

Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds

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SUMMARY

The antibacterial action of honey in infected wounds does not depend wholly on its high osmolality. We tested the sensitivity of 58 strains of coagulase-positive *Staphylococcus aureus*, isolated from infected wounds, to a pasture honey and a manuka honey. There was little variation between the isolates in their sensitivity to honey: minimum inhibitory concentrations were all between 2 and 3% (v/v) for the manuka honey and between 3 and 4% for the pasture honey. Thus, these honeys would prevent growth of *S. aureus* if diluted by body fluids a further seven-fold to fourteen-fold beyond the point where their osmolality ceased to be completely inhibitory. The antibacterial action of the pasture honey relied on release of hydrogen peroxide, which *in vivo* might be reduced by catalase activity in tissues or blood. The action of manuka honey stems partly from a phytochemical component, so this type of honey might be more effective *in vivo*. Comparative clinical trials with standardized honeys are needed.

INTRODUCTION

Honey, whose medicinal uses date from ancient times, has lately been rediscovered as a therapy for wounds^{1,2}. Interest in this approach stems partly from the emergence of antibiotic-resistant pathogens. Many publications attest to honey's antimicrobial properties³ but the mechanisms by which it acts are incompletely studied. Strong solutions of honey or sugar, and sugar pastes, inhibit microbial growth because of their high osmolality⁴, but when used as dressings they become diluted to the point where this action ceases—especially in the case of *Staphylococcus aureus*^{5,6}. But such wounds are rapidly rendered sterile by honey⁷⁻¹⁰ because of its additional antimicrobial activity.

Honey is produced from many sources, and its antimicrobial activity varies greatly with origin and processing¹¹. Manuka honey, for example, has high antibacterial activity associated with an unidentified phytochemical component¹¹. To date, few clinical reports have defined the specific type of honey applied to infected wounds, burns or ulcers. Similarly, *in vitro* investigations of antimicrobial activity have used uncharacterized honey samples. A study by Willix *et al.*¹² did specify the type of honey. They determined the sensitivity of wound pathogens to the non-peroxide antibacterial activity of standardized manuka honey and to a standardized honey in which the

antibacterial activity was primarily due to hydrogen peroxide. All seven species tested were completely inhibited by both types of honey at concentrations below 11% (v/v), with *S. aureus* sensitive to honey concentrations below 5%. However, this study was restricted to laboratory-maintained cultures of individual type species, and so has limited clinical relevance; furthermore, we do not know how much the different strains of *S. aureus* vary in their sensitivity to honey. Therefore in the current study a large number of strains of coagulase-positive *S. aureus* were isolated from infected wounds and tested in the laboratory for their sensitivity to two standardized honeys.

METHODS

Strains of *S. aureus* were isolated from swabs collected from a wide range of infected wounds routinely submitted to the department of medical microbiology and public health at the University Hospital of Wales during a four-week period. 58 isolates were identified as *S. aureus* by standard bacteriological techniques and pure cultures were kindly supplied for this study. These cultures were maintained by subculture on blood agar for up to seven days.

Two New Zealand honeys were used—a manuka honey and a honey of mixed pasture source. Their antibacterial activity was determined by an agar well diffusion bioassay as described previously, phenol being used as a reference standard antiseptic¹³. Activity was determined with and without addition of catalase¹³ to remove any hydrogen peroxide generated by the honey, so allowing the detection of non-peroxide antibacterial activity (i.e. that due to phytochemical components) as well as total antibacterial

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activity. A 25% (w/v) solution of the mixed pasture honey had no detectable antibacterial activity when tested in the presence of catalase but had antibacterial activity equivalent to 14.8% phenol without catalase; thus, bacterial inhibition was mainly due to hydrogen peroxide generation. A 25% solution of the manuka honey had approximately the same antibacterial activity with and without catalase, so its bacterial inhibition was attributable primarily to non-peroxide components. The honeys used were selected to be close to the median antibacterial activity found in a survey of New Zealand honeys obtained from commercial sources¹³. The manuka honey had a non-peroxide antibacterial activity equivalent to 13.2% (w/v) phenol (of 15.5% median for this type of activity). The median for pasture honey was 17.5%. The honeys were stored in the dark at 4 °C to prevent loss of activity, confirmed by re-assay before use.

The minimum inhibitory concentration (MIC) of each honey for the clinical isolates was determined by an agar incorporation technique. Since hydrogen peroxide is generated on dilution of honey³, it was necessary to introduce the clinical isolates to the honey-containing agar without delay. Therefore, honey samples were measured out and diluted in sterile deionized water to prepare a 20% stock honey solution from which further dilutions in agar could be readily prepared. Appropriate volumes of stock honey and deionized water (totalling 10 mL) were dispensed aseptically into 10 mL samples of sterilized double-strength nutrient agar (Oxoid) immediately before pouring into petri dishes to produce a dilution series between 10% and 1% (v/v) of each type of honey in agar.

Subcultures of the *S. aureus* isolates were grown overnight in 10 mL nutrient broth (Oxoid) for sensitivity assays. Undiluted cultures (typically 1.25×10^8 colony forming units per mL) were inoculated as 0.30 μ L spots of culture, via a Mast multipoint inoculator, onto the honey-containing agar plates and were incubated at 37 °C for 24 h before visual assessment of whether or not growth had occurred. An overnight broth culture of a reference Oxford strain (*S. aureus* NCTC 6571) was inoculated onto each plate for comparison. The sensitivity assays were repeated a total of three times at intervals of 3–4 weeks, each time with a different subculture.

Viability of clinical isolates during sensitivity testing was confirmed by inoculation onto nutrient agar without honey.

RESULTS

The results are shown in Table 1. There was a striking similarity between the isolates in their sensitivity to honey: the MIC values observed were all between 2 and 3% for the manuka honey and between 3 and 4% for the pasture honey.

Table 1 Sensitivity of 58 clinical isolates of *S. aureus* to the antibacterial activity of a pasture honey and a manuka honey

	Minimum concentration of honey (v/v) required for complete inhibition of growth for 24 h	
	Pasture honey	Manuka honey
Mean for isolates	3.79%	2.88%
Mean for Oxford <i>S. aureus</i>	3.41%	2.89%
Standard deviation between isolates	0.25	0.15

DISCUSSION

The lowest concentration of sugar that prevents the growth of *S. aureus* has a water activity of 0.86⁴, equivalent to a concentration of 29% (v/v)³. (Water activity is a measure of the consequential effect of the average intermolecular forces between water molecules, being increased when water molecules become oriented on the surface of solute molecules. When numerous molecules are tied up in this way the water molecules are on average less free to act, e.g. to hydrate something, so the 'activity' is lower.) Granulated sugar packed in an abdominal wound becomes diluted by body fluids within 4 h for the water activity to increase to 0.897, which will allow growth of *S. aureus*⁵. This is equivalent to a concentration of honey of 22%. In the present study, both of the honeys inhibited *S. aureus* completely at much greater dilution. This is because their mode of action is not exclusively through their osmolarity. The lack of significant variance in the sensitivity of a large number of clinical isolates collected from a wide range of wounds indicates that there is no mechanism of resistance to either of the additional types of antibacterial activity in honey (phytochemical or hydrogen peroxide). This contrasts with the variations seen in staphylococcal sensitivity to antibiotics¹⁴. Thus, either of these two honeys might be an effective treatment for a wound infected with any strain of *S. aureus*. However, although their MIC values differed little *in vitro*, *in vivo* the hydrogen peroxide produced in mixed pasture honey would be partly inactivated by the catalase in tissues and blood¹⁵ and manuka honey with its non-peroxide antibacterial activity is likely to be more effective. Their relative merits need to be tested in clinical trials.

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