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Comparing the Effectiveness of Alternative and Prescription Antibiotics Against Gram-Positive Bacteria

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Abstract

The rapid emergence of antibiotic-resistant bacteria is a global public health concern that threatens the efficacy of antibiotic drugs. We found that natural remedies, specifically coconut oil, honey and cinnamon essential oil, have the potential to be used as a clinical alternative to treat antibiotic-resistant infections. In this experiment, we performed a disk diffusion test and measured the area of inhibition of each treatment to compare the effectiveness of natural and prescription antibiotics. Cinnamon essential oil showed significantly greater antibiotic activity compared to a prescription treatment, amoxicillin. With bacterial resistance continuously expanding, more work needs to be done to determine how alternative antibiotics might be used in clinical settings.

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Literature Review

The increase in antibiotic resistance is an alarming public health issue that is affecting populations globally. In the last decade alone, there has been a dramatic increase in the number of bacterial pathogens presenting multidrug resistance to antibacterial agents. Each year in the US at least 2 million people become infected with bacteria that are resistant to antibiotics ("Antibiotic/Antimicrobial Resistance," 2017). The number of multidrug resistant pathogens increases every year, largely because of overdosage, self-medication, limited health education, and poverty (Rather et al., 2017). These factors are amplified in countries where antibiotics are readily available without prescriptions and public knowledge about the dangers of antibiotic resistance is limited.

One commonly prescribed antibiotic is amoxicillin (Ready et al., 2013). However, with the rise of antibiotic-resistant strains of bacteria, the effectiveness of amoxicillin and similar drugs is compromised. Researchers are now investigating alternative treatment methods to target these resistant strains. Natural compounds, including coconut oil, honey and cinnamon are emerging as potential alternative treatments. The effectiveness of natural antibiotics that have been used traditionally in indigenous medicine are now being studied by western researchers. However, little is known about the effectiveness of these alternatives and clinical use is considered controversial. Many studies have investigated the effectiveness of natural antibiotics against particular strains of bacteria, but very few comparative studies with multiple treatments have been conducted.

Honey has long been valued for its antimicrobial properties. Honey contains hydrogen peroxide, an effective antibiotic compound. Additionally, the low pH and high sugar concentration also inhibit bacterial growth. In some studies, honey has demonstrated antibiotic activity at concentrations as low as 20% (Shenoy et al., 2012). However, not all types of honey are the same. There is a large variation in the antimicrobial activities of honey because of the spatial and temporal variation in sources of nectar. Several studies have identified Manuka and Tualang honey as the most effective broad-spectrum antibiotics (Mandal & Mandal, 2011).

Coconut oil has also been shown to be an effective antibiotic. The medium-chain fatty acids found in the oil, particularly lauric acid and its derivatives, are the primary antibiotic agents. Monolaurin in particular has significant antimicrobial properties, although other medium-chain fatty acids, including capric acid and caprylic acid, also showed antibacterial activity (Carpo et al., 2007). In one study, transmission electron microscopy (TEM) showed that lauric acid disrupts the cell membrane and cytoplasm of bacteria, suggesting this is the mechanism of its antibiotic activity (Shilling et al., 2013). Coconut oil has almost exclusively been used as an antimicrobial to treat skin infections, and its potential effectiveness against other types of infections is unknown.

Finally, cinnamon essential oil is also known to have antimicrobial properties. Phytochemicals, including cinnamaldehyde, trans-cinnamaldehyde and eugenol are the primary antibiotic agents (Nabavi et al., 2015). Cinnamaldehyde has been shown to disrupt the bacterial cell membrane and organelles, leading to cell death. In addition, trans-cinnamaldehyde is also known to inhibit bacterial acetyl CoA carboxylase, an essential enzyme that catalyses the first committed step in fatty acid biosynthesis. There are a handful of cinnamon varieties, but

previous work has confirmed that *Cinnamomum zeylanicum* is the most potent antimicrobial (Ranasinghe et al., 2013).

In this experiment, the effectiveness of these three alternatives is compared to the antibiotic activity of the prescription drug amoxicillin. Based on previous work, we designed an experiment to compare multiple treatments against the same bacteria culture. These alternatives were selected based on their broad-spectrum antibiotic activity, particularly against antibiotic-resistant microbes, and the substantial amount of literature available for each. All four antibiotics will be tested against gram-positive bacteria isolated from an mouth swab. Based on previous research, we expect to see antibiotic activity in each treatment group. We hypothesize that although the prescription antibiotic will show the most antibiotic activity, the alternative treatments will demonstrate comparable degrees of bacterial inhibition.

Methods

Experimental Procedure

All testing took place in University Hall room 2-142 at Lesley University using materials supplied by the University.

Bacteria sampling. To begin the experiment, we swabbed an individual's mouth with a sterile inoculating loop and transferred the collected bacteria onto an 85 mm plastic petri dish with Carolina Nutrient Agar Catalog No. 785300. The petri dish was covered, turned upside down, and stored in the lab at room temperature. This procedure was repeated a second time, and the colonies were left to grow for 48 hours. After 48 hours, one distinct colony was selected and transferred to another plate with agar for isolation. We transferred the bacteria using a sterilized inoculating loop and streaked the bacteria across the plate. The plate was again left at room temperature to grow for another 48 hours.

Antibiotic preparation. The prescription and natural antibiotics were chosen before the experiment began and included amoxicillin, cinnamon essential oil, coconut oil, and Manuka honey. The amoxicillin was a liquid solution with a 250 mg/5 ml concentration. Healing Solutions 100% cassia cinnamon essential oil, 365 93% MCT coconut oil unflavored, and Manuka Doctor 10+ bioactive Manuka honey were the natural antibiotics procured for this experiment. We used 110 mm diameter #1 Whatman filter paper and a hole punch to create circular paper disks to soak in the antibiotics.

Antibiotic testing. The method used for antibiotic testing was adapted from the Kirby-Bauer Disk Diffusion Susceptibility Test. The Kirby-Bauer Method is used to determine the sensitivity of bacteria to different antimicrobials (Hudzicki, 2009). This method involves placing antibiotic disks on agar inoculated with bacteria. The antibiotic diffuses into the area around the disk, creating an area of inhibition where the bacteria cannot grow. This area is a quantitative measure of antibiotic activity that can be compared among treatments (Hudzicki, 2009). Due to our supply limitations, we were able to create a test resembling this method but unable to complete the Kirby-Bauer protocol to the standard required. The Kirby-Bauer protocol requires Mueller-Hinton agar with a pH range of 7.2 to 7.4, which we did not have access to at the time. We instead used the agar prepared and supplied in the lab. Antibiotic disks are most often purchased for this protocol, however, because of our choice of antibiotics and lack of funds, we created our own disks using filter paper rather than purchasing them. The protocol also requires the preparation of a 0.5 McFarland Standard to determine the turbidity of the bacterial suspension used to inoculate the agar (Hudzicki, 2009). We were unable to create the Standard or the suspension due to a lack of resources and instead transferred the bacteria to the agar with a

sterilized inoculating loop. Finally, we were unable to access an incubator for this experiment and instead opted for an undisturbed location to place the samples at room temperature.

We began antibiotic testing by first acquiring two sterile petri dishes with agar and separating one dish into three sections and the other into two using black sharpie. Each section was labeled with a designated treatment. The sections remained consistent throughout testing. The first pair of plates was an initial test of our adapted disk diffusion method. We used a sterile inoculating loop to transfer the isolated bacteria onto each section of both plates. We then used the hole punch to create circular disks from filter paper and placed the disks in the center of each section. The antibiotics were applied to each disk in their designated section and the control group was left without a disk. We covered and inverted the plates before storing them. We returned 48 hours later to reassess the dishes and found that the method was successful. However, these plates were not included in the data because we modified our final procedure.

We altered the original procedure slightly to create a more consistent method. Instead of dropping the antibiotics onto the disks, we soaked the disks in the antibiotics before placing them on the agar. We transferred the saturated disks from the antibiotic solutions to the plate using tweezers that were cleaned and sterilized between antibiotics. All antibiotics were used in their original concentrations except for the honey, which was mixed with water in a 1:1 ratio. This was done to provide a consistency of the honey solution that the paper disk could absorb. We also used a disk for the control that was left untreated to determine whether the filter paper itself had any inhibiting effect.

This process was repeated with 13 pairs of plates over the course of a month in different sets. The plates were checked 48 hours after inoculation for each trial. The bacteria sample was taken each time from the original colony. After 48 hours, we photographed the plates and

measured the area of inhibition. One pair of plates were measured on 10/20/17, two sets on 10/27/17, seven on 11/03/17, and finally three sets on 11/17/17.

Staining procedures. We initially attempted to identify the isolated bacteria colony using a methylene blue simple stain. We transferred the bacteria onto a slide using a sterile inoculating loop and used ethanol to fix the sample. We then used a dropper to drop methylene blue stain onto the sample. The stain was washed off with distilled water and the slide was placed under a microscope at 1000x magnification. Unfortunately, we were not able to identify anything beyond a possible rod shape to the bacteria.

We then conducted a Gram stain. Bacteria can be differentiated based on how they respond to a Gram stain. Specifically, bacteria are either gram positive or gram negative (Bisen, 2014). This distinction provides information about the outer membrane layer of the bacteria and helps determine the most effective antibiotic treatment. To perform the Gram stain, a slide was prepared with the bacteria sample and heat-fixed. Crystal violet stain was then added and held for 30 seconds and rinsed with water. Then the slide was flooded with iodine and left for 30 seconds before rinsing. Finally, the sample was decolorized with ethanol and stained with safranin for 30 seconds, then rinsed and dried. The slide was then viewed under a microscope at 1000x magnification, and it was determined that the bacteria sample was gram positive (Bisen, 2014). We repeated the Gram stain once more to confirm our results, but fixed the sample with ethanol rather than with heat.

Data Analysis

Once data collection was complete, we used Excel to create tables documenting the diameter of the areas of inhibition for each trial. We calculated the area of inhibition in a seperate table. The normality of the data was assessed using the Shapiro-Wilk Normality Test at

sdittami.altervista.org (Dittami, 2009). A one-way, weighted, independent sample ANOVA test was performed using a calculator provided by vassarstats.net (Lowry, 2017). These results were then organized into graphs and interpreted.

Results

During the testing period, one species of gram-positive bacteria was tested against four treatments in thirteen trials. The bacteria was identified as gram positive, exhibiting rod shaped characteristics based on a Gram stain. Researchers have extensively studied the effects of amoxicillin on gram-positive bacteria. Amoxicillin is typically used to treat gram-positive bacterial infections, as it is not as effective against gram-negative species (Fair & Tor, 2014). In most studies, amoxicillin has shown resistance against gram-positive bacteria (Sallam et al., 2016). Gram-positive bacteria have a permeable membrane that allows for fluid penetration of antibiotics like amoxicillin (Fair & Tor, 2014). Researchers have also examined alternative treatment methods, including the compounds tested in this experiment, and have found promising effects against gram-positive bacteria. For instance, Manuka honey has been found to be effective against several gram-positive bacteria, including, methicillin-resistant *S. aureus* (MRSA), β-haemolytic *Streptococci and* vancomycin-resistant *Enterococci* (Mandal & Mandal, 2011). The medium-chain fatty acids in coconut oil and phytochemicals in cinnamon essential oil extract have shown similar antibiotic properties in vitro (Nabavi et al., 2015; Shilling et al., 2013).

The antibiotic activity of cinnamon essential oil, amoxicillin, coconut oil and honey is summarized in Figure 1. Coconut oil, cinnamon essential oil and amoxicillin all presented significant areas of inhibition across the thirteen trials. Cinnamon essential oil was consistently

the most effective treatment. On the other hand, the Manuka honey and control group demonstrated no inhibition.

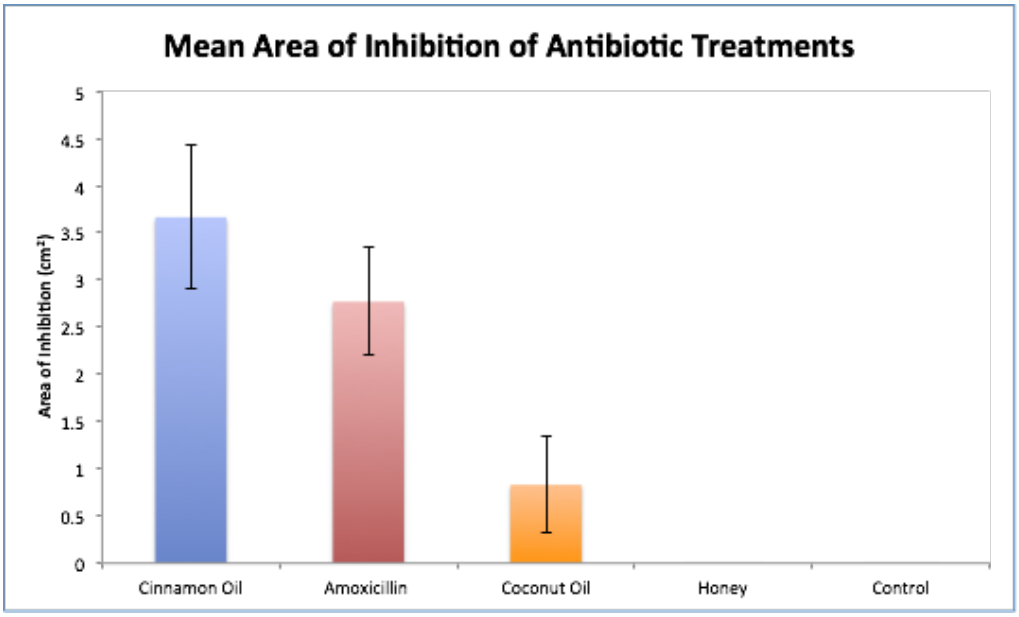

Figure 1: Mean area of inhibition of antibiotic treatments with error bars representing standard deviation.

Among the five samples, cinnamon essential oil, amoxicillin and coconut oil were the only treatments that showed antibiotic activity. Cinnamon essential oil was the most effective treatment tested, with an average area of inhibition of 3.664 cm^2. Amoxicillin was a close second, with a mean area of inhibition of 2.770 cm^2. Coconut oil showed significantly less effectiveness, with a mean area of inhibition of 0.830 cm^2.

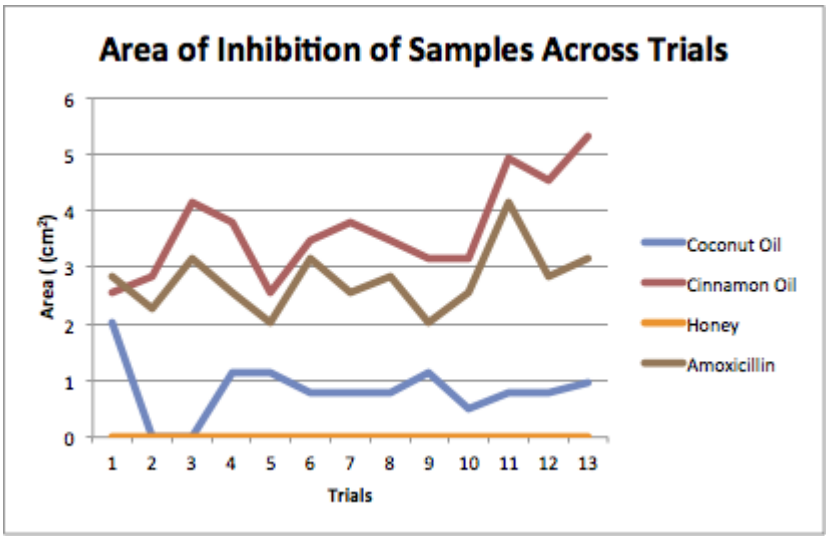

Figure 2: Area of inhibition across samples.

Figure 2 illustrates the variation observed in the area of inhibition measured among trials. Through each trial, cinnamon essential oil and amoxicillin consistently showed the largest areas of inhibition. On the other hand, coconut oil showed more variation among trials, with a large drop off in trials two and three before eventually stabilizing. Coconut oil also exhibited its largest area of inhibition in trial one and did not reach that area again in the remaining trials.

An ANOVA test was conducted to determine the statistical significance of the results. At a .05 level of significance, there was a statistically significant difference in the mean area of inhibition among cinnamon essential oil, amoxicillin and coconut oil ($p < 0.0001$). A Tukey post hoc test revealed that all treatment means were significantly different from all other treatments. However, there was no statistically significant difference between honey and the control group because neither showed any inhibitory activity. The standard deviations of each treatment set were calculated to compare the variation within each group.

Table 1: Area of Inhibition Summary

	Cinnamon Oil	Amoxicillin	Coconut Oil	Honey	Control
Mean	3.664	2.770	0.830	0	0
Std. Dev.	0.871	0.570	0.514	0	0
Minimum	2.545	2.011	0	0	0
Maximum	5.309	4.155	2.011	0	0

Table 2: Area of Inhibition ANOVA Analysis Summary

	Sum of Squares	Degrees of Freedom	Mean Square	F-value	P-value
Variance Between Groups	146.06	4	36.51	135.5	<0.0001
Error	16.17	60	0.27		
Total	162.23	64			

In conclusion, we can reject the initial hypothesis that the alternative treatment methods would have comparable but lesser effects on bacterial inhibition than amoxicillin. Based on the results and findings from the ANOVA analysis, cinnamon essential oil demonstrated significantly more antibiotic effectiveness compared to amoxicillin. Conversely, coconut oil showed significantly less inhibitory activity than amoxicillin.

Discussion

The rapid emergence of antibiotic-resistant bacteria is occurring worldwide, threatening the efficacy of traditional antibiotics. Honey, coconut oil and cinnamon essential oil are all promising alternative treatments for a variety of infections (Shenoy et al., 2012; Carpo et al., 2007; Ranasinghe et al., 2013). However, the antibiotic activity of these treatments vary, and more work needs to be done to determine what alternative antibiotics might be most clinically effective. Here, we investigated the antibiotic activity of three alternatives compared to a

traditional treatment of amoxicillin against a sample of gram-positive human oral bacteria. We show that cinnamon essential oil is more effective against gram-positive bacteria than amoxicillin, and that coconut oil is also effective in inhibiting bacterial growth.

We initially expected the alternative antibiotic products to have a comparable but inferior effectiveness in treating gram-positive oral bacteria compared to amoxicillin. However, we found that cinnamon essential oil was the most effective antibiotic overall, with a significantly higher mean area of inhibition compared to all other treatments. Conversely, the Manuka honey treatment had no effect against the gram-positive bacteria. Overall, there were significant differences in the inhibitory effects among all four treatments.

We expected amoxicillin to be the most effective antibiotic treatment tested in our experiment. Instead, it was significantly less effective than cinnamon essential oil. This may be because the amoxicillin was diluted in a 250 mg/5 mL liquid solution. No other treatments tested, with the exception of honey, were diluted. This may have affected the potency of amoxicillin relative to other treatments.

We found cinnamon essential oil to be the most effective treatment against gram-positive bacteria. The antibacterial activity of cinnamon is a result of chemicals including cinnamaldehyde and eugenol, which are effective against both gram-positive and gram-negative bacteria strains (Nabavi et al., 2015). Previous work has shown that cinnamon bark essential oil has significant antibiotic activity at concentrations as low as 2.9 to 4.8 mg/mL. Other studies have demonstrated that cinnamon extract has an area of inhibition diameter ranging from 22 to 27 mm against drug-resistant *S. aureus* bacteria (Nabavi et al., 2015). We found similar areas of inhibition in our study, suggesting that cinnamon essential oil is consistently an effective antibiotic treatment.

We also found that coconut oil had significant antibiotic activity. The primary antibiotic agent in coconut oil is lauric acid, a long-chain fatty acid. In previous studies, lauric acid demonstrated broad-spectrum sensitivity against gram-positive and gram-negative bacteria. However, this work was done with bacteria strains isolated from skin infections, not oral bacteria (Carpo et al., 2007). One study found that the most effective coconut oil treatment was a 2 mg/mL lauric acid solution isolated from the oil. Perhaps lauric acid is a more effective antibiotic treatment than coconut oil alone, and should be explored in future experiments (Shilling et al., 2013).

Surprisingly, we did not see any antibiotic activity from the Manuka honey treatment. This may be because the honey solution was diluted 1:1 with water. This was done to improve the viscosity of the solution, but may have affected its potency. We selected Manuka honey for this particular study because several studies have concluded that this type of honey has one of the greatest antibiotic properties (Mandal & Mandal, 2011). Manuka is one of many types of honey, all of which vary in their chemical composition and antibiotic properties. (Mandal & Mandal, 2011). As a result, there is a wide range of effectiveness of different types of honey on different types of bacteria. It is possible then that Manuka honey was not the most effective type of honey for the particular strain of gram-positive bacteria used in this study. For example, one study found that the most effective type of honey against *E. coli* bacteria, another gram-positive strain, was pineapple honey and not Manuka (Zainol et al., 2013). Additionally, there is evidence in the literature to suggest that Manuka honey is more effective against gram-negative bacteria, particularly *Pseudomonas aeruginosa* (Zainol et al., 2013).

This preliminary research reveals the exciting possibilities of alternative treatments against antibiotic-resistant bacteria infections. This study is the first of its kind to explore

antimicrobial activity among many types of treatments. Although this experiment is limited by a small sample size, it clearly indicates that alternative antibiotics have potential to be effective treatments. More work needs to be done to explore the potential clinical uses of these compounds. Future studies may explore the effectiveness of isolating the antibiotic agents of each treatment and working to develop more potent antibiotics. Additionally, more work needs to be done to explore the specific mechanisms of antibiotic activity in natural treatments. Ultimately, these alternatives may prove critical to the treatment of life-threatening infections as the global medical community struggles to fight increasingly drug-resistant microbes.

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References

Antibiotic / Antimicrobial Resistance. (2017, August 21). Retrieved from www.cdc.gov/drugresistance/index.html

Antibiotics Aren't Always the Answer. (2017, April 21). Retrieved from http://www.cdc.gov/features/getsmart/index.html

Bisen, P. S. (2014). *Laboratory protocols in applied life sciences.* Boca Raton, FL: CRC Press

Carpo, B. G., Verallo-Rowell, V., & Kabara, J. (2007). Novel antibacterial activity of monolaurin compared with conventional antibiotics against organisms from skin infections: An in vitro study. *Journal of Drugs in Dermatology*, *6*, 991-998.

Dittami, S. (2009). Shapiro-Wilk Normality Test [Software]. Available from http://sdittami.altervista.org/shapirotest/ShapiroTest.html

Fair, R. J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspectives in Medicinal Chemistry, 6*, 25-64. http://doi.org/10.4137/PMC.

Hudzicki, J. (2009). *Kirby-Bauer disk diffusion susceptibility test protocol.* Retrieved from http://www.asmscience.org/content/education/protocol/protocol.3189

Lowry, R. (2017). One-way Analysis of Variance for Independent or Correlated Samples [Software]. Available from http://vassarstats.net/anova1u.html

Mandal, M. D., & Mandal, S. (2011). Honey: Its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*, *1*(2), 154–160.

Nabavi, S. F., Di Lorenzo, A., Izadi, M., Sobarzo-Sánchez, E., Daglia, M., & Nabavi, S. M. (2015). Antibacterial effects of cinnamon: From farm to food, cosmetic and pharmaceutical industries. *Nutrients*, *7*(9), 7729–7748.

Ranasinghe, P., Pigera, S., Premakumara, G., Galappaththy, P., Constantine, G., & Katulanda, P. (2013). Medicinal properties of 'true' cinnamon (*Cinnamomum zeylanicum*): A systematic review. *BMC Complementary & Alternative Medicine, 13*(275). http://doi.org/10.1186/1472-6882-13-275

Rather, I. A., Kim, B.-C., Bajpai, V. K., & Park, Y.-H. (2017). Self-medication and antibiotic resistance: Crisis, current challenges, and prevention. *Saudi Journal of Biological Sciences*, *24*(4), 808–812. http://doi.org/10.1016/j.sjbs.2017.01.004.

Ready, D., Lancaster, H., Qureshi, F., Bedi, R., Mullany, P., & Wilson, M. (2004). Effect of amoxicillin use on oral microbiota in young children. *Antimicrobial Agents And Chemotherapy, 48*(8), 2883-2887.

Sallam, M., Abou-Aisha, K., & El-Azizi, M. (2016). A novel combination approach of human polyclonal IVIG and antibiotics against multidrug-resistant Gram-positive bacteria. *Infection And Drug Resistance, 9*, 301-311.

Shenoy, V. P., Ballal, M., Shivanada, P. G., & Bairy, I. (2012). Honey as an antimicrobial agent against *Pseudomonas Aeruginosa* isolated from infected wounds. *Journal of Global Infectious Diseases, 4*(2), 102–105.

Shilling, M., Matt, L., Rubin, E., Visitacion, M. P., Haller, N. A., Grey, S. F., & Woolverton, C. J. (2013). Antimicrobial effects of virgin coconut oil and its medium-chain fatty acids on *Clostridium difficile*. *Journal of Medicinal Food, 16*(12), 1079–1085. http://doi.org/10.1089/jmf.2012.0303.

Zainol, M. I., Yusoff, K. M., & Yusof, M. Y. (2013). Antibacterial activity of selected Malaysian honey. *BMC Complementary and Alternative Medicine, 13*(129). http://dx.doi.org/10.1186/1472-6882-13-129.