# Aging Manuka Honey Improves Antimicrobial Activity Against Pathogenic Bacteria

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## Abstract

- Manuka honey (MH) has been documented to possess powerful anti-microbial properties through mechanisms such as low pH, high osmolarity, methylglyoxal (MGO) and iron chelation.
- MGO is the source of MH's antimicrobial properties.
- MH minimum inhibitory concentrations (MIC) experiments suggested that some MH antimicrobial mechanisms lose effectiveness over time; tested bacterial species were differentially affected.
- We are interested in discovering which MH antimicrobial mechanism is most effective against three major pathogenic bacterial species, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.
- We sought to analyze how MH age impacted its MIC against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus.*
- Weekly MIC experiments were conducted with newly prepared MH and aging MH.
- It is hypothesized that the conversion of dihydroxyacetone (DHA) to methylglyoxal (MGO) is contributing to the increased potency of MH against *S. aureus.*

## Introduction

- Anti-microbial resistance has developed into a severe issue in modern day society causing a drive to identify new and alternative treatments.
- Manuka honey (MH) is a monofloral honey found in Australia and New Zealand that has a rich history of use as a topical medication.
- Research has established that MH possesses many different anti-microbial mechanisms.
- Gram-positive and gram-negative bacteria are both susceptible to the honey and are both represented in this study.
- Major groups of bacteria present different cellular membrane components, to which MH is differentially effective against; it is hypothesized that different anti-microbial mechanisms may be responsible for its effectiveness against each type.
- MH portrays a fairly large degree of variability in effectiveness between different strains of bacteria; they will have different MH MICs.
- MH is effective against all bacteria for which it has been tested on.
- Resistance to MH has not been detected thus far.

## Hypothesis

The MH MIC against some bacteria will increase as the age of the MH increases (increase in MIC indicates loss of effectiveness).

## Methods

- Overnight cultures of *E. coli*, *P. aeruginosa*, and *S. aureus* with LB media are left in a shaking incubator at 37℃ for ~18 hours.
- Cultures are then diluted and incubated in the 37℃ shaking incubator until they reach an $OD_{600}$ of 0.5 (roughly 2.50 x$10^8$ cells/mL).
- MH and/or growth media was added in appropriate volumes to obtain desired MH % in assay wells. There were approximately 1.0 x $10^6$ cells per well.
- The T=0hr $OD_{600}$ was determined with a spectrophotometer.
- The plate was incubated in the 37℃ incubator for ~18 hours. The T=18hr $OD_{600}$ was also determined with a spectrophotometer.
- The $OD_{600}$ change is plotted in Figure 2.

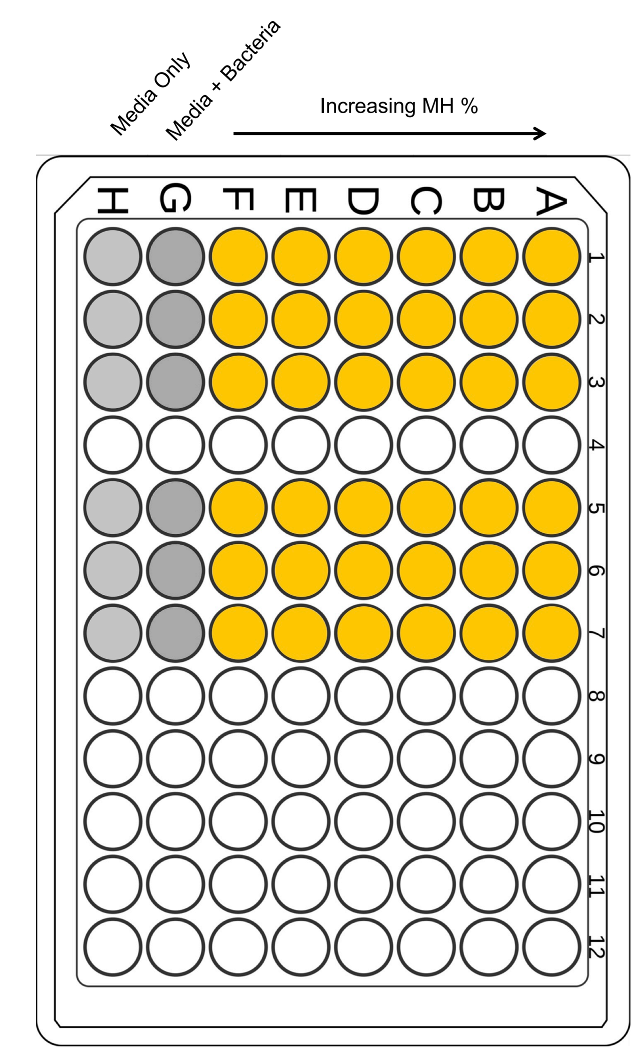


Figure 1. 96-well plate set-up.

## Results

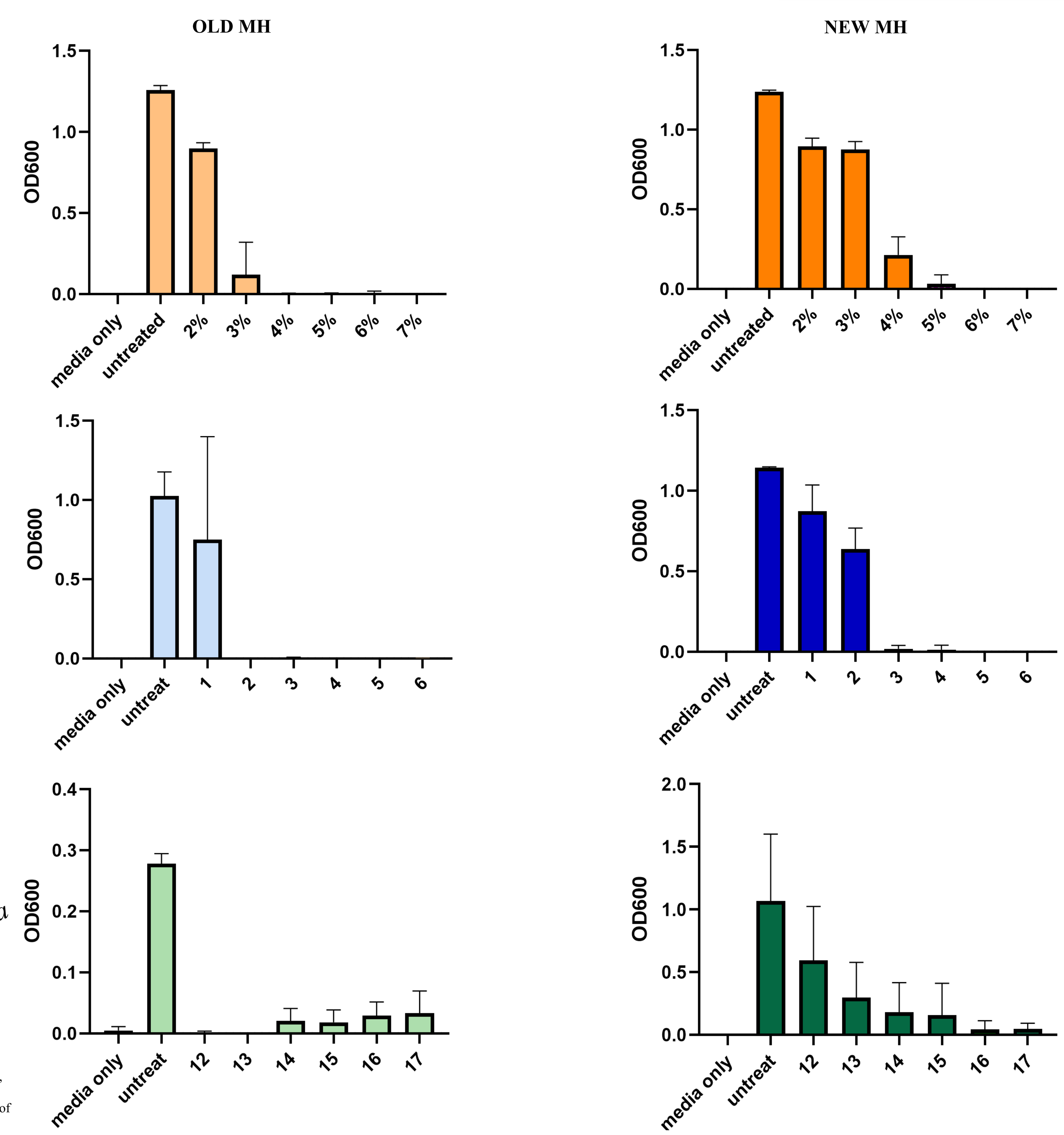


Figure 2. *E. coli*, *S. aureus*, and *P. aeruginosa* treated with different percentages of old or new MH

## Multiple Unpaired T-test Analysis

***E. coli***

| | P value |
|---|---|
| Media | 0.488251 |
| Untreated | 0.329494 |
| 2% MH | 0.978845 |
| 3% MH | 0.003141 |
| 4% MH | 0.031114 |
| 5% MH | 0.376679 |
| 6% MH | 0.269807 |
| 7% MH | 0.281654 |

Table 1. Multiple unpaired t-tests were run for each bacterial strain to determine the p-value of mean aged MH (t = x) MIC and the mean new MH (t = 0).

***S. aureus***

| | P value |
|---|---|
| Media | 0.719479 |
| Untreated | 0.249120 |
| 1% MH | 0.765976 |
| 2% MH | 0.067318 |
| 3% MH | 0.340556 |
| 4% MH | 0.405590 |
| 5% MH | 0.124662 |
| 6% MH | 0.004002 |

***P. aeruginosa***

| | P value |
|---|---|
| Media | 0.337363 |
| Untreated | 0.062236 |
| 12% MH | 0.075714 |
| 13% MH | 0.127786 |
| 14% MH | 0.304394 |
| 15% MH | 0.388712 |
| 16% MH | 0.738375 |
| 17% MH | 0.680717 |

## Conclusions & Future Steps

- Variable differences between new and aged MH MICs have been observed in *S. aureus*, *P. aeruginosa*, and *E. coli*.
- Aged MH seems to increase in its antimicrobial abilities.
- The MIC of *S. aureus* decreased by ~1% with aged MH compared to new MH.
- The MIC of *E. coli* decreased ~1-2% with aged MH compared to new MH.
- *P. aeruginosa* largely remained unchanged in MIC between new and aged, staying consistently within 12-17% MH.
- Compare the chemical composition of newly prepared and aged MH.

## Acknowledgements

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