9
Honey: Antimicrobial Actions and Role in Disease Management

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Abstract

The ancient treatment of dressing infected wounds with honey is rapidly becoming re-established in professional medicine, especially where wounds are infected with antibiotic-resistant bacteria. This is because of the demonstrated sensitivity of such bacteria to the antibacterial activity of honey, which is not influenced by whether or not strains are resistant to antibiotics. Honey has been found to have a very broad spectrum of activity, but its potency of antibacterial activity can vary greatly. In most honeys the antibacterial activity is due to enzymatically produced hydrogen peroxide and thus the potency of its antibacterial activity can be decreased by catalase present in an open wound. Manuka honey has an antibacterial component derived from the plant source. Manuka honey with a quality-assured level of antibacterial activity is being used by companies marketing honey products for wound care that are registered with the medical regulatory authorities in various countries. Such honey can be diluted 10-fold or more and still completely inhibit the usual wound-infecting species. There is a large amount of clinical evidence for the effectiveness of honey in clearing infection in wounds, and some clinical evidence of its effectiveness in treating other infections. Although the antibacterial potency of honey is insufficient to allow its use systemically, there are various clinical applications besides wound care in which it is used topically or where it does not get excessively diluted, such as for treatment of gastritis, enteritis, gingivitis, ophthalmological infections and bronchial infections. In most of these applications the anti-inflammatory activity of honey is of additional benefit in decreasing the inflammation resulting from infection. Additional clinical research is needed to provide better evidence of the effectiveness of honey in these therapeutic applications of honey.

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9.1 Introduction

An Editorial in the *Journal of the Royal Society of Medicine* in 1989 [1], entitled 'Honey – a remedy rediscovered', expressed the view that 'The therapeutic potential of uncontaminated pure honey is grossly under-utilized' and that 'The time has now come for conventional medicine to lift the blinds off this "traditional remedy" and give it its due recognition'. This Editorial noted the many papers being published reporting good results when honey was used as a dressing on infected wounds and when used in an electrolyte solution in a clinical trial on treatment of diarrhea. In many of the published reports on treatment of infected wounds honey was used where antibiotics were failing to clear the infection. The rapidly increasing number of papers published on the use of honey on wounds in more recent years is probably a reflection of the escalation of the problem of bacteria developing resistance to antibiotics. It is probably also a reflection of honey becoming available as various registered sterile wound-care products, especially ones designed for ease of use [2]. This chapter covers the nature and spectrum of the antimicrobial activity of honey, the evidence for its clinical effectiveness in clearing infection, and the other beneficial therapeutic activities that are seen when honey is used as a topical antimicrobial agent.

9.1.1 History

Honey is the oldest medicine known and in many ancient races of people was prescribed by physicians for a wide variety of ailments [3]. The ancient Egyptians, Assyrians, Chinese, Greeks and Romans all used honey, in combination with other herbs and on its own, to treat wounds and diseases of the gut [4]. Its use for the treatment of diarrhea was recommended by the Muslim prophet Mohammed [5]. In Ancient Greece, Aristotle [6] wrote of honey being a salve for wounds and sore eyes and Dioscorides around 50 AD wrote of honey being 'good for sunburn and spots on the face' and 'for all rotten and hollow ulcers' [7]. He also wrote that 'honey heals inflammation of the throat and tonsils, and cures coughs'.

The use of honey as a therapeutic agent has continued into present-day folk medicine. In India, lotus honey is used to treat eye diseases [8]. Other examples of current-day usage of honey in folk medicine are: as a traditional therapy for infected leg ulcers in Ghana [9], as a traditional therapy for earache in Nigeria [10], and as a traditional therapy in Mali for the topical treatment of measles and in the eyes in measles to prevent corneal scarring [11]. Honey also has a traditional folklore usage for the treatment of gastric ulcers [12] and its ancient usage to treat sore throats has continued into the traditional medicine of modern times [13].

However, many medical professionals are of the opinion that honey has no place in modern medicine. An Editorial in *Archives of Internal Medicine* assigned honey to the category of 'worthless but harmless substances' [14] and Editorials in other medical journals have clearly shown a lack of awareness of the research that has demonstrated

the rational explanations for the therapeutic effects of honey [15, 16]. Many physicians are not even aware that honey has an antibacterial activity beyond the osmotic effect of its sugar content [16–23], yet there have been numerous publications over the past 70 years reporting that there are other components of honey that have a much more potent antimicrobial effect.

The first indication that the antimicrobial activity of honey was not just an osmotic effect was in a report by Sackett [24] who observed that the antibacterial potency was increased by limited dilution of honey – an observation that was hard to explain. More intensive study two decades later by Dold *et al.* [25] led to the discovery of an antibacterial factor which they termed 'inhibine' – a term widely used in the literature for the next 26 years until the antibacterial factor was identified as hydrogen peroxide by White *et al.* [26]. The term 'inhibine number' was also used, this being the number of dilution steps a honey could be subjected to and still have antibacterial activity. Subsequent studies have found that where a range of honeys has been tested against a single species of microorganism the minimum inhibitory concentration (MIC) of honey varied widely: 25–0.25 % [27], greater than 50–1.5 [28], 20–0.6 [29] and 50–1.5% (v/v) [30].

This discovery by microbiologists studying honey that different honeys varied markedly in their antimicrobial potency is in effect probably a rediscovery of ancient wisdom. The ancient physicians who prescribed honey for various ailments would have had no knowledge of the principles involved in its medicinal action, just an empirical knowledge gained from its effective usage. However, they were aware that some honeys were better others for medical usage: Dioscorides around 50 AD stated that a pale yellow honey from Attica was the best, being 'good for all rotten and hollow ulcers' [7]; and Aristotle [6], discussing differences in honeys, referred to pale honey being 'good as a salve for sore eyes and wounds'. Present-day folk medicine also recognizes differences in honeys: the strawberry-tree honey of Sardinia is valued for its therapeutic properties [31]; in India, lotus honey is said to be a panacea for eye diseases [8]; honey from the Jirdin valley of Yemen is highly valued in Dubai for its therapeutic properties [32]; manuka honey has a long-standing reputation in New Zealand folklore for its antiseptic properties (K. Simpson, personal communication). This knowledge that honey is not a 'generic medicine' but needs appropriate selection for therapeutic use is not widespread, so until recently most clinical treatment and microbiological studies have been done with honey with an unknown level of antimicrobial activity.

9.2
Nature of the Antimicrobial Activity of Honey

9.2.1
High Osmolarity

The osmolarity of honey alone is sufficient to prevent microbial growth. Granulated honey is a saturated solution of sugars and clear honey is a supersaturated solution.

Although honey with a high water content can spoil because some osmophilic yeasts can live in it, no fermentation occurs if the water content is below 17.1% [33]. The water content of honey is usually 15–21% by weight [34]. Of the solids in honey, 84% is comprised of the monosaccharides fructose and glucose [35]. The strong interaction of these sugar molecules with water molecules leaves very few of the water molecules available for microorganisms. The water molecules that are 'free' water are measured as the water activity (a_w). The mean values of a_w for honey have been reported as 0.562 and 0.589 [36], 0.572 and 0.607 [37], and 0.62 [38]. Many species of bacteria have their growth completely inhibited by the a_w being in the range 0.94–0.99 [39, 40]. Calculated on the basis of the concentration being proportional to $-\log a_w$, these inhibitory values of a_w correspond to solutions of a typical honey (with a_w of 0.6) of concentrations from 12 down to 2% (v/v) [40]. Fungi are generally much more tolerant than bacteria of low a_w [39]. *Staphylococcus aureus* has an exceptionally high tolerance of low a_w; for complete inhibition of growth of *S. aureus* the a_w has to be lowered below 0.86 [39, 41, 42], which would be a typical honey at 29% (v/v). There have been many reports of granulated sugar being used as a wound dressing [43], but it has been reported that infection is not cleared or new infection becomes established in cases where urine or heavy exudate from wounds dilutes the sugar [44]. With honey, the presence of other antimicrobial factors allows it to be inhibitory even when diluted down to an osmolarity that will freely allow growth of microorganisms. With a honey that has a median level of antibacterial activity it is possible to have it diluted to as low a concentration as 2% (v/v) and still have it completely inhibit the growth of *S. aureus* [45]. In a study of methicillin-resistant *S. aureus* (MRSA) [46] with honeys that had near median levels of antibacterial potency it was found that whereas the MIC of the honeys for any of the strains was below 4% (v/v), the MIC for a syrup of a mixture of sugars at the concentrations that occur in honey was above 30% (v/v). Similar studies with coagulase-negative staphylococci [47], *Burkholderia cepacia* [48], enterococci [46] and *Pseudomonas* spp. [49] found MIC values for the honeys of 3–5, 2.1–5, 3.83–9.66 and 4.33–9.0%, respectively, whereas the MIC for the syrup was 27.5–31.7, 17.5–22, 27.7–29.8 and 17–22%, respectively.

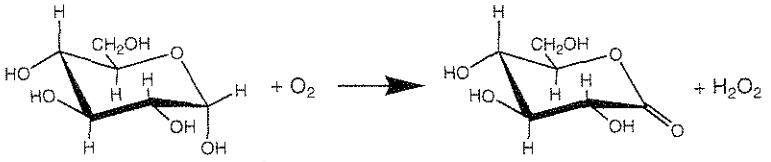
9.2.2 Acidity

The antibacterial activity of honey is partly due also to acidity. Honey is characteristically of a pH between 3.2 and 4.5 [34]. This acidity is due primarily to honey containing 0.23–0.98% (1.8–7.5 mmol/kg) gluconolactone/gluconic acid [35], which is formed by the action of the enzyme glucose oxidase which bees add to the nectar they collect to make honey. However, no correlation has been found between antibacterial activity and the pH of the honey when this has been studied [37, 50–54]. This may be because of different degrees of buffering in different honeys: the pH does not necessarily indicate the titratable acidity, but it is the titratable acidity that determines the final pH when honey is diluted by a neutralizing solution. With such a low concentration of acid in honey there is not

much lowering of the pH when honey is added to culture media or serum. In work with *S. aureus* no inhibition was seen with gluconic acid added to nutrient broth at levels up to 0.25% [55]. However, in a study with *Corynebacterium diphtheriae* the MIC of the honey used was found to be 4.5%, but was 10% when the honey was neutralized [56]. The pH of the nutrient broth containing honey at 4.5% was measured and found to be 6.2. The acidity of honey was found to be of effect in the inhibition of *Bacillus cereus* also: inhibition by 50% honey in an agar diffusion assay was lost if phosphate buffer was added to bring the pH to 6.1–6.5 [57]. The low pH of honey that has not been too much diluted by a neutralizing medium would be at least partially inhibitory to many animal pathogens. The optimum pH for growth of these pathogens is normally between 7.2 and 7.4, although the minimum pH values for growth of some common wound-infecting species are: *Escherichia coli*, 4.3; *Salmonella* species, 4.0; *Pseudomonas aeruginosa*, 4.4; *Streptococcus pyogenes*, 4.5 [58]. The concentration of bicarbonate (the principle buffering ion) in the extracellular fluid of the body is 25 mmol/l, so the dilution of a honey containing a median level of gluconolactone/gluconic acid with an equal volume of extracellular fluid would raise the pH of the honey to 6.8. This means that where honey gets diluted by body fluid the acidity of honey makes a minor contribution to antibacterial activity and it is the other antibacterial components that are primarily responsible for control of infection when honey is used therapeutically.

9.2.3
Hydrogen Peroxide

Hydrogen peroxide is the major antimicrobial factor in most honeys. Adcock [59] found that the antibacterial activity of honey could be removed by the addition of catalase (which catalyzes the destruction of hydrogen peroxide), and White *et al.* [26] demonstrated a direct relationship between the hydrogen peroxide produced and the 'inhibine number' of various honeys. The hydrogen peroxide in honey is produced by the action of the enzyme glucose oxidase, which is secreted into collected nectar from the hypopharyngeal gland of the bees. A similar type of antimicrobial system was discovered when Fleming's work on the antibacterial properties of *Penicillium notatum* was followed up by Coulthard *et al.* [55]. They traced the cause of the erratic results they were obtaining to the potent activity of a second factor, notatin, which was present in addition to penicillin. They found notatin to be a combination of the enzyme glucose oxidase with glucose, and showed the activity of notatin to be due to the hydrogen peroxide produced. Oxygen needs to be available for the reaction:



β-D-glucose + oxygen → δ-gluconolactone + hydrogen peroxide

This means that that this antimicrobial activity from honey can only be of use under aerobic conditions. The production of hydrogen peroxide during the ripening of honey serves to sterilize the honey stored in the comb, but undiluted honey has a negligible level of hydrogen peroxide [26, 60, 61].

Glucose oxidase is practically inactive in full-strength honey, it giving rise to hydrogen peroxide only when the honey is diluted [26]. One explanation for this is that the activity of the enzyme is suppressed by the low pH in ripened honey. The enzyme has an optimum pH of 6.1, with a good activity from pH 5.5 to 8, but the activity drops off sharply below pH 5.5 to near 0 at pH 4 [62]. It is not a case of substrate inhibition of the enzyme, as glucose concentrations beyond those occurring in honey do not suppress the rate of reaction, the optimum substrate concentration for the glucose oxidase in honey being exceptionally high (1.5 mol/l) [62]. This high optimum concentration is well suited to the enzyme's functioning in ripening honey (the concentration of glucose in ripened honey being around 2 mol/l), but will markedly limit the rate of production of hydrogen peroxide in well-diluted honey. The need to dilute honey to get the enzyme active is most likely because of the low water activity of honey, as it is known that enzymes need a sufficiently high water activity to be active [63]. As honey is diluted the activity of glucose oxidase increases to a peak at a concentration around 30–50% (v/v) honey as the water activity is increased, then falls again as the enzyme and substrate concentrations are decreased by further dilution [64]. Honey solutions were found to maintain at least half of the maximum rate of generation of hydrogen peroxide over a wide range of dilution that is concentrations of honey from approximately 15 to 67% (v/v) [64].

Inhibition of the enzyme by high concentrations of honey is not caused by either of the products of the reaction. In a system buffered to prevent inhibition of the enzyme by low pH, no inhibition at all was seen with 10 mmol/l gluconic acid or gluconolactone [62]. Nor does hydrogen peroxide cause inhibition at the levels that are produced in honey [65]. However, studies with honey [26] and with the isolated enzyme [65] found the rate of reaction to be falling off over a short period of time. Adding ascorbic acid to remove the hydrogen peroxide as it was produced gave a fivefold increase in the rate of reaction [65]. Bang *et al.* [64] found that when 50% solutions of honey were incubated, hydrogen peroxide accumulated to a peak level then the concentration of hydrogen peroxide dropped, it becoming zero after 24–48 h. This is probably the result of damage to the enzyme by accumulated hydrogen peroxide, as it has been reported that addition of 68 mmol/l hydrogen peroxide to glucose oxidase isolated from honey caused a significant decline in the enzyme's rate of reaction after 20 min [65]. Whilst this means that honey does not have prolonged antimicrobial activity once it has been diluted, it does have the advantage of preventing hydrogen peroxide from accumulating to levels that are harmful to body tissues. The maximum concentration of hydrogen peroxide achieved when a 50% (v/v) solution of a honey with a high level of antibacterial activity was incubated was found to be 3.65 mmol/l [64], which is 242-fold lower than the 3% (882 mmol/l) solution of hydrogen peroxide typically used as an antiseptic [66]. The use of hydrogen peroxide as an antiseptic has been discouraged because it is cytotoxic [67], but at the low levels that form in honey this is not a problem. Hydrogen

peroxide has gone out of common use as an antiseptic also because it causes inflammation, but the antioxidant content of honey would help prevent inflammation being caused, as it has been found that it oxidative species formed from hydrogen peroxide, rather than hydrogen peroxide itself, that are responsible for the activation of the transcription factor NF-κB involved in the inflammatory response in leukocytes [68]. This activation can be prevented by antioxidants [69].

Although only low levels of hydrogen peroxide accumulate in diluted honey, this is still an effective antimicrobial system because of its continuous production. Hydrogen peroxide has been found to be more effective when supplied by continuous generation by glucose oxidase than when added as a bolus [70]. *E. coli* exposed to a constantly replenished stream of hydrogen peroxide had their growth inhibited by as little as 0.02–0.05 mmol/l hydrogen peroxide, a concentration that was not damaging to fibroblast cells from human skin [71]. Rates of production of hydrogen peroxide in diluted honey that have been reported are: 2.2–5.6 mmol/l/h for 30% (v/v) solutions of eight honeys (three of them blends of 20–30 samples of individual honeys) [64], 0–2.12 mmol/l/h for 14% (v/v) solutions of 90 samples of honey [61], 0–4.8 mmol/l/h for 20% (v/v) solutions of 37 samples of honey [72] and 0.10–0.58 mmol/l/h for 36% (v/v) solutions of 25 samples of honey [59].

Quite low levels of hydrogen peroxide are required for antibacterial activity. It has been reported that *S. aureus* failed to grow in 24 h in nutrient broth containing hydrogen peroxide at 0.29 mmol/l, but grew at 0.15 mmol/l [55]. In other work with *S. aureus* the 20% inhibition of growth over an incubation period of 16 h that was observed corresponded with an accumulation of 0.12 mmol/l hydrogen peroxide from the glucose oxidase–glucose system used to generate it [72]. Others found growth of only one colony of *S. aureus* on a nutrient agar plate containing 0.29 mmol/l hydrogen peroxide and none at the next level tested, 0.5 mmol/l [26].

The level of hydrogen peroxide achieved in diluted honey varies from sample to sample. It can be related to the floral source, as components from some floral sources can affect the enzyme activity that gives rise to hydrogen peroxide and others affect the destruction of hydrogen peroxide. The level of hydrogen peroxide achieved is the result of there being a dynamic equilibrium between the rate of its production and the rate of its destruction [61]. Hydrogen peroxide has been found to rapidly disappear when added to dilute honey [61]. Catalase, an enzyme that destroys hydrogen peroxide, has been shown to be present in honey [73], it coming from the pollen and nectar of certain plants, more from the nectar [74]. Honeys from some floral sources have been found to have very high levels of catalase activity and these accumulate low levels of hydrogen peroxide, whereas those with low levels of catalase activity accumulate high levels of hydrogen peroxide [28, 74]. However, it has been found that hydrogen peroxide disappears when added to honey even if honey is boiled beforehand to inactivate catalase, indicating that loss though chemical reaction is involved as well as through enzymic destruction [26]. Variation between honeys occurs also in the rate of production of hydrogen peroxide. Extraction of honey from the combs and processing to remove wax and other particles requires the honey to be heated. Very large differences have been found between honeys from different floral sources in the thermal stability of the glucose oxidase in them [75] and in the

sensitivity of glucose oxidase to denaturation by light [76]. Thus, the rate of production of hydrogen peroxide will depend on the exposure of honey to heat and light, particularly daylight and the light from fluorescent tubes [61], in its processing and storage, as well as it depending on the floral source of the honey.

9.2.4 Additional Antibacterial Factors

In some honeys there are antimicrobial factors additional to osmolarity, acidity and production of hydrogen peroxide. Reports of antibacterial activity in honey that is stable to heating well in excess of the variation in stability of glucose oxidase indicates that hydrogen peroxide is not the only antibacterial factor in diluted honey. In a study of some Jamaican honeys, the activity of the two most active honeys was not reduced by steam sterilizing, whereas in the others it was reduced or destroyed [77]. Conifer honeydew honey, with exceptionally high activity, was reported to contain a heat-stable as well as a heat-sensitive antibacterial factor [50]. More direct evidence for the existence of antibacterial factors additional to hydrogen peroxide is seen in reports of activity persisting in honeys treated with catalase to remove the hydrogen peroxide activity [57, 59, 72, 78–82]. In one of these studies where substantial antibacterial activity remained it was shown by direct assay of the level of hydrogen peroxide present that the catalase had been completely effective [59]. Lysozyme has been identified in honey, usually occurring at a level of 5–10 μg/ml, occasionally at 35–100 μg/ml if the honey is freshly extracted from the comb, but at much lower levels in older samples [83]. The flavonoid pinocembrin has been identified as an antibacterial component of honey, but at a level only 1–2% of what would be required to account for the observed activity not due to hydrogen peroxide [72]. Some phenolic acid components of manuka (*Leptospermum scoparium*) honey with antibacterial activity have been identified: 3,5-dimethoxy-4-hydroxybenzoic acid (syringic acid), methyl 3,5-dimethoxy-4-hydroxybenzoate (methyl syringate) and 3,4,5-trimethoxybenzoic acid [84], but these were later found to account for no more than 4% of the antibacterial activity of diluted honey not due to hydrogen peroxide [85]. In viper's bugloss (*Echium vulgare*) honey this type of activity was accounted for entirely by its content of 1,4-dihydroxybenzene [85], but the activity was very low compared with that of manuka honey [78].

9.2.5 Manuka Honey

Manuka honey, produced in large quantities in New Zealand, is very unusual in having a high level of antibacterial activity after addition of catalase to destroy hydrogen peroxide, sufficient catalase being added to remove hydrogen peroxide at a level 100 times higher than that with activity equivalent to the most active honey in the study [80]. The possibility was investigated that the activity remaining in manuka honey after the addition of catalase was the result of a component of this honey inhibiting the enzyme, but it was shown that inhibition did not occur [78]. This type of

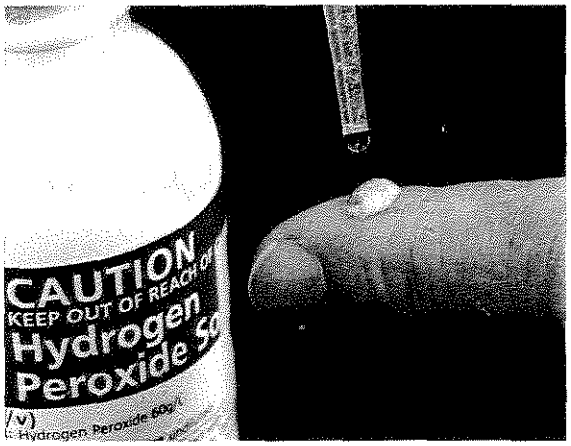


Figure 9.1 Illustration of the rapidity of the breakdown of hydrogen peroxide (to oxygen and water) when it is exposed to the catalase activity in a small drop of blood on a pricked finger.

antibacterial activity is significant for clinical applications because all cells of the body contain the enzyme catalase, so at least part of the antibacterial activity of other types of honey will be destroyed if the honey comes in contact with cells. As hydrogen peroxide freely diffuses across cell membranes this breakdown can be quite rapid, as is illustrated in Figure 9.1, where a drop of 3% hydrogen peroxide solution has been placed on a pricked finger. There will not be complete breakdown of hydrogen peroxide because the enzyme will act more slowly as the concentration of its substrate decreases, so eventually there will be an equilibrium level reached where the rate of production equals the rate of destruction. Thus, although both types of antibacterial activity in assays in agar or broth may appear to be of similar potency, where the honey is exposed to catalase activity in or on the body the activity of other honeys will be less than that of manuka honey. Also, the unusual antibacterial activity in manuka honey is fully effective in undiluted honey, whereas other types of honey need dilution before glucose oxidase becomes active and production of hydrogen peroxide begins. Figure 9.2 shows an illustration of this difference, where wound dressings were prepared from a manuka honey and a clover honey, each with the same level of antibacterial activity when compared as 25% solutions in an agar well diffusion assay. Placing the pieces of dressing against the cut edge of the agar gel seeded with *S. aureus* simulates the situation where honey dressings are placed on an infected open wound. It can be seen that antibacterial activity has diffused deeply into the agar from the manuka honey, but very little antibacterial activity has been produced in the clover honey as there has been little dilution to activate the glucose oxidase enzyme to produce hydrogen peroxide. It is for these reasons that companies marketing honey products for wound care that are registered with the medical regulatory authorities in Australia, Canada, the European Union, Hong Kong, New Zealand and the United States have chosen to use manuka honey or the equivalent honey produced from other *Leptospermum* species in Australia.

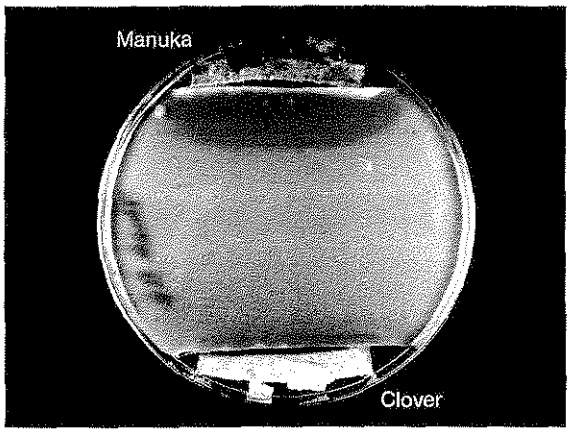


Figure 9.2 Model of honey dressings on an infected wound. One dressing pad was impregnated with manuka honey, the other with clover honey.

Each honey had the same level of antibacterial activity in an agar well diffusion assay. They were placed against the cut edge at each and of the agar which had been seeded with *S. aureus*, then the plate was incubated at 37 °C for 18 h.

9.3 Spectrum and Potency of the Antimicrobial Activity of Honey

There have been many reports published on the sensitivity of a wide range of species of bacteria and fungi to honey. However, in much of this work only a single concentration of honey has been used. Sometimes this concentration has been high enough for the inhibition of microbial growth that has been observed to have probably been due just to the osmotic effect of the honey. Also, with much of the published research, even where MIC values for honey are reported the honey has been arbitrarily chosen, so its antimicrobial potency relative to that of other honey is not known. As mentioned above, the MIC has been found to vary up to 100-fold between different honeys, which means that much of the published data is not a useful indication of the results that could be expected with other honey if the use of honey for infection control is being considered. A review of all the research on the antimicrobial activity of honey published up to 1992 is available [86, 87] for anyone wanting to see the scope of this.

In the present chapter only the findings reported which give information useful for making clinical decisions will be covered. Thus, data are presented which are either the range of MIC values found where numerous different honeys were tested or are the MIC values where the honey used in the research was selected to have a near-median level of antibacterial activity. The antibacterial potency of these selected honeys has been rated against phenol as a standard antibacterial substance, using an agar well diffusion assay with a standard strain of *S. aureus* [78]. Many companies

Table 9.1 MICs of honeys, for various species of bacteria, reported in studies where numerous different honeys were used.

No. of samples	Species	Mean MIC (% v/v)	SD	Reference
60	*Staphylococcus aureus*	21.6	28.1	[29]
22	*Salmonella typhi* H901	4.2	6.1	[27]
	Escherichia coli	4.1	5.5	
	Shigella flexneri Type I	1.4	2.1	
	Proteus morganii	6.0	5.7	
	Staphylococcus aureus Oxford 209	7.9	9.0	
	Bacillus anthracis	10.2	10.7	
27	*Staphylococcus aureus*	5.6	5.0	[30]
	Streptococcus pyogenes Group A	6.1	5.2	
	Streptococcus α-haemolyticus	10.6	6.2	
	Corynebacterium diphtheriae	17.7	10.2	
	Escherichia coli	40.7	20.4	
	Proteus vulgaris	57.0	10.7	
	Pseudomonas pyocyanea	28.3	19.6	
	Klebsiella pneumoniae	30.4	19.9	
	Shigella flexneri	21.4	17.5	
	Bacillus anthracis	16.6	19.6	
	Bacillus mesentericus	27.4	24.1	
	Monilia albicans	60.0	0.0	
18	*Staphylococcus aureus* ATCC 6538	12.7	1.5	[88]
42	*Bacillus subtilis* ATCC 6633	27.8	10.8	[89]
	Escherichia coli ATCC 14948	22.8	11.2	

marketing honey for use as an antibacterial agent are now rating the activity of their honeys in the same way, which allows prediction of their likely clinical effectiveness by reference to the published research findings. The findings from research with numerous samples of honey are summarized in Table 9.1 and those from research using standardized honey are summarized in Table 9.2. The data in Table 9.1 will be less representative than that in Table 9.2 of honey in general, as the studies that are in Table 9.2 have selected honeys that have antimicrobial potency that is near the median level found for honey in a survey of 345 samples of honey, from 26 different floral sources [78]. In studies with smaller numbers of samples the activity of the honeys used may have been unusually low or unusually high.

The failure to take into account the large variance in antibacterial potency of different honeys may explain some of the large differences in results reported between hospitals using honey in similar ways. Some have reported rapid clearance of infection in a range of different types of wound, with wounds all becoming sterile in 3–6 [98, 99], 7 [100–102] or 7–10 days [103]. Others have reported bacteria still present in wounds after 2 [104, 105], 3 [106–108] and 5 weeks [109].

Where antibiotic-resistant strains of bacteria have been studied, their sensitivity to honey has been found to be essentially the same as that of the antibiotic-sensitive strains of the same species [46, 47, 94]. This and the very broad spectrum of

Table 9.2 MICs of honeys for various species of bacteria and fungi, reported in studies where honeys with standardized antibacterial activity were used.

No. of strains	Species of microorganism	Mean MIC (% v/v)	SD	Reference
Manuka honey: nonperoxide activity equivalent to 13.2% phenol				[90]
1	*Escherichia coli*	3.7		
1	*Proteus mirabilis*	7.3		
1	*Pseudomonas aeruginosa*	10.8		
1	*Salmonella typhimurium*	6		
1	*Serratia marcescens*	6.3		
1	*Staphylococcus aureus*	1.8		
1	*Streptococcus pyogenes*	3.6		
Rewarewa honey: hydrogen peroxide activity equivalent to 21.5% phenol				[90]
1	*Escherichia coli*	7.1		
1	*Proteus mirabilis*	3.3		
1	*Pseudomonas aeruginosa*	6.8		
1	*Salmonella typhimurium*	4.1		
1	*Serratia marcescens*	4.7		
1	*Staphylococcus aureus*	4.9		
1	*Streptococcus pyogenes*	2.6		
Manuka honey: nonperoxide activity equivalent to 13.2% phenol				[91]
7	*Helicobacter pylori*	5	0	
Manuka honey: nonperoxide activity equivalent to 13.2% phenol				[92]
1	*Epidermophyton floccosum*	10		
1	*Microsporum canis*	25		
1	*Microsporum gypseum*	50		
1	*Trichophyton mentagrophytes* var. *interdigitale*	25		
1	*Trichophyton mentagrophytes* var. *mentagrophytes*	20		
1	*Trichophyton rubrum*	10		
1	*Trichophyton tonsurans*	25		
Pasture honey: hydrogen peroxide activity equivalent to 14.8% phenol				[92]
1	*Epidermophyton floccosum*	10		
1	*Microsporum canis*	15		
1	*Microsporum gypseum*	20		
1	*Trichophyton mentagrophytes var. interdigitale*	15		
1	*Trichophyton mentagrophytes* var. *mentagrophytes*	15		
1	*Trichophyton rubrum*	5		
1	*Trichophyton tonsurans*	20		
Manuka honey: nonperoxide activity equivalent to 13.2% phenol				[93]
1	*Actinomyces pyogenes*	5		
1	*Klebsiella pneumoniae*	10		
1	*Nocardia asteroides*	5		
1	*Staphylococcus aureus*	5		
1	*Streptococcus agalactiae*	5		
1	*Streptococcus dysgalactiae*	5		
1	*Streptococcus uberis*	5		

Table 9.2 (*Continued*)

No. of strains	Species of microorganism	Mean MIC (% v/v)	SD	Reference
Rewarewa honey: hydrogen peroxide activity equivalent to 21.5% phenol				[93]
1	*Actinomyces pyogenes*	5		
1	*Klebsiella pneumoniae*	10		
1	*Nocardia asteroides*	10		
1	*Staphylococcus aureus*	5		
1	*Streptococcus agalactiae*	10		
1	*Streptococcus dysgalactiae*	10		
1	*Streptococcus uberis*	10		
Manuka honey: nonperoxide activity equivalent to 13.2% phenol				[94]
1	*Enterococcus faecalis*	7		
1	*Escherichia coli*	5		
1	*Klebsiella oxytoca*	5		
1	*Pseudomonas aeruginosa*	6		
1	*Staphylococcus aureus*	3		
1	MRSA	3		
1	*Staphylococcus aureus* NCTC6571	3		
	Escherichia coli NCTC10418	4		
Pasture honey: hydrogen peroxide activity equivalent to 14.8% phenol				[94]
1	*Enterococcus faecalis*	9		
1	*Escherichia coli*	9		
1	*Klebsiella oxytoca*	8		
1	*Pseudomonas aeruginosa*	9		
1	*Staphylococcus aureus*	5		
1	MRSA	4		
1	*Staphylococcus aureus* NCTC6571	3		
	Escherichia coli NCTC10418	7		
Manuka honey: nonperoxide activity equivalent to 13.2% phenol				[95]
20	*Pseudomonas* spp. from wounds	6.9	1.3	
Pasture honey: hydrogen peroxide activity equivalent to 14.8% phenol				[95]
20	*Pseudomonas* spp. from wounds	7.1	1.0	
Manuka honey: nonperoxide activity equivalent to 13.2% phenol				[45]
58	*Staphylococcus aureus* from wounds	2.88	0.15	
1	*Staphylococcus aureus* NCTC6571	2.89		
Pasture honey: hydrogen peroxide activity equivalent to 14.8% phenol				[45]
58	*Staphylococcus aureus* from wounds	3.79	0.25	
1	*Staphylococcus aureus* NCTC6571	3.41		
Manuka honey: nonperoxide activity equivalent to 13.2% phenol				[48]
20	*Burkholderia cepacia* (multiresistant)	2.9	0.94	
Pasture honey: hydrogen peroxide activity equivalent to 14.8% phenol				[48]
20	*Burkholderia cepacia* (multiresistant)	3.6	0.77	

(*Continued*)

Table 9.2 (*Continued*)

No. of strains	Species of microorganism	Mean MIC (% v/v)	SD	Reference
Manuka honey: nonperoxide activity equivalent to 18% phenol				[46]
18	MRSA	2.98	0.14	
7	VSE (*Enterococcus faecalis*)	4.92	0.28	
1	VRE (*Enterococcus avium*)	3.83		
3	VRE (*Enterococcus faecalis*)	4.59	0.52	
15	VRE (*Enterococcus faecium*)	4.72	0.22	
1	VRE (*Enterococcus raffinosus*)	4.86		
Pasture honey: hydrogen peroxide activity equivalent to 13.7% phenol				[46]
18	MRSA	3.07	0.26	
7	VSE (*Enterococcus faecalis*)	9.66	0.46	
1	VRE (*Enterococcus avium*)	5.6		
3	VRE (*Enterococcus faecalis*)	9.43	0.21	
15	VRE (*Enterococcus faecium*)	8.33	0.52	
1	VRE (*Enterococcus raffinosus*)	9.0		
Manuka honey: nonperoxide activity equivalent to 18% phenol				[49]
17	*Pseudomonas* spp. from burns	9.71	0.69	
Pasture honey: hydrogen peroxide activity equivalent to 14.8% phenol				[49]
17	*Pseudomonas* spp. from burns	9.0	1.22	
Manuka honey: nonperoxide activity equivalent to 16.8% phenol				[47]
2	*Staphylococcus capitis*	3.3	0.5	
11	*Staphylococcus epidermidis*	3.5	0.5	
3	*Staphylococcus haemolyticus*	3.3	0.7	
1	*Staphylococcus simulans*	3		
1	*Staphylococcus warneri*	3.3		
Pasture honey: hydrogen peroxide activity equivalent to 17.5% phenol				[47]
2	*Staphylococcus capitis*	3.8	0.6	
11	*Staphylococcus epidermidis*	3.3	0.6	
3	*Staphylococcus haemolyticus*	4.2	0.8	
1	*Staphylococcus simulans*	4		
1	*Staphylococcus warneri*	3.5		
Manuka honey: nonperoxide activity equivalent to ≥18% phenol				[96]
18	*Candida albicans*	39.9	1.7	
10	*Candida glabrata*	42.6	2.7	
10	*Candida dubliniensis*	33.4	2.5	
Medihoney (blend): nonperoxide activity equivalent to ≥18% phenol				[96]
18	*Candida albicans*	38.2	2.9	
10	*Candida glabrata*	43.1	4.2	
10	*Candida dubliniensis*	34.6	2.5	
Jarrah honey: hydrogen peroxide activity equivalent to 30.2% phenol				[96]
18	*Candida albicans*	18.5	2.7	
10	*Candida glabrata*	29.9	2.8	
10	*Candida dubliniensis*	15.4	2.8	

Table 9.2 (*Continued*)

No. of strains	Species of microorganism	Mean MIC (% v/v)	SD	Reference
Manuka honey: nonperoxide activity equivalent to 15% phenol				[97]
1	*Actinobacillus actinomycetemcomitans* NCTC 9709	6.1		
1	*Actinomyces gerencseriae* ATCC 233860	7		
1	*Actinomyces naeslundii* NCTC 10301	9.1		
1	*Eikenella corrodins* ATCC 23834	4.7		
1	*Fusobacterium nucleatum* ATCC 25586	5.1		
1	*Peptostreptococcus micros* ATCC 33270	9		
1	*Porphyromonas gingivalis* ATCC 33277	6.2		
1	*Veillonella parvula* ATCC 17745	7.2		
1	*Candida albicans* ATCC 10261	21.5		
1	*Candida glabrata* CBS 138	40		
Pasture honey: hydrogen peroxide activity equivalent to 18.2% phenol				[97]
1	*Actinobacillus actinomycetemcomitans* NCTC 9709	4.8		
1	*Actinomyces gerencseriae* ATCC 233860	9		
1	*Actinomyces naeslundii* NCTC 10301	4		
1	*Eikenella corrodins* ATCC 23834	5.8		
1	*Fusobacterium nucleatum* ATCC 25586	6.7		
1	*Peptostreptococcus micros* ATCC 33270	9.3		
1	*Porphyromonas gingivalis* ATCC 33277	9		
1	*Veillonella parvula* ATCC 17745	7		
1	*Candida albicans* ATCC 10261	40		
1	*Candida glabrata* CBS 138	40		

The level of antibacterial activity of the honeys used is expressed as the concentration of phenol, w/v, with equivalent activity against *S. aureus* ATCC 25923 in an agar well diffusion assay. For the manuka honeys this was determined with catalase added to destroy hydrogen peroxide, so the antibacterial activity is recorded as 'nonperoxide'. MRSA = methicillin-resistant *S. aureus*; VRE = vancomycin-resistant enterococci; VSE = vancomycin-sensitive enterococci.

antimicrobial activity of honey are features that make honey very convenient for clinical use as a topical agent to control infections, as it is not necessary to first identify the infecting species, nor to find the sensitivity of the microorganisms to antibiotics, before effective treatment can be given.

9.4 Other Actions

The clearance of infection by honey may involve more than the antibacterial activity of honey, as research findings with leukocytes in cell culture indicate that honey may work also by stimulating the activity of the immune system. Peripheral blood B lymphocytes and T lymphocytes in cell culture have been found to be stimulated to proliferate by honey at concentrations as low as 0.1% [110]. This low concentration of honey was also found to activate phagocytes isolated from blood [110]. Others have

reported that honey at a concentration of 1% stimulates monocytes in cell culture to release the cytokines tumor necrosis factor-α, interleukin-1β and interleukin-6 which are intermediates in the immune response [111, 112]. Honey has the potential to further augment the immune response by supplying glucose, which is essential for the 'respiratory burst' in macrophages. The hydrogen peroxide thus generated is the major component of the bacteria-destroying activity of these cells [113]. The functioning of macrophages would be further aided by the supply of sugars from honey as these would provide substrates for glycolysis, which is the major mechanism for energy production in these cells. This would allow macrophages to function in damaged tissues and exudates where the poor oxygen supply would limit aerobic respiration for the supply of energy [113].

Another way in which control of infection may be aided by honey is through the ability of honey to prevent attachment of bacteria to cells. It has been reported that exposure of *Salmonella interitidis* to an 11% solution of honey for 1 h prior to mixing the washed bacteria with intestinal epithelial cells decreased the number of bacteria attaching to the cells by 74% [114]. Honey, at concentrations as low as 0.00025%, has also been found to block the PA-IIL lectin of *P. aeruginosa*, which mediates biofilm formation and adhesion to animal cells by this species of bacteria [115]. It has been found that biofilm formation by *P. aeruginosa* and by a coagulase-negative *Staphylococcus* is almost completely prevented by honey at a concentration of only 20% of its MIC [116].

9.5 Clinical Uses of Honey as an Antimicrobial Agent

The major usage of honey for control of infection has been in wound care [2], but there are reports in the modern medical literature of its successful use in ophthalmology and gastroenterology (see below), and of its effectiveness in a trial on gingivitis [117]. With the reporting that inhalation of an aerosol of a 60% solution of honey causes no adverse effects [118], there is also the possibility of using honey for treatment of bronchial infections. The author is aware of anecdotal reports of such therapy being effective, also of honey being effective in the treatment of infection of the nasal sinuses and the ear canal, and for the treatment of tineas. These are applications which warrant further research.

Although the antimicrobial activity of honey is ample for control of infection where the honey is in direct contact with the site of infection and does not get excessively diluted by body fluids, there would be far too much dilution to achieve anywhere near the MIC systemically even if the antimicrobial factors entered the circulation from the gut. However, within the gut it is feasible that a bolus of honey passing through from oral dosage would retain a concentration in excess of the MIC for gut pathogens. The results from a trial where mortality rates from induced infection of mice with *E. coli* 0157:H7 and *Salmonella typhimurium* were substantially decreased by daily subcutaneous injection of 1 ml honey is more likely to be due to the honey stimulating the immune response than from a systemic direct antibacterial activity because the

dilution into the 25 g of body mass of the mouse would have given a concentration below the MIC for these pathogens for all but the most potent of the honeys they used.

Honey given at a concentration of 5% (v/v) in place of glucose in a rehydration fluid was found to give a statistically significant reduction in the duration of bacterial diarrhea (58 versus 93 h), and give no increase in the duration of nonbacterial diarrhea in a clinical trial conducted on infants and children admitted into hospital with gastroenteritis [119]. In a clinical trial in which 45 patients with dyspepsia were given no medication other than honey substantial reductions were found to result in the number of patients passing blood (from peptic ulcers) in their feces, the number with dyspepsia and the number with gastritis, duodenitis or a duodenal ulcer seen on endoscopy [120]. However, this action of honey may not be by way of its antibacterial activity, as it was found in a clinical trial that it failed to clear *Helicobacter pylori* [121]. It appears to be more likely that it is the anti-inflammatory activity of honey (see below), rather than its antibacterial activity, that is involved in its beneficial effects on gastritis. A series of publications on biochemical studies on induced gastric ulcers in rats have pointed to the effect of honey to be via reduction of inflammation; this has been reviewed by Molan [122].

The anti-inflammatory activity of honey is probably a contributing factor in the effectiveness of honey in ophthalmological applications, besides control of infection. Improvement was reported in 85% of the cases, with no deterioration in any of the other, in a trial of honey on 102 patients with a variety of ophthalmological disorders not responding to conventional treatment, such as keratitis, conjunctivitis and blepharitis [123]. Remission in more than 60% of the cases was reported where honey was used to treat blepharitis, catarrhal conjunctivitis, and keratitis [124]. A review of the use of honey in ophthalmology in Russia [125] describes anti-inflammatory, antibacterial and antifungal actions being seen, honey being used for chemical and thermal burns to the eye, conjunctivitis and infections of the cornea. A transient stinging sensation and redness of the eye soon after putting honey in the eye have been reported, but never enough to stop the treatment [123, 126]. A similar effect is experienced by some patients when honey is used to treat inflamed wounds and this has been attributed to the acidity of honey [2].

9.6 Clinical Evidence for Effectiveness of Honey on Infected Wounds

The very large body of clinical evidence for the effectiveness of honey in healing wounds has been reviewed [127]. The evidence covered in that review, plus that from trials published since the review was published [128–134], is from 23 randomized controlled trials involving a total of 2257 participants, seven clinical trials of other forms involving 142 participants treated with honey, four case studies where there were multiple wounds allowing comparison of honey with other treatment and 16 trials of honey on a total of 533 wounds in animal models (which rule out a placebo effect). Mostly the wounds involved were infected. Where details were given in the reports about the clearance of infection by honey these are listed in Table 9.3.

Table 9.3 Reported details of clearance of infection in wounds when the wounds were dressed with honey.

Type of wound	Outcome of honey treatment	Reference
Superficial burns	91% of wounds treated with honey became sterile within 7 days with honey, compared with 7% treated with silver sulfadiazine	[135]
Fresh partial-thickness burns	eight cases infected after 8 days with honey, compared with 17 treated with OpSite	[136]
Superficial burns	honey gave, better control of infection than silver sulfadiazine did	[106]
Moderate burns, 1/6th total burn area being full thickness	after 7 days of honey treatment the original 44 cases giving positive swab cultures decreased to four, but with silver sulfadiazine there was no change in the 42 cases giving positive swab cultures at the start	[137]
Superficial burns	honey gave better control of infection	[138]
Severe postoperative wound infections following abdominal surgery	mean time to get negative swab cultures was 6 days with honey treatment compared with 14.8 days with washing with 70% ethanol then applying povidone-iodine	[139]
Fournier's gangrene (necrotizing fasciitis on the scrotum)	within 1 week with honey all swabs were negative: there was no need to change from the routine antibiotics to ones to which the bacteria were found to be sensitive	[100]
Large infected surgical wounds on infants	with honey treatment, marked clinical improvement was seen in all cases after 5 days, and all wounds were closed, clean and sterile after 21 days; whereas the wounds had failed to heal with treatment of at least 14 days using intravenous antibiotics (vancomycin plus cefotaxime, subsequently changed according to bacterial sensitivity), fusidic acid ointment; and wound cleaning with aqueous 0.05% chlorhexidine solution	[108]
Multiple chronic leg ulcers, on both legs	after 10 days of dressing the ulcers with honey signs of infection had cleared and the green exudate had ceased, whereas with the ulcers dressed with Aquacell there was copious leakage of green fluid.	[140]

Table 9.3 (*Continued*)

Type of wound	Outcome of honey treatment	Reference
Recalcitrant wounds and ulcers of varied etiology	no signs of healing in 1–24 months of conventional treatment (such as Eusol toilet and dressings of Acriflavine, Sofra-Tulle or Cicatrin, or systemic and topical antibiotics), but after honey treatment the 51 wounds with bacteria present became sterile within 1 week and the others remained sterile; burn wounds treated early healed quickly, not becoming colonized by bacteria.	[101]
Broken-down wounds from radical vulvectomy with lymphadectomy	wounds became free from bacteria in 3–6 days	[99]
Surgical wounds, mostly dehiscent or infected	wounds became sterile within 1–4 days, on patients with profound immunosuppression because of chemotherapy	[141]
Disrupted abdominal wounds from Caesarean section	wounds were made sterile within 1 week	[102]

9.7 Resistant Bacteria

Because of its high osmolarity honey is not a medium in which bacteria could survive and thus have evolved genes for resistance by selection of mutant individuals with genes conferring resistance to the antibacterial factors that are effective in diluted honey. The period in which bacteria could live and have strains multiply during the production of honey in the hive would be short; then the selectively bred surviving bacterial strains would be terminated by prolonged exposure to high osmolarity. In a study designed to select for resistant mutants, by continuous exposure of cultures of *P. aeruginosa* and *S. aureus* to increasing concentrations of an antibacterial agent, no increased resistance to honey was developed yet under the same experimental conditions marked increases in resistance to antibiotics were developed [116]. Similar resistance training experiments with manuka honey and several wound pathogens are being conducted elsewhere, but have not yet succeeded in recovering honey-resistant bacteria (R. Cooper, University of Wales Institute, personal communication).

Owing to the increasing problem of bacteria almost inevitably developing resistance to antibiotics where these are extensively used, the low probability of resistance to honey developing makes the use of honey an attractive alternative for topical control of infection. As an example, although the incidence of catheter-associated blood-stream infections in dialysis patients with honey-treated catheter exit sites was

found in a trial [142] to be a bit higher than in those treated with mupirocin (0.97 versus 0.85 episodes per 1000 catheter-days, not significantly different), the low likelihood of selecting for resistant strains of bacteria using honey compared with the high likelihood with continuous use of mupirocin makes the use of honey for chemoprophylaxis in patients with central venous catheters a better option.

With most life-threatening infections with antibiotic-resistant bacteria being acquired by bacteria entering the bloodstream via catheters or open wounds, there is potential for preventing cross-infection in hospitals with antibiotic-resistant strains of bacteria, by dressing all open wounds or catheter exit sites with honey. As well as the trial mentioned above there has been another trial which also has shown honey to be effective in chemoprophylaxis in patients with central venous catheters [143]. In this, the incidence of bloodstream infections in dialysis patients with honey-treated exit sites was found to be a bit lower than in those treated with povidone-iodine (12 versus 19 episodes per 1000 catheter-days, not significantly different). With wounds, the reports of cases where honey was effective in clearing established infection with MRSA and vancomycin-resistant *Enterococcus* (VRE) [140, 141, 144–148] indicate that it is likely to be effective prophylactically. If such an approach to infection control were tried, even if it were not successful it would at least give the best healing conditions for the wounds because of the other features of honey which promote wound healing.

9.8
Benefits Apart from Control of Infection in Topical Treatment with Honey

Apart from its antibacterial activity honey has a potent anti-inflammatory activity, rapidly brings about autolytic debridement of slough and necrotic tissue from wounds, rapidly deodorizes malodorous wounds, speeds up the healing process, and gives healing with minimal scarring: references to the many reports of observations of these features are given by Molan [149]. Antiseptics in common use are all cytotoxic and so slow the healing process [150]. Silver also is cytotoxic [151] and can cause poisoning systemically when absorbed from wound dressings [151]. Honey, however, is not only not toxic, but actually stimulates the growth of cells involved in wound healing [152–154] and stimulates the production of the components of the extracellular matrix [155, 156].

As is so aptly stated about honey by the Muslim prophet Mohammed (around 570–632 AD) in verse 69 of *Surah 16* ('The bee') of the Holy Qu'ran: 'From its belly cometh forth a fluid of varying hues, *wherein there is healing for mankind*'.

9.9
Future Directions

More research is needed to obtain further data on the sensitivity to honeys with standardized activity of some of the multiresistant infecting species of bacteria which infect wounds and catheter exit sites, such as *Acinetobacter baumanii* and

Stenotrophomonas maltophilia. There is also a need for research to find the sensitivity to honeys with standardized activity of untested species which cause ophthalmic, bronchial and gut infections, to establish if clinical treatment of such infections with honey is worth trying. Good clinical trials are needed to establish with certainty how effective honey is for treating such infections. There is also a need for more good clinical trials to be conducted on honey as a treatment for chronic infected wounds, as much of the large body of work that has been done to date has been carried out on acute wounds and/or has had some defects in the design of the trials. There is also a need to measure in these trials the effectiveness of honey in clearing infection, as many of the trials conducted so far have assessed healing rather than specifically assessing clearance of infection, but the healing may have resulted from other bioactivities of the honey such as the anti-inflammatory and debriding actions and the stimulation of growth of repair tissues.

References

1 Zumla, A. and Lulat, A. (1989) *Journal of the Royal Society of Medicine*, **82**, 384–385.

2 Molan, P.C. and Betts, J.A. (2004) *Journal of Wound Care*, **13**, 353–356.

3 Ransome, H.M. (1937) *The Sacred Bee in Ancient Times and Folklore*, George Allen & Unwin, London.

4 Stomfay-Stitz, J. (1960) *Science Counsellor*, **23**, 110–125.

5 Al-Bukhari, M. (1976) *Sahih Al-Bukhari*, 3rd edn revised, Kazi Publications, Chicago, IL.

6 Aristotle (1910) *Historia Animalium. The Works of Aristotle*, vol. IV, (eds J.A. Smith and W.D. Ross), Oxford University Press, Oxford.

7 Gunther, R.T. (1934) *The Greek Herbal of Dioscorides (Reprinted 1959)*, Hafner, New York.

8 Fotidar, M.R. and Fotidar, S.N. (1945) *Indian Bee Journal*, **7**, 102.

9 Ankra-Badu, G.A. (1992) *East African Medical Journal*, **69**, 366–369.

10 Obi, C.L., Ugoji, E.O., Edun, S.A., Lawal, S.F. and Anyiwo, C.E. (1994) *African Journal of Medicine Science*, **23**, 257–260.

11 Imperato, P.J. and Traoré, D. (1969) *Tropical and Geographical Medicine*, **21**, 62–67.

12 Kandil, A., El-Banby, M., Abdel-Wahed, K., Abdel-Gawwad, M. and Fayez, M. (1987) *Journal of Drug Research (Cairo)*, **17**, 103–106.

13 Beck, B.F. and Smedley, D. (1944) *Honey and Your Health*, 2nd edn, McBride, New York.

14 Soffer, A. (1976) *Archives of Internal Medicine*, **136**, 865–866.

15 Editorial (1974) *South African Medical Journal*, **56**, 2300.

16 Condon, R.E. (1993) *Surgery*, **113**, 234–235.

17 Bose, B. (1982) *Lancet*, **i**, 963.

18 Green, A.E. (1988) *British Journal of Surgery*, **75**, 1278.

19 Keast-Butler, J. (1980) *Lancet*, **ii**, 809.

20 Mossel, D.A.A. (1980) *Lancet*, **ii**, 1091.

21 Seymour, F.I. and West, K.S. (1951) *Medical Times*, **79**, 104–107.

22 Somerfield, S.D. (1991) *Journal of the Royal Society of Medicine*, **84**, 179.

23 Tovey, F.I. (1991) *Journal of the Royal Society of Medicine*, **84**, 447.

24 Sackett, W.G. (1919) Honey as a carrier of intestinal diseases. *Bulletin of the Colorado State University Agricultural Experimental Station*, **252**.

25 Dold, H., Du, D.H. and Dziao, S.T. (1937) *Zeitschrift fur Hygiene und Infektionskrankheiten*, **120**, 155–167.

26 White, J.W., Subers, M.H. and Schepartz, A.I. (1963) *Biochimica et Biophysica Acta*, **73**, 57–70.

27 d'Agostino Barbaro, A., La Rosa, C. and Zanelli, C. (1961) *Quaderni della Nutrizione*, **21**, 30–44.

28 Dustmann, J.H. (1979) *Apiacta*, **14**, 7–11.

29 Buchner, R. (1966) *Südwestdeutscher Imker*, **18**, 240–241.

30 Christov, G. and Mladenov, S. (1961) *Comptes Rendus de l'Academie Bulgare des Sciences*, **14**, 303–306.

31 Floris, I. and Prota, R. (1989) *Apicoltore Moderno*, **80**, 55–67.

32 Abbas, T. (1997) *Living in the Gulf Magazine*, 50–51.

33 Amor, D.M. (1978) *Composition, Properties and Uses of Honey – A Literature Survey*, The British Food Manufacturing Industries Research Association, Leatherhead.

34 White, J.W. (1975) Composition of honey, in *Honey: A Comprehensive Survey*, (ed. E. Crane), Heinemann, London, pp. 157–206.

35 White, J.W. (1975) Physical characteristics of honey, in *Honey: A Comprehensive Survey*, (ed. E. Crane), Heinemann, London, pp. 207–239.

36 Rüegg, M. and Blanc, B. (1981) *Lebensmittel-Wissenschaft und Technologie*, **14**, 1–6.

37 Bogdanov, S., Rieder, K. and Rüegg, M. (1987) *Apidologie*, **18**, 267–278.

38 Tysset, C., Rousseau, M. and Durand, C. (1980) *Apiacta*, **15**, 51–60.

39 Leistner, L. and Rödel, W. (1975) The significance of water activity for micro-organisms in meats, in *Water Relations of Foods* (ed. R.B. Duckworth), Academic Press, London, pp. 309–323.

40 Scott, W.J. (1957) *Advances in Food Research*, **7**, 83–127.

41 Chirife, J., Scarmato, G. and Herszage, L. (1982) *Lancet*, **i**, 560–561.

42 Christian, J.H.B. and Waltho, J.A. (1964) *Journal of General Microbiology*, **35**, 205–218.

43 Topham, J. (2000) *Journal of Tissue Viability*, **10**, 86–89.

44 Bhanganada, K., Kiettiphongthavorn, V. and Wilde, H. (1986) *Journal of the Medical Association of Thailand*, **69**, 358–366.

45 Cooper, R.A., Molan, P.C. and Harding, K.G. (1999) *Journal of the Royal Society of Medicine*, **92**, 283–285.

46 Cooper, R.A., Molan, P.C. and Harding, K.G. (2002) *Journal of Applied Microbiology*, **93**, 857–863.

47 French, V.M., Cooper, R.A. and Molan, P.C. (2005) *Journal of Antimicrobial Chemotherapy*, **56**, 228–231.

48 Cooper, R.A., Wigley, P. and Burton, N.F. (2000) *Letters in Applied Microbiology*, **31**, 20–24.

49 Cooper, R.A., Halas, E. and Molan, P.C. (2002) *Journal of Burn Care and Rehabilitation*, **23**, 366–370.

50 Daghie, V., Cîrnu, I. and Cioca, V. (1971) Contribution to the study of the bacteriostatic and bactericidal action of honey produced by *Physokermes* sp. in the area of coniferous trees, in Proceedings of the XXIIIrd International Apicultural Congress, pp. 593–594.

51 Lindner, K.E. (1962) *Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, **115**, 720–736.

52 Plachy, E. (1944) *Zentralblatt für Bakteriologie: International Journal of Medical Microbiology*, **100**, 401–419.

53 Rychlik, M. and Dolezal, M. (1961) *Pszczelnicze Zeszyty Naukowe*, **5**, 53–64.

54 Stomfay-Stitz, J. and Kominos, S.D. (1960) *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, **113**, 304–309.

55 Coulthard, C.E., Michaelis, R., Short, W.F., Sykes, G., Skrimshire, G.E.H., Standfast, A.F.B., Birkinshaw, J.H., Raistrick, H. (1945) *Biochemical Journal*, **39**, 24–36.

56 Pothmann, F.J. (1950) *Zeitschrift für Hygiene und Infektionskrankheiten*, **130**, 468–484.

57 Roth, L.A., Kwan, S. and Sporns, P. (1986) *Journal of Food Protection*, **49**, 436–441.

58 Thimann, K.V. (1963) *The Life of Bacteria*, 2nd edn, Macmillan, New York.

59 Adcock, D. (1962) *Journal of Apicultural Research*, **1**, 38–40.

60 Radwan, S.S., El-Essawy, A.A. and Sarhan, M.M. (1984) *Zentralblatt fur Mikrobiologie*, **139**, 249–255.

61 White, J.W. and Subers, M.H. (1963) *Journal of Apicultural Research*, **2**, 93–100.

62 Schepartz, A.I. and Subers, M.H. (1964) *Biochimica et Biophysica Acta*, **85**, 228–237.

63 Alston, M.J. and Freedman, R.B. (2002) *Biotechnology and Bioengineering*, **77**, 641–650.

64 Bang, L.M., Buntting, C. and Molan, P.C. (2003) *Journal of Alternative and Complementary Medicine*, **9**, 267–273.

65 Schepartz, A.I. (1966) *Biochimica et Biophysica Acta*, **118**, 637–640.

66 Lineaweaver, W., McMorris, S., Soucy, D. and Howard, R. (1985) *Plastic and Reconstructive Surgery*, **75**, 394–396.

67 Thomas, S. (1990) *Wound Management and Dressings*, The Pharmaceutical Press, London.

68 Schreck, R., Rieber, P. and Baeuerle, P.A. (1991) *EMBO Journal*, **10**, 2247–2258.

69 Grimble, G.F. (1994) *New Horizons*, **2**, 175–185.

70 Pruitt, K.M. and Reiter, B. (1985) Biochemistry of peroxidase system: antimicrobial effects, in *The Lactoperoxidase System: Chemistry and Biological Significance* (eds K.M. Pruitt and J.O. Tenovuo) Marcel Dekker, New York, pp. 144–178.

71 Hyslop, P.A., Hinshaw, D.B., Scraufstatter, I.U., Cochrane, C.G., Kunz, S., Vosbeck, K. (1995) *Free Radical Biology and Medicine*, **19**, 31–37.

72 Bogdanov, S. (1984) *Lebensmittel-Wissenschaft und Technologie*, **17**, 74–76.

73 Schepartz, A.I. (1966) *Journal of Apicultural Research*, **5**, 167–176.

74 Dustmann, J.H. (1971) *Zeitschrift fur Lebensmittel-Untersuchung und -Forschung*, **145**, 294–295.

75 White, J.W. and Subers, M.H. (1964) *Journal of Apicultural Research*, **3**, 45–50.

76 White, J.W. and Subers, M.H. (1964) *Journal of Food Science*, **29**, 819–828.

77 James, O.B.O., Segree, W. and Ventura, A.K. (1972) *West Indian Medical Journal*, **21**, 7–17.

78 Allen, K.L., Molan, P.C. and Reid, G.M. (1991) *Journal of Pharmacy and Pharmacology*, **43**, 817–822.

79 Hodgson, M.J. (1989) Investigation of the antibacterial action spectrum of some honeys, MSc thesis, University of Waikato.

80 Molan, P.C. and Russell, K.M. (1988) *Journal of Apicultural Research*, **27**, 62–67.

81 Russell, K.M. (1983) The antibacterial properties of honey, MSc thesis, University of Waikato.

82 Willix, D.J., Molan, P.C. and Harfoot, C.J. (1992) *Journal of Applied Bacteriology*, **73**, 388–394.

83 Mohrig, W. and Messner, B. (1968) *Acta Biologica et Medica Germanica*, **21**, 85–95.

84 Russell, K.M., Molan, P.C., Wilkins, A.L. and Holland, P.T. (1988) *Journal of Agricultural and Food Chemistry*, **38**, 10–13.

85 Molan, P.C., Allen, K.L., Tan, S.T. and Wilkins, A.L. (1989) Identification of components responsible for the antibacterial activity of Manuka and Viper's Bugloss honeys, in *Annual Conference of the New Zealand Institute of Chemistry*, Paper Or1.

86 Molan, P.C. (1992) *Bee World*, **73**, 5–28.

87 Molan, P.C. (1992) *Bee World*, **73**, 59–76.

88 Miorin, P.L., Levy, N.C.J., Custodio, A.R., Bretz, W.A. and Marcucci, M.C. (2003) *Journal of Applied Microbiology*, **95**, 913–920.

89 Brudzynski, K. (2006) *Canadian Journal of Microbiology*, **52**, 1228–1237.

90 Willix, D.J., Molan, P.C. and Harfoot, C.J. (1992) *Journal of Applied Bacteriology*, **73**, 388–394.

91 Al Somai, N., Coley, K.E., Molan, P.C. and Hancock, B.M. (1994) *Journal of the Royal Society of Medicine*, **87**, 9–12.

92 Brady, N.F., Molan, P.C. and Harfoot, C.G. (1997) *Journal of Pharmaceutical Sciences*, **2**, 1–3.

93 Allen, K.L. and Molan, P.C. (1997) *New Zealand Journal of Agricultural Research*, **40**, 537–540.

94 Cooper, R.A. (1998) *Journal of Medical Microbiology*, **47**, 1140.

95 Cooper, R.A. and Molan, P.C. (1999) *Journal of Wound Care*, **8**, 161–164.

96 Irish, J., Carter, D.A., Shokohi, T. and Blair, S.E. (2006) *Medical Mycology*, **44**, 289–291.

97 Svenson, E.L. (2004) Investigating the potential of using honey for therapy of periodontal disease. MSc Thesis, University of Waikato.

98 Braniki, F.J. (1981) *Annals of The Royal College of Surgeons of England*, **63**, 348–352.

99 Cavanagh, D., Beazley, J. and Ostapowicz, F. (1970) *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **77**, 1037–1040.

100 Efem, S.E.E. (1993) *Surgery*, **113**, 200–204.

101 Efem, S.E.E. (1988) *British Journal of Surgery*, **75**, 679–681.

102 Phuapradit, W. and Saropala, N. (1992) *Australian and New Zealand Journal of Obstetrics and Gynaecology*, **32**, 381–384.

103 Armon, P.J. (1980) *Tropical Doctor*, **10**, 91.

104 Harris, S. (1994) *Primary Intention*, **2**, 18–23.

105 Ndayisaba, G., Bazira, L., Habonimana, E. and Muteganya, D. (1993) *Journal of Orthopaedic Surgery*, **7**, 202–204.

106 Subrahmanyam, M. (1998) *Burns*, **24**, 157–161.

107 Dumronglert, E. (1983) *Journal of the National Research Council of Thailand*, **15**, 39–66.

108 Vardi, A., Barzilay, Z., Linder, N., Cohen, H.A., Paret, G., Barzilai, A. (1998) *Acta Paediatrica*, **87**, 429–432.

109 Wadi, M., Al-Amin, H., Farouq, A., Kashef, H. and Khaled, S.A. (1987) *Arab Medico*, **3**, 16–18.

110 Abuharfeil, N., Al-Oran, R. and Abo-Shehada, M. (1999) *Food and Agricultural Immunology*, **11**, 169–177.

111 Tonks, A., Cooper, R.A., Price, A.J., Molan, P.C. and Jones, K.P. (2001) *Cytokine*, **14**, 240–242.

112 Tonks, A.J., Cooper, R.A., Jones, K.P., Blair, S., Parton, J., Tonks, A. (2003) *Cytokine*, **21**, 242–247.

113 Ryan, G.B. and Majno, G. (1977) *Inflammation*. Kalamazoo, Upjohn, MI.

114 Alnaqdy, A., Al-Jabri, A., Al Mahrooqi, Z., Nzeako, B. and Nsanze, H. (2005) *International Journal of Food Microbiology*, **103**, 347–351.

115 Lerrer, B., Zinger-Yosovich, K.D., Avrahami, B. and Gilboa-Garber, N. (2007) *ISME Journal*, **1**, 149–155.

116 Cokcetin, N. (2007) The effect of honey on problematic pathogens. BSc Thesis, University of Sydney.

117 English, H.K., Pack, A.R. and Molan, P.C. (2004) *Journal of the International Academy of Periodontology*, **6**, 63–67.

118 Al-Waili, N. (2003) *European Journal of Medical Research*, **8**, 295–303.

119 Haffejee, I.E. and Moosa, A. (1985) *British Medical Journal*, **290**, 1866–1867.

120 Salem, S.N. (1981) *Bulletin of Islamic Medicine*, **1**, 358–362.

121 McGovern, D.P.B., Abbas, S.Z., Vivian, G. and Dalton, H.R. (1999) *Journal of the Royal Society of Medicine*, **92**, 439.

122 Molan, P.C. (2001) *Bee World*, **82**, 22–40.

123 Emarah, M.H. (1982) *Bulletin of Islamic Medicine*, **2**, 422–425.

124 Popescu, M.P., Paloş, E. and Popescu, F. (1985) *Oftalmologia (Buchar)*, **19**, 53–60.

125 Mozherenkov, V.P. (1984) *Oftalmologicheskii Zhurnal*, 188.

126 Osaulko, G.K. (1953) *Vestnik Oftalmologii*, **32**, 35–36.

127 Molan, P.C. (2006) *International Journal of Lower Extremity Wounds*, **5**, 40–54.

128 Güneş, Ü.Y. and Eşer, I. (2007) *Journal of Wound, Ostomy and Continence Nursing*, **34**, 184–190.

129 Ingle, R., Levin, J. and Polinder, K. (2006) *South African Medical Journal*, **96**, 831–835.

130 McIntosh, C.D. and Thomson, C.E. (2006) *Journal of Wound Care*, **15**, 133–136.

131 Moolenaar, M., Poorter, R.L., van der Toorn, P.P., Lenderink, A.W., Poortmans, P., Gerardus Egberts, A.C. (2006) *Acta Oncologica*, **45**, 623–624.

132 Mphande, A.N., Killowe, C., Phalira, S., Jones, H.W. and Harrison, W.J. (2007) *Journal of Wound Care*, **16**, 317–319.

133 Nagane, N.S., Ganu, J.V., Bhagwat, V.R. and Subramanium, M. (2004) *Indian Journal of Clinical Biochemistry*, **19**, 173–176.

134 Tahmaz, L., Erdemir, F., Kibar, Y., Cosar, A. and Yalcyn, O. (2006) *International Journal of Urology*, **13**, 960–967.

135 Subrahmanyam, M. (1991) *British Journal of Surgery*, **78**, 497–498.

136 Subrahmanyam, M. (1993) *British Journal of Plastic Surgery*, **46**, 322–323.

137 Subrahmanyam, M., Sahapure, A.G., Nagane, N.S., Bhagwat, V.R. and Ganu, J.V. (2001) *Ann Burns Fire Disasters*, **XI**, 143–145.

138 Nagra, Z.M., Fayyaz, G.Q. and Asim, M. (2002) *Professional Medical Journal*, **9**, 246–251.

139 Al-Waili, N.S. and Saloom, K.Y. (1999) *European Journal of Medical Research*, **4**, 126–130.

140 Natarajan, S., Williamson, D., Grey, J., Harding, K.G. and Cooper, R.A. (2001) *Journal of Dermatological Treatment*, **12**, 33–36.

141 Simon, A., Sofka, K., Wiszniewsky, G., Blaser, G., Bode, U. and Fleischhack, G. (2006) *Support Care Cancer*, **14**, 91–97.

142 Johnson, D.W., van Eps, C., Mudge, D.W., Wiggins, K.J., Armstrong, K., Hawley, C.M., Campbell, S.B., Isbel, N.M., Nimmo, G.R., Gibbs, H. (2005) *Journal of the American Society of Nephrology*, **16**, 1456–1462.

143 Mutjaba Quadri, K.H. (1999) *Seminars in Dialysis*, **12**, 397–398.

144 Chambers, J. (2006) *Palliative Medicine*, **20**, 557.

145 Dunford, C., Cooper, R., Molan, P.C. and White, R. (2000) *Nursing Standard*, **15**, 63–68.

146 Dunford, C.E. (2001) *British Journal of Nursing*, **10**, 1058–1065.

147 Eddy, J.J. and Gideonsen, M.D. (2005) *Journal of Family Practice*, **54**, 533–535.

148 Visavadia, B.G., Honeysett, J. and Danford, M.H. (2008) *British Journal of Oral and Maxillofacial Surgery*, **45**, 55–56.

149 Molan, P.C. (2002) *Ostomy/Wound Management*, **48**, 28–40.

150 Tatnall, F.M., Leigh, I.M. and Gibson, J.R. (1991) *Journal of Hospital Infection*, **17**, 287–296.

151 Poon, V.K. and Burd, A. (2004) *Burns*, **30**, 140–147.

152 Bergman, A., Yanai, J., Weiss, J., Bell, D. and David, M.P. (1983) *American Journal of Surgery*, **145**, 374–376.

153 Gupta, S.K., Singh, H., Varshney, A.C. and Prakash, P. (1992) *Indian Journal of Animal Sciences*, **62**, 521–523.

154 Kumar, A., Sharma, V.K., Singh, H.P., Prakash, P. and Singh, S.P. (1993) *Indian Veterinary Journal*, **70**, 42–44.

155 Suguna, L., Chandrakasan, G., Ramamoorthy, U. and Joseph, K.T. (1993) *Journal of Clinical Biochemistry and Nutrition*, **14**, 91–99.

156 Suguna, L., Chandrakasan, G. and Thomas Joseph, K. (1992) *Journal of Clinical Biochemistry and Nutrition*, **13**, 7–12.