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An Exploration of a Honey-Ginger Supplement as an Antimicrobial Agent

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Summary

Many bacterial strains have become resistant to antibiotics, such as methicillin, novobiocin, vancomycin, and penicillin. Due to the increase in antimicrobial resistance, alternative medicinal therapies are being explored. Studies have shown that honey and ginger alone have antimicrobial effects on the genera *Staphylococcus* and *Escherichia*, including *S. epidermidis* and *E. coli*. This study tested whether a honey-ginger supplement, Jengimiel™, could be used as an antimicrobial agent against *S. epidermidis* and *E. coli K-12*. The data showed that Jengimiel™ exhibited no antimicrobial activity against either *S. epidermidis* or *E. coli K-12*, and therefore should not be used as an antimicrobial agent against these organisms. The limitations of this study include the fact that perhaps this supplement does not contain a high enough concentrations of honey and/or ginger in order to observe an antimicrobial effect in our studies. Future testing should explore whether or not different concentrations of this supplement would inhibit the growth of these organisms, as well as other bacterial species. It would also be worth exploring whether or not this supplement serves as a prebiotic, stimulating the growth of these bacterial strains.

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Introduction

Escherichia coli and *Staphylococcus epidermidis* are among the many strains of bacteria found within the human microbiome. *E. coli* is a gram-negative bacillus and is a mutualistic inhabitant of the mammalian intestinal tract, where it aides in the breakdown of lactose and produces vitamin K, as well as clotting factors (8). While it is usually not pathogenic, there are some strains that are known to cause urinary tract infections, traveler's diarrhea, and serious food-borne disease. Additionally, food and water sources can be easily contaminated with *E. coli* when proper sanitization procedures are not followed. *S. epidermidis* is a coagulase-negative, gram-positive commensal bacterium that is part of the skin microflora, aiding in proper immune system development by keeping potential pathogens out of the body through the process of competitive inhibition (8). While *S. epidermidis* is also not usually pathogenic, this organism does have the ability to become a pathogen, particularly through the development of biofilms. A biofilm is a microbial community that can form a slimy layer on a surface (8). *S. epidermidis* often forms biofilms on implanted medical devices, such as catheters (1).

While both *E. coli* and *S. epidermidis* are considered to be part of the normal, human microflora, there are harmful strains of *E. coli* and *S. epidermidis* that are able to overcome the immune system and pose a threat to the host organism's well-being. When this is the case, the normal treatment for infections caused by these organisms is the use of antibiotics, such as tetracycline for *E. coli* infection (2) and erythromycin for *S. epidermidis* infection (8). The issue, however, is the increasing resistance to antibiotics that is occurring within bacteria, including *E. coli* and *S. epidermidis*. This has led to investigations for alternative treatments for infection. Prebiotics, food substances that have selective effects on the growth of bacteria (8), are a growing field of exploration in terms of antimicrobial therapeutics due to their ready availability and thus far, the lack of antimicrobial resistance observed with these compounds. Both honey and ginger are prebiotic foods that have been explored as possible antimicrobial agents against various bacterial strains of organisms, including several clinical pathogens.

Honey has been shown to be an effective antimicrobial agent against coagulase-negative *Staphylococci* species, such as *S. epidermidis*. In a study performed by French *et al.*, two natural honeys were tested for antimicrobial activity against 18 clinical isolates of coagulase-negative *Staphylococci* (3). The results of the study indicated that honey in its natural form was indeed effective at inhibiting the growth of the coagulase-negative *Staphylococci* species, and that the antimicrobial activity of the honey was likely due to a combination of sugar content, along with hydrogen-peroxide activity, and unknown phytochemical components. Honey has

also been shown to be an effective antimicrobial agent against *E. coli*. A study performed by Mandal *et al.* aimed to determine the partial inhibitory, minimal inhibitory, and minimal bacteriocidal concentrations of honey against three gram-negative bacterial pathogens: *Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella enterica* serovar *Typhi* (6). The results of the study showed partial inhibition of *E. coli* growth at a honey concentration of 0.75%-1.50%. An inhibitory effect was observed at a 1.00% honey concentration, and a bacteriocidal effect was observed at a concentration of 3.00% honey.

Prebiotic spices such as ginger have also been shown to be effective antimicrobial agents. Common cooking spices contain phytochemical and/or essential oil components that inhibit bacterial growth, making them ideal for preventing food-borne illness in areas with limited access to adequate food and water sanitization procedures (4). A study performed by Gull *et al.* compared aqueous, ethanol, and methanol extractions of ginger and their potential antimicrobial activity against both *S. epidermidis* and *E. coli*. The results of this study indicated that all of the ginger extracts were successful at preventing the bacterial growth of both organisms, however, the extracts were more successful against gram-positive organisms.

As demonstrated, both ginger and honey have been shown to have an inhibitory effect on the growth of both *E. coli* and *S. epidermidis*, but the question remains as to how these two compounds would work if they were tested together against these organisms. There are several nutritional supplements available for purchase at retail establishments that contain both ginger and honey. One such supplement is Jengimiel™. Jengimiel™ is a homeopathic, proprietary blend of honey and ginger root, containing 8 grams of the proprietary mixture per tablespoon of liquid. Jengimiel™ is marketed to clear the throat and aid in digestion (5). Since this product contains both honey and ginger, the question as to whether or not this product could serve as an antimicrobial agent against *E. coli* and *S. epidermidis* was explored. For this study, *E. coli K-12* and *S. epidermidis* were grown along with disc containing undiluted Jengimiel™ on Mueller-Hinton agar. It was predicted that the ginger-honey supplement, Jengimiel™, would inhibit the growth of *E. coli K-12* and *S. epidermidis.* Testing indicated that despite Jengimiel™ containing both ginger and honey, the product did not appear to exhibit any antimicrobial activity against *E. coli K-12* nor *S. epidermidis*. These findings are significant because the apparent lack of antimicrobial activity of Jengimiel™ against *E. coli K-12* and *S. epidermidis* may guide consumers to seek something other than Jengimiel™ to treat infections against *E. coli* or *S. epidermidis*.

Results

The question being explored was whether Jengimiel™ is an effective antimicrobial agent since it contains both honey and ginger, which are known antimicrobial compounds. In order to test this, Jengimiel™ was grown alongside *E. coli K-12* and *S. epidermidis* so that the effectiveness of this product against both a Gram-positive and Gram-negative organism could be determined. Negative and positive control plates were set up alongside the Jengimiel™ plates. The negative control plates consisted of the organism, either *E. coli K-12* or *S. epidermidis*, grown on a Mueller-Hinton agar at a 0.5 McFarland standard, with a blank, sterile paper disc. This was done so that the growth of the organisms could be observed, but since there was no chemical present on the sterile, paper disc, no zone of inhibition (ZOI) should be observed. The positive control plates consisted of the organism, either *E. coli K-12* or *S. epidermidis*, grown on a Mueller-Hinton agar at a 0.5 McFarland standard, with a paper disc containing a known, effective antibiotic which inhibits the growth of the organism. The antibiotic chosen for *E. coli K-12* was tetracycline, and the antibiotic chosen for *S. epidermidis* was erythromycin. This was done so that the growth of the organisms could be observed in the presence of these antibiotics, and the ZOI could be compared with known National Committee for Clinical Laboratory Standards (NCCLS) antimicrobial sensitivity values for these organisms.

The zones of inhibition were measured for all

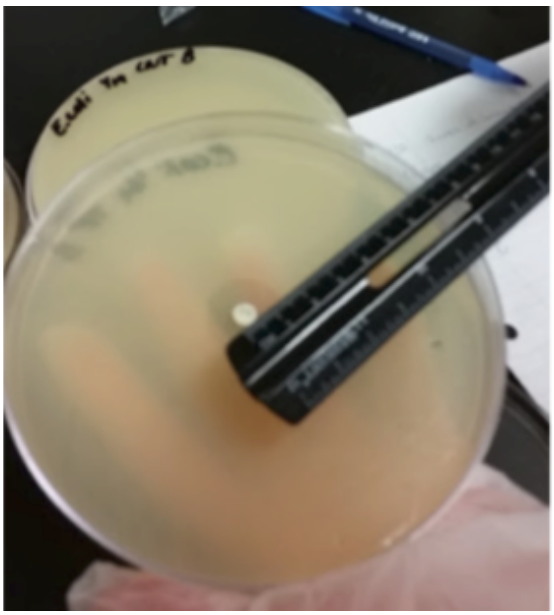

Figure 1. Zone of Inhibition (ZOI) measurements. The ZOI for each organism versus each product was measured. A standard ruler is used to measure the diameter of the ZOI, in millimeters, across the disc, from where growth of the organism stops to where it resumes.

plates, in millimeters, by using a standard metric ruler to measure across the diameter of the ZOI, from the area where the growth stopped to where the growth resumed. **Figure 1** shows the determination of the ZOI. All three of the sterile, blank paper discs for *E. coli K-12* had a ZOI of 6 mm, which indicated growth directly up to the disc. The ZOI for E. *coli K-12* containing tetracycline 30 µg were 25 mm, 25 mm, and 27 mm. For all three Jengimiel™ discs, the ZOI was 6 mm, indicating no antimicrobial effect against *E. coli K-12* (**Table 1**). Each of the negative controls for *S. epidermidis* had a ZOI of 6 mm. Two of the positive control trials of Erythromycin 15 µg against *S. epidermidis* had a ZOI of 33 mm, and the third positive control trial of Erythromycin 15 µg against *S. epidermidis* had a ZOI of 37 mm. Each test of Jengimiel™ against *S. epidermidis* had a ZOI of 6 mm, showing that Jengimiel™ had no antimicrobial effect against *S. epidermidis* (**Table 1**).

Discussion

The quality control zones of inhibition for *E. coli* being treated with tetracycline indicate sensitivity to the antibiotic if the ZOI is ≥ 23 mm (3), and since in our studies *E. coli K-12* had zones of inhibition for tetracycline of 25 mm, 25 mm, and 27 mm, it can be said that the tetracycline was an effective antimicrobial agent against *E. coli K-12.* The quality control zones of inhibition for erythromycin indicate sensitivity if the ZOI is ≥ 19 mm (3), and since *S. epidermidis* had zones of inhibition for Erythromycin of 33 mm, 33 mm, and 37 mm, it can be said that the erythromycin was an effective antimicrobial agent against *S. epidermidis.* Since growth of the microorganisms occurred up to the disc containing the Jengimiel™ honey-ginger supplement, it was determined that there was no observed antimicrobial activity against *E. coli K-12* and *S. epidermidis* when grown in the presence of Jengimiel™. The results of this experiment contradict previous studies exploring the efficacy of honey and ginger together as antimicrobial agents. A study by Omoya and Akharaiyi previously showed that honey, ginger, and extracts of honey and ginger combined were all effective antimicrobial agents against various bacteria, including *E. coli* and *S. aureus* (7); however, Jengimiel™, which contains both honey and ginger, did not show the same efficacy. This may be attributed to the preparations of the extracts used in the Omoya and Akharaiyi study versus those used to prepare Jengimiel™ and warrants further exploration.

However, there are limitations within this study. One limitation is the small sample size. Only three trials were conducted for each bacterial strain. Another limitation could be that this supplement is ineffective against *E. coli K-12* and *S. epidermidis*, but it may be effective against other bacterial species. Future directions should investigate whether this supplement is an effective antimicrobial agent against other strains of bacteria, such as those found within the pharynx, as this supplement claims to “clear and refresh the throat” (5). A final limitation of this study could be that the concentrations of both the honey and the ginger found within Jengimiel™ are too dilute to be effective antimicrobial agents against *E. coli K-12* and *S. epidermidis*. Future investigations should explore the effectiveness of Jengimiel™ as an antimicrobial agent at higher honey and ginger concentrations. Additionally, it would be worth exploring whether or not this supplement serves as a prebiotic, meaning it would stimulate bacterial growth. Despite the limitations of this study, analyses show that Jengimiel™ was not effective at inhibiting the growth of *E. coli K-12* or *S. epidermidis*.

Materials and Methods

First, a sterile 0.85% Isotonic Buffered Blood Bank Saline tube (Thermo Scientific) was inoculated to a 0.5 McFarland standard with *Escherichia coli K-12* (Carolina Biological) using visual inspection and comparing the inoculum to a 0.5 McFarland latex standard. Next, a sterile swab was dipped into the inoculated saline tube. Following this, a Mueller-Hinton (MH) plate was inoculated by rolling the swab across the plate using X, Y,

Organism	Trial #	Negative control – Blank Disc (mm)	Positive Control – Tetracycline 30 µg / Erythromycin 15 µg (mm)	Jengimiel™ (mm)
E. coli K-12	1	6	25	6
	2	6	25	6
	3	6	27	6
S. epidermidis	1	6	33	6
	2	6	33	6
	3	6	37	6

Table 1. Zone of Inhibition measurements for *Escherichia Coli K-12* and *Staphylococcus epidermidis. E. coli K-12* and *Staphylococcus epidermidis* zones of inhibition (ZOI) for the negative control, positive control, and Jengimiel™. ZOI measurements were taken in millimeters and compared with NCCLS antimicrobial guidelines. Quality control (QC) is a procedure set forth by NCCLS to verify the identification of an organism. The positive control tests fell within the QC guidelines.

Z quadrants, creating a lawn. This process was repeated for two additional saline tubes and two additional Mueller-Hinton plates. As a negative control, a blank sterile disc (Blank disc Biogram Lot SDO-1507) was placed in the center of the Mueller-Hinton plate containing *E. coli K-12*. As a positive control, a disc containing 30 µg tetracycline (BBL Sensi-Disc) was placed in the center of the second inoculated MH plate. Finally, a blank sterile disc was dipped directly into undiluted Jengimiel™, the honey-ginger supplement solution (Natural Ginger Corp Lot 140602), and then placed onto the center of the third inoculated MH plate. All plates were incubated at 35°C for 24 hours. The above procedure was repeated using *S. epidermidis,* however, the use of 30 µg tetracycline (BBL Sensi-Disc Lot 4064344) was replaced with 15 µg erythromycin (BBL Sensi-Disc Lot 4132962) as the positive control. The following day each MH plate was observed to measure the zones of inhibition (ZOI), in millimeters. Zones of inhibition are measured using a standard metric ruler to measure across the diameter of the ZOI, from the area where growth stops to where the growth resumes. The entire procedure was performed for a total of three trials.

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