics. Bacteria can be tested for their properties by conducting a gram stain to determine its structure. A gram stain can display pink cells under a microscope, which means it is gram negative. For example, *Escherichia coli* (*E. coli)* is gram negative and this overall means that it contains a thin cell wall. Whereas gram positive bacteria -like *Staphylococcus epidermidis (S. epidermidis)* - display purple cells under a microscope and contain thicker cell walls. Using both gram negative and gram-positive bacterial cells for testing with honey can allow for more data and a better understanding of honey's effects on both types of bacteria, which can allow for more implications to be applied. Before the lab was conducted the bacteria was tested using gram staining to ensure that the correct bacteria were in use, and as seen in Fig 1-2 the purple coccus - sphere shaped - cells are *S. epidermidis, and* the pink bacillus -rod shaped- cells are *E. coli.*

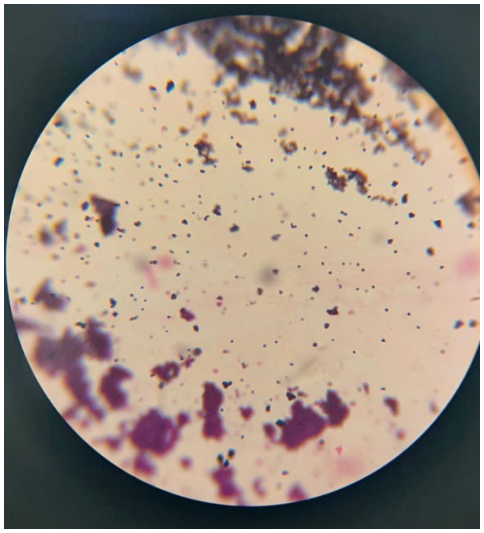

Figure 1. Gram Stain of *S. epidermidis*

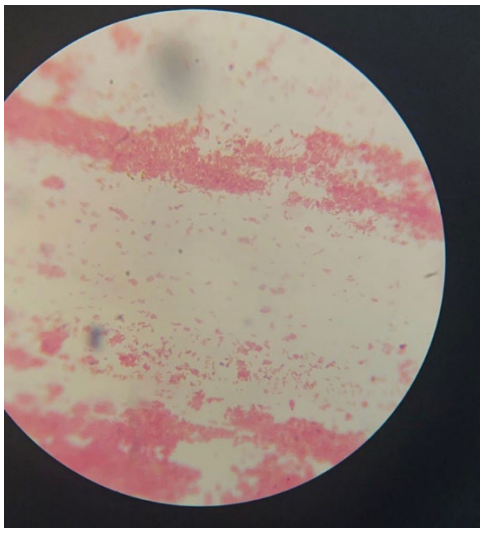

Figure 2. Gram Stain of *E. coli.*

Gram negative bacteria - like *E. coli* - are frequently identified from infections of the skin and soft tissues, but little research has been done on their potential for pathogenicity (Petkovsek et al. 2009). It not only is seen growing in humans but also in the small intestine which can lead to many stomach issues (Dowshen 2017). *E. coli* and other human infections have been proven to be successfully combated by honey in previous studies like the one conducted by Manisha Deb Mandal (Mandal and Mandal 2011). Due to the direct impact topical treatments have on E. coli and the implications of E. coli growth, honey should be assessed for the implications that it acquires given that it is known to have antibacterial properties.

S. epidermidis, a gram-positive bacteria, is one of the microorganisms most commonly found colonizing healthy human skin. Although it is natural for *S. epidermidis* to live on human skin and in human mucosa, it is also possible to reach the bloodstream after passing through the prosthetic device and invade the human body (Lee and Anjum 2020). In addition, *S. epidermidis* is a type of bacteria that has presented numerous difficulties in the therapeutic process (Namvar et al. 2014). With both *E. coli and S. epidermidis* being potential infectious bacteria to human health, it is important to evaluate both gram negative and gram positive bacteria against various honey to be able to apply implications towards both types of bacteria for certain skin conditions and inflammatory issues.

Hence, the inquiry "To what extent does the type of honey, sourced from one type of pollen, affect the growth of *E. coli* and *S. epidermidis*?" is seen. The effects of several one-sourced pollinated honey on S. epidermidis and *E. coli* are examined in this study in connection to their different characteristics. When examining the usage of honey for medical therapies, which can treat a variety of illnesses including bacterial infections, it is critical to consider these consequences (Mandal and Mandal 2011). The effects of honey on participants that are suffering from these types of infections can help them towards healing, which is why the effects of various honey should be evaluated.

Review of Literature Review

Human Bacteria and Honey Usage

Despite a lack of a thorough understanding of the precise processes underlying these effects, the antibacterial properties of honey have been known in practice for more than a century. According to a study conducted by Najla A. Albaridi, an assistant professor at Abdulrahman University, honey has many different properties that allow it to acquire antimicrobial activities, including glucose oxidase, catalase, peroxidase, and many more (Albaridi 2019). Mandal

(2011), a professor at KPC Medical College and Hospital, agrees with this statement as they claimed that honey's antibacterial properties are primarily provided by hydrogen peroxide, the concentration of which is determined by the ratio of glucose oxidase. In addition to these properties that honey contains, it also can contain different proteins that have been detected in different honey varieties, predominantly related to different types of plants/flowers (Albaridi 2019). As claimed by Mandal, the concentration of peroxidase is determined by the ratio of glucose oxidase, which is determined by the type of pollen in the honey (Mandal and Mandal 2011). Saad Almasaudi also agrees with this statement as he claimed that the pasture where the bees were bred, the climate, and the natural makeup of the floral nectar are all likely to have an impact on the antimicrobial properties of honey (Almasaudi 2020). A study conducted by Gianluca Morroni, a doctoral researcher at Marche Polytechnic University, evaluated the antimicrobial activities of 4 different honey that all came from different countries. Morroni came to a conclusion and found that the honey that came from Kenya was least effective at being resistant to bacteria (Morroni et al. 2018). Morroni's findings prove how honey originated from different environmental factors can contribute to the differences in the antimicrobial activities that each type of honey acquires. Comparing different types of honey from several countries shows how large of a difference each type of honey can have and the different properties they contain on a much larger scale. Although a comparison was made throughout different countries, it does not confirm if different honey produced from one single pollination source in one region have different properties than other honey produced in the same region as a more narrowed approach.

Honey Specific Research

Many different types of honey have been researched throughout history, and many studies that have tested various honey claimed that honey's antimicrobial properties were due to the concentration of hydrogen peroxide. Although some of these studies do acknowledge that the concentration of hydrogen peroxide in honey is determined by the ratio of glucose oxidase, which is determined by the source of pollination of the honey, there has not been any research conducted that tests specific types of honey sourced from one type of pollen from one region. This can be seen from Mandal's (2011) previous claim which also agrees with the claim about how depending on the honey's floral source, there are variations in its antibacterial properties, which was claimed by Khalid Mehmood (Wadi 2022). As the source of pollination of various honeys can vary depending on where it is produced, this can overall affect the antimicrobial activities that it acquires, which in turn has not been explored yet, overall demonstrating the gap in the literature.

Antibiotic resistance is making it more difficult and expensive to treat hazardous bacteria on the surface and inside the human body (Albaridi 2019). As honey is used regularly in society, it is crucial to investigate how different types of honey impact the human body and skin microbiome, especially for people who may already have skin issues. So the inquiry is once again raised: "To what extent does the type of honey, sourced from one type of pollen, affect the growth of *E. coli* and *S. epidermidis*?" since the effects of honey generated from one single pollination source to another single sourced honey have not yet been evaluated.

Methods

Because of the dependence on primary, quantitative data and experimental controls, an experimental technique of inquiry was used for testing. A related study that measured the antibacterial effects of diluted honey and propolis similarly used the method employed to gather quantitative data (Mama et al. 2019): the Kirby Bauer test. The Clinical Laboratory Standard Institute's guidelines for determining an organism's susceptibility support the standardization of the Kirby Bauer test. (Mama et al. 2019). Agar plates are inoculated with a bacterial lawn for each plate. After placing disks containing the tested honey on the agar, the presence and extent of an inhibition zone surrounding the disks, where no bacterial growth is visible, serve as indicators of the honey's inhibitory zones. The Kirby-Bauer test's main

goal is to provide consistent, repeatable data regarding the antibacterial capabilities of diverse substances by using data from inhibition zones.

Procedural Alterations

Honey can be classified based on the type of plant it was pollinated from or just as general as "honey" if it contained multiple types of pollen. This variety may be caused by the varied regions of production and may result from the various antibacterial activity of honey in various geographic regions as bees prepare their valuable honey (Mama et al. 2019). As the type of pollination in honey varies significantly, the pollination type plays a very important role in the antibacterial properties of each honey. However, pollination by each bee can not be tracked in nature, which is why all honey will always contain different types of pollen. This study compared the general pollination source and antibacterial properties of several types of honey.

E. coli and *S. epidermidis* are both known to have prominent fast growth, which can be grown on various types of agar. As a result of accessibility in my school's lab, Mueller-Hinton agar was substituted with nutritional agar. The concentration of both bacteria when inoculated on the agar was taken into account for how much growth is expected to be seen on the agar plates. 0.5 McFarland is the standard for the concentration of the bacteria (Stagos et al. 2018). This allows for a standardized number of cells in the bacterial broth before inoculation.

Materials Used

Supplies including an autoclave, beakers, bunsen burner, stir plates, graduated cylinders, distilled water, erlenmeyer flasks, 85mm petri dishes, sterile paper towel, forceps, and watch dishes were already available for use in this study. Refer to the following list of materials in Table 1, which denotes the necessary items needed for an experiment to be conducted for replication.

Table 1. Materials used.

Product Name	Company	Quantity	Cost
Buckwheat	Spencer Apiaries	1 container	$20
Palmetto	Bee Natural	1 container	$3.10
Avocado	Bee Natural	1 container	$3.05
Star Thistle (donated)	Sleeping Bears	1 container	$0
Blueberry (donated)	Hardy Honey	1 container	$0
Orange Blossom (donated)	Hardy Honey	1 container	$0
Black Locust (donated)	Hardy Honey	1 container	$0
Clover	Bee Natural	1 container	$3.10
Nutrient Agar	Carolina	1 pack	$43.45
Nutrient Agar	Carolina	1 pack	$22.20
Filter Paper	United Specific	1 pack	$7.68
E. coli, Nutrient Broth	Carolina	1 tube	$12.25
S. epidermidis, Nutrient Broth	Carolina	1 tube	$12.25

Pre-experimental Preparation

Agar, diffusion disks, and broth were required to be obtained and sterilized before the primary procedure could be carried out. The Nutrient agar, as well as the nutrient broth, were prepared by the instructions according to the container from Carolina. By hole-punching filter paper, 6mm disks were made for the honey and an antibiotic was chosen

based on what type of bacteria it was being tested on: neomycin for the plates tested against *E. coli* and Erythromycin for the plates tested against *S. epidermidis*. In addition, there was a dry control on every agar plate to illustrate the growth of the bacteria.

After this preparation, all utensils were autoclaved to ensure that everything was sterile upon the start of the experiment. Lastly, the bacterial broth for each species - *E. coli* and *S. epidermidis*, was made by inoculating a small portion of the bacteria from an agar slant culture into a tube of nutrient broth aseptically following the 0.5 McFarland standard, and incubated for 37 degrees Celsius in order for the bacteria to grow before the day of the experiment and were 24 hours fresh. Aseptic techniques were followed throughout the whole procedure to ensure that there were no contaminations as well as sterile environments.

Main Procedure

32 85mm diameter agar plates 4 mm deep were made, where 16 of them were poured with just nutrient agar, and the other 16 were poured with agar and 500 um of the required bacterial species in the plate. Spread plates were inoculated with 1 ml of bacteria on the surface of the agar after the agar was settled solidly in the plate. Whereas the pour plates already had 500um of bacterial broth inoculated in the liquid agar, then was settled. In addition, 16 of the plates were designated for *E. coli* and the other 16 were designated for *S. epidermidis*. Each type of honey was tested on 4 different agar plates as two variables were being tested against each honey in this experiment: the type of bacteria and the type of inoculation. Plates were labeled with the type of honey the species, and the type of inoculation.

To create a uniform surface growth, the spread-plate method included spreading the 1mL broth inoculum throughout the hardened nutritional agar. This was done with a sterilized glass rod in order to evenly distribute the bacterial broth on the surface of the agar to allow for even growth. The pour-plate method required micro pipetting 500 um of bacterial broth within the liquid agar in each plate, then swirling the dish gently to create even growth within the agar. Both of these types of inoculation were used because they allowed for a comparison between bacteria within the agar and on the surface of the agar. The pour plate method was utilized as it is known for being a great representation of the skin microbiome and the deep dermis, whereas the spread plates allowed for a demonstration of bacteria on the surface of the skin. To obtain a more thorough assessment of each honey's impact on bacterial surface growth and deeper growth, both techniques were used.

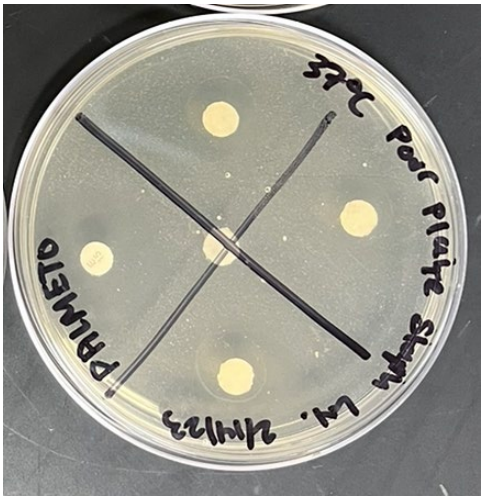

Figure 3. Standard Plate Setup.

Honey discs were created on the day of the experiment by blotting a thin layer on both sides of the filter disc with a sterile paper towel. With a dry control in the center, an antibiotic- Erythromycin used for *S. epidermidis* and

Neomycin used for *E. coli*-was placed in one of the quadrants as the plates were then divided into quadrants Fig. 3. Lastly, the remaining 3 quadrant spaces each received one disc of the 3 replica honey discs. Using the same forceps for each honey and species, which were autoclaved, the disks were positioned in their designated quadrant. Finally, the plates were placed in the incubator at 37 degrees Celsius. The same overall technique was done for both the pour plates and spread plates because the filter paper discs were all made the same according to each honey. Refer to Fig 3. as it illustrates the standard setup of all the plates made in my experiment. An overview of all the pour plates in my experiment can be seen in Fig. 4. which follows the setup of spread plates in my experiment. Refer to Table 2 as it indicates the composition of the plates made for this experiment.

Figure 4. Setup for Experiment for Pour Plates.

Table 2. Plate setups

Honey Type	Bacterial Species	Method	Number of Plates
Buckwheat	*E. coli*	Pour-plate	1
Buckwheat	*E. coli*	Spread-plate	1
Buckwheat	*S. epidermidis*	Pour-plate	1
Buckwheat	*S. epidermidis*	Spread-plate	1
Palmetto	*E. coli*	Pour-plate	1
Palmetto	*E. coli*	Spread-plate	1
Palmetto	*S. epidermidis*	Pour-plate	1
Palmetto	*S. epidermidis*	Spread-plate	1
Avocado	*E. coli*	Pour-plate	1
Avocado	*E. coli*	Spread-plate	1
Avocado	*S. epidermidis*	Pour-plate	1
Avocado	*S. epidermidis*	Spread-plate	1
Star Thistle	*E. coli*	Pour-plate	1
Star Thistle	*E. coli*	Spread-plate	1
Star Thistle	*S. epidermidis*	Pour-plate	1
Star Thistle	*S. epidermidis*	Spread-plate	1
Orange Blossom	*E. coli*	Pour-plate	1

Honey Type	Bacterial Species	Method	Number of Plates
Orange Blossom	*E. coli*	Spread-plate	1
Orange Blossom	*S. epidermidis*	Pour-plate	1
Orange Blossom	*S. epidermidis*	Spread-plate	1
Blueberry	*E. coli*	Pour-plate	1
Blueberry	*E. coli*	Spread-plate	1
Blueberry	*S. epidermidis*	Pour-plate	1
Blueberry	*S. epidermidis*	Spread-plate	1
Black Locust	*E. coli*	Pour-plate	1
Black Locust	*E. coli*	Spread-plate	1
Black Locust	*S. epidermidis*	Pour-plate	1
Black Locust	*S. epidermidis*	Spread-plate	1
Clover	*E. coli*	Pour-plate	1
Clover	*E. coli*	Spread-plate	1
Clover	*S. epidermidis*	Pour-plate	1
Clover	*S. epidermidis*	Spread-plate	1

Results

All conditions were kept constant and every 24 hours for two days, plates were taken out of the incubator, and the inhibition zones were measured by taking the distance of the radius in millimeters — not including the paper disc — was recorded following the standards of the Kirby Bauer test. This is seen on the agar plates by the area, which contains no bacterial growth. When there was no zone, it was recorded as 0 as its zone. The accompanying tables and figures display the zones of inhibition recorded for the various honey tested after a 48-hour incubation period.

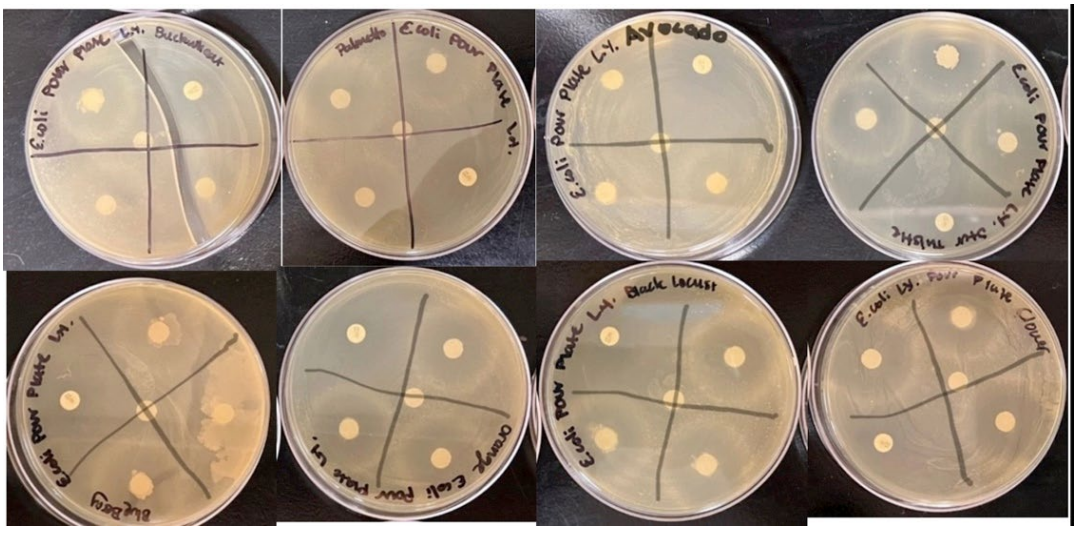

Figure 5. Honey Pour Plates after 48 Hours of Growth against *E. coli.*

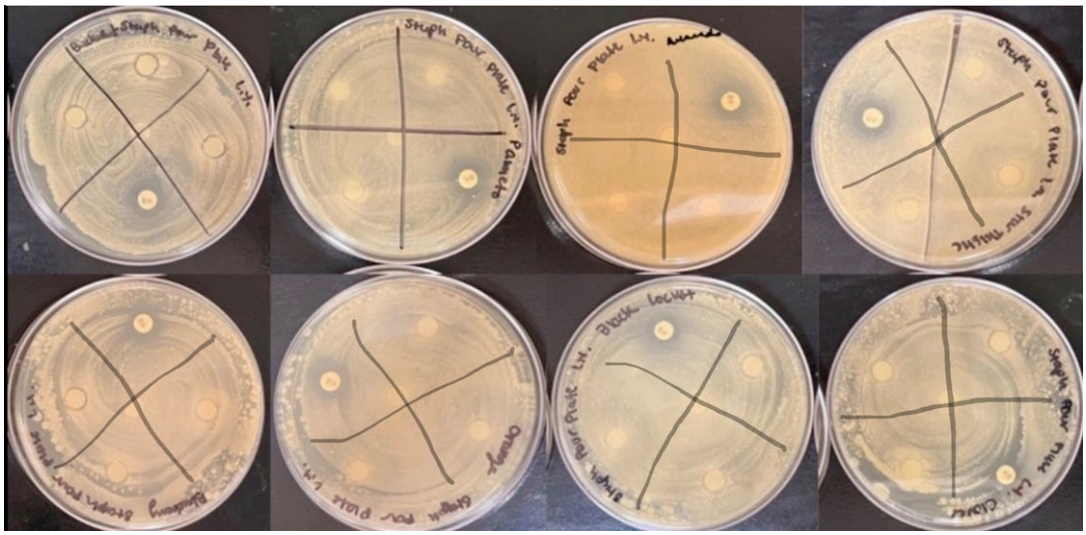

Figure 6. Honey Pour Plates after 48 Hours of Growth against *S. epidermidis.*

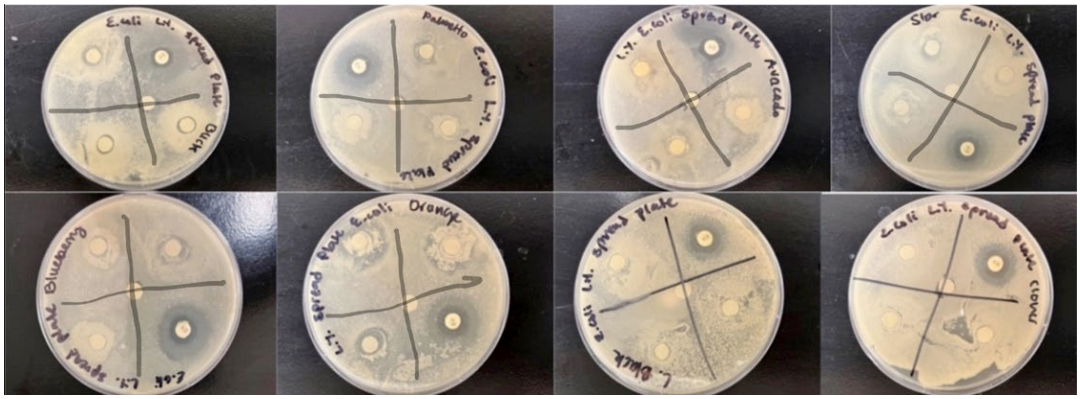

Figure 7. Honey Spread Plates after 48 Hours of Growth against *E. coli.*

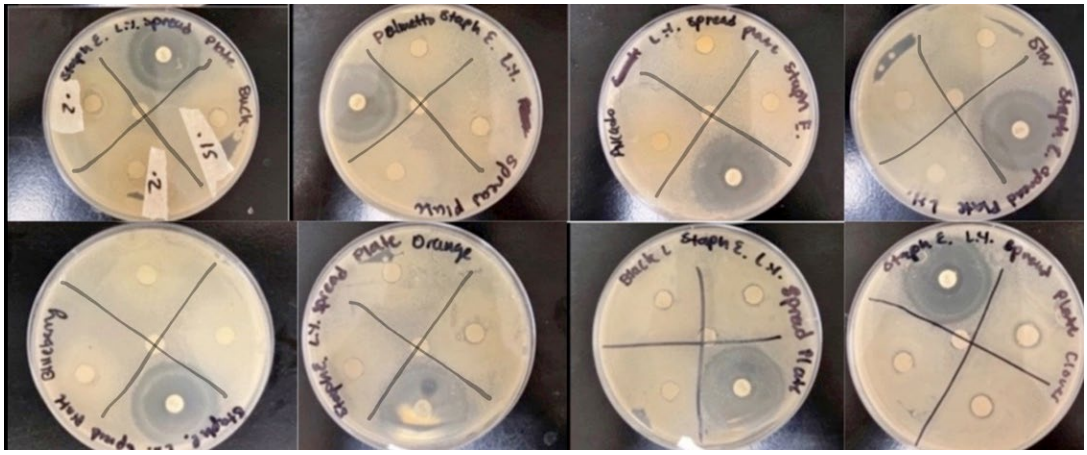

Figure 8. Honey Spread Plates after 48 Hours of Growth against *S. epidermidis.*

Only images of the plates were shown after 48 hours because there were minor to no changes to the inhibition zones from 24 hours of growth to 48 hours of growth, which illustrates how time may not be a large factor in the experiment. The order of the plates is in the order of the honey type from left to right seen in the order of the honey type from all tables for convenience of reading Fig. 5-8; each plate is labeled with the condition it is in and with each type of honey. For instance, all of the figures start with Buckwheat and end with Clover because it is determined by the order of each honey type in the tables. In addition, each figure has a title that describes what type of species was inoculated on those plates and what technique was used for inoculation. There were some contaminants in some of the plates which caused there to be a rerun on some of the plates. Plates that contained contamination and caused a re-run are denoted by the highlighted rows in Tables 3-6. One specific honey that had a contaminant in every trial was the Orange Blossom honey, but it may not be visible in the image because of possible blur or size. Because of the recurring contamination of the plates with Orange Blossom honey a clean agar plate was inoculated with just the Orange Blossom Honey to test for microbes. Below Fig. 9. shows the Orange Blossom isolation plate after 48 hours of incubation.

Figure 9. Orange Blossom Isolation Plate

As seen in Fig. 9. there was prominent growth from the Orange Blossom itself which is a possible reason why every trial of Orange Blossom contained contaminants. The Standard Deviation (SD) of each honey was as well as the standard error (SE), which will be discussed in further detail later on, were calculated and is seen for every plate in Tables 3-6.

Table 3. Zones of inhibition for honey with pour plates against *E. coli*, 48 hours

Plate	SD	Dry	Neomycin	Honey (disc #1)	Honey (disc #2)	Honey (disc #3)	SE
Buckwheat	.058	0	.2	.8	.7	.7	.033
Palmetto	.058	0	.2	.6	.5	.5	.033
Avocado	.058	0	.2	.4	.3	.3	.033
Star Thistle	.058	0	.2	.9	.8	.8	.033
Blueberry	.058	0	.2	.4	.3	.3	.033
Orange	.058	0	.2	.6	.5	.5	.033
Black Locust	.058	0	.2	.6	.5	.5	.033
Clover	.058	0	.2	.9	9	.8	.033

Table 4. Zones of inhibition for honey with pour plates against *S. epidermidis*, 48 hours

Plates	SD	Dry	Erythromycin	Honey (disc #1)	Honey (disc #2)	Honey (disc #3)	SE
Buckwheat	.58	0	.2	.2	.2	.15	.033
Palmetto	0	0	.2	.01	.01	.01	0
Avocado	0	0	.2	.01	.01	.01	0
Star Thistle	0	0	.2	.02	.02	.02	0
Blueberry	.012	0	.2	.07	.05	.05	.007
Orange	0	0	.2	.02	.02	.02	0
Black Locust	0	0	.2	.05	.05	.05	0
Clover	.1	0	.2	.3	.2	.1	.058

Table 5. Zones of inhibition for honey with spread plates against *E. coli*, 48 hours

Plates	SD	Dry	Neomycin	Honey (disc #1)	Honey (disc #2)	Honey (disc #3)	SE
Buckwheat	.058	0	.3	.3	.2	.2	.033
Palmetto	.189	0	.3	.4	.1	.05	.109
Avocado	.179	0	.3	.4	.2	.05	.103
Star Thistle	.173	0	.3	.4	.3	.2	.1
Blueberry	.179	0	.4	.4	.2	.05	.103
Orange	.116	0	.2	.3	.3	.1	.067
Black Locust	.154	0	.2	.3	.1	.1	.089
Clover	.076	0	.3	.2	.1	.05	.044

Table 6. Zones of inhibition for honey with spread plates against *S. epidermidis*, 48 hours

Plates	SD	Dry	Erythromycin	Honey (disc #1)	Honey (disc #2)	Honey (disc #3)	SE
Buckwheat	.055	0	.2	.2	.2	.15	.032
Palmetto	.029	0	.2	.1	.1	.05	.017
Avocado	.029	0	.2	.1	.1	.05	.017
Star Thistle	.05	0	.2	.1	.05	0	.029
Blueberry	.014	0	.2	.05	.03	.03	.008
Orange	0	0	.2	.1	.1	.1	0
Black Locust	.029	0	.2	.1	.1	.05	.017
Clover	.03	0	.2	.2	.15	.15	.017

Analysis

The zones of inhibition on all of the plates represent the area where there is no bacterial growth around each disc, which is the basis of the Kirby Bauer test results. Although this may not necessarily translate to similar effects on the skin, it does provide definitive recommendations for the use of one honey over another. The results of this study are intended to lay a solid basis for further investigation or in vivo tests by presenting an understanding of the mechanisms of the action of the antibacterial properties of various honey produced by one source of pollen, and responding to the

inquiry of "To what extent does the type of honey, sourced from one type of pollen, affect the growth of *E. coli* and *S. epidermidis*?"

Each honey plate was tested with three replicate discs in their quadrant to allow for the establishment of accurate standard deviations. The standard deviation can allow for calculations to discover the standard (Cumming et al. 2007). The error bars on each line are determined by the standard error values of that honey in its conditions, and its standard error value can be found in tables (3-6).

The graphs that follow are condensed, where the zones of inhibition were on the y-axis and the disc number on each plate was on the x-axis. Each honey was given a color and can be found in the legend on each graph.

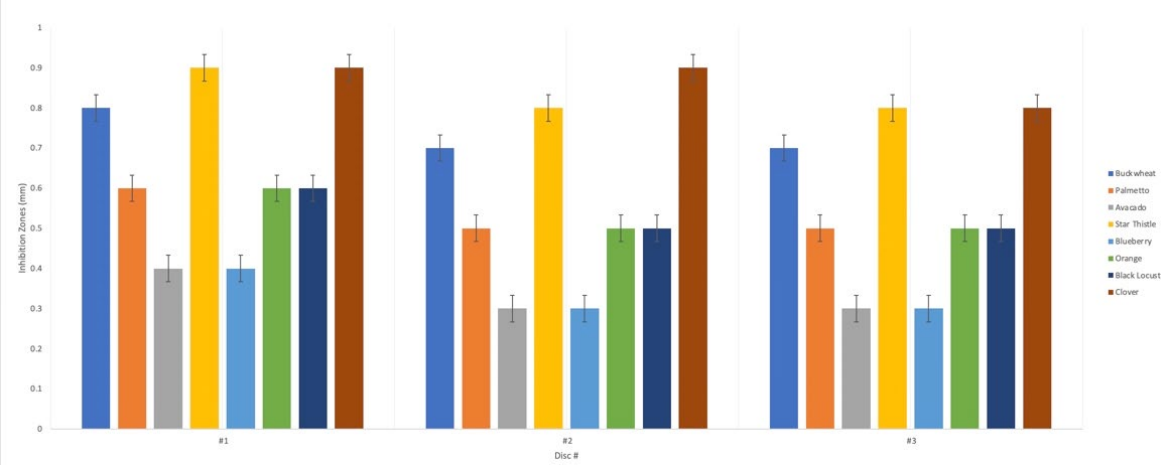

Figure 10. Zones of Inhibition for Honey with Pour Plates against *E. coli*, 48 hours

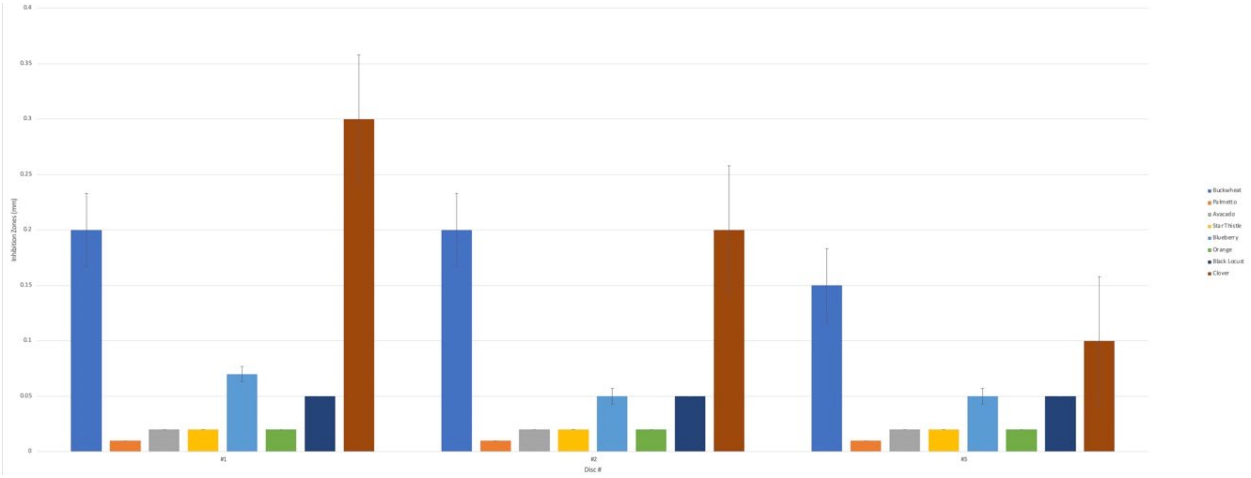

Figure 11. Zones of Inhibition for Honey with Pour Plates against *S. epidermidis*, 48 hours

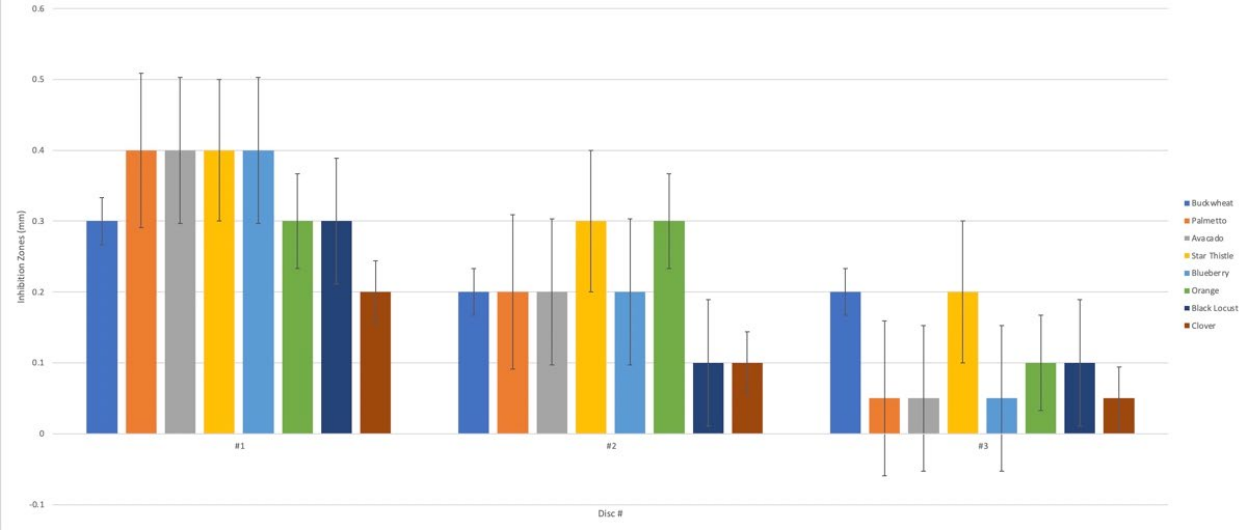

Figure 12. Zones of Inhibition for Honey with Spread Plates against *E. coli*, 48 hours

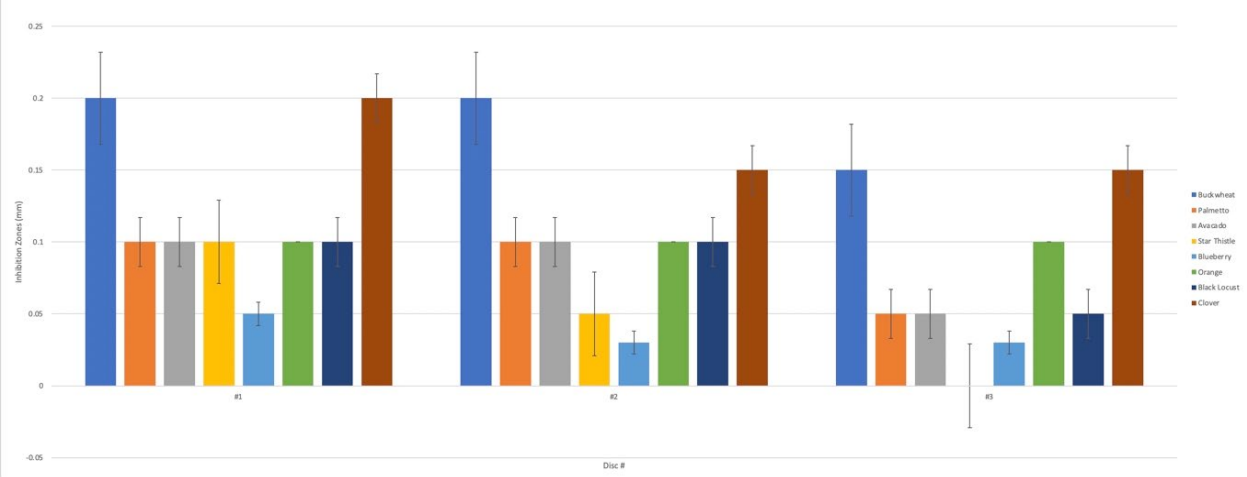

Figure 13. Zones of Inhibition for Honey with Spread Plates against *S. epidermidis*, 48 hours

Overall Analysis

The antibacterial effects of all the honey occurred within the 24 hours of growth in the incubator and had minimal to zero changes at 48 hours of growth which indicates how the inhibition zones were not affected by time, but rather the type of honey within 24 hours of growth. Even if there was no growth beneath the disc, honey discs without any zones of inhibition were recorded as 0. This factor was constant for all of the data, which illustrates why some of the zones of inhibition were insignificant.

All of the honey demonstrated mostly all of its antibacterial effects within the first 24 hours of growth, but some honey would continuously have greater antibacterial action against one kind of bacteria than the other. In addition, the honey's antibacterial actions performed differently depending on how the bacteria were inoculated into the plates: pour plates or spread plates. As seen in Fig. 10-11 there is a drastic difference in the size of the inhibition zones, specifically Fig. 10 having larger zones than Fig .11. This illustrates how the honey exerts more antibacterial action when it is present on *E. coli* rather than *S. epidermidis*. This can also be seen in Fig. 12-13 as the honey on average had larger inhibition zones in Fig. 12 than in Fig. 13. So overall, within the honey that was tested, they all demonstrated

more antibacterial action against *E. coli* than *S. epidermidis*. Examining the effects of zones of inhibition based on the type of inoculation shows that the pour method exhibited much larger inhibition zones than the spread plates as the graphs seen in Figures 10-11 have greater inhibition zones on average compared to the inhibition zones in Fig 12-13. Thus, the extent to which honey sourced from one type of pollen affects the growth of *E. coli* and *S. epidermidis* varies on the type of honey, the type of bacteria, and the method of inoculation of the bacteria.

Analysis by Honey

Each honey varied within how much antibacterial actions they illustrated against each bacterium, but there were some individual honey that performed better than others. When each honey was placed against *E. coli* on pour plates Fig. 10, Star Thistle and Clover demonstrated very high and similar amounts of antibacterial activities based on their inhibition zones, but Clover did end up having larger inhibition zones than Star Thistle. In addition, when the honey was placed against *S. epidermidis* on pour plates Fig. 11, Clover also ended up having very high inhibition zones, but Star Thistle did not. This might signify the antibacterial actions of Star Thistle and how it is more effective at killing *E. coli* than *S. epidermidis* within a microbiome. The antibacterial actions of Star Thistle against *E. coli* on spread plates Fig. 12 also demonstrate the large inhibition zones compared to when it was placed on a spread plate of *S. epidermidis* Fig. 13. In addition, Star Thistle had the largest inhibition zones on average compared to the other honey when placed on spread plates against *E. coli* Fig. 12. This demonstrates how Star Thistle is more effective at killing *E. coli* than *S. epidermidis* both on a surface and within a microbiome. Buckwheat also had large inhibition zones when it was placed against *S. epidermidis* on pour plates Fig. 11. Clover also did have larger inhibition zones against these variables, but in this case, Buckwheat exhibited larger inhibition zones than Clover indicating that Buckwheat is more effective at killing gram positive bacteria, like *S. epidermidis,* on the surface and Clover is more effective at killing bacteria within a microbiome like skin.

Implications of Trends

Overall, the figures indicate how Clover, Buckwheat, and Star Thistle exhibited the largest inhibition zones on average depending on the type of bacteria and the type of inoculation of the bacteria. In addition, the inhibition zones were seen within the first 24 hours of incubation which indicates that time is not a large factor in the antibacterial actions of each honey. Although within all of the honey tested, there was an overall larger effect on inhibition zones when they were tested on pour plates rather than spread plates, which can indicate how honey is more effective at killing bacteria within a microbiome like skin. This illustrates the impact that honey has on the skin, which can allow for new ways to treat skin conditions that need to go deep into the epidermis of the skin microbiome. Because the antibacterial activities of honey depend on the type of pollination, the results indicate that Clover, Buckwheat, and Star Thistle demonstrated the largest antibacterial activity in certain conditions compared to the other types of honey in the experiment. This implies that clover flowers, buckwheat flowers, and Knapweed flowers - which are the source of star thistle honey - are the most effective at killing bacteria compared to the other types of pollen in the honey used in this experiment. When comparing these top three honey, Clover honey had the largest inhibition zones compared to others, which illustrates how it contained the strongest antibacterial activities among the other 7 honey. Therefore, I can get an answer to my initial research question, "To what extent does the type of honey, sourced from one type of pollen, affect the growth of *E. coli* and *S. epidermidis*?" from the antibacterial effects seen from the inhibition zones; the antibacterial effects of honey depend on the type of honey, the type of bacteria, and the method of inoculation of the bacteria; among all 8 of the honey tested in my experiment, Clover honey exhibited the largest inhibition zones, which means it experienced the most amount antibacterial activities, where the results of the experiment are reliant on the type of bacteria used in the experiment, how the bacteria sits on the medium, and the type of honey.

Limitations

The procedure for this experiment was conducted in sterile environments with aseptic techniques, but there are some factors that may need to be altered for future recreations of the procedure. The error resulted in some contamination of the plates, which caused there to be a rerun of the experiment; the contamination did not affect the experiment significantly to the point where it might have caused determinants to the data, but without contamination, clearer results could have been found.

The opacity of each honey may be a reason why one honey was more effective at killing bacteria than another because each type of honey may have been able to sink into the agar more than one honey. Each honey was made from a different type of pollen which caused each honey to have different physical properties. This also caused there to be a small difference in the amount of honey that was placed on each filter paper disc as there was no way of measuring the amount of honey used. All the honey had too thick of a consistency, which caused it to not be able to micropipette.

Lastly, only 8 different types of honey were tested in this experiment, which is double the amount used in Morroni et al. (2018)'s study, but there are so many different types of honey that can be tested against each other to get more specific results. Having a larger variety can allow for more data and comparison, and overall get more results for clarification.

Conclusion

There is still much that needs to be researched and this can be done in various methods. In addition, there are many more different types of honey that are sourced from one type of pollination, so researching a larger variety of honey could also make more conclusions. This can also be taken into account when looking at the type of conditions that honey can help treat. For example, wounds inflamed by *E. coli* may benefit more when getting treated by Clover honey as it exhibited the largest inhibition zones, which means it demonstrates larger antibacterial effects than other types of honey used in the experiment. Although within all of the honey tested, there was an overall larger effect on inhibition zones when they were tested on pour plates rather than spread plates, which can indicate how honey is more effective at killing bacteria within a microbiome like skin. In addition, of all of the honey tested, they were all more effective at killing *E. coli* rather than *S. epidermidis* as they had larger inhibition zones. This can allow for further research, which can possibly create new methods for treating skin conditions that are prone to gram negative bacteria like *E. coli*. There currently is research about the antibacterial effects of honey such as Morroni et al. (2018)'s study on the difference in antibacterial effects of honey that came from four different countries, which can also be recreated with a more narrowed focus on which type of honey and what specific plant they are pollinated from. While such studies have not taken this into account, results from my study illustrate how the type of pollination that sources the honey does significantly affect the antibacterial effects it exhibits on the growth of bacteria, and should therefore be taken into consideration for future research.

References

Albaridi NA. 2019. Antibacterial potency of honey. International Journal of Microbiology.:1–10. Doi: HTTPs://doi.org/10.1155/2019/2464507. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6589292/.

Almasaudi S. 2020. The antibacterial activities of honey. Saudi Journal of Biological Sciences. 28(4). Doi: HTTPs://doi.org/10.1016/j.sjbs.2020.10.017.

Brown MM, Horswill AR. 2020. Staphylococcus epidermidis—Skin friend or foe? Kline KA, editor. PLOS Pathogens. 16(11):e1009026. doi: https://doi.org/10.1371/journal.ppat.1009026.

Cumming G, Fidler F, Vaux DL. 2007. Error bars in experimental biology. The Journal of Cell Biology. 177(1):7–11. doi:https://doi.org/10.1083/jcb.200611141.

Dowshen S. 2017. E. Coli (for Teens) - KidsHealth. Kidshealthorg. https://kidshealth.org/en/teens/e-coli.html.

Guruvu N. 2020. Antimicrobial activity of different types of honeys against wound pathogens. National Journal of Physiology, Pharmacy and Pharmacology.(0):1. doi:https://doi.org/10.5455/njppp.2021.11.09257202012102020.

Khan SU, Anjum SI, Rahman K, Ansari MJ, Khan WU, Kamal S, Khattak B, Muhammad A, Khan HU. 2018. Honey: Single food stuff comprises many drugs. Saudi Journal of Biological Sciences. 25(2):320–325. doi:https://doi.org/10.1016/j.sjbs.2017.08.004. https://www.sciencedirect.com/science/article/pii/S1319562X17302073.

Lee E, Anjum F. 2020. Staphylococcus Epidermidis. PubMed. https://www.ncbi.nlm.nih.gov/books/NBK563240/.

Mama M, Teshome T, Detamo J. 2019 Apr 3. Antibacterial Activity of Honey against Methicillin-Resistant Staphylococcus aureus: A Laboratory-Based Experimental Study. International Journal of Microbiology. https://www.hindawi.com/journals/ijmicro/2019/7686130/.

Mandal MD, Mandal S. 2011. Honey: its medicinal property and antibacterial activity. Asian Pacific Journal of Tropical Biomedicine. 1(2):154–160. doi:https://doi.org/10.1016/s2221-1691(11)60016-6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3609166/.

Morroni, G., Alvarez-Suarez, J. M., Brenciani, A., Simoni, S., Fioriti, S., Pugnaloni, A., Giampieri, F., Mazzoni, L., Gasparrini, M., Marini, E., Mingoia, M., Battino, M., & Giovanetti, E. (2018, June 6). *Comparison of the antimicrobial activities of four honeys from three countries (New Zealand, Cuba, and Kenya)*. Frontiers. Retrieved March 27, 2023, from https://www.frontiersin.org/articles/10.3389/fmicb.2018.01378/full

Namvar AE, Bastarahang S, Abbasi N, Ghehi GS, Farhadbakhtiarian S, Arezi P, Hosseini M, Baravati SZ, Jokar Z, Chermahin SG. 2014. Clinical characteristics of Staphylococcus epidermidis: a systematic review. GMS hygiene and infection control. 9(3):Doc23. doi:https://doi.org/10.3205/dgkh000243. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4184040/.

Petkovsek Z, Elersic K, Gubina M, Zgur-Bertok D, Starcic Erjavec M. 2009. Virulence Potential of Escherichia coli Isolates from Skin and Soft Tissue Infections. Journal of Clinical Microbiology. 47(6):1811–1817. doi:https://doi.org/10.1128/jcm.01421-08. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2691073/.

Rahman M, Richardson A, Sofian-Azirun M. 2010. Antibacterial activity of propolis and honey against Staphylococcus aureus and Escherichia coli. African Journal of Microbiology Research. 4(16):1872–1878. [accessed 2023 Mar 28]. https://academicjournals.org/journal/AJMR/article-full-text-pdf/8FA325D14281.

Stagos D, Soulitsiotis N, Tsadila C, Papaeconomou S, Arvanitis C, Ntontos A, Karkanta F, Adamou-Androulaki S, Petrotos K, Spandidos DA, et al. 2018. Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. International Journal of Molecular Medicine. 42(2):726–734. doi:https://doi.org/10.3892/ijmm.2018.3656. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6034916/.

Wadi MA. 2022. In Vitro Antibacterial Activity of Different Honey Samples against Clinical Isolates. Mehmood K, editor. BioMed Research International. 2022:1–8. doi:https://doi.org/10.1155/2022/1560050.