

The use of honey as an antiseptic in managing *Pseudomonas* infection

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A laboratory study was undertaken to extend existing knowledge about the effectiveness of the antibacterial properties of honey against pseudomonads. To date, sensitivity testing has used non-standardised honeys, which may vary greatly in their antibacterial potency. Pure cultures of *Pseudomonas spp*, isolated from swabs from 20 infected wounds, were inoculated on the surface of nutrient agar plates containing various concentrations of honey in the medium. Two types of honey were used, a manuka honey and a pasture honey, each selected to have antibacterial activity close to the median for each type. The minimum inhibitory concentration of the manuka honey for the 20 isolates ranged from 5.5-8.7% (v/v) (mean 6.9% (v/v), standard deviation 1.13). The minimum inhibitory concentration of the pasture honey for the 20 isolates ranged from 5.8-9.0% (v/v) (mean 7.1% (v/v), standard deviation 1.0). Honeys with an average level of antibacterial activity could be expected to be effective in preventing the growth of pseudomonads on the surface of a wound even if the honey were diluted more than ten-fold by exudation from the wound.

The nature of the polymicrobial flora of wounds is well established, and the factors which determine

whether those organisms colonise or infect a wound have been discussed¹. Many species of bacteria have been recovered from wounds, but *Staphylococcus aureus* is the most frequently isolated wound pathogen². *Pseudomonas aeruginosa* was detected in only 4% of wounds in hospital patients² but it is generally accepted to be an important pathogen in chronic wounds and burns. Its presence in wounds has been demonstrated in numerous studies³⁻⁵, and has been found in one-third of chronic leg ulcers⁶. Its importance in burns has been emphasised⁷ and it has been shown that extensive burns acquire this organism more readily than minor burns⁸.

The effect of pseudomonads on wound healing rates has been underestimated. It was deduced that *Pseudomonas aeruginosa* was more likely to be isolated from pressure sores that were healing than from those that were not³. However, pseudomonads of clinical importance produce a range of destructive enzymes to assist invasiveness⁹. During normal growth, these virulence factors are packaged into membrane-bound vesicles that are generated at the surface of the bacteria and subsequently fuse with the membranes of epithelial cells¹⁰. Hence

Honey; Pseudomonas; Wound infection potent bacterial hydrolytic enzymes are delivered to the interior of host cells and give rise to

potent bacterial hydrolytic enzymes are delivered to the interior of host cells and give rise to host cell damage. It has been concluded that staphylococci and pseudomonads retard ulcer healing rates¹¹, and it has been suggested that pseudomonads and β -haemolytic streptococci reduce the success of skin grafts to leg ulcers¹². Monitoring of microbial flora and ulcer size on a weekly basis has confirmed that necrosis and delayed healing are correlated with the presence of *Pseudomonas aeruginosa*¹³. These observations are not surprising in the light of the above elucidation of the mechanism of *Pseudomonas* virulence.

Pseudomonas species are notoriously resistant to antimicrobial therapy, due to the presence of a relatively impermeable outer membrane layer and antibiotic efflux systems¹⁴. Treatment usually relies on aminoglycosides, new β -lactams and ciprofloxacin. Monotherapy or combination therapy of two of these agents is usual. Adverse patient effects (ototoxicity and nephrotoxicity) have been linked to the use of aminoglycosides. Gentamicin was recently shown to increase by three to five times the production of surface-derived membrane vesicles from *Pseudomonas aeruginosa*¹⁰. Endotoxin embedded within the membranes of these vesicles is thought to be

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responsible for some of the side-effects seen in patients treated with aminoglycosides¹⁰. Another problem associated with antibiotic treatment of bacterial infections is the possibility of selecting antibiotic-resistant strains. In a retrospective review of bacteria isolated from chronic leg ulcers⁶, most of the bacteria were sensitive to the antibiotics commonly used in systemic therapy; 15% of the *Pseudomonas spp* included in the study were resistant to ciprofloxacin and 25% to aztreonam.

Results obtained by clinicians using honey as an alternative wound dressing material, have been reported¹⁵, particularly its use on surgical and traumatic wounds, abscesses, chronic ulcers and burns. Many of these publications report on infected wounds, often with pseudomonads present, being rendered bacteriologically sterile by honey within one week¹⁶⁻²⁰; others report bacteria surviving²¹⁻²⁴, pseudomonads being commonly detected. In a study where 12 different species of bacteria isolated from infected wounds were tested for their sensitivity to honey, *Clostridium oedematiens* and *Pseudomonas aeruginosa* were the only species found to be resistant²⁵.

These variable results may reflect differences in the antibacterial potency of the honey used. It has been suggested that, for the evaluation of a clinical trial of honey, the antibacterial activity of the batch of honey might be important²⁶. To date, clinical reports on the use of honey as an antiseptic dressing on infected wounds have given little or no regard to the type of honey used. However, the antibacterial activity of different batches of honey can vary by a factor of up to one hundred²⁷.

Honey is produced from many floral sources, and its antimicrobial activity varies markedly with origin and processing²⁷. The variation can be in the amount of hydrogen peroxide produced enzymically in different honeys, and in the presence of additional antibacterial components derived from the nectar source²⁷. This may also explain why there is so much variation in the *in vitro* testing of the sensitivity of wound-infecting bacteria to honey²⁸. Only one study has reported testing the sensitivity of wound-infecting species of bacteria to standardised honeys²⁹, but only single strains of laboratory-maintained cultures of individual species were tested, thus limiting the clinical relevance of the results.

The present study was undertaken with a wide range of *Pseudomonas* strains isolated from infected wounds. These were tested in the laboratory to assess their sensitivity to two standardised honeys so that the potential usefulness of honey as an antiseptic dressing for wounds infected with pseudomonads could be determined. The two honeys used were selected to have antibacterial activity close to

the median for each type. One was representative of honey in which the antibacterial activity is due primarily to hydrogen peroxide. The other was a representative manuka honey, a type which has substantial non-peroxide antibacterial activity associated with an unidentified phytochemical component²⁷.

Materials and method

Strains of *Pseudomonas spp* were isolated from swabs which were routinely submitted during a four-week period in 1997 for laboratory examination by the Department of Medical Microbiology and Public Health at University Hospital of Wales Cardiff. These swabs were taken from a wide variety of 20 different wounds, including: chronic venous ulcers on the leg, malleolus and foot; a pressure ulcer on the hip; an acutely infected traumatic wound on the ear; acutely infected surgical wounds on the abdomen and groin. The isolates were identified as *Pseudomonas spp* by standard bacteriological techniques. Pure cultures, randomly selected, were donated and maintained by weekly subculture on nutrient agar (Oxoid).

Two types of honey were used and were selected to be close in antibacterial activity to the median found in a survey of a large number of New Zealand honeys obtained from commercial sources, tested against *Staphylococcus aureus* (ATCC 25923)³⁰. The manuka honey used had a non-peroxide antibacterial activity (with catalase added to remove hydrogen peroxide) equivalent to 13.2% (w/v) phenol (cf 15.5% median for this type of activity). The other honey, from a mixed-pasture source, was chosen because of its high level of hydrogen peroxide activity and undetectable non-peroxide activity. Its antibacterial activity was equivalent to 14.8% (w/v) phenol (cf 17.5% median for this type of activity).

Solutions of each honey, 20% (v/v) in sterile de-ionised water, were prepared using aseptic technique, and dispensed with appropriate volumes of sterile de-ionised water totalling 10mL into 10mL aliquots of sterilised double-strength nutrient agar (Oxoid) immediately before pouring into Petri dishes to produce a dilution series from 10% to 1% (v/v) of each type of honey in agar. A control plate of nutrient agar without honey was included on each occasion to confirm the viability of isolates. Sub-cultures of the *Pseudomonas* isolates were grown overnight in 10mL nutrient broth (Oxoid) for assaying sensitivity to honey. Undiluted cultures (typically 1.25×10^8 cfu/mL) were inoculated as 0.30µL spots of culture, using a Mast multipoint inoculator, on to the honey-containing agar plates, which were then incubated at 37°C. After 24 hours, the plates were assessed visually for growth. Sensitivity assays were repeated at intervals of three to four weeks, each using a

Table 1. The sensitivity of 20 clinical isolates of *Pseudomonas spp* to the antibacterial activity of a pasture honey and a manuka honey with median levels, respectively, of hydrogen peroxide and non-peroxide activity.

Minimum concentration of honey required for complete inhibition of growth for 24 h	Pasture honey	Manuka honey
	Mean for isolates	7.1% (v/v)
Range	5.8% - 9.0%	5.5% - 8.7%
Standard deviation between isolates	1.0	1.3

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different sub-culture. On one or more occasions, replicates were used: two of the isolates were tested in duplicate on each of two dates; five were tested on three dates (in duplicate on each occasion apart from one, which was in duplicate on only two of the dates); 13 of the isolates were tested on four dates (11 in duplicate on three occasions, and two in duplicate on all four).

The minimum inhibitory concentration of each type of honey for each isolate was taken as the lowest concentration of honey on which there was no sign of growth where the inoculum spot had been placed. Visible growth at concentrations of honey lower than this also served as a control to demonstrate that the cultures were all viable.

Results

Consistent results were obtained between duplicate determinations carried out on the same date, but there was some variation seen (by up to two steps of 1% in concentration) between results obtained with different sub-cultures on different dates: the average deviation between all replicated determinations for an isolate was 0.52. The results are shown in Table 1. Little difference was found between the 20 isolates in their sensitivity to honey: the coefficient of variance was 16.6%. The similarity in effectiveness of the two types of honey in inhibiting pseudomonads was seen across all of the individual isolates: the correlation coefficient between the minimum inhibitory concentrations of the manuka honey and the pasture honey was 0.84.

Discussion

There have been widely different values reported for the sensitivity of pseudomonads to the antibacterial action of honey. The minimum inhibitory concentration of honey has variously been reported to be (as % v/v): for *Pseudomonas spp*, 10%³¹; for *Pseudomonas aeruginosa*, 3%³², 3.1-6%³³, 5%^{34,36}, 5-6%³⁷, 13%³⁸, 25%³⁹, 30%⁴⁰, 36%⁴¹; for *Pseudomonas fluorescens*, 8.3%⁴² and 25%³⁹. This variance could reflect differences in sensitivity

between different strains of *Pseudomonas*, or could reflect differences in the antibacterial potency of the honeys used in the research. A study in which several different honeys were used reported that the minimum inhibitory concentration against *Pseudomonas aeruginosa* ranged from 8% to 20% (w/w)²⁶.

The lack of substantial variance found in the present study in the sensitivity of a reasonably large number of clinical isolates, collected from a wide range of wounds, indicates that the variation in findings reported previously is probably not due to differences in sensitivity. It also suggests that there is no mechanism of resistance to either of the types of antibacterial activity in honey (phytochemical and hydrogen peroxide) present in pseudomonads, in which antibiotic resistance is widespread⁴³. However, a much larger sample would be needed before this could be concluded.

The variance that was found between replicate determinations (mainly between determinations made at longer intervals rather than those made on the same day), may have been due to the bacteria being at a different stage of their growth cycle at the time of inoculation, or may have been due to mutations occurring during maintenance of the cultures. However, even if the extreme outliers are considered, a honey concentration of 9% (v/v) would be sufficient to completely inhibit any of the isolates. This shows substantially more resistance than the extreme outlier in the other direction, 5% (v/v), but it is well within the concentration of honey that could be expected to be achieved in the fluid bathing a wound bed after application of a honey dressing. It should also be remembered that the honeys used in this study were of average activity, and honeys of much higher activity are available^{27,44}.

When honey is not excessively diluted by wound exudate, its high osmolarity would be sufficient to inhibit pseudomonads regardless of the level of any other antibacterial components contained in the honey. The minimal water activity necessary for multiplication of strains of *Pseudomonas* has been reported to be higher than that for other species that commonly infect wounds⁴⁵, which indicates that pseudomonads are more susceptible to the high osmolarity of honey. However, pseudomonads may be expected to grow in the presence of diluted honey if there are no additional antibacterial factors involved. Experiments conducted with honey-simulating sugar solutions have found that the minimum inhibitory concentration against *Pseudomonas aeruginosa* of this 'artificial honey' was 20% (w/w) compared with 8% (w/w) for a lime honey²⁶.

On testing two of the isolates in the present study with a similar artificial honey we found

KEY ISSUES FOR PRACTICE

- Pseudomonads present in wounds may cause necrosis and delayed healing. They are difficult to clear as they are notoriously resistant to antimicrobial therapy.
- This study has shown the sensitivity and range of pseudomonads to the antibacterial action of honey, a traditional wound dressing material for which there is good, modern, clinical evidence of effectiveness.
- This study has indicated a treatment option for wounds in which the presence of pseudomonads cause a problem, but the honey used should be selected to have a high level of antibacterial activity. This would be facilitated if either quality-assured honey or honey-containing dressings were made available by a supplier of medical ethicals

the minimum inhibitory concentrations to be 21.5% and 22% (v/v), compared with values of 7.3% and 5.6% for the pasture honey, and 5.6% and 5.5% for the manuka honey, respectively (unpublished results). This shows the importance of factors additional to osmolarity, and thus the importance of selecting honeys that are to be used as therapeutic agents. The minimum inhibitory concentrations of around 7% found in the present study, compared with the minimum concentration of more than 21% that would be found in the honey with no antibacterial activity other than that due to its sugar content, shows that honeys selected to have just average levels of bacterial activity would be three times more potent in controlling infection due to pseudomonads.

The findings in this study indicate that there is little difference on average between the two different types of honey in their effectiveness against pseudomonads, and there was no individual strain that showed a significantly greater sensitivity to one or the other honey; the degree of sensitivity to one was correlated with the degree of sensitivity to the other. This suggests that either of these two honeys is likely to be an effective treatment for a wound infected with a pseudomonad.

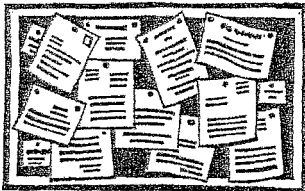
However, there is a possibility that the hydrogen peroxide produced in the pasture

honey will be at least partly inactivated in the bed of the wound. Tissue cells and serum exudate contain the enzyme catalase which breaks down hydrogen peroxide. Manuka honey, which has a high non-peroxide antibacterial activity that is not affected by catalase, may thus prove to be more effective than other types of honey.

There is also the possibility that one type of antibacterial activity will penetrate deeper into a wound, and thus be more effective where bacteria have invaded below the wound surface. Further comparative clinical trials are required to provide evidence for selection of honey type.

Conclusion

When applied directly to a wound, honey with an average level of antibacterial activity may be expected to be effective in preventing the growth of pseudomonads on the wound surface and possibly deeper into the wound. The inhibitory effect of the honey is maintained on the wound surface when the honey is diluted more than 10-fold by wound exudate. However, further clinical trials are needed to find which type of honey gives the best therapeutic results, and what minimum level of antibacterial activity is necessary in the honey for it to be effective as a topical antiseptic treatment for infected wounds. ■

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