The antibacterial activity of honey and its role in treating diseases

Disclaimer: The content of this document is for general information purposes only and does not constitute, nor does it intend to constitute, medical or other professional advice.

IN BRIEF:

- Honey has an antimicrobial activity that is effective against all types of bacteria and some fungi
- It is fully effective against antibiotic-resistant strains of bacteria (the so-called "superbugs")
- It is effective against bacteria in biofilms and prevents formation of biofilms
- The antimicrobial activity is partly due to the high sugar content and the acidity of honey, but mostly to hydrogen peroxide formed by enzymic activity when honey is diluted
- Some honeys also have antibacterial activity due to non-peroxide components. Manuka honey can have a high level of this.
- Some honeys have as much as 100 times more antibacterial potency than others
- There is much clinical evidence for honey clearing infection in wounds
- Honey is effective only when in localised contact with bacteria, not after infection has penetrated into the blood-stream
- The antimicrobial action of honey is also used for treating eye infections and has potential for treating nasal infections, gum disease, gastroenteritis, fungal infections of the skin, and mastitis in dairy cows and goats

CONTENTS OF THIS ARTICLE:

Introduction

In an editorial in the *Journal of the Royal Society of Medicine* in 1989[1], titled "Honey - a remedy rediscovered" the view was expressed that "The therapeutic potential of uncontaminated pure honey is grossly under-utilised" and that "The time has now come for conventional medicine to lift the blinds off this 'traditional remedy' and give it its due recognition." It referred to the many papers published reporting good results when honey was used as a dressing on infected wounds. In many of these honey was used where antibiotics were failing to clear the infection. The number of papers published on the use of honey on wounds has been rapidly increasing in more recent years. This probably

reflects the rapid increase in the problem of bacteria developing resistance to antibiotics. This article describes the nature of the antimicrobial activity of honey, the spectrum of bacterial species against which it has been found to be active, and the evidence for its clinical effectiveness in clearing infection.

History

Honey was used by the ancient Egyptians, Assyrians, Chinese, Greeks and Romans to treat wounds and diseases of the gut[2]. Its use for the treatment of diarrhoea was recommended by the Muslim prophet Mohammed[3]. Aristotle[4] wrote of honey being a salve for wounds and sore eyes and Dioscorides c.50 AD wrote of honey being "good for all rotten and hollow ulcers"[5].

The use of honey to treat infections has continued into present-day folk-medicine. In India, lotus honey is traditionally used to treat eye diseases[6]; in Ghana to treat infected leg ulcers[7]; in Nigeria to treat earache[8]; in Mali it is applied on the spots of measles, and in the eyes in measles to prevent scarring of the cornea[9]. Honey also has a traditional usage for the treatment of gastric ulcers[10]. Its ancient usage to treat sore throats has continued into the traditional medicine of modern times[11].

Discovery of the nature of the antibacterial activity of honey

It used to be assumed that it was the high sugar content of honey that was responsible for it killing bacteria. Sugar is used as a preservative of fruit in jam/jelly conserves, where by osmosis it draws water out of bacterial cells and thus deprives them of something essential for life. The first indication that there was something more involved than osmosis was the discovery by Sackett in 1919[12] that the antibacterial potency of honey was increased rather than decreased by dilution of honey with water, an observation that was hard to explain. Two decades later research by Dold *et al.*[13] led to the discovery of an antibacterial factor which they termed "inhibine". This term was widely used for the next 26 years until the antibacterial factor was identified as hydrogen peroxide by White *et al.*[14].

The antibacterial components of honey

High sugar content

The osmolarity of honey, due to about 80% of its composition being sugar, is on its own sufficient to prevent growth of bacteria and fungi. Granulated honey is a saturated solution of sugars, and clear honey is a super-saturated solution. Honey with a high water content can spoil because some species of yeasts can live in it. But no fermentation occurs if the water content is below 17.1%[15]. The water content of honey is usually 15-21%[16]. Bacteria are far less tolerant of high levels of sugar than fungi are. Many species of bacteria will not survive in the osmolarity of honey diluted to about 10% honey. However, *Staphylococcus aureus*, the most common wound-infecting species, has an exceptionally high tolerance of osmolarity and will survive in the osmolarity of honey at concentrations up to 30%.

There have been many reports of granulated sugar being used as a wound dressing[17], but it has been reported that infection is not cleared or new infection gets established in cases where urine or heavy outflow of fluid from wounds dilutes the sugar[18]. This means that dressings have to be changed very frequently, as would be the case using honey which did not have a good level of additional antibacterial components.

Acidity

Part of the antibacterial activity of honey is due also to its acidity being sufficient to stop bacteria from growing. Honey usually has a pH between 3.2 and 4.5[16], owing primarily to its content of gluconolactone/gluconic acid[19]. This is formed by the action of the enzyme glucose oxidase which bees add to the nectar they collect to make honey (see below).

But the concentration of the acid in honey is low, so there is neutralisation of the acidity when honey is mixed with wound fluid or saliva. The concentration of bicarbonate in the fluid surrounding the cells of the body is such that the

dilution of an average honey with an equal volume of extracellular fluid would raise the pH of the honey to near neutrality (pH 6.8). This means that where honey gets diluted by body fluid the acidity of honey makes a minor contribution to antibacterial activity.

Hydrogen peroxide

Hydrogen peroxide is the major antimicrobial factor in most honeys. Adcock[20] found that the antibacterial activity of honey could be removed by the addition of catalase (an enzyme which catalyses the destruction of hydrogen peroxide). White *et al.*[14] demonstrated a direct relationship between the hydrogen peroxide produced and the potency of the antibacterial activity 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. Oxygen needs to be available for the reaction:

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

The antimicrobial activity from hydrogen peroxide can only be of use where honey is exposed to air. The production of hydrogen peroxide during the ripening of honey serves to sterilise the honey stored in the comb, but undiluted ripened honey has a negligible level of hydrogen peroxide[14, 21, 22]. This is because hydrogen peroxide that has been formed in honey disappears as a result of reaction with other components of the honey.

Glucose oxidase is practically inactive in full-strength honey. It becomes active to form hydrogen peroxide only when the honey is diluted[14]. 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 pH 8, but the activity drops off sharply below pH 5.5 to near zero at pH 4[23]. Another possible explanation is that that enzymes need a sufficiently high level of free water to be active[24], and in undiluted honey the water that is present is almost all bound up on the sugar molecules.

As honey is diluted the activity of glucose oxidase increases to a peak at a concentration around 30–50% (v/v) honey as the level of free water is increased, then falls again as the enzyme and glucose concentrations are decreased by further dilution[25]. Honey solutions have been 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% (v/v) to 67% (v/v)[25].

It was found that when 50% solutions of honey were incubated, hydrogen peroxide accumulated to a peak level then the concentration of hydrogen peroxide dropped. It dropped to zero after 24–48 hours[25]. This is probably the result of damage to the enzyme by accumulated hydrogen peroxide, as it was reported that addition of hydrogen peroxide to glucose oxidase isolated from honey caused a significant decline in the enzyme's rate of reaction after 20 minutes[26]. This means that honey in which the antibacterial activity is due to hydrogen peroxide has antimicrobial activity for a limited time once it has been diluted. This has implications for the use of such honey in wound dressings which are not changed frequently – the honey may cease to have antibacterial activity after it has been diluted by fluid oozing from the wound.

However, having a peak for accumulation means that hydrogen peroxide does not accumulate to levels that are harmful to body tissues. The use of hydrogen peroxide as an antiseptic has been discouraged because it is toxic to wound tissues[27], but at the low levels that form in honey this is not a problem. The maximum concentration of hydrogen peroxide achieved when a 50% solution of a honey with a high level of antibacterial activity was incubated was found to be 242 times less than in the 3% solution of hydrogen peroxide normally used as an antiseptic[25].

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. It has been found that it is oxidative free radicals formed from hydrogen peroxide, rather than hydrogen peroxide itself, that trigger the inflammatory response in white blood cells[28]. This can be prevented by antioxidants[29].

The low levels of accumulation of hydrogen peroxide that occurs in diluted honey still gives 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 in one go[30]. Bacteria (*Escherichia 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[31]. Rates of production of hydrogen peroxide in diluted honey have been reported to be in the range of 0–0.6 mmol/l/h[20, 22, 25, 32].

The level of hydrogen peroxide achieved in diluted honey varies from honey to honey. Some floral sources can affect the enzyme activity that gives rise to hydrogen peroxide and others affect the destruction of hydrogen peroxide that has formed. The level of hydrogen peroxide achieved is a balance between the rate of its production and the rate of its destruction[22]. Catalase, an enzyme that destroys hydrogen peroxide, has been shown to be present in honey[33]. It comes from the pollen and nectar of certain plants, more so from the nectar[34]. The disappearance of hydrogen peroxide added to honey which has been boiled beforehand to inactivate catalase shows that loss though chemical reaction is involved as well as loss through destruction by catalase[14].

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 heat-stability of the glucose oxidase in them[35]. Very large differences are also seen in the sensitivity of glucose oxidase to damage by exposure to light[36], particularly daylight and the light from fluorescent tubes[22].

So, to summarise, the antibacterial activity in honey that is due to hydrogen peroxide will depend on the exposure the honey has had to heat and light in its processing and storage, as well as it depending on the floral source of the honey.

Additional antibacterial components

In some honeys there are other substances responsible for antibacterial activity besides the sugar content, acidity and hydrogen peroxide. Reports of antibacterial activity in honey that is stable to heating well in excess of the variation in stability of glucose oxidase indicate that hydrogen peroxide is not the only antibacterial factor in diluted honey. Conifer honeydew honey, with exceptionally high antibacterial activity, has been reported to contain a heat-stable antibacterial factor as well as the heat-sensitive enzyme which produces hydrogen peroxide[37]. There have been many reports of antibacterial activity persisting in honeys treated with catalase to remove hydrogen peroxide[20, 32, 38-42].

The bacteria-destroying enzyme lysozyme has been identified in honey, with higher levels if the honey is freshly extracted from the comb, but at much lower levels in older samples[43]. 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[32]. Some phenolic acid components of manuka (*Leptospermum scoparium*) honey with antibacterial activity have been identified[44] but these were later found to account for no more than 4% of the non-peroxide antibacterial activity of diluted honey[45]. In viper's bugloss (*Echium vulgare*) honey this type of activity was accounted for entirely by its content of 1,4-dihydroxybenzene[45], but the activity was very low compared with that of manuka honey[38].

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. There are several reasons why this is likely to be more effective in treating infections than honey with antibacterial activity due to hydrogen peroxide is. (See "What's special about *Active Manuka Honey*?" http://www.academia.edu/attachments/30239537/download_file .)

Variation in the potency of the antibacterial activity of honey

It was found that honeys varied in the potency of their antibacterial activity, and in 1955 the term "inhibine number" was coined to indicate the potency[46]. The "inhibine number" was the number of dilution steps a honey could be

subjected to and still have antibacterial activity. More recent research has found that where a wide range of different honeys has been tested against a single species of microorganism the potency varies much more than that. The minimum inhibitory concentration (MIC) of honey is the lowest concentration to which it can be diluted and still stop bacteria from growing. The MIC of honey (with the concentration expressed as % honey in solution) has been reported from this research to range from 25% to 0.25%[47]; from more than 50% to 1.5%[48]; from 20% to 0.6%[49]; and from 50% to 1.5%[50].

The ancient physicians who prescribed honey for various ailments were aware that some honeys were better others for medical usage: Aristotle c. 350 BC[4], discussing differences in honeys, referred to pale honey being "good as a salve for sore eyes and wounds". Dioscorides c.50 AD stated that a pale yellow honey from Attica was the best[5] In present-day traditional medicine in some countries specific types of honey are recognised as being the best to use: the strawberry-tree honey of Sardinia[51], lotus honey in India[6] honey from the Jirdin valley of Yemen (which is sidr honey) in the Middle East[52].

Generally it has not been known that honey varies in antibacterial potency and that there should therefore be selection of honey for use as an antibacterial agent. So most clinical treatment and microbiological studies published have been done with honey with an unknown level of antimicrobial activity.

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 sugar in 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 as much as 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 used to treat infections.

There are now many producers selling honey which has the potency of its antibacterial activity rated in comparison to that of a standard antiseptic, phenol. (See http://www.academia.edu/attachments/30239604/download_file.) If these honeys with standardised antibacterial activity are used, the purchaser can relate the potency of their honey to research results published for honey with standardised antibacterial activity. Take for example a published MIC for a species of bacteria that was found to be 4% honey in research where the standardised honey had activity equivalent to 20% phenol. If a consumer bought a standardised honey which had activity stated to be equivalent to only 10% phenol (*i.e.* with a potency only half of that used in the research) then it could be diluted only half as much and still stop bacteria from growing (*i.e.* its MIC would be 8% honey).

Research using standardised honey with a potency that is about mid-range for honey (which is equivalent to about 15% phenol) has found that the MIC for bacteria is mostly below 10% honey. That means that even if the honey gets diluted ten times the solution of honey will still have enough antibacterial potency to stop the growth of bacteria. If a standardised honey were purchased which had a potency rated as equivalent to 5% phenol this could be diluted only about three times. But often quite a lot of dilution of honey by body fluids occurs when honey is used to treat infections, and there are other reasons for which it is important to have a level of antibacterial activity greater than the MIC. (See article on selection of honey: http://www.academia.edu/attachments/30239728/download_file.) The results obtained in studies by various researchers on the sensitivity of various species of bacteria and fungi are shown in the table on the following page.

Table showing the minimum inhibitory concentration (MIC) of honeys (shown as %, by volume, of honey in solution) found for various species of bacteria and fungi

These are results reported in research studies where honeys with standardised antibacterial activity were used. The rating of the antibacterial activity of the honeys used is shown as the concentration of phenol (%) with equivalent activity (see "How the antibacterial activity of manuka honey is rated": http://www.academia.edu/attachments/30239604/download_file). For the manuka honeys the antibacterial activity rating is shown as "NPA" (*i.e.* the special "non-peroxide activity" of manuka honey). Most of the results in this table obtained with manuka honey were in assays with catalase added to destroy hydrogen peroxide. Where the MIC of manuka honey was measured without catalase added the antibacterial activity may have been augmented with hydrogen peroxide produced in the honey: these results are marked with an asterisk. Average values are shown where the results are from studies where there were multiple strains/isolates of the bacteria.

Species of microorganism	MIC	Type of honey	Activity rating	Reference no.
Antibiotic-resistant strains of bacteria:				
Pan-resistant *Acinetobacter baumannii*	8*	Medihoney® (blend)	NPA ≥18	[53]
Burkholderia cepacia (multi-resistant)	2.9	Manuka	NPA 13.2	[54]
"	3.6	Pasture	14.8	[54]
ESBL (extended-spectrum beta-lactamase) strains:				
Escherichia coli	6.6	Manuka	NPA 16.5	[55]
"	6.4*	Medihoney® (blend)	NPA ≥18	[53]
Klebsiella pneumoniae	6.2*	Medihoney® (blend)	NPA ≥18	[53]
Enterobacter cloacae	9.2	Manuka	NPA 16.5	[55]
Enterobacter species	8.1	Manuka	NPA 16.5	[55]
MRSA (methicillin-resistant *Staphylococcus aureus*):	3	Manuka	NPA 13.2	[56]
"	2.98	Manuka	NPA 18	[57]
"	4*	Medihoney® (blend)	NPA ≥18	[53]
"	3.07	Pasture	13.7	[57]
"	4	Pasture	14.8	[56]
Multi-resistant *Stenotrophomonas maltophilia*	14.1*	Manuka	NPA ≥15	[58]
VRE (vancomycin-resistant *Enterococcus* species):				
Enterococcus avium	3.83	Manuka	NPA 18	[57]
"	5.6	Pasture	13.7	[57]
Enterococcus faecalis	4.59	Manuka	NPA 18	[57]
"	9.43	Pasture	13.7	[57]
" (VanA/B strains)	8*	Medihoney® (blend)	NPA ≥18	{George, 2007 #1729

Enterococcus faecium	4.72	Manuka	NPA 18	{Cooper, 2002 #318}
"	8.33	Pasture	13.7	[57]
" (VanA/B strains)	8*	Medihoney® (blend)	NPA ≥18	[53]
Enterococcus raffinosus	4.86	Manuka	NPA 18	[57]
"	9.0	Pasture	13.7	[57]

Coagulase-negative Staphylococci (many strains antibiotic-resistant):

Staphylococcus capitis	3.3	Manuka	NPA 16.8	[59]
"	3.8	Pasture	17.5	[59]
Staphylococcus epidermidis	3.5	Manuka	NPA 16.8	[59]
"	3.3	Pasture	17.5	[59]
Staphylococcus haemolyticus	3.3	Manuka	NPA 16.8	[59]
"	4.2	Pasture	17.5	[59]
Staphylococcus simulans	3	Manuka	NPA 16.8	[59]
"	4	Pasture	17.5	[59]
Staphylococcus warneri	3.3	Manuka	NPA 16.8	[59]
"	3.5	Pasture	17.5	[59]

Gram-negative species of bacteria:

Enterobacter cloacae	6*	Medihoney® (blend)	NPA ≥18	[53]
Escherichia coli	5	Manuka	NPA 13.2	[56]
"	3.7	Manuka	NPA 13.2	[42]
"	9	Pasture	14.8	[56]
"	7.1	Rewarewa	21.5	[42]
Klebsiella oxytoca	5	Manuka	NPA 13.2	[56]
"	8	Pasture	14.8	[56]
Proteus mirabilis	7.3	Manuka	NPA 13.2	[42]
"	3.3	Rewarewa	21.5	[42]
Pseudomonas aeruginosa	10.8	Manuka	NPA 13.2	[42]
"	6	Manuka	NPA 13.2	[56]
"	13.7*	Medihoney® (blend)	NPA ≥18	[53]

"	9	Pasture	14.8	[56]
"	6.8	Rewarewa	21.5	[42]
Pseudomonas species from burns	9.71	Manuka	NPA 18	[60]
"	9.0	Pasture	14.8	[60]
Pseudomonas species from wounds	6.9	Manuka	NPA 13.2	[61]
"	7.1	Pasture	14.8	[61]
Serratia marcescens	6.3	Manuka	NPA 13.2	[42]
"	4.7	Rewarewa	21.5	[42]
Gram-positive species of bacteria which infect wounds:				
Enterococcus faecalis	7	Manuka	NPA 13.2	[56]
"	4.92	Manuka	NPA 18	[57]
"	8.2*	Manuka	NPA 18	[62]
"	9.66	Pasture	13.7	[57]
"	9	Pasture	14.8	[56]
Staphylococcus aureus	3	Manuka	NPA 13.2	[56]
"	2.9	Manuka	NPA 13.2	[63]
"	1.8	Manuka	NPA 13.2	[42]
"	5	Manuka	NPA 13.2	[64]
"	4*	Medihoney® (blend)	NPA ≥18	[53]
"	3.8	Pasture	14.8	[63]
"	5	Pasture	14.8	[56]
"	4.9	Rewarewa	21.5	[42]
"	5	Rewarewa	21.5	[64]
Streptococcus pyogenes	3.6	Manuka	NPA 13.2	[42]
"	8.5*	Manuka	NPA 18	[62]
"	2.6	Rewarewa	21.5	[42]
Streptococcus equisimilis	7.9*	Manuka	NPA 18	[62]
Streptococcus dysgalactiae subspecies *equisimilis*	7.6*	Manuka	NPA 18	[62]
Streptococcus porcinus	6.4*	Manuka	NPA 18	[62]
Bacteria which cause gastrointestinal infections:				
Campylobacter coli	1.1*	Manuka	NPA 29.4	[65]

Campylobacter jejuni	1.0*	Manuka	NPA 29.4	[65]
Escherichia coli	7.5	Manuka	NPA 16.5	[55]
Helicobacter pylori	5	Manuka	NPA 13.2	[66]
Yersinia enterocolitica	7.5	Manuka	NPA 16.5	[55]
Salmonella typhimurium	6	Manuka	NPA 13.2	[42]
"	4.1	Rewarewa	21.5	[42]
Salmonella typhimurium Phage type 4	6.8	Manuka	NPA 16.5	[55]
Salmonella typhimurium DT104	7.5	Manuka	NPA 16.5	[55]
Salmonella mississippi	8.5	Manuka	NPA 16.5	[55]
Salmonella enteritidis	6.8	Manuka	NPA 16.5	[55]
Enterobacter aerogenes	16.6	Manuka	NPA 16.5	[55]
Enterobacter cloacae	16.0	Manuka	NPA 16.5	[55]
Shigella flexneri	9.4	Manuka	NPA 16.5	[55]
Shigella sonnei	8.9	Manuka	NPA 16.5	[55]
Anaerobic bacteria that cause periodontal disease:				
Actinobacillus actinomycetemcomitans	6.1	Manuka	NPA 15	[67]
"	4.8	Pasture	18.2	[67]
Actinomyces gerencseriae	7	Manuka	NPA 15	[67]
"	9	Pasture	18.2	[67]
Actinomyces naeslundii	9.1	Manuka	NPA 15	[67]
"	4	Pasture	18.2	[67]
Eikenella corrodins	4.7	Manuka	NPA 15	[67]
"	5.8	Pasture	18.2	[67]
Fusobacterium nucleatum	5.1	Manuka	NPA 15	[67]
"	6.7	Pasture	18.2	[67]
Peptostreptococcus micros	9	Manuka	NPA 15	[67]
"	9.3	Pasture	18.2	[67]

Porphyromonas gingivalis	6.2	Manuka	NPA 15	[67]
"	9	Pasture	18.2	[67]
Veillonella parvula	7.2	Manuka	NPA 15	[67]
"	7	Pasture	18.2	[67]

Bacteria causing mastitis in dairy animals:

Actinomyces pyogenes	5	Manuka	NPA 13.2	[64]
"	5	Rewarewa	21.5	[64]
Klebsiella pneumoniae	10	Manuka	NPA 13.2	[64]
"	10	Rewarewa	21.5	[64]
Nocardia asteroides	5	Manuka	NPA 13.2	[64]
"	10	Rewarewa	21.5	[64]
Streptococcus agalactiae	5	Manuka	NPA 13.2	[64]
"	10	Rewarewa	21.5	[64]
Streptococcus dysgalactiae	5	Manuka	NPA 13.2	[64]
"	10	Rewarewa	21.5	[64]
Streptococcus uberis	5	Manuka	NPA 13.2	[64]
"	10	Rewarewa	21.5	[64]

Yeasts:

Candida albicans	21.8	Manuka	NPA 15	[67]
"	39.9	Manuka	NPA ≥18	[68]
"	38.2	Medihoney® (blend)	NPA ≥18	[68]
"	40	Pasture	18.2	[67]
"	18.5	Jarrah	30.2	[68]
Candida dubliniensis	33.4	Manuka	NPA ≥18	[68]
"	34.6	Medihoney® (blend)	NPA ≥18	[68]
"	15.4	Jarrah	30.2	[68]
Candida glabrata	40	Manuka	NPA 15	[67]
"	42.6	Manuka	NPA ≥18	[68]
"	43.1	Medihoney® (blend)	NPA ≥18	[68]

"	40	Pasture	18.2	[67]
"	29.9	Jarrah	30.2	[68]

Fungi which cause tineas:

Epidermophyton floccosum	10	Manuka	NPA 13.2	[69]
"	10	Pasture	14.8	[69]
Microsporum canis	25	Manuka	NPA 13.2	[69]
"	15	Pasture	14.8	[69]
Microsporum gypseum	50	Manuka	NPA 13.2	[69]
"	20	Pasture	14.8	[69]
Trichophyton mentagrophytes var. interdigitale	25	Manuka	NPA 13.2	[69]
"	15	Pasture	14.8	[69]
Trichophyton mentagrophytes var. mentagrophytes	20	Manuka	NPA 13.2	[69]
"	15	Pasture	14.8	[69]
Trichophyton rubrum	10	Manuka	NPA 13.2	[69]
"	5	Pasture	14.8	[69]
Trichophyton tonsurans	25	Manuka	NPA 13.2	[69]
"	20	Pasture	14.8	[69]

Antibiotic-resistant bacteria

As can be seen in the table, the antibiotic-resistant strains of bacteria that have been studied have been found to have just about the same sensitivity to honey as the antibiotic-sensitive strains of the same species. There is concern with the use of any antibacterial agent that strains of bacteria will develop resistance to it as has happened with antibiotics. Resistance to antibiotics is the result of selective breeding, and cross-breeding, producing strains of bacteria with high levels of activity from ancient genes. Because of its high osmotic activity due to its sugar content 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. Studies have been carried out that were designed to select for resistant mutants. This was done by continuous exposure of cultures of bacteria to gradually increasing concentrations of honey that were just sub-lethal. Various species of bacteria which are notorious for developing resistance to antibiotics were used. In one study no increased resistance to honey was developed yet under the same experimental conditions marked increases in resistance to antibiotics were developed[70] In the other study reduced sensitivity to manuka honey in the training with increasing concentrations was found, but this was not a permanent change. Mutant strains resistant to honey were not detected. It was concluded that the risk of bacteria developing resistance to honey will be low if high concentrations of honey are maintained in situations in the body where it is used clinically[71].

Effectiveness on bacteria in biofilms

Some species of bacteria in some situations form biofilms. These are bacteria embedded in a gel composed of protein and/or polysaccharide. The film coats the surface of solid material and enables the bacteria to remain attached to the surface. It also provides protection for the bacterial cells. A well-known example of a biofilm is the plaque which forms on the surface of teeth.

Biofilms form on the surface of open wounds and the nasal sinuses. The protection that they provide for the bacterial cells is thought to decrease the effectiveness of treatment with antibacterial agents. Research has found that manuka honey is effective against bacteria in biofilms.

A 33% solution of manuka honey (NPA 28) has been reported to kill *Staphylococcus aureus* in a biofilm[72]. Medihoney® (NPA ≥18) at a concentration of 6% has been reported to kill MRSA and MRSE (methicillin-resistant *Staphylococcus epidermidis*) in biofilms, and at a concentration of 12% has been reported to kill *Pseudomonas aeruginosa* and ESBL *Klebsiella pneumoniae* in biofilms[73]. The formation of biofilms by these species of bacteria was prevented at concentrations of honey half of these[73]. In another study, killing of *Pseudomonas aeruginosa* in biofilms was seen with 40% solutions of Medihoney® but a 20% concentration of the honey was less effective. Medihoney® in solution at a concentration of 10% was found to cause some killing of *Streptococcus pyogenes* in biofilms and release of bacterial cells, decreasing the biomass in the biofilm by 72%: at a concentration of 20% it decreased the formation of biofilms by 90%[74]. Manuka honey and sidr honey, with their level of antibacterial potency not stated, were found to kill most strains of the bacteria when biofilms formed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* were exposed to honey in solution at a concentration of 50%[75].

Clinical uses of honey as an antimicrobial agent

When antibiotics are used to treat infections the usual practice is to first identify the species of the infecting bacterium and to test its sensitivity to antibiotics. This is done to ensure that an antibiotic that may be used for treatment is appropriate for the infecting species, and to ensure that the particular strain of bacteria is not resistant to the antibiotic. This is not necessary when honey is used. The very broad spectrum of antimicrobial activity of honey and the absence of strains of bacteria with resistance to it means that it is almost certain to be effective if a honey with an appropriate standardised level of antibacterial potency is used.

There is a serious and increasing problem of bacteria almost inevitably developing resistance to antibiotics where these are extensively used. For this reason efforts are being made to decrease the usage of antibiotics. The low chance of resistance to honey developing makes the use of honey an attractive alternative for control of infection in situations such as wounds where honey can be applied. For example, patients with kidney failure who have indwelling catheters for dialysis treatment have a risk of getting blood-stream infections from coagulase-negative Staphylocci which grow on the catheters. The usual way of protecting patients from this risk is to apply the antibiotic mupirocin, but this long-term use of the antibiotic makes it likely that strains of bacteria resistant to mupirocin will develop. Honey has been found to be as effective as mupirocin in preventing blood-stream infections in these patients and removes the risk of developing antibiotic-resistant strains of bacteria[76].

When patients in hospitals get life-threatening infections with antibiotic-resistant bacteria this cross-infection usually gets into their body via catheters or open wounds. Cross-infection in hospitals with antibiotic-resistant strains of bacteria can potentially be prevented by dressing all open wounds or catheter exit sites with honey. The effectiveness of honey in killing antibiotic-resistant strains of bacteria has been demonstrated not only in the laboratory studies listed in the table above but also in clinical cases. Honey has been reported to be effective in clearing established infection with MRSA and VRE[77-83].

The major usage of honey for treatment of infection has been in wound care[84], but there increasing interest in its use to treat infections in nasal sinuses[72, 75, 85-87]. Honey has been registered with the regulatory authorities in Australia for use in the eyes, and a small clinical trial has established its effectiveness in treating gingivitis[88]. The publication of a report that inhalation of an aerosol of a 60% solution of honey causes no adverse effects[89] has raised the possibility of using honey for treatment of lung infections[54].

There is a large amount of evidence from clinical trials that demonstrates the effectiveness of honey in clearing infection in wounds. This has been reviewed in papers which can be downloaded which cover **trials published up to 2005** [Link: http://researchcommons.waikato.ac.nz/handle/10289/229] and **trials published 2006–2011** [Link: http://hdl.handle.net/10289/6095]. There have also been published papers each reporting honey clearing infection in multiple cases that were treated: Fournier's gangrene (necrotising fasciitis on the scrotum)[90]; large infected surgical wounds on infants, not responding to antibiotic treatment[91]; various types of wounds and ulcers not responding to antibiotic treatment[92]; broken down wounds following surgery (vulvectomy)[93]; surgical wounds on patients with immunosuppression because of chemotherapy[82]; broken down wounds following surgery (caesarian section)[94].

Limitations to usage of honey to treat infections

Many websites and the popular literature on health and self-treatment of ailments give the impression that honey can be taken to cure almost anything, but a rational consideration would suggest that the antimicrobial activity would be insignificant when an oral dose of honey becomes diluted after absorption from the gut into the many litres of fluid in the circulation and tissues of the body. Realistically, the potential for honey as an antimicrobial agent in medicine is where it is in direct contact with the site of infection rather than as a systemic agent, although there are some situations such as gastrointestinal infections or mastitis in dairy cows and goats where the honey could remain localised and thus not become too dilute to be effectively antibacterial.

In the very large number of cases that have been reported of treatment with honey of open wounds, and the numerous cases of treatment of the eyes, there have been no reports of adverse effects other than a stinging sensation. The stinging has been found to be due to the acidity of honey and is experienced most when there is inflammation present. This is because inflammation sensitises the nerve endings which detect acidity. The stinging can be severe enough to prevent some people from being able to tolerate honey dressings being applied. The honey dressings that are on sale which have the honey in a gel or covered by a layer of gel decrease the amount of stinging experienced because the gel slows the release of the acidity of the honey into the body tissue. Applying a layer of gel to a wound before applying a honey dressing will also decrease the stinging experienced.

REFERENCES

1. Zumla A. & Lulat A. (1989) Honey - a remedy rediscovered. *Journal of the Royal Society of Medicine* **82**(7):384-5.
2. Stomfay-Stitz J. (1960) Honey: an ancient yet modern medicine. *Science Counsellor* **23**:110-25.
3. Al-Bukhari M. Sahih Al-Bukhari. Third, revised ed. Chicago: Kazi Publications; (c. 740 A.D.) 1976.
4. Aristotle. Historia Animalium. Smith JA & Ross WD, editors. Oxford, U.K.: Oxford University; 1910.
5. Gunther R. T. The Greek Herbal of Dioscorides. New York: Hafner; 1934 (Reprinted 1959).
6. Fotidar M. R. & Fotidar S. N. (1945) 'Lotus' honey. *Indian Bee Journal* **7**:102.
7. Ankra-Badu G. A. (1992) Sickle cell leg ulcers in Ghana. *East African Medical Journal* **69**(7):366-9.
8. Obi C. L., Ugoji E. O., Edun S. A., Lawal S. F.*et al.* (1994) The antibacterial effect of honey on diarrhoea causing bacterial agents isolated in Lagos, Nigeria. *African Journal of Medical Sciences* **23**:257-60.
9. Imperato P. J. & Traoré D. (1969) Traditional beliefs about measles and its treatment among the Bambara of Mali. *Tropical and Geographical Medicine* **21**:62-7.
10. Kandil A., El-Banby M., Abdel-Wahed K., Abdel-Gawwad M.*et al.* (1987) Curative properties of true floral and false nonfloral honeys on induced gastric ulcer. *Journal of Drug Research (Cairo)* **17**(1-2):103-6.
11. Beck B. F. & Smedley D. Honey and Your Health. Second ed. New York: McBride; 1944.
12. Sackett W. G. Honey as a carrier of intestinal diseases. Bulletin of the Colorado State University Agricultural Experimental Station, 1919:18.
13. Dold H., Du D. H. & Dziao S. T. (1937) Nachweis antibakterieller, hitze- und lichtempfindlicher Hemmungsstoffe Inhibine im Naturhonig Blütenhonig. *Zeitschrift für Hygiene und Infektionskrankheiten* **120**:155-67.
14. White J. W., Subers M. H. & Schepartz A. I. (1963) The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. *Biochimica et Biophysica Acta* **73**:57-70.
15. Amor D. M. Composition, properties and uses of honey - a literature survey. Leatherhead, U.K.: The British Food Manufacturing Industries Research Association1978. Report No.: Scientific and Technical Surveys No.108.
16. White J. W. Composition of honey. In: Crane E (Ed). Honey: a Comprehensive Survey. London: Heinemann, 1975:157-206.
17. Topham J. (2000) Sugar for wounds. *Journal of Tissue Viability* **10**(3):86-9.
18. Bhanganada K., Kiettiphongthavorn V. & Wilde H. (1986) The use of super-saturated sucrose solution for chronic skin ulcers (Resurrection of an old remedy). *Journal of the Medical Association of Thailand* **69**(7):358-66.
19. White J. W. Physical characteristics of honey. In: Crane E (Ed). Honey: a Comprehensive Survey. London: Heinemann, 1975:207-39.
20. Adcock D. (1962) The effect of catalase on the inhibine and peroxide values of various honeys. *Journal of Apicultural Research* **1**:38-40.
21. Radwan S. S., El-Essawy A. A. & Sarhan M. M. (1984) Experimental evidence for the occurrence in honey of specific substances active against microorganisms. *Zentralblatt für Mikrobiologie* **139**(4):249-55.
22. White J. W. & Subers M. H. (1963) Studies on honey Inhibine. 2. A chemical assay. *Journal of Apicultural Research* **2**(2):93-100.
23. Schepartz A. I. & Subers M. H. (1964) The glucose-oxidase of honey. I. Purification and some general properties of the enzyme. *Biochimica et Biophysica Acta* **85**:228-37.

24. Alston M. J. & Freedman R. B. (2002) The water-dependence of the catalytic activity of bilirubin oxidase suspensions in low-water systems. *Biotechnology and Bioengineering* **77**(6):651-7.

25. Bang L. M., Buntting C. & Molan P. C. (2003) The effect of dilution on the rate of hydrogen peroxide production in honey and its implications for wound healing. *Journal of Alternative and Complementary Medicine* **9**(2):267-73.

26. Schepartz A. I. (1966) The glucose-oxidase of honey. IV. Some addition observations. *Biochimica et Biophysica Acta* **118**:637-40.

27. Thomas S. Wound management and dressings. Thomas S, editor. London: The Pharmaceutical Press; 1990.

28. Schreck R., Rieber P. & Baeuerle P. A. (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kB transcription factor and HIV-1. *EMBO Journal* **10**(8):2247-58.

29. Grimble G. F. (1994) Nutritional antioxidants and the modulation of inflammation: theory and practice. *New Horizons* **2**(2):175-85.

30. Pruitt K. M. & Reiter B. Biochemistry of peroxidase system: antimicrobial effects. In: Pruitt KM & Tenovuo JO (Eds). The Lactoperoxidase System: Chemistry and Biological Significance. New York: Marcel Dekker, 1985:144-78.

31. Hyslop P. A., Hinshaw D. B., Scraufstatter I. U., Cochrane C. G.*et al.* (1995) Hydrogen peroxide as a potent bacteriostatic antibiotic: implications for host defense. *Free Radical Biology and Medicine* **19**(1):31-7.

32. Bogdanov S. (1984) Characterisation of antibacterial substances in honey. *Lebensmittel-Wissenschaft und -Technologie* **17**(2):74-6.

33. Schepartz A. I. (1966) Honey catalase: occurrence and some kinetic properties. *Journal of Apicultural Research* **5**(3):167-76.

34. Dustmann J. H. (1971) Über die Katalaseaktivität in Bienenhonig aus der Tracht der Heidekrautgewächse (Ericaceae). *Zeitschrift für Lebensmittel-Untersuchung und -Forschung* **145**:294-5.

35. White J. W. & Subers M. H. (1964) Studies on honey Inhibine. 3. Effect of heat. *Journal of Apicultural Research* **3**(1):45-50.

36. White J. W. & Subers M. H. (1964) Studies on honey Inhibine. 4. Destruction of the peroxide accumulation system by light. *Journal of Food Science* **29**(6):819-28.

37. Daghie V., Cîrnu I. & Cioca V., editors. Contribution to the study of the bacteriostatic and bactericidal action of honey produced by *Physokermes* sp. in the area of coniferous trees. XXIIIrd International Apicultural Congress; 1971; Moscow: Apimondia Publishing House, Bucharest, Romania.

38. Allen K. L., Molan P. C. & Reid G. M. (1991) A survey of the antibacterial activity of some New Zealand honeys. *Journal of Pharmacy and Pharmacology* **43**(12):817-22.

39. Hodgson M. J. Investigation of the antibacterial action spectrum of some honeys [M.Sc.]. Hamilton, New Zealand: University of Waikato; 1989.

40. Molan P. C. & Russell K. M. (1988) Non-peroxide antibacterial activity in some New Zealand honeys. *Journal of Apicultural Research* **27**:62-7.

41. Roth L. A., Kwan S. & Sporns P. (1986) Use of a disc-assay system to detect oxytetracycline residues in honey. *Journal of Food Protection* **49**(6):436-41.

42. Willix D. J., Molan P. C. & Harfoot C. J. (1992) A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. *Journal of Applied Bacteriology* **73**:388-94.

43. Mohrig W. & Messner B. (1968) Lysozym als antibakterielles Agens im Bienenhonig und Bienengift. *Acta Biologica et Medica Germanica* **21**:85-95.

44. Russell K. M., Molan P. C., Wilkins A. L. & Holland P. T. (1990) The identification of some antibacterial constituents of New Zealand Manuka honey. *Journal of Agricultural and Food Chemistry* **38**:10-3.

45. Molan P. C., Allen K. L., Tan S. T. & Wilkins A. L., editors. Identification of components responsible for the antibacterial activity of Manuka and Viper's Bugloss honeys. Annual Conference of the New Zealand Institute of Chemistry; 1989.

46. Dold H. & Witzenhausen R. (1955) Ein Verfahren zur Beurteilung der örtlichen inhibitorischen (keimvermehrungshemmenden) Wirkung von Honigsorten verschiedener Herkunft. *Zeitschrift für Hygiene und Infektionskrankheiten* **141**:333-7.

47. d'Agostino Barbaro A., La Rosa C. & Zanelli C. (1961) Atttività antibatterica di mieli Siciliani. *Quaderni della Nutrizione* **21**(1/2):30-44.

48. Dustmann J. H. (1979) Antibacterial effect of honey. *Apiacta* **14**(1):7-11.

49. Buchner R. (1966) Vergleichende Untersuchungen über die antibakteriellen Wirkung von Blüten- und Honigtauhonigen. *Südwestdeutscher Imker* **18**:240-1.

50. Christov G. & Mladenov S. (1961) Propriétés antimicrobiennes du miel. *Comptes rendus de l'Académie bulgare des Sciences* **14**(3):303-6.

51. Floris I. & Prota R. (1989) Sul miele amaro di Sardegna. *Apicoltore Moderno* **80**(2):55-67.

52. Abbas T. Royal treat. Living in the Gulf. 1997:50-1.

53. George N. M. & Cutting K. F. (2007) Antibacterial honey (Medihoney ™): in-vitro activity against clincal isolates of MRSA, VRE and other multiresistant gram negative organisms including Pseudomonas aeruginosa. *Wounds* **19**(9):231-6.

54. Cooper R. A., Wigley P. & Burton N. F. (2000) Susceptibility of multiresistant strains of *Burkholderia cepacia* to honey. *Letters in Applied Microbiology* **31**:20-4.

55. Lin S. M., Molan P. C. & Cursons R. T. (2011) The controlled *in vitro* susceptibility of gastrointestinal pathogens to the antibacterial effect of manuka honey. *European Journal of Clinical Microbiology & Infectious Diseases* **30**:569-74.

56. Cooper R. A. (1998) The inhibition of bacteria isolated from chronic venous leg ulcers by honey. *Journal of Medical Microbiology* **47**:1140.

57. Cooper R. A., Molan P. C. & Harding K. G. (2002) The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. *Journal of Applied Microbiology* **93**:857-63.

58. Majtan J., Majtanova L., Bohova J. & Majtan V. (2011) Honeydew honey as a potent antibacterial agent in eradication of multi-drug resistant *Stenotrophomonas maltophilia* isolates from cancer patients. *Phytotherapy Research* **25**(4):584-7.

59. French V. M., Cooper R. A. & Molan P. C. (2005) The antibacterial activity of honey against coagulase-negative staphylococci. *Journal of Antimicrobial Chemotherapy* **56**(1):228-31.

60. Cooper R. A., Halas E. & Molan P. C. (2002) The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns. *Journal of Burn Care and Rehabilitation* **23**(6):366-70.

61. Cooper R. A. & Molan P. C. (1999) The use of honey as an antiseptic in managing *Pseudomonas* infection. *Journal of Wound Care* **8**(4):161-4.

62. Cooper R. A., Lindsay E. & Molan P. C. (2011) Testing the susceptibility to manuka honey of streptococci isolated from wound swabs. *Journal of ApiProduct and ApiMedical Science* **3**(3):117 - 22.

63. Cooper R. A., Molan P. C. & Harding K. G. (1999) Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. *Journal of the Royal Society of Medicine* **92**(6):283-5.

64. Allen K. L. & Molan P. C. (1997) The sensitivity of mastitis-causing bacteria to the antibacterial activity of honey. *New Zealand Journal of Agricultural Research* **40**:537-40.

65. Lin S. M., Molan P. C. & Cursons R. T. (2009) The in vitro susceptibility of Campylobacter spp. to the antibacterial effect of manuka honey. *European Journal of Clinical Microbiology & Infectious Diseases* **28**(4):339-44.

66. Al Somai N., Coley K. E., Molan P. C. & Hancock B. M. (1994) Susceptibility of *Helicobacter pylori* to the antibacterial activity of manuka honey. *Journal of the Royal Society of Medicine* **87**(1):9-12.

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

68. Irish J., Carter D. A., Shokohi T. & Blair S. E. (2006) Honey has an antifungal effect against Candida species. *Medical Mycology* **44**(3):289-91.

69. Brady N. F., Molan P. C. & Harfoot C. G. (1997) The sensitivity of dermatophytes to the antimicrobial activity of manuka honey and other honey. *Pharmaceutical Sciences* **2**:1-3.

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

71. Cooper R. A., Jenkins L., Henriques A. F. M., Duggan R. S.*et al.* (2010) Absence of bacterial resistance to medical-grade manuka honey. *European Journal of Clinical Microbiology & Infectious Diseases* **29**(10):1237-41.

72. Jervis-Bardy J., Foreman A., Bray S., Tan L.*et al.* (2011) Methylglyoxal-infused honey mimics the anti-*Staphylococcus aureus* biofilm activity of manuka honey: potential implication in chronic rhinosinusitis. *Laryngoscope* **121**(5):1104-7.

73. Merckoll P., Jonassen T. Ø., Vad M. E., Jeansson S. L.*et al.* (2009) Bacteria, biofilm and honey: A study of the effects of honey on 'planktonic' and biofilm-embedded chronic wound bacteria. *Scandinavian Journal of Infectious Diseases* **41**(5):341-7.

74. Maddocks S. E., Lopez M. S., Rowlands R. S. & Cooper R. A. (2012) Manuka honey inhibits the development of *Streptococcus pyogenes* biofilms and causes reduced expression of two fibronectin binding proteins. *Microbiology* **158**:781-90.

75. Alandejani T., Marsan J., Ferris W., Slinger R.*et al.* (2009) Effectiveness of honey on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. *Otolaryngology–Head and Neck Surgery* **141**(1):114-8.

76. Johnson D. W., van Eps C., Mudge D. W., Wiggins K. J.*et al.* (2005) Randomized, controlled trial of topical exit-site application of honey (Medihoney) versus mupirocin for the prevention of catheter-associated infections in hemodialysis patients. *Journal of the American Society for Nephrology* **16**(5):1456-62.

77. Chambers J. (2006) Topical manuka honey for MRSA-contaminated skin ulcers. *Palliative Medicine* **20**(5):557.

78. Dunford C., Cooper R., Molan P. C. & White R. (2000) The use of honey in wound management. *Nursing Standard* **15**(11):63-8.

79. Dunford C. E. (2001) Treatment of a wound infection in a patient with mantle cell lymphoma. *British Journal of Nursing* **10**(16):1058-65.

80. Eddy J. J. & Gideonsen M. D. (2005) Topical honey for diabetic foot ulcers. *Journal of Family Practice* **54**(6):533-5.

81. Natarajan S., Williamson D., Grey J., Harding K. G.*et al.* (2001) Healing of an MRSA-colonized, hydroxyurea-induced leg ulcer with honey. *Journal of Dermatological Treatment* **12**:33-6.

82. Simon A., Sofka K., Wiszniewsky G., Blaser G.*et al.* (2006) Wound care with antibacterial honey (Medihoney) in pediatric hematology-oncology. *Supportive Care in Cancer* **14**(1):91-7.

83. Visavadia B. G., Honeysett J. & Danford M. H. (2008) Manuka honey dressing: An effective treatment for chronic wound infections. *British Journal of Oral and Maxillofacial Surgery* **46**(1):55-6.

84. Molan P. C. & Betts J. A. (2004) Clinical usage of honey as a wound dressing: an update. *Journal of Wound Care* **13**(9):353-6.

85. Chang E. H., Alandejani T., Akbari E., Ostry A.*et al.* (2011) Double-blinded, randomized, controlled trial of medicated versus nonmedicated Merocel sponges for functional endoscopic sinus surgery *Journal of Otolaryngology - Head & Neck Surgery* **40**:S14-S9.

86. Kilty S. J., Duval M., Chan F. T., Ferris W.*et al.* (2011) Methylglyoxal: (active agent of manuka honey) in vitro activity against bacterial biofilms. *International Forum of Allergy & Rhinology* **1**(5):348-50.

87. Thamboo A., Philpott C., Javer A. & Clark A. (2011) Single-Blind Study of Manuka Honey in Allergic Fungal Rhinosinusitis. *Journal of Otolaryngology-Head & Neck Surgery* **40**(3):238-43.

88. English H. K., Pack A. R. & Molan P. C. (2004) The effects of manuka honey on plaque and gingivitis: a pilot study. *Journal of the International Academy of Periodontology* **6**(2):63-7.

89. Al-Waili N. (2003) Intrapulmonary administration of natural honey solution, hyperosmolar dextrose or hypoosmolar distill water to normal individuals and to patients with type-2 diabetes mellitus or hypertension: their effects on blood glucose level, plasma insulin and C-peptide, blood pressure and peaked expiratory flow rate. *European Journal of Medical Research* **8**(7):295-303.

90. Efem S. E. E. (1993) Recent advances in the management of Fournier's gangrene: Preliminary observations. *Surgery* **113**(2):200-4.

91. Vardi A., Barzilay Z., Linder N., Cohen H. A.*et al.* (1998) Local application of honey for treatment of neonatal postoperative wound infection. *Acta Paediatrica* **87**(4):429-32.

92. Efem S. E. E. (1988) Clinical observations on the wound healing properties of honey. *British Journal of Surgery* **75**:679-81.

93. Cavanagh D., Beazley J. & Ostapowicz F. (1970) Radical operation for carcinoma of the vulva. A new approach to wound healing. *Journal of Obstetrics and Gynaecology of the British Commonwealth* **77**(11):1037-40.

94. Phuapradit W. & Saropala N. (1992) Topical application of honey in treatment of abdominal wound disruption. *Australian and New Zealand Journal of Obstetrics and Gynaecology* **32**(4):381-4.