THE ANTIBACTERIAL ACTIVITY OF HONEY
1. The nature of the antibacterial activity

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Introduction

Honey has been used as a medicine since ancient times in many cultures[61, 86](fig. 1), and is still used in 'folk medicine'[55]. The use of honey as a therapeutic substance has been rediscovered by the medical profession in more recent times, and it is gaining acceptance as an antibacterial agent for the treatment of ulcers and bed sores, and other surface infections resulting from burns and wounds[4, 135]. In many of the cases in the cited reports, honey was used on infections not responding to standard antibiotic and antiseptic therapy. It was found in almost all of the cases to be very effective in rapidly clearing up infection and promoting healing. Honey has also been found to be effective in treating bacterial gastroenteritis in infants[47].

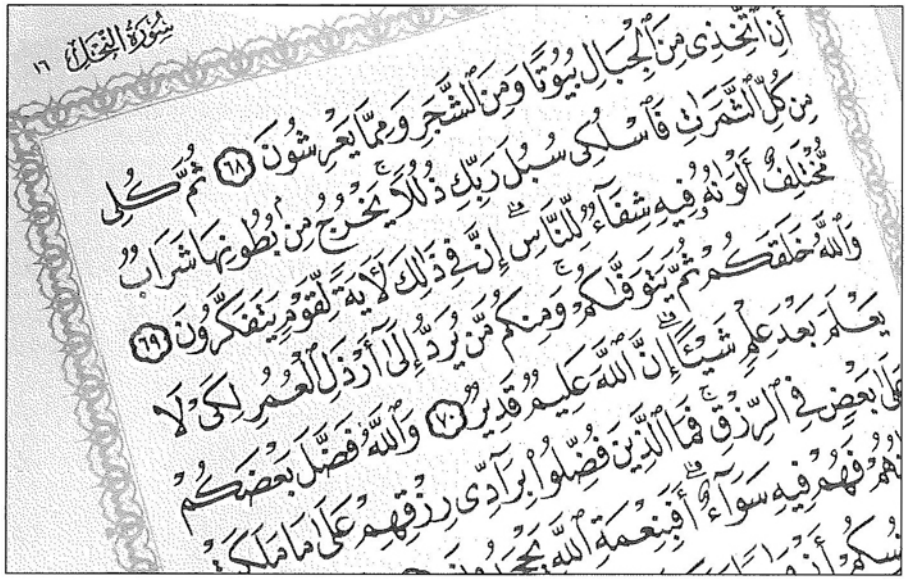

FIG. 1. The Koran, *circa* 590 AD.

Translation:
68. And thy Lord taught the Bee
To build it cells in the hills,
On trees, and in (men's) habitations;
69. Then to eat all ...../.....: there issues
From within their [the bees] bodies
A drink of varying colours,
Wherein is healing for men.

Some infections caused by some of the species of bacteria that have been found to be sensitive to the antibacterial activity of honey[78]

Pathogen	Infection caused
Bacillus anthracis	anthrax
Corynebacterium diphtheriae	diphtheria
Escherichia coli	diarrhoea, septicaemia, urinary infections, wound infections
Haemophilus influenzae	ear infections, meningitis, respiratory infections, sinusitis
Klebsiella pneumoniae	pneumonia
Listeria monocytogenes	meningitis
Mycobacterium tuberculosis	tuberculosis
Pasteurella multocida	infected animal bites
Proteus species	septicaemia, urinary infections, wound infections
Pseudomonas aeruginosa	urinary infections, wound infections
Salmonella species	diarrhoea
Salmonella cholerae-suis	septicaemia
Salmonella typhi	typhoid
Salmonella typhimurium	wound infections
Serratia marcescens	septicaemia, wound infections
Shigella species	dysentery
Staphylococcus aureus	abscesses, boils, carbuncles, impetigo, wound infections
Streptococcus faecalis	urinary infections
Streptococcus mutans	dental caries
Streptococcus pneumoniae	ear infections, meningitis, pneumonia, sinusitis
Streptococcus pyogenes	ear infections, impetigo, puerperal fever, rheumatic fever, scarlet fever, sore throat, wound infections
Vibrio cholerae	cholera

In the ancient use of honey as a medicine there was no knowledge of it having antibacterial properties — it was just known to work. In more recent times, now that it is known that festering wounds are the result of infection by micro-organisms, honey is used on the basis of it being an antibacterial substance, but the nature and extent of its antibacterial activity is not widely known. A large amount of research work has been done on the antibacterial activity of honey, but the results of this remain unknown to most users of honey because the work is so widely spread over time, and is published in different journals and in different languages. Because it is important to be aware of the research findings to realize the full potential of honey as a therapeutic substance, this review has been prepared to bring together what is known about the antibacterial activity of honey.

Reports of antimicrobial activity of honey

Experimental approach

The antibacterial activity of honey appears to have been reported first by van Ketel in 1892 (cited by Dustmann[35]). The next report was by Sackett in 1919[95]. He also reported that the antibacterial potency was increased by limited dilution of honey, an observation that was hard to explain. More intensive study did not commence until the work of Dold *et al.* in 1937[27]. They introduced the term 'inhibine' for the antibacterial activity of honey, a term which has been widely used since in the literature on honey.

Since then there have been many reports. Some have been of simple testing that

FIG. 2. Honey solutions being pipetted into wells in an agar plate (the agar is impregnated with *Staphylococcus aureus*).

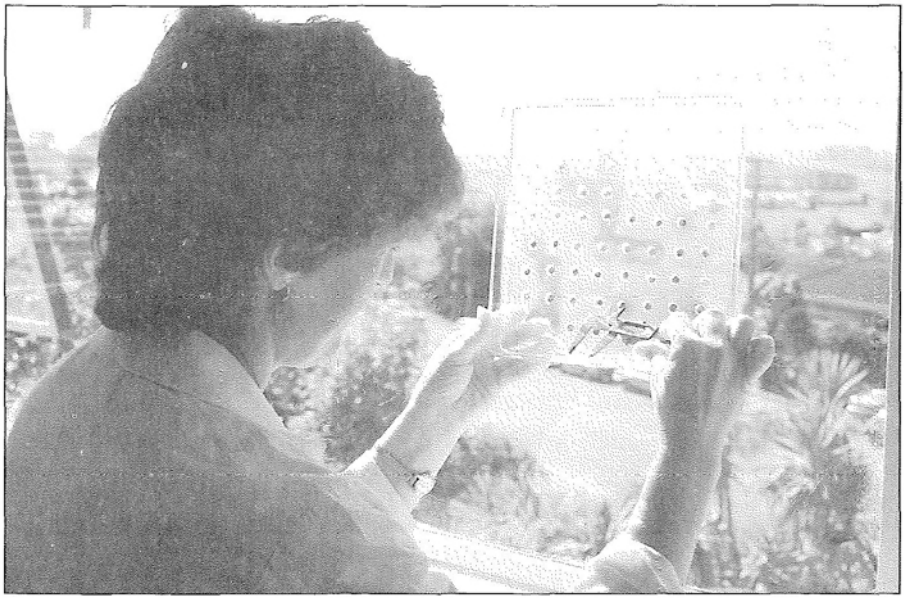

FIG. 3. Measuring the size of zones of inhibition of growth on the agar plate.

has shown honey to have antibacterial activity: these have often been done without recognition of the prior discovery of this by others. Most, however, have involved investigation of the activity spectrum of honey (i.e. determining which species of micro-organism are sensitive to the action of honey), or comparison of different types of honey for the potency of their action against one or more species of bacteria. Also there have been many investigations of the nature of the antibacterial substances present.

In studies where the potency of the antibacterial activity of honeys has been measured, this has involved the use of one form or another of two standard microbiological techniques. In the agar diffusion assay technique, a small quantity of honey, or a solution of honey, is applied to a nutrient agar plate inoculated with a microbial culture (fig. 2). While the plate is incubating, the honey diffuses out into the agar from its point of application. Where the concentration of honey in the agar is high enough to inhibit growth of the culture no colonies develop, and a clear zone is seen around the point of application of the honey. The size of the clear zone is a measure of the potency of the honey (fig. 3). However, because the honey is diluted as it diffuses into the agar, the effective antibacterial concentration of the honey in this type of assay is always lower than the concentration of the solution applied. In the other type of assay, honey is incorporated in the nutrient agar or in the nutrient broth in which the culture is grown. By using a series of different concentrations of honey it is possible to find the minimum inhibitory concentration for each honey. Whether diluted by extensive diffusion in the first method, or as a further step in a dilution series in the second, the more potent the antibacterial activity of a honey, the more it can be diluted and still retain its inhibitory action.

None of the methods mentioned can show whether the action of honey is bactericidal (i.e. lethal to the bacteria). If no colony development occurs in the period of incubation, it can only be taken as a bacteriostatic action (i.e. inhibition of growth of the bacteria). Demonstration of bactericidal activity requires subsequent culturing in fresh nutrient medium to see if the test micro-organisms survived exposure to the honey.

Species found to be susceptible

The microbial species that have been found to be sensitive to the antimicrobial activity of honey are listed in table 1. Many of the reports, especially the older ones, use names no longer in common use for many of the bacterial species: the currently used names for these species are listed in table 1, as identified from past and present editions of *Bergey's manual of determinative bacteriology*[12, 13].

Table 1 also shows the lowest concentration of honey reported to show an antibacterial effect against each species in each study. In many of the studies this concentration is not necessarily the minimum inhibitory concentration. In some cases the testing for susceptibility was done with a single concentration of honey. In others, where a dilution series was used, activity was found at the lowest concentration in the series. It is possible that activity could have been detected at lower concentrations in all of these instances, if lower concentrations had been used in the testing.

In some of the reports, results are given of the testing of susceptibility to more than one type of honey. In these instances the results presented in table 1 are those obtained with the most active honey used. The decision to do this was based on the finding in many other studies that honeys vary very widely in their antibacterial potency, many having no detectable antibacterial activity (see later). As one of the aims of this review is to show the potential of honey for use as an antibacterial agent, the results are therefore presented of what can be achieved with honeys of high activity, rather than what is achieved if unselected honeys are used.

The concentrations of honey used in the assays of antibacterial activity are given in most of the reports as percentages, but in many of the reports there is no notation of whether it is grams of honey per 100 g of solution (% wt/wt), grams of honey per 100 ml of solution (% wt/vol), or millilitres of honey per 100 ml of solution (% vol/vol). As honey is a liquid of high density, the way the percentage is calculated makes a substantial difference to the value given. Where it cannot be deduced from the description given of the way the solutions were prepared, it is assumed that the values given are % vol/vol. If in any instance the assumption is incorrect, the actual concentration of honey that caused the observed antibacterial effect would have been lower than the value given in table 1. To facilitate comparison between the reports, all values for the concentration of honey used are given in this review as % vol/vol, these being calculated on the basis of honey having a density of 1.4 g/ml[125].

Antifungal activity

Although an earlier brief review[49] of the biological effects of honey expressed the

TABLE 1. A summary of the reports of the antimicrobial activity of honey, showing the species affected and the concentration (% by volume) of honey used in the testing. Results for the most active honey are shown where more than one honey was used. The lowest concentration with activity is shown if more than one concentration was used.

§ signifies an agar diffusion assay — the active concentration is lower than the value given, the honey being diluted by diffusion into the agar.
Values are in brackets where completeness of inhibition was not stated (the results were reported as "sensitive to honey").
? signifies that the actual concentration used was not given in the report.

Species inhibited	Conc. of honey (%) for complete microbicidal action	Conc. of honey (%) for complete inhibition of growth	Conc.of honey (%) for partial inhibition of growth
BACTERIA			
Alcaligenes sp.		10§[54]; 100§[82]	
Alcaligenes faecalis	7.4[95]		
Bacillus sp.	50[15]		
Bacillus alvei		extract[42, 118]	
Bacillus anthracis	2.5[20, 57]	1.3[20, 57]; 5§[3]; 17[27]; 20[40]; 100§[43]	
Bacillus cereus		17[27]; 42§[90]; ≤100§[106]; 100§[82]	
Bacillus cereus var. mycoides		17[27, 83]; 100§[82]	8[27]
Bacillus larvae			extract[42]
Bacillus megaterium		extract[118]	
Bacillus pumilus	1.3[20, 57]	1.3[20, 57]; 13[27]; 17[83]; 25§[67]; ?§[88]	8[27]; 25[61]
Bacillus stearothermophilus		42§[90]	
Bacillus subtilis	[50 partial][69]	10§[54]; 10[42]; 13[27]; 17[83]; 20[17]; 42§[90]; ≤100§[106]; 100§[69, 103, 119]; (?)[70]; distillate[59, 79]; extract[8, 42, 118]	5[17]; 8[27]; 25[61]

TABLE 1. (continued)

Species inhibited	Conc. of honey (%) for complete microbicidal action	Conc. of honey (%) for complete inhibition of growth	Conc.of honey (%) for partial inhibition of growth
Citrobacter freundii		3.6[50]; 10§[92]	
Corynebacterium diphtheriae	5[20, 57]; 17[84]	2.5[20, 57]; 10§[54]; 25§[67]; 25[28]	
Edwardsiella tarda	99[115]		
Escherichia coli	7.4[95]; 9[84]; 20[40]; 30[15]; 99[115]; [25 partial][69]	0.25§[3]; 2[25]; 3.1[30]; 3.6[50]; 4.5[131]; 5[21]; 5–6[52]; 6.25[94]; 7.6[81]; 10§[54, 92]; 10[51]; 12.5[44]; 20[17, 20, 40, 57]; ≤25[77]; 25§[67, 85, 92]; 25[61]; 40[56]; 100§[37, 43, 69, 82, 103, 112, 119]; (10)[53]; (?)[70]; ?[133]; ?§[88]; distillate[79, 113]	0.7[131]; 1.4[50]; 5[17]; 10[54]; 17[27, 83]; extract[42]; distillate[101]
Haemophilus influenzae		10[56]	
Klebsiella sp.		10§[54]; (10)[53]	
Klebsiella pneumoniae	15[95]; 20[20, 57]	10[20, 57]; 25§[67]; 25[61]; 50[56]; 100§[119]; distillate[79, 113]	40[56]
Listeria monocytogenes		≤25[77]; 30[56]	
Micrococcus sp.		10§[54]	
Micrococcus luteus		10§[106]; 42§[90]	
Mycobacterium tuberculosis	100[105]	4.5[83]	1.2[83]
Neisseria sp.		10§[54]	
Pasteurella multocida		(?)[70]	
Proteus sp.		10§[54]; 20[17]; (10)[53]; extract[42]	5[17]
Proteus mirabilis	30[15]	3.6[131]; 6.4[50]; 20[17]; 40[56]; 100§[37]; distillate[79]	1.4[131]; 5[17]

TABLE 1. (continued)

Species inhibited	Conc. of honey (%) for complete microbicidal action	Conc. of honey (%) for complete inhibition of growth	Conc.of honey (%) for partial inhibition of growth
Proteus morganii		0.25§[3]	
Proteus vulgaris	23[95]; 99[115]	0.6[25]; 5–6[52]; 10§[92]; 20[20, 57]; ≤36[35]; extract[42]	10[54]
Pseudomonas sp.		(10)[53]; 100§[82]	
Pseudomonas aeruginosa	10[20, 57]; 20[52]; 99[115]	3[25]; 3.1–6[44]; 5[20, 21, 57]; 5-6[52]; 6.4[50]; 10§[54]; 13[27]; ≤25[77]; 25[61]; 30[56]; ≤36[35]; 100§[119]; distillate[79, 113]; extract[118]	0.7[131]; 8[27]; 17[83]; 100§[37]; extract[42]
Pseudomonas fluorescens		8.3[81]; 25[61]	
Salmonella sp.		10§[54]; 25§[85]; 40[56]; 100§[43]	30[56]; extract[42]
Salmonella cholerae-suis	7.4[95]		
Salmonella dublin			extract[42]
Salmonella enteritidis	7.4[95]; 99[115]		
Salmonella gallinarum		10[17]; ≤36[35]	5[17]; extract [42]
Salmonella paratyphi-A	7.4[95]	17[83]; extract[118]	
Salmonella pullorum		≤36[35]	extract[42]
Salmonella schottmuelleri	7.4[95]	25§[67]	17[27, 83]
Salmonella typhi	99[115]	0.25§[3]; 10[54]; 30[56]; extract[118]	20[56]
Salmonella typhimurium	99[115]	5[21, 131]; 10§[92]; ≤25[77]; 100§[82]; extract[118]	
Salmonella typhosa	7.4[95]; 20[40]	17[27, 83]; 20[40]; 25§[67]	
Sarcina lutea		10§[106]; 25[61]; ≤36[35]; 50§[92]; ≤100§[87]; 100§[82]	
Sarcina orangea		25§[67]	
Serratia marcescens	99[115]	5[131]; 10§[54]; 13[27]; 25§[67, 92]; 25[61]; 50[56]; distillate[79]	0.7[131]; 8[27]; 17[83]; 40[56]
Shigella sp.		10§[54]	

TABLE 1. (continued)

Species inhibited	Conc. of honey (%) for complete microbicidal action	Conc. of honey (%) for complete inhibition of growth	Conc.of honey (%) for partial inhibition of growth
Shigella boydii		40[56]	
Shigella dysenteriae	7.4[95]	6.9[66]; 8.3[108]; 17[27]; 20[40]	17[27]
Shigella flexneri	5[20, 57]	0.5§[3]; 1[25]; 2.5[20, 57]; 5[52]; 10§[92]; 10[51]	17[27, 83]
Shigella sonnei		0.8[25]; 5–6[52]; 17[83]	10[54]
Staphylococcus sp.	20[52]; 30[15]	3[25]; 5–6[52]; 9[51]	8[51]
Staphylococcus aureus (albus)		10[17]; 25[61]; (10)[53]; extract[118]	5[17]; 17[27, 83]
Staphylococcus aureus	1.3[20, 57]; 1.5[14]; 9[84]; 20[40]; 50[1, 69]	0.3[35]; 0.5§[3]; 0.6[20, 57]; 1[72]; 1.56[94]; 2.9[50]; 3[96, 127, 128, 130]; 3.1[30]; 3.6§[4]; 3.6[1]; 4[132]; 4.5[122]; 5[21, 31, 121]; 3.1–6[44]; 6[129]; 6.3[73]; 9[81]; 10[29]; 10§[54, 92]; (10)[53]; 20[40]; ≤25[77]; 25§[67]; 25[61]; 50§[8, 16, 17]; 50[56]; 100§[37, 43, 82, 103, 112, 119]; (?)[70]; ?§[88]; distillate[79, 113]; extract[118]	0.4[131]; 1.4[50]; 17[27, 83]; 20[8]
Streptococcus sp.	2.5[20, 57]; 30[15]; 33[84]	2.5[20, 57]; 5[21]; 5.4[81]; 30[56]; ≤36[35]; (10)[53]	20[56]
Streptococcus faecalis		6.9[66]; 7.1[50]; 8.3[108]; 10§[54]; 20[17]; 25§[92]; 40[56]; 100§[82]; distillate[79]	5[17]; 30[56]
Streptococcus mitis		10§[54]	
Streptococcus mutans		100§[36]	
Streptococcus pneumoniae		10§[54]	

TABLE 1. (continued)

Species inhibited	Conc. of honey (%) for complete microbicidal action	Conc. of honey (%) for complete inhibition of growth	Conc.of honey (%) for partial inhibition of growth
Streptococcus pyogenes	0.6[20, 57]	0.6[20, 57]; 2.9[131]; 10§[54]; 20[56]; 100§[37, 103]	0.7[131]; 10[56]
Streptococcus salivarius		25§[67]	
Streptomyces sp.			25[61]
Vibrio cholerae		17[27]; 20[56]	
Vibrio cholerae biotype *Proteus*		17[27]	
FUNGI			
Aspergillus flavus		60[123]; 75[85]	25[85]
Aspergillus fumigatus		3.1[30]	
Aspergillus niger		75[85]; distillate[79]	25[85]
Aspergillus parasiticus		60[123]	
Candida albicans	100[15]; distillate[79]	1.6[30]; ≤100§[87]; distillate[80, 113]	
Candida pseudotropicalis	10[15]		
Candida reukaufii	50[15]		
Candida stellatoidea	50[15]		
Candida tropicalis	50[15]		
Candida utilis		(?)[70]	
Penicillium sp.		3.1[30]; distillate[79]; ?§[88]	
Penicillium chrysogenum		75[85]	25[85]
Saccharomyces sp.	[50 partial][69]		

opinion that honey had no effect on fungi beyond its osmotic action, the data in table 1 show that some honeys, at least, must have antifungal factors present, as some fungi are inhibited under conditions where the sugar content of the honey is clearly not responsible.

Non-specific reports

Two studies have been carried out on the antimicrobial activity of honey against unidentified micro-organisms in soil, water and air. Growth of colonies from 70–90% of the bacteria and 30–60% of the fungi from sewage, soil, air and tap water was found to be prevented by 25% honey[85]. Growth of colonies from airborne contaminants was found to be prevented completely by 20% honey and partially by 2% honey, the survivors being mainly fungi[54].

Differences in susceptibility between species

The relative sensitivity of various species of micro-organisms to honey is of great interest, as more resistant species may be able to overcome the inhibitory effects of the honey in areas of an infection where the honey is at lower concentrations. However, the nature of the studies carried out so far limit the accuracy of quantitative comparisons between species in their sensitivity to the antibacterial effect of honey. Because of this, and because the values given are not necessarily the minimum inhibitory concentrations, comparison of the sensitivity of various species is not possible by reference to the values given in table 1.

The major differences in findings on the sensitivity of each species are more likely, however, to be due to differences in the honeys used. Many workers have demonstrated that not all honey samples have the same degree of antibacterial activity (see later), therefore the sensitivity of species cannot be compared using the results from different studies, as the honeys used in the studies may have had widely differing antibacterial activity. The sensitivity of species relative to each other can be validly determined within a single study in which the same honey and same test conditions are used. Even so, the relative sensitivity of species could be found to be different within another study because species could respond differently to the different types of antibacterial factor that may be present in a different honey. This difference in ranking of sensitivity has been demonstrated by Willix[131] in a specific study of this point using two honeys known to have different types of antibacterial factors present. It was also observed by Popeskovik *et al.*[82], and further evidence of it can be seen in the data of others who worked with larger numbers of honeys[3, 52, 94].

Where the effect of a honey, or a group of honeys, on a number of species has been assayed under the same conditions within one study, sensitivities can be compared and the relative sensitivity of the species tested ranked. *Staphylococcus aureus*, a species included in most of these comparative studies, can be seen to be one of the species most sensitive to honey[17, 20, 35, 40, 50, 52, 54, 57, 61, 69, 79, 82, 84, 92, 103, 118, 119, 131]. (This is of medical significance because this species, as a result of its wide resistance to antibiotics, has become the major cause of wound infections and septicaemia in hospitals[62]). The relative sensitivity of other species is not so discernible because of

the marked variation from study to study. This almost certainly reflects the differences in the antibacterial factors in the honeys used in the various studies.

Explanation of the antibacterial activity of honey

Osmotic effect

Honey is a saturated or super-saturated solution of sugars, the water content usually being only 15–21% by weight[124]. Of the solids in honey, 84% is a mixture of the monosaccharides fructose and glucose[125]. The strong interaction of these sugar molecules with water molecules leaves very few of the water molecules available for micro-organisms. This 'free' water is what is measured as the *water activity* (a_w): mean values for honey have been reported as 0.562 and 0.589[91], 0.572 and 0.607[10], and 0.62[117]. Although some yeasts can live in honeys that have a high water content, causing spoilage of the honey, the a_w of ripened honey is too low to support the growth of any species, no fermentation occurring if the water content is below 17.1%[5].

Many species of bacteria have their growth completely inhibited by the a_w being in the range 0.94–0.99[60, 102]. These values correspond to solutions of a typical honey (a_w of 0.6) of concentrations from 12% down to 2%, calculated on the basis of the concentration being proportional to $-\log a_w$[102]. On the other hand, some species have their maximum rate of growth when the a_w is 0.99[102], so inhibition by the osmotic (water-withdrawing) effect of dilute solutions of honey obviously depends on the species of bacteria.

Fungi are generally much more tolerant of low a_w than bacteria are[60], so the reports of antifungal activity with diluted honey indicate that there is more involved than just the sugar content of the honey. Likewise, *Staphylococcus aureus* has an exceptionally high tolerance of low a_w, yet is one of the species most sensitive to the antibacterial activity of honey. For complete inhibition of growth of *S. aureus* the a_w has to be lowered below 0.86[18, 19, 60], which would be a typical honey at 29%. There have been many reports of complete inhibition of *S. aureus* by honeys much more dilute than that.

The results of some experiments have demonstrated quite clearly that there is much more than an osmotic effect involved. In one study with *S. aureus*, honeys were dialysed to remove the sugar, yet complete inhibition was observed with some at dilutions down to 1.5% honey[35]. In another study[4], honeys were tested at a concentration of 18% in an agar diffusion assay, where the activity of many honeys was below the level of detection: the activity of others was up to 20 times higher than the minimum detectable. In a similar study[72] a honey of low antibacterial activity showed no activity against *S. aureus* when tested at a concentration of 50% in an agar diffusion assay that allowed activity to be detected in an active honey diluted to a concentration of 1%. The range of a_w found in honey (0.47–0.70[91]) could account for only a two-fold difference in activity due to osmotic effects.

Further indication that the antibacterial activity of honey is due to a lot more than just the removal of water from bacteria is seen in the results of the many studies in which the antibacterial activity of honey has been compared with that of 'artificial honey' (a solution of sugars of the same proportions as typically in honey).