

# Methylglyoxal-Infused Honey Mimics the Anti-*Staphylococcus aureus* Biofilm Activity of Manuka Honey: Potential Implication in Chronic Rhinosinusitis

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**Objectives/Hypothesis:** Low pH, hydrogen peroxide generation, and the hyperosmolarity mechanisms of antimicrobial action are ubiquitous for all honeys. In addition, manuka honey has been shown to contain high concentrations of methylglyoxal (MGO), contributing the relatively superior antimicrobial activity of manuka honey compared to non-MGO honeys. In high concentrations, manuka honey is effective in killing *Staphylococcus aureus* biofilms in vitro. Lower concentrations of honey, however, are desirable for clinical use as a topical rinse in chronic rhinosinusitis in order to maximize the tolerability and practicality of the delivery technique. This study, therefore, was designed to evaluate the contribution of MGO to the biofilm-cidal activity of manuka honey, and furthermore determine whether the antibiofilm activity of low-dose honey can be augmented by the addition of exogenous MGO.

**Study Design:** In vitro microbiology experiment.

**Methods:** Five *S. aureus* strains (four clinical isolates and one reference strain) were incubated to form biofilms using a previously established in vitro dynamic peg model. First, the biofilm-cidal activities of 1) manuka honey (790 mg/kg MGO), 2) non-MGO honey supplemented with 790 mg/kg MGO, and 3) MGO-only solutions were assessed. Second, the experiment was repeated using honey solutions supplemented with sufficient MGO to achieve concentrations exceeding those seen in commercially available manuka honey preparations.

**Results:** All honey solutions containing a MGO concentration of 0.53 mg/mL or greater demonstrated biofilm-cidal activity; equivalent activity was achieved with  $\geq 1.05$  mg/mL MGO solution.

**Conclusions:** MGO is only partially responsible for the antibiofilm activity of manuka honey. Infusion of MGO-negative honey with MGO, however, achieves similar cidal activity to the equivalent MGO-rich manuka honey.

**Key Words:** Manuka honey, methylglyoxal, *Staphylococcus aureus*, rhinosinusitis, biofilms.

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## INTRODUCTION

Chronic rhinosinusitis (CRS) is a common, debilitating condition with a potential for medical and surgical recalcitrance. In surgically recalcitrant cases, *Staphylococcus aureus* is often the implicated organism.<sup>1</sup> The biofilm form of *S. aureus* is increasingly recognized as relevant in CRS pathogenesis and is frequently associated with poorer postoperative outcomes.<sup>2</sup> Given the persistence of this organism in the postoperative period,<sup>3</sup> additional medical therapies are required to treat this organism if we want to improve patient outcomes, espe-

cially in surgically recalcitrant cases. Numerous antibiofilm topical treatments have recently been proposed, with many specifically targeting *S. aureus* biofilms.<sup>4–7</sup>

Manuka (*Leptospermum scoparium*) honey is active against a broad spectrum of gram-positive and gram-negative bacteria.<sup>8</sup> Using an in vitro model of *S. aureus* (and *Pseudomonas aeruginosa*) biofilms, Alandejani et al. recently demonstrated the biocidal activity of manuka honey at a concentration of 33% v/v (equivalent to approximately 50% w/v).<sup>6</sup> The phenol compound methylglyoxal (MGO) has recently been shown to be uniquely present in manuka honey,<sup>9</sup> as well as certain other selected honeys, affording greater antibacterial activity compared to non-MGO honeys.

Promisingly, in vitro attempts at inducing resistance to manuka honey have similarly not been successful.<sup>10</sup> Manuka honey, therefore, may be an ideal topical agent for use in surgically recalcitrant *S. aureus*-positive CRS.

In order to maximize the affordability and tolerability of manuka honey, lower concentrations of honey are preferred than those tested by Alandejani et al. provided the antibiofilm activity is preserved. Additionally, the

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TABLE I.  
pH and Methylglyoxal Concentration of Tested honeys.

	Capilano/Non-MGO Honey	Manuka Honey
pH	4.0	3.8
MGO concentration, mg/kg	<10.0	790

MGO = methylglyoxal.

contribution of MGO to the antibiofilm activity of manuka honey has not previously been established. This study, therefore, was designed to evaluate this contribution, and furthermore determine whether the antibiofilm activity of low-dose honey can be augmented by the addition of exogenous MGO.

## MATERIAL AND METHODS

### Bacterial Strains

Four clinical isolates taken from patients with severe CRS were selected. The *in vivo* presence of *S. aureus* biofilms on sinonasal mucosa harvested during surgery was previously determined in all patients using an established fluorescence *in situ* hybridization protocol.<sup>11</sup> Bacterial strains were isolated from each patient either at the time of tissue harvest or in the early post-operative period and were stored in 80% glycerol at  $-80^{\circ}\text{C}$  for future use. American Type Culture Collection (ATCC) reference strain 25923, a known biofilm-forming strain,<sup>5</sup> was used for the purpose of quality control.

### Honey

Manuka honey was supplied by Watson & Sons (Masterton, New Zealand). Non-MGO honey was purchased from Capilano Honey (Inala, Queensland). Independent MGO concentration and pH analysis of both honeys was performed by Hill Laboratories (Hamilton, New Zealand) (Table I). After delivery, all honey was stored at  $4^{\circ}\text{C}$  in the dark prior to use.

### Biofilm Assay

All biofilms were grown using a modified version of the Calgary biofilm device protocol<sup>12</sup> as outlined below.

### Bacterial Inoculation

Prior to inoculation of the device strains were transferred from stock cultures to Columbia horse blood agar (Oxoid, Adelaide, Australia). After incubation for 24 hours at  $37^{\circ}\text{C}$ , a single colony was inoculated in 1.5 mL of 0.45% saline and adjusted to a turbidity of 2.0 to 2.2 McFarland opacity standard. Individual wells of a sterile 96-well plate (Nunc, Roskilde, Denmark) were inoculated with 16.67  $\mu\text{L}$  of bacteria solution and 133.33  $\mu\text{L}$  of cerebrospinal fluid (CSF) broth (Oxoid). A sterile 96-pin plate lid (Innovotech, Calgary, Canada) was inserted into the inoculated wells, and incubated at  $35^{\circ}\text{C}$  on a gyro-rotary platform (Ratek, Melbourne, Australia) at 70 rpm for 30 hours.

### Honey Challenge

Manuka honey and non-MGO honey in CSF-broth solutions were prepared immediately prior to use. Additional honey solutions augmented with MGO (Sigma-Aldrich, St. Louis, MO) solutions were also prepared, representing quadruple-strength manuka honey, augmented non-MGO honey equivalent to manuka honey, and augmented non-MGO honey equivalent to quadruple-strength manuka honey.

A challenge plate was constructed by inoculating a new 96-well plate with 200  $\mu\text{L}$  of the five honey solutions at 66.00%, 33.00%, 16.50%, and 8.25% w/v concentrations in CSF broth. Wells containing MGO (in CSF broth) and CSF broth only were also prepared. The 96-pin lid was removed from the bacterial inoculation plate, washed twice with physiological saline, inserted into the challenge plate, and returned to the incubated gyro-rotator for 24 hours at  $35^{\circ}\text{C}$ .

### Recovery

Following the challenge, the 96-pin lid was washed twice with physiological saline and placed into a new 96-well plate with wells containing 150  $\mu\text{L}$  CSF broth and sonicated for 5 minutes to dislodge the peg-bound biofilm. The pin-lid was then removed and replaced with a standard 96-well lid and incubated at  $37^{\circ}\text{C}$  for 24 hours under static conditions.

### Assessment of Biocidal Activity

Following incubation, recovery wells were visually assessed and verified using a microplate spectrophotometer (BioRad, Hercules, CA) for turbidity and macroscopic biofilm. Nonturbid wells reflected antibiofilm activity/biocidal activity of the corresponding challenge solution well.

All treatments were run in duplicate and the experiment was performed twice to ensure consistency of results.

## RESULTS

The biocidal or nonbiocidal activity of all honey and/or MGO solutions was uniform for all strains tested, suggesting that the antibiofilm activity of honey and MGO may be broadly effective against a range of *S. aureus* strains.

### Manuka Honey

Biocidal activity against all strains was seen at a 66.00% and 33.00% w/v concentration of manuka honey (equivalent MGO concentrations 0.53 mg/mL and 0.26 mg/mL), and 16.50% w/v (0.13 mg/mg MGO) was not biocidal against any strains (Table II).

### Non-MGO Honey

Biocidal activity was not demonstrated against any strains at any concentration tested (Table II).

TABLE II.  
Biocidal activity of Various Honeys at Differing Concentrations in Cerebrospinal Fluid Broth.

% Honey Concentration (w/v)	Equivalent solid honey				
	CH (<10mg/kg MGO) only	MH (790mg/kg MGO) only	CH + 790mg/kg MGO	MH +2,370mg/kg MGO	CH +3,160 mg/kg MGO
66	☐	☐	☐	-	-
33	☐	☐	☐	☐	☐
16.50	☐	☐	☐	☐	☐
8.25	☐	☐	☐	☐	☐

Cell color corresponds to equivalent MGO-only concentration as seen in TABLE III.

CH = capilano/non-MGO honey; MGO = methylglyoxal; MH = manuka honey; ☐ = not biocidal; ☐ = biocidal; - = not tested.

### MGO Only

Biocidal activity against all strains was uniformly demonstrated at concentrations  $\geq 1.05$  mg/mL MGO (Table III).

### Manuka and Non-MGO Honey Augmented With MGO

Non-MGO honey augmented with MGO demonstrated equivalent biocidal activity to manuka honey. Biocidal activity against all strains was achieved with 16.50% w/v augmented honey solution, with addition of MGO to an equivalent concentration of that found in 66.00% w/v manuka honey (0.53 mg/mL) (Table II).

## DISCUSSION

Overall, all honey solutions containing a MGO concentration of 0.53 mg/mL or greater demonstrated biofilm-cidal activity; equivalent activity was achieved with  $\geq 1.05$  mg/mL MGO-only solution. Manuka honey at 33.00%, or both honeys at 16.50% with additional MGO, were therefore biocidal. Non-MGO honey was not biocidal at any concentration tested.

An increased appreciation of the role of *S. aureus* biofilms in CRS has simultaneously generated interest in potential biocidal agents to treat this condition.<sup>5,7,13,14</sup> *S. aureus*-associated CRS is often recalcitrant to our current medical and surgical paradigms, making the presence of this organism a marker for severe disease. This subgroup may well require additional targeted therapy to improve their outcomes. Several novel treatments have been proposed to serve this role. Nasal lavage with mupirocin, for example, has been proposed as an efficacious, well-tolerated topical treatment, with a recent pilot study reporting impressive subjective and objective outcomes following a 4-week treatment protocol.<sup>4</sup>

However, mupirocin-resistance, first reported in 1987,<sup>15,16</sup> is an emerging concern. Although rates of mupirocin resistance are thought to be low in the community setting,<sup>17</sup> in selected population groups rates up to 13.2% have been reported.<sup>18</sup> With increasing interest in mupirocin as a methicillin-resistant *S. aureus* decolonizer of the nasal vestibule,<sup>19</sup> similar high rates of mupirocin-resistance may potentially be seen in the future. The ideal alternative to mupirocin, therefore, should be likewise well tolerated and efficacious as a nasal lavage, with minimal or no potential for the development of resistance; manuka honey certainly fulfills the latter criteria.<sup>10</sup>

Honey is not a new topical antibacterial agent. By contrast, the understanding of manuka honey's enhanced and unique antibacterial activity,<sup>20</sup> now shown to be proportional to the MGO concentration,<sup>9</sup> is relatively new. MGO targets protein and DNA synthesis. Relatively resistant bacteria generally have an intrinsic ability to withstand the toxic effects of MGO by possessing a robust capacity for DNA repair and sufficient levels of detoxification enzymes.<sup>21,22</sup> The exact mechanisms involved in MGO activity against *S. aureus* and the reasons why *S. aureus* is particularly sensitive to MGO-rich honey<sup>8</sup> have not been explored.

This study has shown that the biocidal activity of MGO is enhanced when in honey solution, despite the nonbiocidal activity of non-MGO honey. The reasons for this enhanced activity are unclear. In the clinical setting, therefore, a combined MGO and honey solution (whether from manuka or fortified non-MGO honey) yields a stronger biocidal activity compared to an equivalent MGO-only solution and would hence be the preferred treatment option in CRS. Given the hyperosmolarity and acidity of concentrated honey solutions, a 16.50% honey solution augmented with MGO is likely to be better tolerated as a nasal lavage compared to a 33.00% manuka honey solution, although both solutions are similarly

TABLE III.  
Biocidal activity of Methylglyoxal (MGO)-only Solution.

MGO Concentration (mg/ml)	Biocidal activity
2.11	☐
1.05	☐
0.53	☐
0.26	☐
0.13	☐
0.06	☐
<0.01	☐

☐ = biocidal; ☐ = not biocidal.

biocidal. A pilot study conducted by our department suggests that 16.50% honey is the upper-limit of tolerability when delivered by nasal lavage (unpublished data).

## CONCLUSION

MGO is only partially responsible for the antibiofilm activity of manuka honey. Non-MGO honey augmented with MGO, however, achieves similar biocidal activity to the equivalent MGO-rich manuka honey. Clinical trials assessing the tolerability and efficacy of manuka honey at 33.00%, or both honeys at 16.50% with additional MGO, are suggested.

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