Antibacterial Activity of Honey Against *N. gonorrhoeae and S. aureus*

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Abstract

This study aims to evaluate the antibacterial efficacy of honey against wild-type *N. gonorrhoeae* and *S. aureus* via zone of inhibition (ZOI), comparative analysis of the antibacterial components within honey, and the differing inhibitory effects exerted by honey on gram-positive and gram-negative bacterium. This study's findings contribute foundational knowledge to the development of accessible, integrative techniques utilizing bioactive honey, offering a critical step toward combating the growing threat of antibiotic resistance.

Introduction

Antibiotic resistance is a rapidly escalating global health crisis, necessitating research aimed at finding alternative treatments to resistant pathogens. Antibiotic resistance can develop in a bacteria strain after being exposed to an antibiotic over a period, with the microbes that failed to be terminated developing adaptations to bypass the antibiotic's specific mechanism [1]. Antibiotics are prone to resistance developing against them due to the single-action mechanisms used to target one aspect of bacterial replication and protein synthesis. *Neisseria gonorrhoeae* and *Staphylococcus aureus* have both demonstrated severe resistance against first-line antibiotic treatments. ***N. gonorrhoeae*** is a gram-negative diplococcus bacteria and the 2nd most common bacterial sexually transmitted infection (STI) [2,3]. ***S. aureus,*** a gram-positive coccus bacteria, is the leading cause of hospital-acquired infections and a diverse number of potentially life-threatening infections [4]. **Gram-positive bacteria** are often more susceptible to antibacterial agents than **gram-negative bacteria**, as they lack the protective external membrane covering the peptidoglycan layer that gram-negative bacteria have [5]. Honey has exerted potent antimicrobial effects on numerous pathogens, serving as an effective inhibitory and bactericidal agent. The **synergistic effects** of low pH, high osmolarity, phytochemical profile, and methylglyoxal (MGO) or hydrogen peroxide content within honey are known to inhibit numerous bacterial species without inducing resistance [5]. The key distinction between Manuka and local Texas honeys lies in their primary antibacterial mechanisms; Manuka honey is a non-peroxide honey that relies on methylglyoxal as its main antibacterial agent, while Texas honey typically generates hydrogen peroxide from the enzyme glucose oxidase.

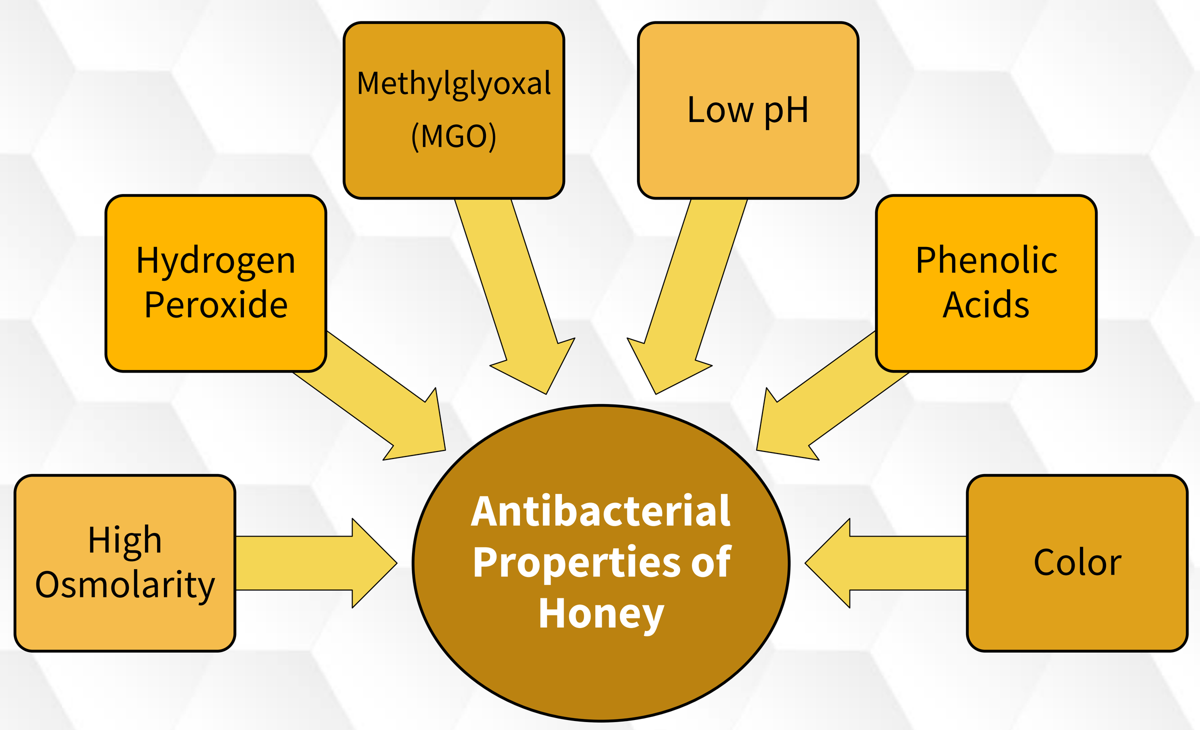

Figure 1. Antibacterial Properties of Honey.

Materials and Methods

Honey samples were collected from New Zealand, New Jersey, and TX beekeepers during 2022 and 2023. The antimicrobial efficiency of the honey samples was assessed through the agar well-diffusion method. *S. aureus* was cultured in Tryptic Soy Broth and incubated at 37ºC for 24 hours aerobically. The culture was then adjusted to 1.5×10^8 cfu/ml and uniformly dispersed to the entire surface of Mueller Hinton Agar plate. 8 mm wells were created for the honeys, and each honey was carefully transferred into their respective wells. Manuka honey samples, M-225, M-252, and M600+, with varying MGO levels were used for comparisons. Following incubation, the average zone of inhibition (ZOI) of each sample was precisely measured using a digital caliper. *N. gonorrhoeae* (ATCC 49226), bacteria was cultured from frozen stock by streaking onto Thayer-Martin agar and incubating for 24 hours at 32°C in a 1% CO_2 atmosphere. After visible growth, bacteria were collected using a sterile inoculating loop and suspended in 1000 µL of phosphate-buffered saline (PBS). From this suspension, 100 µL was dispensed onto each plate and spread evenly using a sterile inoculating loop. 8 mm wells were created for the honeys, and each honey was added to their respective wells. Plates were incubated at 32°C under 1% CO_2 for 24 hours, and the average ZOIs were measured.

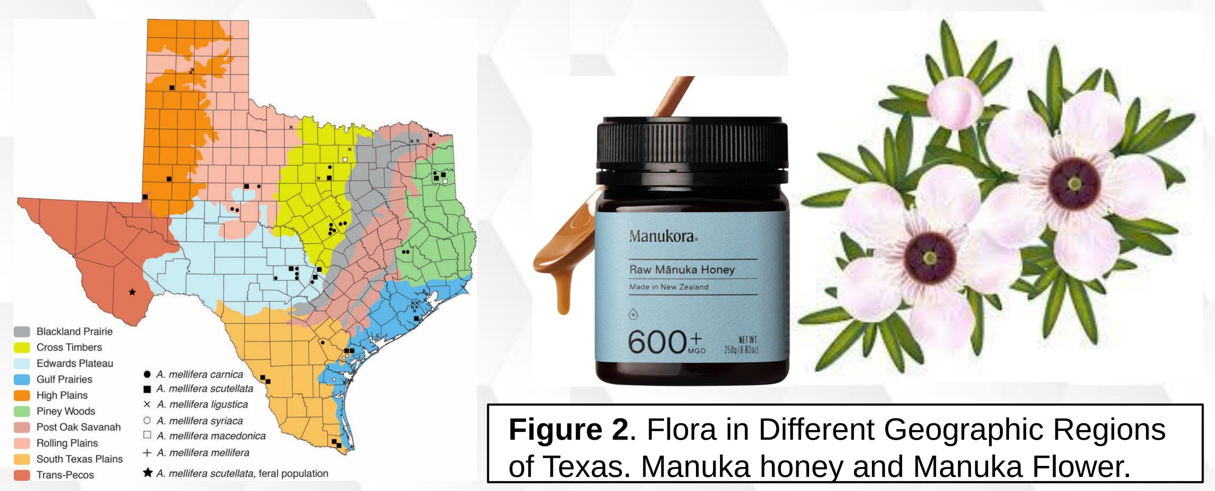

Figure 2. Flora in Different Geographic Regions of Texas. Manuka honey and Manuka Flower.

Results

The agar well-diffusion assays against *N. gonorrhoeae* showed that Manuka honey showed the strongest inhibition, with average ZOIs up to 23.02 mm. Several Texas honeys also performed well, surpassing the artificial and negative controls. On average, *S. aureus* was more effectively inhibited by the honey samples than *N. gonorrhoeae,* with the exception of the Manuka sample, M-600+. Overall, a positive trend between honey pFund values (honey color) and ZOIs was observed. However, honey color is not a perfect indicator of antibacterial activity, since many factors ultimately contribute to a honey's inhibitory efficacy.

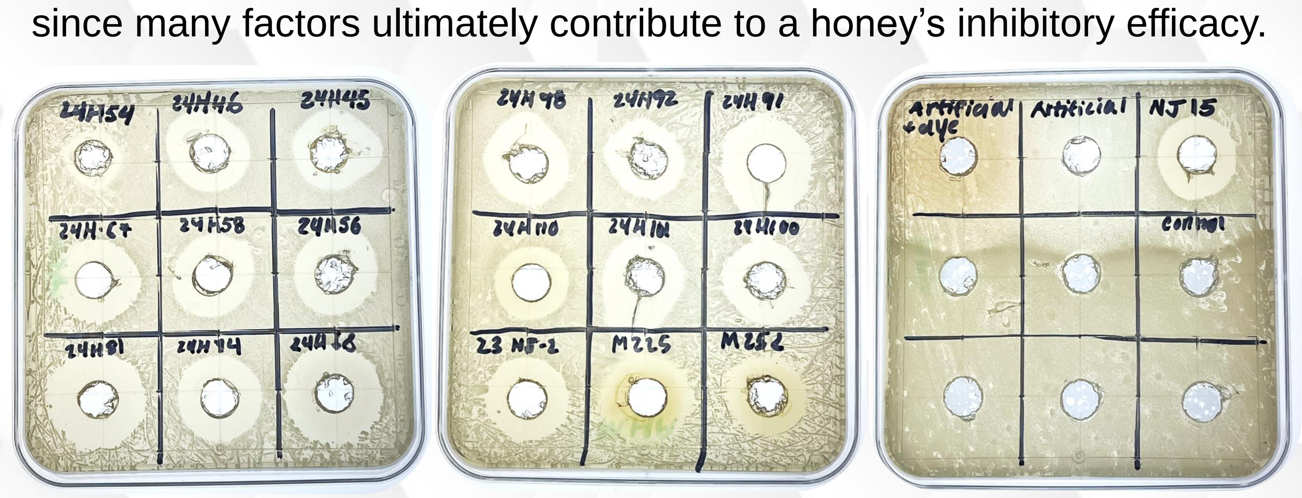

Figure 3. The ZOIs produced by 20 different honey samples, including local Texas honeys, Manuka honeys, New Jersey honey varieties, and an artificial sugar composite honey, against *S. aureus* are displayed above. ZOIs > 16 mm are considered highly inhibitory. The honey samples exhibiting the largest ZOIs include 24H-68, 24H-81, 24H-91, and 24H-98, which produced ZOIs of 23.0 mm, 23.0 mm, 22.0 mm, and 22.0 mm, respectively. The 15 most inhibitory honey samples were further tested against *N. gonorrhoeae* for comparison.

Results Cont.

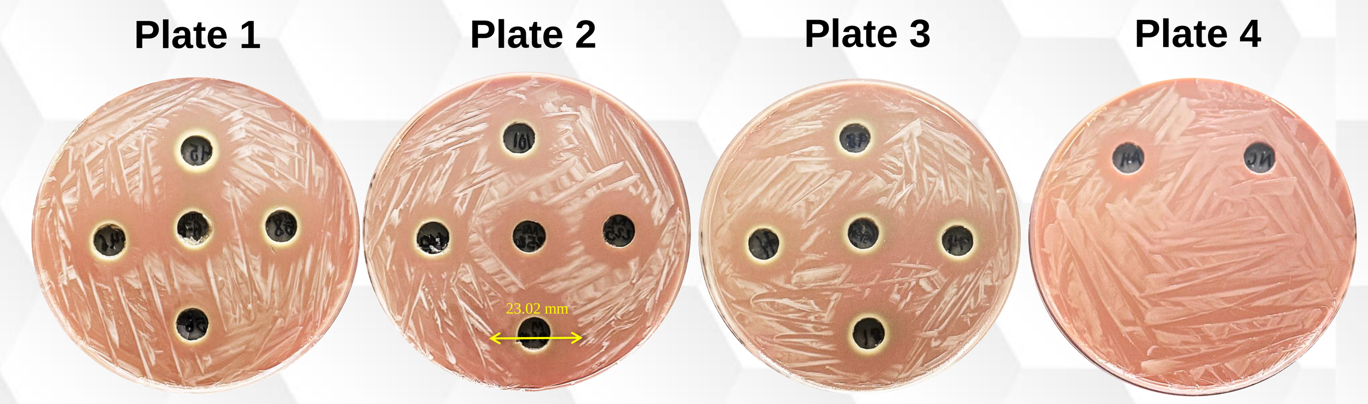

Figure 4. The ZOIs produced by the honey samples tested against *N. gonorrhoeae* are displayed above. **Plate 1** contains samples 24H-45, 24H-46, 24H-56, 24H-58, and 24H-67. **Plate 2** contains samples 24H-100, 24H-101, M-225, M-252, and M600+. **Plate 3** contains samples 24H-68, 24H-74, 24H-81, 24H-91, and 24H-98. **Plate 4** contains the artificial honey sample and negative control, producing ZOIs of 14.75 mm and 8 mm, respectively. The Manuka samples, M-252, M-225, and M-600+ produced the highest average ZOIs of 19.59 mm, 20.01 mm, and 23.02 mm, respectively.

Honey ZOIs Against *N. gonorrhoeae* & *S. aureus*

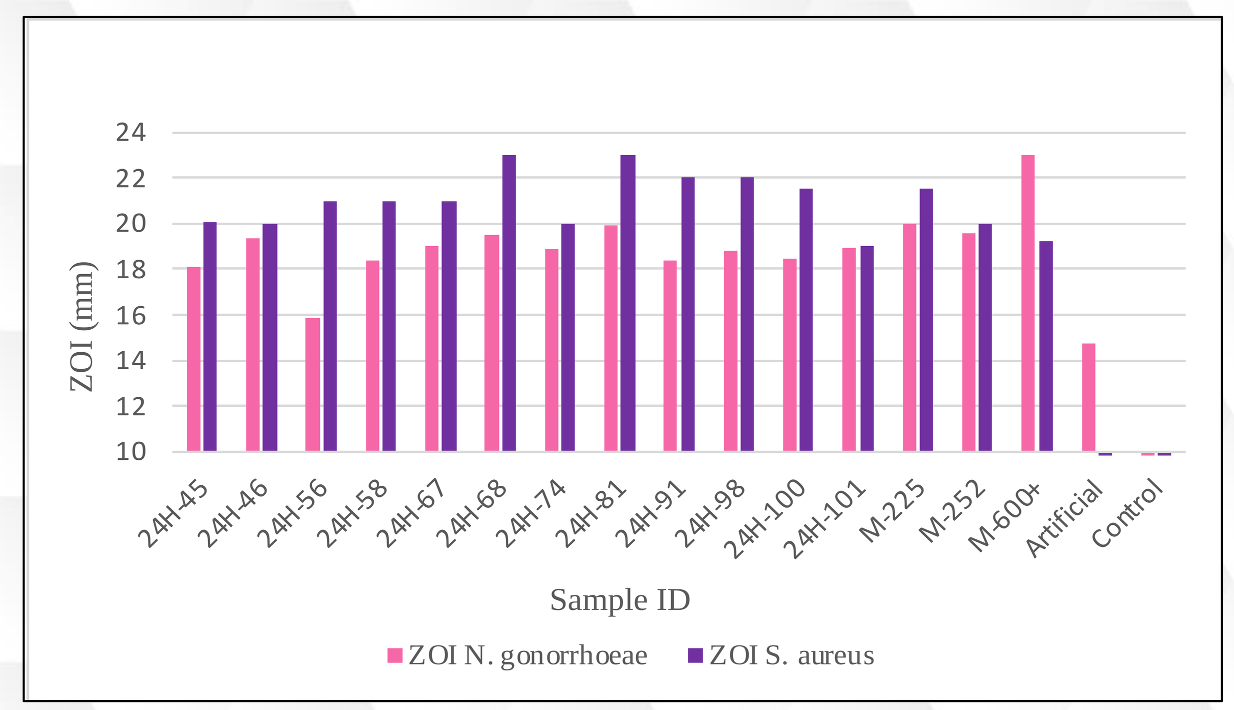

Figure 5. The ZOIs produced by the honey samples tested against *S. aureus* and *N. gonorrhoeae* are compared above. On average, the honey samples exhibited greater ZOIs against *S. aureus* than *N. gonorrhoeae,* highlighting the difference between the antibacterial efficacy of honey against gram-positive and gram-negative bacteria.

Texas Honey ZOIs vs pFund Against *S. aureus*

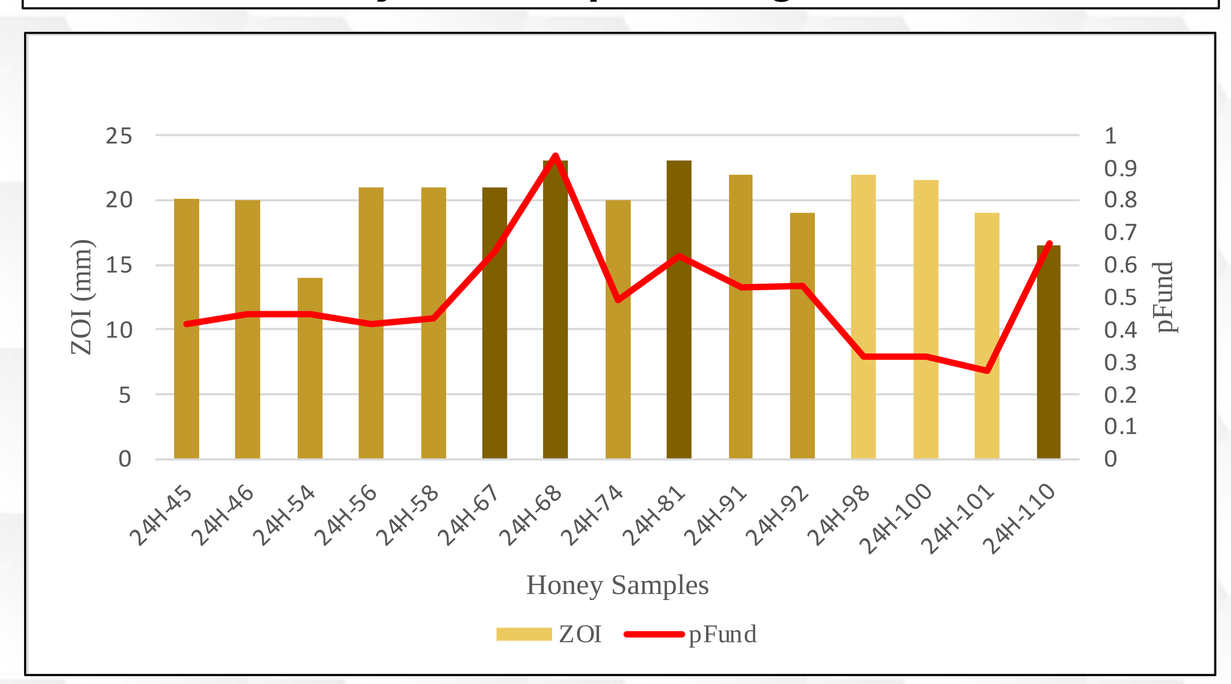

Figure 6. The ZOIs produced by Texas honey samples against *S. aureus* are compared to the samples' respective pFund–or honey color–values. These results show a general trend of darker honeys producing higher ZOIs than lighter color varieties, with samples 24H-67, 24H-68, and 24H-81 exhibiting the highest pFund values and ZOIs.

Conclusions

- Manuka honey largely demonstrated the strongest inhibition against *N. gonorrhoeae*, whereas local Texas honeys largely demonstrated the greatest inhibition against *S. aureus*, highlighting the different cellular mechanisms and cytological variations between gram-negative and gram-positive bacteria.
- Gram-positive bacteria is more susceptible to the antibacterial action of hydrogen peroxide-producing honeys than gram-negative bacteria due to the differences in cell wall structure and peptidoglycan layer accessibility [5].
- The antibacterial activity of honey is not attributable to high osmolarity alone, but rather to the synergistic effects of the numerous unique phytochemical profile and bioactive compounds found within natural honeys.
- The relationship observed between honey color and ZOI illustrates another influence upon the antibacterial activity of honey, with darker color honeys exerting stronger antibacterial activity from the phytochemicals and bioactive characteristics determining honey color.

Broader Impact and Future Exploration

These findings provide baseline support towards the potential implementation of bioactive honey in treatment of resistant *N. gonorrhoeae and S. aureus* infections. Future research should focus on further evaluating the inhibitory and bactericidal effects of Manuka honey against *N. gonorrhoeae*, given their superior efficacy in this study. Additionally, future experiments will be needed to determine the **minimum inhibitory concentration (MIC)** and **minimum bactericidal concentration (MBC)** of the most effective honey samples to define precise bactericidal thresholds.

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