The antimicrobial effects of royal jelly, propolis and honey against bacteria of clinical significance in comparison to three antibiotics

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Abstract

In recent years a marked increase in antibiotic resistance by certain pathogenic bacteria has been seen. This increase is due to the overuse and misuse of antibiotics and has resulted in several highly resistant strains of bacteria such as MRSA—a strain of *Staphylococcus aureus* frequently causing nosocomial infections, against which only the antibiotic Vancomycin is currently effective. In order to assist in combating bacterial infections, alternative methods to traditional antibiotics should be explored. This study was designed to ascertain the effectiveness of propolis, royal jelly and honey against non-MRSA *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis* and *Bacillus cereus.* Each product's relative potency was compared to antibiotics currently in use through the comparison of zones of inhibition produced by each. While the antimicrobial properties of honey, royal jelly and propolis have been examined in previous studies, their effectiveness has not been compared to each other as well as to antibiotics within the same study. Honey, propolis and royal jelly were each found to have inhibitory effects on bacterial growth and were at times, comparable in strength to the antibiotic comparisons. This indicates a potential for their application as viable methods of bacterial control; particularly as a first-line treatment for mild infections or as preventative treatments.

Introduction

Over the last decade, a rapid increase in antibiotic-resistant strains of pathogenic bacteria has been seen [1,2]. This has resulted in increased difficulty for the maintenance of sterile environments by medical facilities and medical practitioners. This in turn has increased the severity of hospital-acquired infections and bacterial illnesses overall. The primary causes of this increased resistance are suggested to be the overuse and misuse of antibiotics in situations where antibiotics are either unnecessarily prescribed or when medical directives for their proper use are not followed [1,3]. Attentions should therefore be placed on exploring alternatives to antibiotic treatments in order to have substitutes available and to potentially stall the further progression of antibiotic resistance.

Honey has historically been used as a therapeutic agent [4]. In addition, propolis and royal jelly—also products of the common honeybee (*Apis mellifera*)—have become available as supplements as new research has emerged suggesting their curative properties.

Despite the lack of a lymphatic-based immune system, many insects, including *A. mellifera*, are able to ward off bacterial infections [5, 6], which suggests a mechanism for the production of potent antimicrobials by these insects [7]. Three of the major known mechanisms of microbial control used by honey bees are: honey, propolis and royal jelly [8].

Since bees neither migrate nor hibernate, honey is produced by bees as an alternative food source during winter months when their primary food supply, nectar, is not available [9]. The properties of honey that prevent its spoiling are its low pH, hydrogen peroxide content, flavonoids and high sugar content, which results in high osmotic pressure [8]. Honey has been effectively used as an ulcer treatment when conventional ulcer treatment methods failed and showed impressive chemical debridement action [10]. While many characteristics of honey are constant, the exact composition of honey varies, however, due to variations in pollen content, the taxa of which indicates the floral source used by that hive to produce the honey [11,12].

Royal jelly is also a food source that is exclusively given to queen bee larva, which are genetically identical to other female bees in the hive but are phenotypically different due to differentiation caused by the queen's royal jelly diet [4,9,11]. Royal jelly has been shown to possess antibacterial properties against both Gram-positive and Gram-negative bacteria [7, 13] and may serve to give the queen bee a broad-spectrum immunity towards harmful bacteria.

Propolis has also been shown to have similar effects [14,15,16] against Gram-positive bacteria, certain fungi and as an antiviral, but with limited success against Gram-negative bacteria [14,16]. It is primarily used to seal the walls and entrances of the hive, strengthen honeycomb walls and "embalm" dead invaders to prevent putrefaction [17].

With certain mainstream companies incorporating natural ingredients, such as honey into their products [18,19], it is important to determine the best usage for such components—an area in which there is a dearth of scientifically credible research.

Adding to the significance of this topic is the steadily increasing rate of antibiotic resistance by bacteria [1]. Infections caused by resistant strains of bacteria have become unresponsive to conventional treatments and may begin to impact not only the effective control of infectious diseases, but also increase the overall cost of health care as more expensive therapies become necessary and funding to establish new treatments becomes critical [2] .

The World Health Organization (WHO) has brought attention to this topic over the last few years, particularly by selecting the struggle against antimicrobial resistance as their theme for World Health Day in 2011 [2]. The organization has issued a call for concerted action to combat antibiotic resistance. In a statement the WHO's Director-General, Dr Margaret Chan, emphasized that, "The

emergence and spread of drug-resistant pathogens has accelerated. More and more essential medicines are failing. The therapeutic arsenal is shrinking. The speed with which these drugs are being lost far outpaces the development of replacement drugs" [20].

Materials and Methods

Samples of *Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis* and *Bacillus cereus* were obtained from Becton Dickinson Baltimore Biological Labs (BD BBL) and were maintained as stock cultures on slants of Tryptic Soy Agar, with weekly transfers to new tubes. Sock cultures were used to inoculate Tryptic Soy broth cultures which served as "working cultures" in the experiments. Cultures were initially incubated for 24 hours at 37°C and then refrigerated to stall growth.

The bacteria selected were chosen based on their frequent occurrence in infections.

Honey and Royal Jelly

The bacterium to be tested was swabbed from a broth culture onto a Muller Hinton agar plate and a well was made within the agar. For each trial 0.05 mL of either honey or royal jelly, which had been warming in a 35-40°C water bath, was pipetted into the well. The plate was incubated for 24 hours at 37°C. After incubation the zone of inhibition surrounding each well was measured. (Figure 1)

Wells were made in the agar after it was inoculated by inverting a glass 5mL pipette and inserting the tip into the plunger. The agar was pierced, forming a circle, and the agar was drawn up by the plunger.

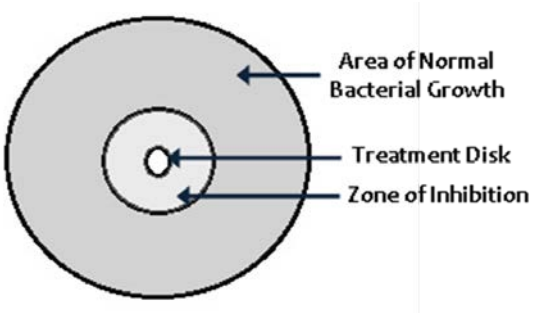

Figure 1: Graphical representation of a treatment disk/well in agar and zone of inhibition produced.

Propolis

A tincture (61 wt/wt%) using an ethanol solvent was made and infused into sterile 6mm disks which were then dried to remove the ethanol. The disks were placed onto inoculated Muller Hinton agar plates and were also incubated for 24 hours at 37°C.

To determine the possible effects of the ethanol solvent, disks saturated with ethanol were added to inoculated agar after 24 hours of drying and an additional control using black sterile disks was similarly run.

Antibiotics

Antibiotic comparison tests were run, using a purchased set of antibiotic infused disks—Kanamycin (30µg), Penicillin (10 units) and Tetracycline (30µg)—from Fischer Scientific. The 6mm disks were placed onto inoculated Mueller Hinton Agar plates, which were subsequently incubated for 24 hours at 37°C. After incubation the zones of inhibition were measured.

Results

Honey and Royal Jelly

Honey and royal jelly displayed a varying degree of bacterial inhibition. Zones of inhibition produced after honey treatment were over 25mm for *S. aureus*, *E.coli* and *B. cereus* while the effectiveness was drastically reduced against *S. epidermidis* (Figure 2a). The rims of the produced zones were graduated rather than distinct, with the zone fading into the area of normal growth.

S. epidermidis was the bacterium most affected by royal jelly, producing a 29.0 mm mean zone of inhibition ±0.9mm standard deviation. The remaining bacteria also experienced some growth inhibition but each with a diameter under 16mm. (Figure 2a)

B. cereus inoculated plates developed a zone of enhanced growth surrounding the zone of inhibition produced by the honey treatment. The zone of enhanced growth was typically 3-4 mm which was then surrounded by an area of normal growth throughout the rest of the plate.

A zone of enhanced growth was also seen in plates inoculated with *S. aureus* and treated with royal jelly.

Propolis

The bacteria tested showed the least amount of susceptibilty to propolis with each producing a zone of inhibition less than 15mm (Figure 2a). *S. aureus* showed a 9.9mm zone of inhibition ±1.6mm standard deviation, *E. coli* 0.4mm ±1.7mm, *S. epidermidis* 13.3mm ±2.7mm, and *B. cereus* 12.4mm ±2.5mm.

The ethanol and blank disk controls run for each bacterium produced no zones of inhibition, making the effects of the ethanol solvent used to create the propolis tincture negligible in their influence.

Antibiotics

Of the antibiotics tested, Kanamycin was the most effective against all four bacteria, except for *S. aureus* which was most susceptible to Tetracycline (Figure 2b).

Of the three, Penicillin displayed the weakest inhibitory effects with the largest mean zone of inhibition produced being 10.5 mm against *S. aureus.*

Discussion

The honey, royal jelly and propolis used exhibited a large range of bacterial inhibitory potentials (Table 2) with the natural honeybee products dispersed between antibiotics

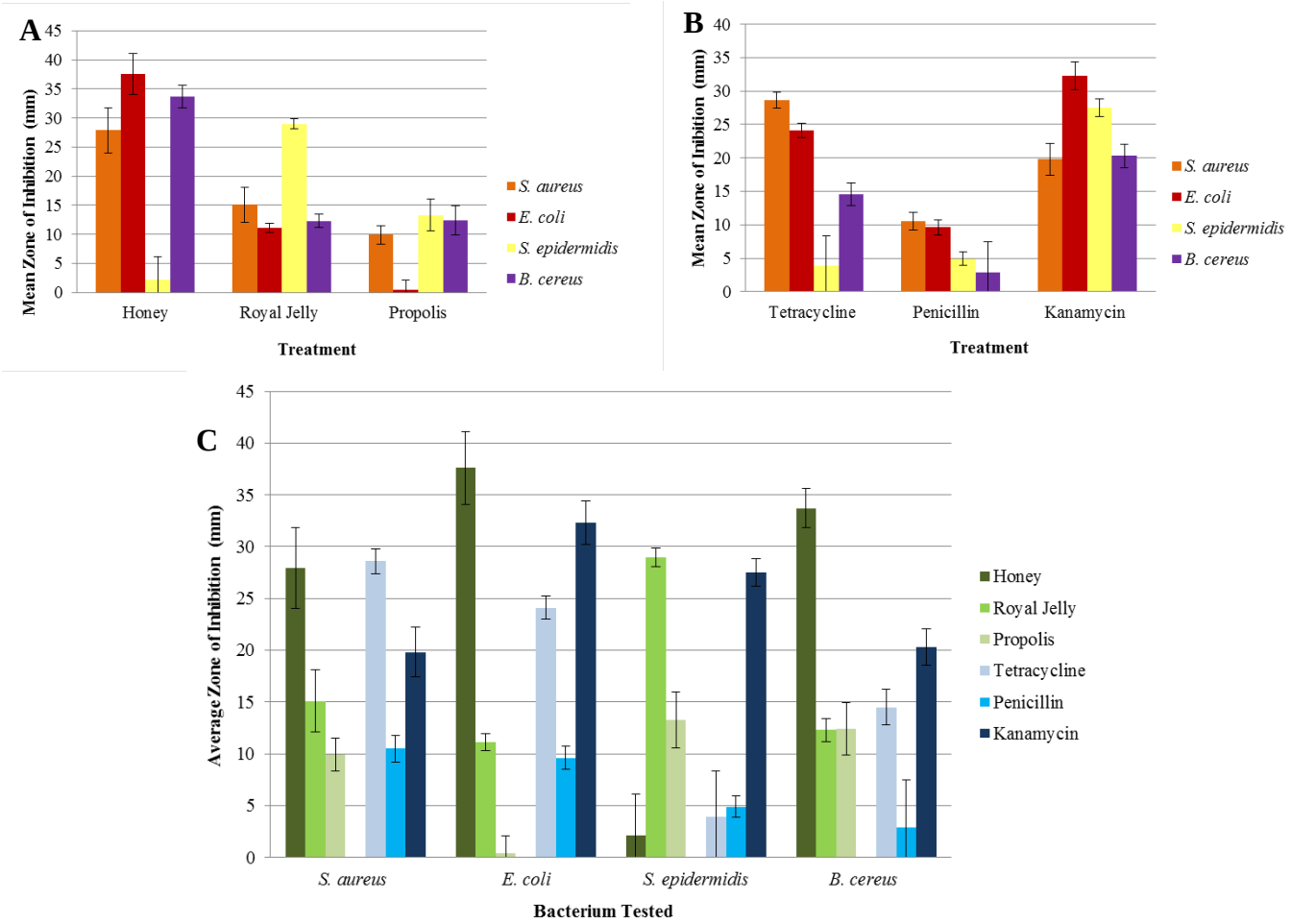

Figure 2: a) Mean values for zones of inhibition produced by honey, propolis and royal jelly for each bacterium b) Mean values for zones of inhibition produced by Tetracycline, Penicillin and Kanamycin c) Comparison of the effects of both the *Apis melifera* products and antibiotics. Sample size of 22-23. Error bars represent standard deviation.

in the spectrum of effectiveness (Table 1). Of the natural products the honey and royal jelly were most inhibitory and were in certain instances, comparable to Tetracycline and Kanamycin.

Kanamycin and Tetracycline were the most effective treatments overall; however, for *Staphylococcus epidermidis* and *Escherichia coli*, honey and royal jelly, respectively, were the most effective products tested.

These and other natural products may have the potential to serve as complementary methods of bacterial inhibition to those already in use by traditional medicine.

The bacteria chosen—*Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis* and *Bacillus cereus*—are common infectious bacterial organisms. *S. aureus* is a common agent in skin infections, food poisonings, and toxic shock syndrome [21]. *E. coli*, while part of the body's natural intestinal flora, can be infectious and produce toxins which, like *S. aureus*, result in food and water-borne poisonings [22]. *S. epidermidis*- of the same genus as *S. aureus*—is another bacterium part of natural flora that, when pathogenic, is prevalent in nosocomial infections. *S. epidermidis* is particularly resilient in its ability to produce a biofilm, making it difficult to remove from contaminated objects [23]. *B. cereus* is another food-borne pathogen that is prevalent in rice, cream sauces and soups [24].

To treat such infections, honey, royal jelly, and propolis have the potential to be applied as mechanisms of either pre-infection control of contaminated foods and surfaces or as a post-infection treatment.

The bacteria used in this experiment were all positive for the catalase enzyme and the group consisted of three Gram-positive and one Gram-negative bacteria. It would be beneficial to more thoroughly test the extent of effectiveness that honey, propolis, and royal jelly have by testing bacteria that are more diverse in their metabolic and physical properties.

The lack of Gram-negative bacteria may explain the dearth of inhibition displayed by the propolis. Additionally, bacterial spirochetes—a bacterial genera not examined in this project—may have exhibited a different susceptibility.

Order of Effectiveness						
Bacterium	**Most**					**Least**
S. aureus	Tetracycline	Honey	Kanamycin	Royal Jelly	Penicillin	Propolis
E. coli	Honey	Kanamycin	Tetracycline	Royal Jelly	Penicillin	Propolis
S. epidermidis	Royal Jelly	Kanamycin	Propolis	Penicillin	Tetracycline	Honey
B. cereus	Honey	Kanamycin	Tetracycline	Propolis	Royal Jelly	Penicillin

Table 1: Ranking of effectiveness for each treatment per bacterium based on mean zone of inhibition.

When examining the issue of an individual product producing a zone of enhanced growth surrounding the area of inhibition it can be theorized that the product became diluted as it spread through the agar, thereby decreasing its effectiveness. The spread resulted in the loss of its inhibitory effects and bacteria in the area with certain metabolic capabilities used the component parts as nutrient sources. Honey enlists several properties to induce bacterial inhibition such as low osmotic pressure, H2O2, etc. However, honey also contains several sugars and bacteria which can digest carbohydrates and may have been able to do so after the product had been diluted.

While more research is required to examine the antimicrobial spectrum of bee products, this research demonstrates that honey, royal jelly, and propolis contain antibacterial derivatives, which could plausibly be prescribed to treat mild bacterial infections and be adopted as standard first-line cures for mild illnesses. They could also be easily incorporated into commercial products such as mouth washes, lotions, antibacterial gels, ear-drops and similar products to enhance effectiveness against microorganisms. This makes them not only curative agents, but also potential preventative measures against disease. Verifying the antimicrobial spectrum of these natural products can result in their frequent, effective use as natural antibiotics, antiseptics and disinfectants.

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Acknowledgements

Sincerest thanks to Professor Donna Frost for her guidance on this project and to Cheryl Lyon, Hank Voegeli and Michael Rossi for their additional input and advisement. Also to the UNH Board of Governors and SURF sponsors for making this and other projects available for undergraduates at the University of New Haven.

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