

ORIGINAL RESEARCH–SINONASAL DISORDERS

Effectiveness of honey on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms

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ABSTRACT

OBJECTIVES: Biofilms formed by *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) have been shown to be an important factor in the pathophysiology of chronic rhinosinusitis (CRS). As well, honey has been used as an effective topical antimicrobial agent for years. Our objective is to determine the in vitro effect of honey against biofilms produced by PA and SA.

STUDY DESIGN: In vitro testing of honey against bacterial biofilms.

METHODS: We used a previously established biofilm model to assess antibacterial activity of honey against 11 methicillin-susceptible SA (MSSA), 11 methicillin-resistant SA (MRSA), and 11 PA isolates. Honeys were tested against both planktonic and biofilm-grown bacteria.

RESULTS: Honey was effective in killing 100 percent of the isolates in the planktonic form. The bactericidal rates for the Sidr and Manuka honeys against MSSA, MRSA, and PA biofilms were 63–82 percent, 73–63 percent, and 91–91 percent, respectively. These rates were significantly higher ($P < 0.001$) than those seen with single antibiotics commonly used against SA.

CONCLUSION: Honey, which is a natural, nontoxic, and inexpensive product, is effective in killing SA and PA bacterial biofilms. This intriguing observation may have important clinical implications and could lead to a new approach for treating refractory CRS.

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Chronic rhinosinusitis (CRS) is among the three most common chronic diseases in North America, affecting about 31 million patients in the United States annually.¹ The costs associated with CRS are substantial and increasing, with direct health expenditures estimated at \$4.3 billion annually.²

The existence of biofilms on the mucosa of patients with CRS has been well established.^{3–5} In two recent studies^{6,7} patients with biofilms formed by *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) had statistically

worse postoperative symptoms, mucosal outcomes, and unfavorable evolution after endoscopic sinus surgery (ESS) for CRS. These observations concluded that biofilms may indeed play an active role in perpetuating inflammation in CRS patients and may explain the recurrent and recalcitrant nature of this disease.

The fact that conventional oral antimicrobial therapy is frequently ineffective in eradicating bacteria in the biofilm form has led to the study of topical therapies. In a recent study⁸ biofilms were treated with citric acid/Zwitterionic surfactant (CAZS) delivered by hydrodynamic force, and another study⁹ demonstrated the effect of topical moxifloxacin on SA biofilms. However, the use of antibiotics is not without risk, as there has been an increasing prevalence of bacterial resistance to antibiotics and there is always the possibility of allergic reactions.

The need for novel therapies has renewed interest in natural products exhibiting antibacterial properties. Honey is an ancient remedy for the treatment of infected wounds. Clinical application has proven to be specifically beneficial in the treatment of wounds that are nonresponsive to conventional therapies, such as diabetic ulcers, and wounds infected with antibiotic-resistant bacteria.^{10,11} Numerous studies describe the antimicrobial effect of honey against bacteria in the planktonic form.^{10–13}

Honey has numerous advantages. It is a natural, inexpensive, and nontoxic product and has no adverse effects on the healing process of tissues.^{10,11} To our knowledge, no research has studied the antimicrobial effect of honey on biofilms.

MATERIALS AND METHODS

Bacterial Strains

Bacteria were obtained from the clinical microbiology laboratory of The Children's Hospital of Eastern Ontario (CHEO). All bacterial isolates were identified in previous studies, done in the same laboratory, and were capable of producing, in vitro, biofilms. After bacteria were grown on

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Table 1
Template of honey and bacteria tested (for both planktonic and biofilms)

Honey	1	2	3	4	5	6	7	8	9	10	11	Sterility
Sidr	S	S	S	S	S	S	S	S	S	S	S	MHB II
Sidr	R	R	R	R	R	R	R	R	R	R	R	MHB II
Sidr	PA	MHB II										
Manuka	S	S	S	S	S	S	S	S	S	S	S	MHB II
Manuka	R	R	R	R	R	R	R	R	R	R	R	MHB II
Manuka	PA	MHB II										

S, MSSA; R, MRSA; PA, *Pseudomonas aeruginosa*; MHB II, Muller Hinton broth with cations.

polystyrene pins, biofilm formation was confirmed by scanning electron microscopy and/or colony counts following sonication of the bacterial biofilms off the pins. Results confirmed that all these bacteria formed biofilms on the polystyrene pins.¹⁴⁻¹⁶ Originally the PA isolates were recovered from the respiratory secretions of patients with cystic fibrosis (CF) and the SA isolates were recovered from patients with multidrug-resistant prosthetic device infections. One quality control strain for each bacteria was obtained from the American Type Culture Collection (ATCC). In total, 11 isolates of methicillin-susceptible SA (MSSA), 11 isolates of methicillin-resistant SA (MRSA), and 11 isolates of PA were studied.

As all the bacterial isolates were taken directly from the laboratory and studied in vitro using anonymous numbers, no patient information, codes, or names were revealed and no patient charts were identified or reviewed; therefore, no institutional review board was necessary.

Type and Concentration of Honey

Preliminary studies were performed on four types of honey to decide which type of honey to use and at which concentrations. The Manuka honey from New Zealand (Comvita Ltd, Bay of Plenty, New Zealand) is known as the most therapeutically potent honey.¹² It is available commercially, after it has been treated with gamma rays,¹⁷ a process that kills clostridial spores that it may contain. The Canadian Clover and Buckwheat honeys (Farmboy Inc., Ottawa, Canada) were chosen since they were shown to have good antimicrobial activity among 40 other Canadian honeys in a study done by Brudzynski.¹³ The Sidr honey (local producer, Yemen) from Yemen has long been known for its antibacterial activity and clinical uses for the treatment of infected wounds. The Sidr, Clover, and Buckwheat honeys had to be filtered using a nalgene filter (pore size 0.45 micrometers) to remove clostridial spores. The examiner was blinded to the type of honey in these preliminary studies. Initially, each type of honey was tested against biofilms formed by one MSSA, one MRSA, and two PA isolates in 10 different concentrations, starting from 1 in 2 dilution (so the honey can be pipetted), then doubling dilutions to well 10. Well 11 was for growth control (bacteria and broth) and well 12 was for sterility control (broth only).

The Sidr honey was cidal to the MSSA, MRSA, and one isolate of PA, and the Manuka honey was cidal to MRSA, both at 1 in 2 dilutions. The two Canadian honeys had no cidal effects on the bacterial biofilms. Therefore we decided to continue our study with Sidr and Manuka honey at a 1 in 2 dilution.

Planktonic Microtiter/Honey Procedure

Honey (Sidr and Manuka honey tested only) was diluted in Muller Hinton broth with cations (MHB II; Becton Dickinson, Oakville, Ontario, Canada) and placed into its appropriate well of a 96-well round-bottom microtiter plate (Nunc Inc., Roskilde, Denmark). Then a suspension of the appropriate organism was added to the wells for a final concentration of 5×10^5 cfu/mL, and incubated at 35°C for 24 hours. The minimal inhibitory concentration (MIC) used was 1 in 2 dilution, and the effect was determined as the wells with no turbidity after incubation read on a mirrored plate reader. Table 1 represents the template that was used for testing bacteria in both the planktonic and biofilm forms.

Biofilm Microtiter/Honey Procedure

All isolates were grown as biofilms using a modified version of the Calgary biofilm device shown previously to be both reliable and reproducible for growing SA and PA as biofilms.^{14-16,18} Each isolate was placed into its appropriate well (Table 1), then a transferable solid-phase (TSP) pin lid (NUNC, Roskilde, Denmark) was placed into the microtiter plate and incubated overnight at 35°C on a rocking table (Bellco Glass Inc., Vineland, NJ) to produce a shearing force to grow bacterial biofilms. The TSP pin lid with grown biofilms was then removed and placed into a new microtiter plate containing honey in a 1 in 2 dilution with MHB II and placed on the rocking table for 24 hours at 35°C. The TSP pin lid was removed again and placed into another microtiter plate containing fresh sterile MHB II and sonicated for five minutes to remove bacterial biofilms on the pins into the sterile MHB II broth. After that, the TSP pin lid was discarded and replaced by a sterile microtiter lid, and the microtiter plate with the removed biofilms in the sterile MHB II broth was incubated overnight at 35°C. The minimal biofilm eradication concentration (MBEC) was determined as the wells showing no turbidity read on a mirrored plate

reader. With results indicated in our preliminary studies, we limited testing at a 1 in 2 dilution of honey only for the biofilms. Serial doubling dilution tests were not performed. A growth control microtiter plate was also done with the same technique, with grown biofilms on the TSP pin lid being placed in the microtiter plate containing the sterile MHB II broth only. All the wells containing isolates in the growth control microtiter plate grew, demonstrating the validity of the test.

Statistical Analysis

The Fisher exact test was used for comparison of results. A *P* value of 0.05 or less was considered significant.

RESULTS

All of the planktonically grown bacteria were inhibited by each Sidr and Manuka honey at 1 in 2 dilution. Biofilm-grown cultures from all three groups of organisms were found to be more resistant compared to the planktonic form. There was no statistical difference ($P > 0.05$) between the effects of the two types of honey, nor between the MSSA, MRSA, and PA susceptibilities. Table 2 shows that the Sidr honey was cidal to seven of 11 MSSA biofilms and the Manuka honey was cidal to nine of 11 MSSA biofilms, with a control rate of 63 percent and 82 percent, respectively. Table 3 shows the results of MRSA biofilm testing with a cidal rate of 73 percent (8/11) and 63 percent (7/11) for the Sidr and Manuka honeys, respectively. Surprisingly, PA biofilms were most susceptible where each of the Sidr and Manuka honey killed 10 of 11 isolates with a control rate of 91 percent for each (Table 4). Comparing our results with a study done in the same laboratory and using the same isolates for testing MSSA and MRSA biofilm susceptibilities, in vitro, against commonly used antibiotics for SA infections,¹⁴ honey was more superior to antibiotics. In that

Table 2
Susceptibility of MSSA biofilms to honey

Isolate number	Sidr honey	Manuka honey
MSSA 1	+	–
MSSA 2	+	–
MSSA 3	+	+
MSSA 4	–	–
MSSA 5	–	–
MSSA 6	–	–
MSSA 7	–	–
MSSA 8	–	+
MSSA 9	–	–
MSSA 10	–	–
MSSA 11 (ATCC-25293)	+	–
Sterility control	–	–

MSSA, methicillin-susceptible SA; (–) indicates no growth; (+) indicates growth.

Table 3
Susceptibility of MRSA biofilms to honey

Isolate number	Sidr honey	Manuka honey
MRSA 1	–	+
MRSA 2	–	–
MRSA 3	+	–
MRSA 4	+	+
MRSA 5	–	–
MRSA 6	–	–
MRSA 7	–	+
MRSA 8	–	–
MRSA 9	–	–
MRSA 10	+	–
MRSA 11 (ATCC-29247)	–	+
Sterility control	–	–

MRSA, methicillin-resistant SA; (–) indicates no growth; (+) indicates growth.

study, only rifampin had a cidal effect against the biofilms, with a rate of 18 percent (2/11) for MSSA and 42 percent (5/12) for MRSA, with a *P* value of 0.08 and 0.21 compared to Sidr honey, and 0.008 and 0.41 compared to Manuka honey. Unfortunately, because organisms develop resistance to rifampin more rapidly, it is usually combined with other antibiotics, which limits its use. None of the other antibiotics tested (cefazolin, oxacillin, vancomycin, azithromycin, fusidic acid, gentamicin, and linezolid) were cidal to any of the biofilms.

DISCUSSION

Despite recent advances in antimicrobial chemotherapy and surgical procedures, CRS remains a challenging disease to treat. Even after successful endoscopic sinus surgery (ESS) and oral and topical antibiotics, all these procedures have

Table 4
Susceptibility of PA biofilms to honey

Isolate number	Sidr honey	Manuka honey
PA 1	–	–
PA 2	–	–
PA 3	–	–
PA 4	–	+
PA 5	–	–
PA 6	–	–
PA 7	–	–
PA 8	–	–
PA 9	–	–
PA 10	–	–
PA 11 (ATCC-27853)	+	–
Sterility control	–	–

PA, *Pseudomonas aeruginosa*; (–) indicates no growth; (+) indicates growth.

failed to cure the disease.¹ The existence of biofilms on the mucosa of patients with CRS has been well established,³⁻⁵ and it is thought that they may play an active role in the pathophysiology of the CRS and explain the recurrent and recalcitrant nature of the disease. Looking for a novel method to remove and/or inhibit biofilm growth has been an active area of research. An ideal method and/or agent would be one that is practical, nontoxic, having few side effects, and inexpensive. Honey is an agent that meets all these criteria. In addition, it is a natural product and other studies have shown that it also promotes wound healing.^{10,11}

From our study, we have been able to conclude that: 1) Manuka and Sidr honeys have very strong antibacterial activity against SA and PA biofilms; 2) the MBEC for both honeys is 1 in 2 dilution; and 3) we confirmed increased resistance of SA and PA in the biofilm form compared to the planktonic form.

This preliminary work opens a new door for further research in treating biofilm-associated infections caused by SA and PA, and these favorable results urge us to continue testing with honey. Multiple questions arise that will require further clinical studies; some of these questions are: 1) Will honey application improve the clinical outcome of patients with CRS and other biofilm-associated infections caused by SA and PA? 2) What is the ideal mode of delivery, the duration, and the frequency of treatment? 3) Can honey be an adjuvant treatment or mixed with topical steroids and/or antibiotics? 4) Does honey have antimicrobial effects on biofilms formed by other organisms such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, and fungi? 5) Can honey be used as a topical treatment for MRSA carriers?

CONCLUSION

Many commonly used antimicrobials have been developed from naturally occurring substances over the years. We have shown that honey, and in particular Manuka honey from New Zealand and Sidr honey from Yemen, have in vitro bactericidal properties for SA and PA bacterial biofilms. These bactericidal properties are superior to those of most commonly used antimicrobials. This intriguing observation may have important clinical implications that could lead to a new approach in the management of biofilm-related infections. Further clinical trials and safety studies are needed.

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AUTHOR CONTRIBUTIONS

Talal Alandejani, main author, research coordinator and presenter; **Joseph Marsan**, study design–clinical significance; **Wendy Ferris**, laboratory work, bacteria and biofilm preparation and analysis; **Robert Slinger**, study design and clinical microbiology significance; **Frank Chan**, study design–laboratory organization.

DISCLOSURES

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