
The Efficacy of Honey in Inhibiting Strains of *Pseudomonas Aeruginosa* From Infected Burns

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Because there is no ideal therapy for burns infected with *Pseudomonas aeruginosa*, there is sufficient need to investigate the efficacy of alternative antipseudomonal interventions. Honey is an ancient wound remedy for which there is modern evidence of efficacy in the treatment of burn wounds, but limited evidence for the effectiveness of its antibacterial activity against *Pseudomonas*. We tested the sensitivity of 17 strains of *P. aeruginosa* isolated from infected burns to two honeys with different types of antibacterial activity, a pasture honey and a manuka honey, both with median levels of activity. All strains showed similar sensitivity to honey with minimum inhibitory concentrations below 10% (vol/vol); both honeys maintained bactericidal activity when diluted more than 10-fold. Honey with proven antibacterial activity has the potential to be an effective treatment option for burns infected or at risk of infection with *P. aeruginosa*. (J Burn Care Rehabil 2002;23:366-370)

Perturbation of skin by thermal injury allows ingress of microbial species to vulnerable tissue and initiates a complex series of interactions¹ that result in transient wound contamination, wound colonization, or clinical infection. Normally the initial microbial burden of burns is low and the aim in patient care is to promote rapid repair without an increase in microbial density or diversity. However, accounts of burns infection date from the 17th century,² and although the causative agents were not then recognized, they have since been extensively monitored.^{1,3} Distinguishing between microbial effects and the consequences of thermal injury on wound healing has been difficult. The pathogenicity of *Staphylococcus aureus* (often the primary colonizer of burns) and of β -hemolytic streptococci is undisputed, but the role of the gram-negative bacteria and the yeasts and fungi is less certain. The contribution of *P. aeruginosa* to graft failure, delayed healing, systemic complications, and fatality after burn colonization was documented in 1951.⁴

However, survival rates in burns patients have since increased by successive improvements in antimicrobial therapy, critically ill patient support techniques, innovative dressing materials, better wound assessment and surveillance methods, and rigorous cross-infection precautions. The epidemiology of burns infections has reflected these changes.^{1,3} Topical prophylactics and antipseudomonal antibiotics have reduced the frequency of *P. aeruginosa* infections in many hospitals,⁵ but this virulent opportunist pathogen can still have a significant impact on patient morbidity and mortality.⁶ In some situations it is still the predominant burn isolate.^{7,8} *P. aeruginosa* displays complex patterns of virulence that include the production of hydrolytic enzymes, exotoxin A, and the release of endotoxin from the lipopolysaccharide component of its cell wall.⁹ The expression of such factors is influenced by cell-to-cell signaling mechanisms that are dependent on bacterial population size.¹⁰ Although the regulation of virulence determinants is not completely elucidated, the necessity of minimizing the numbers of pseudomonads in burns is implied. Unfortunately, antibiotic use has selected for resistant bacteria, and the recent emergence of multiresistant strains of *P. aeruginosa*¹¹ has further increased the urgency to discover more effective inhibitory agents.

Honey has long been known to have antibacterial properties, and has a long established usage as a wound dressing.¹² There have been many reports published of the sensitivity to honey of a wide range

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of bacterial species, and some of these studies have included *P. aeruginosa*.¹² However, little account has been taken in these studies of the very large variation that exists in the antibacterial potency of honey.¹² The aim of the present study was to investigate the efficacy of honey in inhibiting strains of *P. aeruginosa* isolated from infected burn wounds, using honeys with median levels of antibacterial activity. There are two different types of antibacterial component that have been identified as being responsible for the antibacterial activity of honey beyond that caused by its high osmolarity. One is hydrogen peroxide produced by the action of the enzyme glucose oxidase that the bees add to the nectar that they collect.¹² The other is a phytochemical component of the nectar from certain species of plants.¹² Manuka (*Leptospermum scoparium*) honey has an exceptionally high level of this type of activity,¹³ and has been found to be effective in clearing infection in lesions resulting from meningococcal septicemia.¹⁴ Hence, honeys with each type of antibacterial component were used in this study. An "artificial honey" (a sugar mixture simulating honey) was also used so that the effects of the antibacterial components of the honeys could be distinguished from the osmotic effect.

MATERIALS AND METHODS

Strains of *P. aeruginosa* were isolated from swabs being routinely processed by the Department of Medical Microbiology and Public Health Laboratory Service at Singleton Hospital, Swansea, that had been collected from burns patients at Morriston Hospital. Pure cultures with reported antibiotic sensitivities were provided on nutrient agar slopes. The identity of all strains was confirmed by API 20NE (bioMérieux, Marcy l'Etoile, France). For this study isolates were labelled A to Q.

Two New Zealand honeys were used in this study. One was a mixed pasture honey (PS3) with antibacterial activity caused by hydrogen peroxide, equivalent to that of 14.8% (wt/vol) phenol¹³ (*cf* a median of 17.5% for this type of activity in honey.¹³) The other was a manuka honey (M108) with antibacterial activity due primarily to a phytochemical component equivalent to 18% (wt/vol) phenol¹³ (*cf* a median of 15.5% for this type of activity in honey.¹³) Pasture honey generates hydrogen peroxide on dilution, but manuka honey is a nonperoxide honey whose mode of inhibition has been attributed to phytochemicals.¹³ An artificial honey (100 g) was prepared by dissolving 1.5 g sucrose, 7.5 g maltose, 40.5 g fructose, and 33.5 g glucose in 17 ml sterile deionized water.

The minimum inhibitory concentration (MIC) of each honey was determined by an agar incorporation method previously described.¹⁵ Briefly, honey was weighed and dissolved in sterile deionized water to prepare a stock solution of 20% (vol/vol) honey immediately before use. Further dilutions were prepared by pipetting stock solution and sterilized deionized water to sterile 10ml aliquots of molten double-strength nutrient agar (Oxoid Limited, Basingstoke, United Kingdom) at 50°C to produce a range of plates incorporating honey at concentrations between 0 and 10% (vol/vol). Plates were dried at 37°C for 15 min before use. Clinical isolates were grown overnight in nutrient broth at 37°C and inoculated as 0.3- μ l spots using a multipoint inoculator (Mast Diagnostics Limited, Merseyside, United Kingdom). Plates were incubated at 37°C for 24 hours and observed for growth (confluent spot) or no growth. MIC was recorded as the lowest concentration of honey to prevent growth of each isolate. The natural honeys were each assayed in duplicate on three occasions, the artificial honey was tested once.

Using a tube dilution method with liquid media, MIC was compared with minimum bactericidal concentration (MBC) for each natural honey in order to investigate the mode of inhibition. Double-strength nutrient broth (Oxoid) was diluted with appropriate volumes of sterile deionized water and 20% (vol/vol) freshly prepared stock honey solution to produce a range of final concentrations in 1% (vol/vol) intervals between 1 and 10% (vol/vol). Tubes were inoculated with overnight broth cultures of clinical isolates (approximately 3.5×10^6 cfu/ml in each assay tube) and incubated at 37°C for 24 hours. Tubes were observed for bacterial growth (turbidity) to deduce MICs. The viability of bacteria in those tubes with no apparent turbidity was explored by spreading 100 μ l onto a nutrient agar plate (Oxoid) and incubated at 37°C for 24 hours. The lowest honey concentration that failed to allow the survival of viable bacteria was recorded as the MBC.

RESULTS

Fourteen of the isolates exhibited a similar antibiogram, ie sensitive to four of the eight antibiotics tested (Table 1). Only three strains were resistant to ciprofloxacin; strain P was resistant to seven of the eight antibiotics tested, and was therefore a multiresistant isolate. All of the isolates, however, showed similar patterns of sensitivity to the honeys tested (Table 2). The mean MIC for artificial honey was 19.2% (vol/vol), ranging between 17 and 22, but mean MIC values for manuka and pasture honey respec-

Table 1. Antibiotic sensitivities of burns isolates

Strain	Cip	Pip	CxM	Aml	CN	W	Im	Aug
A-J, L-N, and Q	S	S	R	R	S	R	S	R
O	R	S	R	R	S	R	S	R
K	R	S	R	R	R	R	S	R
P	R	R	R	R	S	R	R	R

Cip, 1 µg ciprofloxacin; Pip, 75 µg piperacillin; CxM, 30 µg cefuroxime; Aml, 10 µg amoxicillin; CN, 10 µg gentamicin; W, 1.25 µg trimethoprim; Im, 10 µg imipenem; Aug, 30 µg augmentin; S, sensitive; R, resistant.

Table 2. Minimum inhibitory concentrations (% vol/vol)

Strain	Artificial Honey, Mean (n = 1)	Manuka Honey, Mean ± SD (n = 6)	Pasture Honey, Mean ± SD (n = 6)
A	20	8.66 ± 0.52	7.33 ± 1.36
B	19	6.83 ± 0.75	5.83 ± 1.33
C	20	8.50 ± 0.54	7.66 ± 1.03
D	20	8.66 ± 0.52	7.66 ± 1.03
E	19	7.33 ± 0.52	6.17 ± 1.84
F	20	6.83 ± 0.75	7.50 ± 0.55
G	20	7.83 ± 0.41	7.00 ± 0.63
H	20	6.50 ± 0.55	5.83 ± 1.33
I	NT	8.17 ± 0.41	7.83 ± 0.98
J	22	8.50 ± 0.84	7.50 ± 1.22
K	20	7.83 ± 0.41	7.33 ± 0.52
L	20	6.67 ± 0.52	5.67 ± 1.03
M	17	6.50 ± 0.55	5.83 ± 0.98
N	17	7.50 ± 0.55	7.00 ± 1.26
O	17	4.00 ± 1.55	4.33 ± 1.03
P	18	8.00 ± 0.89	7.67 ± 0.52
Q	18	9.00 ± 0	7.33 ± 0.52
Mean of means	19.2 ± 1.42	7.49 ± 1.22	6.79 ± 0.99

NT, not tested; n, number of assays.

tively were 7.5 and 6.8% (vol/vol; Table 2). On average, therefore, the natural honeys were 2.5 and 2.8 times more effective than artificial honey in inhibiting pseudomonads in in-vitro tests. Mean MBC values for manuka and pasture honey were only marginally higher than MIC values in tube dilution tests (Table 3), indicating a bactericidal rather than bacteriostatic mode of action in the in vitro assays.

DISCUSSION

Previous studies have reported MIC values for *P. aeruginosa* ranging from 3 to 36% (vol/vol) and MBC values from 10 to 99% (vol/vol) honey.¹² However, not all previous studies have specified the floral origin of the honey used in the research, or standardized its antibacterial potency. Sensitivity tests

Table 3. Mean minimum bactericidal concentration (% vol/vol)

	Manuka Honey	Pasture Honey
	9.71 ± 0.69*	9.00 ± 1.22*
Range	8-10	6-10

* 17 clinical isolates tested.

may have utilized organisms taken from type cultures, rather than clinical infections, and their relevance to clinical practice is questionable. This study seeks to address these limitations by using honeys of known potency, with median levels of activity typical of honeys available commercially. Similar honeys have been used in a previous study where pseudomonads isolated from wounds other than burns were tested, but neither their antibiotic sensitivities nor MBCs were determined.¹⁶ Here it can be seen that burns isolates with a variety of antibiotic sensitivities (Table 1) showed relatively consistent susceptibilities to two natural honeys of median levels of antibacterial potency (Table 2). Hence the effectiveness of honeys was independent of antibiotic susceptibility. Since pseudomonads are recalcitrant to antibiotic therapy, the ability of honey to inhibit test strains irrespective of their antibiotic resistances has important implications for clinical practice.

In the routine testing of antibiotic sensitivity, doubling dilution series are usually employed. Because a narrower range of honey concentrations was used in this study (1-10%, vol/vol), more precise estimations of MICs and MBCs were obtained. When MBC exceeds its corresponding MIC by a factor of four or more, a bacteriostatic mode of action is inferred. The proximity of MICs for both manuka and pasture honeys (Tables 2 and 3) to their respective MBCs (Table 3) indicates bactericidal activity rather than bacteriostatic.

All of the 17 strains of *P. aeruginosa* that were isolated from burns patients were inhibited by similar concentrations of the two natural honeys, but significantly higher concentrations of artificial honey were required to achieve an equivalent effect (Table 2).

The mode of action of each of manuka and pasture honey in inhibiting *P. aeruginosa* at concentrations below 17% (vol/vol) is not therefore exclusively via osmolality. When honey is applied to wounds, it will be diluted by body fluids, and if honey is to be an effective wound antiseptic, it must retain inhibitory activity on dilution. In this study the pasture and manuka honeys tested each retained bactericidal activity in vitro after dilution by a factor of 10. Since many of the bacteria that may be recovered from wounds possess catalase, and plasma contains catalase, it is probable that pasture honey may be less effective in vivo, than manuka honey. There is a need to monitor bacterial numbers in wounds after topical application of honey.

Despite the current range of antipseudomonal therapies, *Pseudomonas* infections remain notoriously difficult to cure. Their occurrence in burns and wounds still contributes to extended treatment times and costs by retarding healing rates,^{17,18} causing skin grafts to fail,¹⁹ and increasing risks of septicaemia.⁶ The continued emergence of multiresistant strains has complicated wound care regimes,²⁰ and heightened risks of cross infection,¹¹ such that unusual antimicrobial agents continue to be suggested.²¹⁻²³ Honey is an ancient remedy that might have a place not only in limiting multiresistant species of microorganisms in wounds, but in promoting healing.

In prospective, randomized, controlled clinical trials carried out to evaluate the effectiveness of honey as a dressing for partial-thickness burns,²⁴⁻²⁷ it was observed that honey gave effective control of clinical infection, much better than that achieved with silver sulfadiazine.²⁴ However, in a prospective, randomized, controlled trial on deep burns it was found that the control of infection was not as good with honey dressing as with early tangential excision and skin grafting,²⁸ but in this trial the honey used was not standardized and may have been of low antibacterial potency, and thus possibly better results would be obtained if honey with a higher potency were used.

In the clinical trials on partial-thickness burns honey was found to promote the healing of burn wounds.²⁴⁻²⁷ This has been reported also in animal studies on burn wounds^{29,30} where infection was not involved, indicating a direct stimulatory effect rather than removal of inhibition of healing by bacteria present in the wound. A review of the literature on the use of honey on other types of wounds cites numerous other observations on the stimulation of wound repair by honey.³¹ It has been proposed that because of its growth-promoting action on wound tissues, honey be used in place of expensive recombinant growth factors to stimulate the healing of burns.³²

The faster healing of burns dressed with honey also may result from the anti-inflammatory effect of honey. There are numerous reports of this effect being observed clinically in a variety of types of wound as a reduction in edema and pain.³¹ This is not just a secondary effect of clearing infection and debriding the wound, as it also has been observed, as a reduction in the number of inflammatory cells infiltrating the wound tissue, in experimental burn^{29,30} and surgical^{33,34} wounds in animal models in which there was no infection involved. The anti-inflammatory action could be expected to prevent further necrosis caused by the oxidative free radicals formed in the inflammatory process,³⁵ as would the significant capacity of honey to scavenge free radicals.³⁶ This would also account for the observation that honey reduces hypertrophic scarring resulting from burns,^{24,26} as prolonged production of oxidative free radicals is responsible for fibrosis occurring.³⁷

CONCLUSION

These beneficial effects of honey, and its lack of adverse effects on wounds,³¹ considered along with the findings from the present study, indicate that honey with standardized antibacterial activity has the potential to be a very useful treatment option for burns infected or at risk of infection with *Pseudomonas aeruginosa*.

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