Determination of the Minimum Concentration of Manuka Honey for the Inhibition of Growth of *S. aureus*

Lawrence Technological University
COLLEGE OF ARTS + SCIENCES
Quest Program

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Introduction

The innate immune system includes critical but non-specific barriers to infection like mucus membranes, earwax and skin. When the barrier of the skin is breached, this is called a wound. Open wounds are a potential entry point for microbial infection, such as that caused by *Staphylococcus aureus* (the causal agent of Staph infections). Staph infections are a significant public health threat. The ability to speed wound healing while preventing microbial growth using tissue engineering scaffolds could improve patient outcomes and reduce costs. One of the biggest threats to modern medicine is the presence of drug resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas*, and *Acinetobacter baumannii*. Honey possess many different antimicrobial properties that contribute to the healing process and infection prevention. There are many different types of honey, but for the sake of this research we will focus on Manuka honey. Manuka honey is produced from nectar of the Manuka tree, native to New Zealand and some parts of Australia. This is a strain of honey that has been shown to have higher levels of phenolic compounds, polyphenols and flavonoids that result in antioxidant activity. It has also been shown to inhibit bacterial growth at levels higher than other types of honey.

Aims

- In this study, our goal was to determine the minimum inhibitory concentration (MIC) of Manuka honey required to inhibit bacterial growth by at least a three log fold reduction.
- Our secondary goal was to find an alternative method to disk/well diffusion that would give a better resolution.

Method

Low Resolution Methods Used in Prior Work

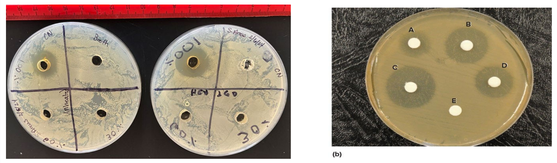


Figure 1A & 1B: Well diffusion Assay and Kirby Bauer Disk Diffusion

Culturing *S. aureus* in varying concentrations of Manuka Honey(MH)

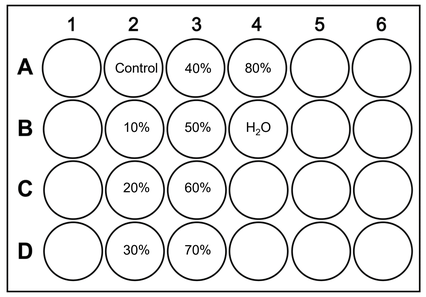


Figure 2: Honey Concentration in each well.

% of MH:NB	µl of 80% MH	ul of Nutrient broth to make 500 µl
70%	437.5	62.5
60%	375	125
50%	312.5	187.5
40%	250	250
30%	187.5	312.5
20%	125	375
10%	62.5	437.5

Figure 3: Amount of 80% honey to Water

For Inoculum plating – 10% honey & (Sa in water) - 5 plates

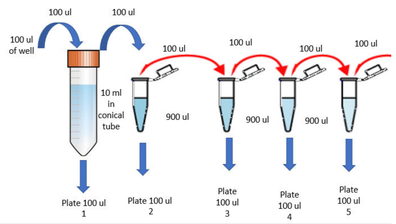


Figure 4: An example of the Serial Dilution method used.

Method

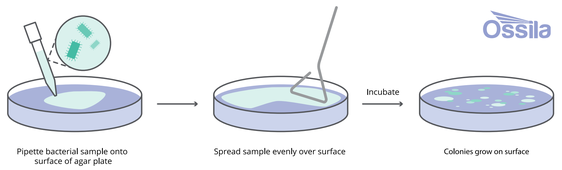
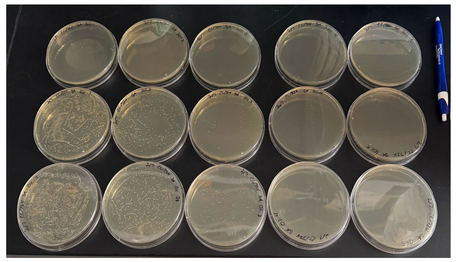


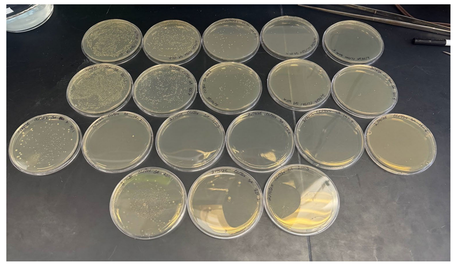
Figure 5: Spread plate method used to distribute *S. aureus* at different concentrations

Results

Serial dilutions for 2/7/25 *S. aureus* and liquid honey cultures



Serial dilutions for 2/14/25 *S. aureus* and liquid honey cultures



% Honey	Log_{10} Fold Reduction
0%	0
10%	2.21

Figure 8: Log Fold Reduction 2/7/2025
One Log fold reduction indicates that only 10% of the bacteria survive

% Honey	Log_{10} Fold Reduction
0%	0
10%	1.74
15%	5.77
20%	6.00

Figure 9: Log Fold Reduction 2/14/2025

Growing *S. aureus* in liquid cultures allows for greater detection of small changes

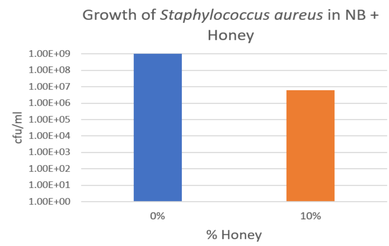


Figure 10:Growth Of *S. aureus* in NB+Honey 2/7/2025 0%-10%

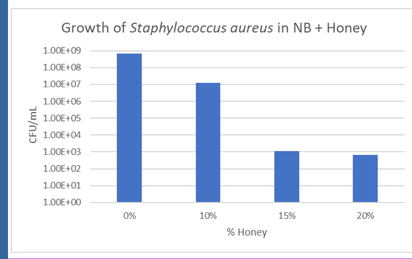


Figure 11:Growth Of *S. aureus* in NB+Honey 2/714/2025 0%-20%

Conclusion

- Our work demonstrated that liquid culture of *S. aureus* with nutrient broth supplemented with honey could be used to determine the MIC with greater resolution.
- We also showed that Manuka honey concentrations of 15% or greater were able to inhibit the growth of *S. aureus* in liquid culture.
- Both 15% and 20% Manuka honey were able to achieve greater than 3 log fold reductions.
- We were unable to directly measure the log fold reduction created by Manuka honey concentrations at 30% or greater because all *S. aureus* were killed (but this does indicate that these concentrations would also be effective).

Acknowledgements

This work was generously supported by a Quest Award. We would also like to thank the Dept of Natural Sciences. We would also like to thank other members of the research team Sandra Zori, Connor Nowak, and Andrew Ulaszek.

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