The antibacterial activity of honey against coagulase-negative staphylococci

Short title: **Honey *vs* coagulase-negative staphylococci**

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Key words: invasive medical devices; antibiotic-resistant; minimum active dilution; manuka honey

Synopsis

Objectives

Development of antibiotic-resistant strains of coagulase-negative staphylococci has complicated the management of infections associated with the use of invasive medical devices, and innovative treatment and prophylactic options are needed. Honey is increasingly being used to treat infected wounds, but little is known about its effectiveness against coagulase-negative staphylococci. The aim of this study was to determine the minimum active dilution of two standardized, representative honeys for 18 clinical isolates of coagulase-negative staphylococci.

Methods

An agar incorporation technique was used to determine the minimum active dilution, with dilution steps of 1% (v/v) honey (or steps of 5% (v/v) of a sugar syrup matching the osmotic effect of honey). The plates were inoculated with 10 μl spots of cultures of the isolates.

Results

The honeys were inhibitory at dilutions down to 3.6 (± 0.7) % (v/v) for the pasture honey, 3.4 (± 0.5) % (v/v) for the manuka honey, and 29.9 (± 1.9) % (v/v) for the sugar syrup.

Conclusions

Typical honeys are about eight times more potent against coagulase-negative staphylococci than if bacterial inhibition were due to their osmolarity alone. Therefore honey applied to skin at the insertion points of medical devices may have a role in the treatment or prevention of infections by coagulase-negative staphylococci.

Introduction

Until the 1960s coagulase-negative staphylococci were regarded as saprophytic commensals of low pathogenicity that normally inhabit human skin and mucosal membranes. However, increased recovery rates from clinical specimens prompted a re-evaluation of their clinical status to opportunist pathogens and they are now among the five most frequently isolated causative agents of hospital-acquired infection,[1, 2] often associated with the use of temporary and permanent invasive medical devices (*e.g.* intravenous catheters, continuous ambulatory peritoneal dialysis catheters, urethral stents, endotracheal tubes, cerebrospinal fluid shunt mechanisms, prosthetic heart valves and orthopaedic prostheses) because of their ability to adhere to synthetic polymeric biomaterials and form biofilms.[1] Although *Staphylococcus epidermidis* is the coagulase-negative staphylococcus most commonly isolated from clinical specimens, other species have also emerged as opportunist pathogens.[2, 3] The emergence of methicillin-resistant coagulase-negative staphylococci and strains with multiple antibiotic resistance has made this an increasingly difficult group to treat.[2] Management of infections associated with medical devices usually requires removal of the prosthetic device and the administration of systemic antibiotics.[1, 4] Although all devices are sterilised at the outset, cross-infection from skin is probably responsible for contamination of devices during implantation or subsequent use.

The severe consequences to patients with such infections demands effective strategies designed to minimise and eliminate infections.[1, 4] Although use of electric fields to improve antibiotic therapy, modification of polymeric biomaterial to reduce bacterial adherence, and incorporation of antimicrobial agents into devices to reduce bacterial growth have been explored[1], a solution has yet to be found.

Honey is increasingly being used in the management of infected wounds where conventional pharmaceutical products are failing, especially now that CE-marked

sterile honey and honey-impregnated dressings are available,[5] thus it is reasonable to consider prophylactic application of honey *in situ* at device exit sites.

Although susceptibility to the antibacterial activity of honey of other pathogens is established *in vitro*,[6-8] coagulase-negative staphylococci have not been tested. This study was undertaken to determine their susceptibility to honey *in vitro*. The antibacterial activity of honey varies not only between floral sources but even within one floral source,[9] so representative honeys with median levels of activity were used. The antimicrobial activity in most honeys is due to the enzymic production of hydrogen peroxide, but honey from some *Leptospermum* species, such as manuka, can also have a high antimicrobial activity due to an unidentified phytochemical component:[9] both types of activity were studied. Also, to distinguish these activities from any osmotic inhibition of bacteria, a syrup simulating honey was included in the study as a control.

Materials and methods

Honey

The two natural honeys used were selected to be close to the median antibacterial activity for each type of activity, tested against *Staphylococcus aureus* (ATCC 25923): [10] a manuka honey with non-peroxide activity equivalent to 16.8% (w/v) phenol and a pasture honey with hydrogen peroxide activity equivalent to 17.5% (w/v) phenol. The simulated honey was prepared by combining 38.4 g fructose, 30.3 g glucose, 1.3 g sucrose, 8.6 g maltose and 1.4 g maltodextrin with 17.2 mL distilled water.

Bacterial isolates

Isolates of coagulase-negative staphylococci were obtained from eighteen Waikato Hospital patients. Cultures were isolated from midstream and catheter urines, peritoneal fluid, cerebrospinal fluid, breast aspirate, a peritoneal catheter tip and blood cultures. The isolates were identified using a range of biochemical and

morphological techniques, and the Vitek automated bacterial identification instrument (McDonnell Douglas Health System Company).

The isolates were stored on Protect Bacterial Preserver Beads (LabSupply Pierce) at -70°C. We confirmed identity of the isolates to species level by means of BBL Crystal gram positive kits (Becton Dickinson N.Z.).

Microbiological materials

Trypticase soy broth (TSB) was obtained from Difco Laboratories. Nutrient agar was obtained from Scharlau Laboratories. Blood agar base was obtained from Merck Laboratories, and 5% sterile defibrinated sheep's blood (Life Technologies N.Z.) was added.

Determination of minimum active dilution

Prior to testing, each isolate was cultured from preserver beads by inoculating two beads into 9 mL of TSB and incubating for approximately 16 h at 37°C. Cultures obtained were diluted with TSB to obtain 2–3 x 10^7 cfu/mL, the minimum to produce confluent growth at inoculation positions.

The minimum active dilution of each honey for each of the clinical isolates was determined by an agar incorporation technique. Nutrient agar was made up at double strength, measured out into 25 mL aliquots and autoclaved. To prepare the plates it was melted and tempered in a 50°C water-bath until poured. Solutions of the two natural honey samples (at a concentration of 20% v/v) and the simulated honey (at a concentration of 70% v/v) were prepared in sterile de-ionised water immediately prior to performing an assay and diluted with different volumes of sterile de-ionised water to give double the final concentration required in a volume of 25 mL. These solutions were then also tempered at 50°C then each mixed with one of the 25 mL lots of double-strength nutrient agar. The various agar-honey mixtures were then poured into duplicate petri-dishes.

A dilution series with honey concentrations ranging from 1% to 10% (v/v) final honey concentration, in 1% increments, was used for the susceptibility assays for the natural honeys, and from 5% to 35% (v/v) final honey concentration, in 5% increments, for the simulated honey. Duplicate control plates of nutrient agar with no honey were included in each susceptibility assay to confirm the viability and density of the cultures.

Samples (10 µl) of each culture were inoculated onto the agar plates in three rows using an eight-channel auto-pipettor with tips attached to channels # 1, 3 and 5, to get nine strains inoculated per plate as evenly spaced spots. Duplicate plates were inoculated and assays were repeated on two subsequent days, with fresh subcultures each occasion.

The inoculated plates were incubated at 37°C for 16 h, then growth, partial inhibition or complete inhibition was recorded at each inoculation position. The minimum active dilution was taken to be the lowest concentration of honey at which bacterial growth was completely inhibited, and the mean value for the minimum active dilution was calculated from the six replicates for each isolate.

Analysis of variance was carried out using GenStat (Lawes Agricultural Trust).

Results

In Table 1 it can be seen that the growth of all eighteen coagulase-negative staphylococcus isolates was inhibited by manuka and pasture honeys at concentrations of 2.7–5% (v/v). By contrast, simulated honey inhibited the various isolates at concentrations of 27.5–31.7% (v/v), showing that the antibacterial activity observed with the natural honeys was 5.5–11.7 times greater than that due to the osmotic effect of the sugar content of honey.

The mean values for the results in various groupings of the data are shown in Table 2. There was no significant difference between the two types of natural honey for all

eighteen isolates (p=0.44), nor between the antibiotic-resistant and antibiotic-susceptible isolates (p=0.35), nor between any of the species of bacteria (p=0.66).

Discussion

The results of this study clearly show that honey has the potential to be used as an antibacterial agent to prevent and control infection with coagulase-negative staphylococci. The lack of significant difference in susceptibility to honey between any of the isolates tested (p=0.13) indicates that other isolates are likely to be equally as susceptible. The similarity in susceptibility to honey between antibiotic-resistant and antibiotic-susceptible strains was also seen with *Staphylococcus aureus.*[7]

These findings show that coagulase-negative staphylococci are very similar to *S. aureus*[6, 7] in their susceptibility to honey of similar antibacterial potency and more susceptible than *Pseudomonas aeruginosa*[8] and *Enterococcus* species (VRE and VSE),[7] thus they can be expected to be controlled by honey *in vivo* since there are many reports of honey rapidly healing wounds infected with *S.aureus* and pseudomonads.[5] The results show that honey could be diluted by exudate up to twenty-fold and still inhibit their growth, thus honey would be suitably active for both therapeutic and prophylactic application. The *Leptospermum* honey on sale as licensed products for wound care in Australia, Europe and New Zealand[5] has a standardised level of antibacterial activity close to that of the honey samples used in this study, and so the results are relevant to clinical usage.

There are other advantages in applying honey to the traumatised tissue around medical devices. Its anti-inflammatory activity can be expected to prevent serous exudates which can provide a medium for bacteria to colonise.[11] Also its physical properties provide moist conditions ideal for healing, it has a stimulatory action on growth of wound repair tissues, and unlike other antiseptics it has no harmful effects on tissues, the slow enzymic production of hydrogen peroxide giving about one thousandth of that in a 3% hydrogen peroxide solution.[11]

The development of honey in the form of a rubbery gel which can be moulded to conform to any shape[5] will further increase the practicality of use with medical devices beyond that with the honey-impregnated dressings currently available. It remains for further clinical evaluation to be tried.

Acknowledgements

We thank the staff of the Microbiology Department at Waikato Hospital for identifying and supplying the cultures of coagulase-negative staphylococci for this study, and determining their antibiotic susceptibility; we thank Kerry Allen for confirming the identity of the isolates to species level and for determining their susceptibility to methicillin; also we thank the Waikato Medical Research Foundation for a research grant that provided a study award for V. M. French.

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