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Critical Review

Antibacterial Components of Honey

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Summary

The antibacterial activity of honey has been known since the 19th century. Recently, the potent activity of honey against antibiotic-resistant bacteria has further increased the interest for application of honey, but incomplete knowledge of the antibacterial activity is a major obstacle for clinical applicability. The high sugar concentration, hydrogen peroxide, and the low pH are well-known antibacterial factors in honey and more recently, methylglyoxal and the antimicrobial peptide bee defensin-1 were identified as important antibacterial compounds in honey. The antibacterial activity of honey is highly complex due to the involvement of multiple compounds and due to the large variation in the concentrations of these compounds among honeys. The current review will elaborate on the antibacterial compounds in honey. We discuss the activity of the individual compounds, their contribution to the complex antibacterial activity of honey, a novel approach to identify additional honey antibacterial compounds, and the implications of the novel developments for standardization of honey for medical applications. © 2011 IUBMB
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INTRODUCTION

Honey is well known for its antibacterial activity, which was first reported in 1892 (as cited by Dustmann in 1919). Since ancient times, honey has been used for treatment and prevention of wound infections. With the advent of antibiotics, the clinical application of honey was abandoned in modern Western medicine, though in many cultures it is still used. For all antibiotic classes, including the major last resort drugs, resistance is increasing worldwide (*1, 2*) and even more alarming, very few new antibiotics are being developed. The potent activity of honey against antibiotic-resistant bacteria (*3–5*) resulted in renewed interest for its application. Several honeys have been approved for clinical application. The incomplete knowledge of the antibacterial compounds involved and the variability of antibacterial activity are however major obstacles for applicability of honey in medicine. In recent years, the knowledge on the antibacterial compounds in honey has expanded. In this review, we will give an overview of the current knowledge on the antibacterial components in honey, and we will discuss the implications for standardization of the antibacterial activity of honey.

MEDICAL-GRADE HONEY

Raw honey can contain bacterial spores, mainly those of *Bacillus* spp., and spores of the notorious pathogen *Clostridium botulinum*, which can cause wound botulism or gangrene, are incidentally detected (*6, 7*). Medical-grade honey intended for clinical application therefore must be sterilized to destroy potentially present bacterial spores. This is generally achieved by gamma-irradiation (*8*). Manuka and Revamil® are the major medical-grade honeys currently approved for clinical application. Manuka honey is produced from the manuka bush (*Leptospermum scoparium*) indigenous to New Zealand and Australia. The honey used as a source for medical-grade manuka honey is collected from its natural environment. The honey used as a source for Revamil®, in this review designated as RS honey, is produced by a standardized process in greenhouses. The manufacturer does not disclose further details on the origin of this honey.

The antibacterial activity of manuka honey is often expressed by an industry standard phenol-equivalent scale, the so-called unique manuka factor (UMF). This factor represents the concentration of a phenol solution yielding a similar zone of growth inhibition as the honey, when tested in a radial diffusion assay with *Staphylococcus aureus* as the target microorganism (*9*). Because of the large batch-to-batch variation in antibacterial activity (*9*), batches of manuka honey are individually tested for activity. Although the UMF designation suggests that the indicated level of activity is due to a manuka-specific com-

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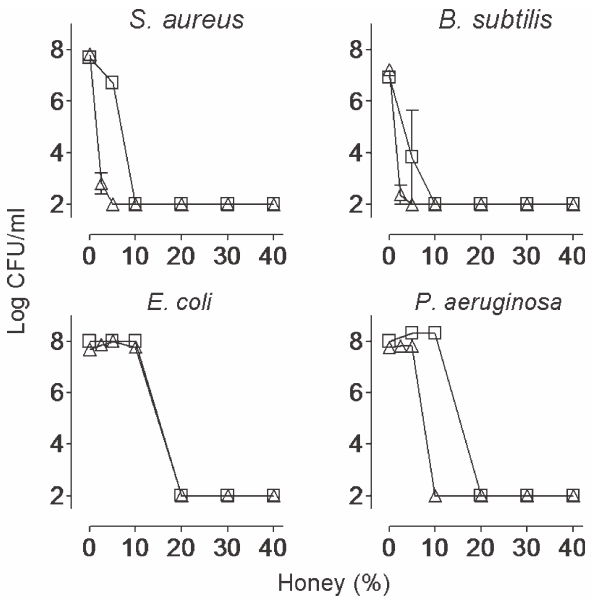

Figure 1. Bactericidal activity of RS and manuka honey. The indicated bacteria were incubated in various concentrations of RS (squares) or manuka (triangles) honey. After 24 h, numbers of surviving bacteria were determined. Data were reproduced from the Refs. *16* and *22* with permission from The Federation and Public Library of Science, respectively.

pound, the UMF assay only measures the level of antibacterial activity but is not informative regarding the identity of the components involved.

Revamil® honey is registered as a medical device for applications in wound healing and not as an antimicrobial agent. Consequently, the antimicrobial activity is not specified for individual batches of this honey. We however characterized the antimicrobial activity of this honey and demonstrated that 11 batches had less than a factor of two differences in the minimal concentration required for bactericidal activity against *Bacillus subtilis* (*10*). In a quantitative liquid bactericidal assay, both RS honey and manuka honey have potent bactericidal activity (Fig. 1). Manuka honey has bactericidal activity at up to twofold to fourfold higher dilutions than RS honey against *S. aureus*, *B. subtilis*, and *P. aeruginosa*, while these honeys have identical activity against *E. coli* (Fig. 1). The factors contributing to antimicrobial activity of honeys identified to date are the high sugar concentration, hydrogen peroxide, methylglyoxal, the antimicrobial peptide bee defensin-1, and the low pH. As manuka honey and RS honey are the best characterized honeys, we will focus on the contribution of these factors and possible other compounds to the antibacterial activity of these honeys in this review.

ANTIBACTERIAL COMPONENTS IN HONEY

Ripened honey consists of 80% sugars, mainly glucose and fructose and some sucrose and maltose, and contains <18% water. The high concentration of sugars combined with a low moisture content causes osmotic stress, which prevents spoilage of honey by microorganisms. Only slight dilution of honey can already result in yeast growth, but the sugar content of honey is sufficient to retain antibacterial activity of honey when diluted to approximately 30–40%. At higher dilutions, the antibacterial activity is due to other compounds than sugar.

In the 1960s, H_2O_2 was identified as a major antibacterial compound in honey (*11–13*). The enzyme glucose oxidase—added by honey bees to the collected nectar during production of honey—is activated on moderate dilution of honey and converts glucose into H_2O_2 and gluconic acid. However, various honeys have substantial antibacterial activity due to nonperoxide components. Recently, methylglyoxal and bee defensin-1 have been identified in manuka honey and RS honey, respectively, as antibacterial compounds in honey (*14–16*). Several studies yielded conflicting results regarding the contribution of the low pH (generally between 3.2 and 4.5) for the antibacterial activity of honey (*13*), but we have conclusively shown a role for the pH in this activity (*16*). In addition, there are clear indications for the presence of additional honey antibacterial compounds of which the identity remains to be elucidated.

HYDROGEN PEROXIDE

Glucose oxidase, one of the carbohydrate-metabolizing enzymes added to nectar by bees, converts glucose into hydrogen peroxide and gluconic acid under aerobic conditions (*17, 18*). The presumed function of H_2O_2 is prevention of spoilage of unripe honey when the sugar concentration has not yet reached levels able to prevent microbial growth. During ripening of honey glucose oxidase is inactivated but it regains activity on dilution of honey. H_2O_2 accumulation is highest in the range of 30–50% honey and declines rapidly below 30% honey due to the relatively low affinity of honey bee glucose oxidase for its substrate glucose (*19*).

In a screening of 90 honeys, the mean and maximal levels of H_2O_2 accumulation in 20% honey solutions after 1 h were 12 ± 19 and 72 μg/mL, respectively (*17*). Several honeys do not accumulate any H_2O_2 at all (*17, 20*). RS honey produces 22.5 ± 1.3 μg/mL in 30% honey after 2 h and up to 148.4 ± 27.8 μg/mL after 24 h (*21*), whereas the manuka honey we tested did not accumulate detectable levels of H_2O_2 (*22*). The contribution of H_2O_2 to the antibacterial activity of honey can be determined by the effect of neutralization of this compound by the addition of catalase. Neutralization of H_2O_2 reduces the antibacterial activity of the majority of honey samples tested, indicating the important role of H_2O_2, but a substantial number of honey samples retain activity after H_2O_2-neutralization (*9, 23*).

Factors known to affect H_2O_2 accumulation are inactivation of the H_2O_2-producing enzyme glucose oxidase by exposure to heat or light (*24, 25*) or degradation of H_2O_2 by honey (*12, 26*). It has been suggested that catalase originating from pollen, nectar, or microorganisms would be responsible for the

enzymatic H_2O_2-neutralizing activity of honey (*26, 27*); however, to the best of our knowledge, catalase has never been identified in honey.

As in honey, H_2O_2 is also a major antimicrobial defense system in plant nectar (*28*) and substantial variation in accumulation of H_2O_2 also exists among nectar samples (*29, 30*). Interestingly, peroxidases are among the most abundant proteins in petunia nectar and the level of H_2O_2 accumulation in tobacco and petunia nectar is inversely related to the level of peroxidase activity in these nectars (*30, 31*) . Possibly nectar-derived peroxidases rather than catalase might be a cause of variation in H_2O_2-neutralizing capacity of different honeys.

Another explanation for the variation in H_2O_2 accumulation in honeys could be differences in activity of glucose oxidase. To our knowledge, no studies have been performed to assess the concentration or activity of glucose oxidase in different honeys.

METHYLGLYOXAL

Various honeys have substantial nonperoxide antibacterial activity (*9, 13*). Manuka honey has been most extensively subjected to identification of nonperoxide antimicrobial components. This honey is produced from nectar of the manuka tree (*Leptospermum scoparium*), which is indigenous to New Zealand and is renowned for its nonperoxide antibacterial activity. Recently, exceptionally high levels of the antimicrobial compound methylglyoxal (MGO) have been found in manuka honey (*14, 15*). In general, MGO is formed from sugars during heat treatment or prolonged storage of carbohydrate-containing foods and beverages (*32*). However, the high levels of MGO in manuka honey are formed by conversion of dihydroxyacetone (DHA) present at exceptionally high concentrations in the nectar of *L. scoparium* flowers (*33*). This conversion occurs nonenzymatically at a slow rate during storage of honey. It is unknown how DHA is formed in nectar and why it is present in such large amounts in nectar of manuka trees. Concentrations of MGO in various foods in the range of 3–47 mg/kg have been reported, while manuka honey contains much higher concentrations [ranging from 38 mg/kg to 1,541 mg/kg (0.74–30.0 mM)] (*14, 15, 34*). MGO is also present in honeys from other plant species, but in screenings of 106 different samples, the concentrations did not exceed 24 mg/kg (*14–16*).

Based on a strong correlation between the MGO levels and the potential of honey to inhibit the growth of *S. aureus*, it has been suggested that MGO is fully responsible for the nonperoxide antibacterial activity of manuka honey (*14*). To verify this, we assessed the effect of neutralization of MGO on the activity of a manuka honey with a high level of MGO (10.9 ± 1.7 mM) in a quantitative bactericidal assay. Neutralization of MGO abolished the activity of manuka honey against *S. aureus* and substantially reduced the activity against *B. subtilis* but did not affect the activity against *E. coli* and *P. aeruginosa* (*22*). Thus, MGO is not fully responsible for manuka nonperoxide antimicrobial activity.

BEE DEFENSIN-1

We recently identified the antimicrobial peptide bee defensin-1 in RS honey (*16*). This peptide (also known as royalisin) was previously identified in honeybee hemolymph, the insect equivalent of blood (*35*), in honeybee head and thoracic glands (*36*) and in royal jelly, the major food of queen bee larvae (*37*) but had never been detected in honey. Bee defensin-1 has potent activity but only against Gram-positive bacteria including *B. subtilis*, *S. aureus*, and *Paenibacillus larvae* (*16, 38*). The latter species is the causative agent of the devastating bee larval disease American Foulbrood.

Invertebrates strongly rely on antimicrobial peptides (AMPs) as part of their innate immune system for defence against microorganisms. In honeybees, four types of AMPs are produced in the hemolymph after experimental infection with *E. coli*, that is, hemenoptecin, bee defensin-1, apidaecin and the group of abaecin peptides. Each of these AMPs has a distinct spectrum of antimicrobial activity, and collectively these peptides cover all major classes of microorganisms (*35*).

American foulbrood is a devastating disease that specifically affects bee larvae. Infection with *P. larvae* occurs via the digestive tract and results in severe mortality among larvae during the first 48 h following egg hatching (*39*). Bee defensin-1, but none of the other honey AMPs, has been identified in royal jelly and honey, the major food sources for bee larvae. Although this is speculative, the presence of bee defensin-1 in royal jelly and honey may contribute to protection of bee brood against American Foulbrood.

Although bee defensin-1 was readily detectable in RS honey, we could not detect this peptide in a manuka honey (*22*). The presence of bee defensin-1 in different honeys has not yet been systematically investigated, and quantitative data on the concentration of this peptide in honey have not yet been established. Proteinaceous antibacterial compounds were previously reported for six of 26 honeys, but identification of these proteins was not pursued (*23, 40*). For four of those honeys, the reported antibacterial spectrum of the proteinaceous compounds strongly resembles the spectrum we observed for bee defensin-1 (i.e., potent activity against *Bacillus* spp. but no activity against *S. aureus*). It is therefore possible that these honeys contain bee defensin-1, but this obviously requires further investigation.

Bee defensin-1 is secreted by the honeybee hypopharyngeal gland (*16*). Bees use secretions of the hypopharyngeal gland for production of royal jelly and honey (*41, 42*). The amount of bee defensin-1 in royal jellies (therein referred to as "royalisin") and in honeys varies strongly (*22, 38*), with some samples completely devoid of this peptide. This implies that bee defensin-1 expression in hypopharyngeal glands and/or the amount of gland secretions added vary strongly. As bee defensin-1 is active against *Paenibacillus larvae* (see above), the cause of American foulbrood, it will be interesting to investigate whether variation in bee defensin-1 expression levels is related to susceptibility of honeybees to this disease.

PRESENCE OF ADDITIONAL COMPONENTS

In many studies, the presence of nonperoxide antibacterial activity in honey has been reported (*9, 13, 23*). It is difficult to deduct to what extent MGO and bee defensin-1 or as yet unidentified components might contribute to the activity. Some indications for the presence of additional compounds can however be obtained by comparing the nonperoxide antibacterial properties of those honeys with the properties of MGO and of bee defensin-1 described above. Mundo et al. reported that after neutralization of H_2O_2 and degradation of proteinaceous compounds, several honeys retain activity against *Bacillus stearothermophilis* but not against several other microorganisms including the highly MGO-susceptible *S. aureus* (*23*). This suggests that the additional activity in these honeys is exerted by components other than H_2O_2, bee defensin-1, or MGO.

Phenolic compounds originating from plant nectar have been proposed as important factors for the nonperoxide antibacterial activity of honey. Several antibacterial phenolic compounds have been identified in honeys (*13, 43–45*), but their contribution to the overall activity of honey remains unclear. The activity of individual phenolics isolated from honey is too low to substantially contribute to the antibacterial activity (*13, 44*). Complex phenolic fractions of several Malaysian honeys exert antibacterial activity, but the identity of the compound(s) responsible for this activity is unknown (*46*). Perhaps the combination of different phenolics instead of individual compounds might contribute substantially to the activity of honey. This however remains to be investigated using purified compounds instead of undefined partially purified fractions.

Microorganisms present in honey have been proposed as a potential source of antimicrobial compounds in honey. Bacteria isolated from honey can indeed produce antimicrobial compounds when cultured *in vitro*, but it remains unclear whether such compounds actually are present in honey (*47*).

CHARACTERIZATION OF BACTERICIDAL COMPONENTS IN MEDICAL-GRADE HONEY

To identify the bactericidal components in RS honey, the source for Revamil® medical-grade honey, we used a novel approach of successive neutralization of individual honey bactericidal components. First, we enzymatically neutralized the established honey bactericidal components H_2O_2 and MGO by addition of catalase and glyoxalase I, respectively. The bactericidal activity of honey prior to and after neutralization of these compounds was quantified for a representative panel of bacteria and compared with the activity of a honey-equivalent sugar solution (*16*). Neutralization of H_2O_2 and MGO markedly reduced the bactericidal activity of RS honey, but this honey retained substantial activity. We used activity-guided isolation to identify components contributing to the remaining activity. Using size separation, cation-exchange chromatography, and native acid-urea polyacrylamide gel-electrophoresis, we isolated the cationic antimicrobial peptide bee defensin-1 from honey. The activity of this peptide was specifically neutralized by addition of a polyclonal anti-bee defensin-1 antibody or by addition of the polyanionic compound sodium polyanetholesulfonate. After neutralization of H_2O_2, MGO, bee defensin-1, and subsequent titration of honey to neutral pH, the activity of RS honey was reduced to that of the honey-equivalent sugar solution (*16*). We thus were the first to identify all components contributing to the bactericidal activity of a specific honey.

The contribution of MGO to the antibacterial activity of manuka honey was previously estimated by correlation of the MGO content and antibacterial activity of manuka honey (*14*). Using our quantitative bactericidal assay with neutralization of individual honey bactericidal compounds, we confirmed that MGO is indeed the major component for the activity of manuka honey against *S. aureus* but additional compounds contributed to the activity against *B. subtilis*, *E. coli*, and *P. aeruginosa* (*22*). We did not detect H_2O_2 or bee defensin-1 in the investigated manuka honey and demonstrated that the non-MGO activity was due to a combination of cationic and noncationic as yet unidentified compounds (*22*). In the studies that correlated the MGO content and the antibacterial activity, the activity of non-neutralized manuka honey was determined against the highly MGO-susceptible species *S. aureus* (*14*). As a consequence, the activity of potentially present additional components would be obscured by the activity of MGO and could only have been detected after MGO-neutralization. This underlines the importance to eliminate the activity of established bactericidal components when searching for additional components contributing to the activity of honey. The contribution of all major honey antibacterial compounds to the bactericidal activity of RS and manuka honey against a panel of representative bacteria is shown in Table 1.

For antimicrobial compounds present at low concentrations, it may not be possible to determine their contribution to the activity of honey using the method described above. Such compounds might be detected in honey extracts or in concentrated fractions obtained after purification. Although the contribution of such compounds with low abundance to the antimicrobial activity of honey may be limited, these compounds might be interesting as leads for development of novel antimicrobials.

METHODS TO ASSESS THE BACTERICIDAL ACTIVITY OF HONEY

The agar diffusion assay with *S. aureus* (*9*), currently the most commonly used method to estimate the antibacterial activity of medical-grade honey, has several major limitations. First, antibacterial activity of honey against *S. aureus* is not representative for activity against other bacteria, because different species have varying susceptibility to honey and to honey antibacterial components. Second, in the agar diffusion assay, the activity of honey is estimated by the size of the growth inhibition zone. Obviously, the size of such zones depends not only on the antimicrobial activity but also on the rate of diffusion of

Table 1
Contribution of bactericidal factors to activity of RS and Manuka honey

	H_2O_2		Bee defensin-1		MGO		pH		Additional cationic		Additional noncationic	
	RS[a]	Man	RS	Man	RS	Man	RS	Man	RS	Man	RS	Man
B. subtilis	+	−	+	−	+	+	+	+	−	+	−	+
MRSA	+	−	+	−	+	+	−	−	−	−	−	−
E. coli	+	−	+	−	+	+	−	+	−	+	−	+
P. aeruginosa	+	−	+	−	+	+	+	−	−	+	−	−

Contribution is defined as ≥ 1 log reduction in numbers of CFU after 24 h of incubation.
[a]RS: RS honey, Man: manuka honey.

antibacterial components through the agar matrix. Honey with potent antibacterial activity due to compounds with relatively high molecular weight, which have limited migration in the agar, may thus erroneously be characterized as having low activity. Third, the agar diffusion test does not discriminate between growth inhibiting and bactericidal activity and does not allow quantification of bactericidal activity or kinetics of killing. These limitations are overcome by the use of a quantitative liquid bactericidal assay with a panel of representative bacterial species. This quantitative liquid assay is also more suited to assess the contribution of individual bactericidal components using the approach of neutralization of specific components.

CONTRIBUTION OF INDIVIDUAL COMPONENTS TO THE ANTIBACTERIAL ACTIVITY OF HONEY

To assess the role of individual bactericidal components to the activity of RS honey, we neutralized the contributing components individually and in different combinations and quantified the effect on the bactericidal activity (*16*). All bacteria tested were susceptible to the concentration of honey tested (20%), and all activities were abolished when H_2O_2, MGO, and bee defensin-1 were neutralized (Fig. 2). The role of the different compounds for the bactericidal activity of different bacterial species is also shown in Fig. 2. Neutralization of MGO or bee defensin-1 alone did not affect the activity against *S. aureus*, but combined neutralization of these compounds resulted in ~2 logs increased survival. This indicates that MGO and bee defensin-1 both contribute to the activity of RS honey against *S. aureus* but due to redundancy, neutralization of either of these compounds alone does not result in an observable reduction in bactericidal activity. Strikingly, bee defensin-1 in a partially purified preparation, in the absence of other honey antibacterial compounds, lacked activity against *S. aureus* (*21*). However, this peptide is essential for activity of MGO-neutralized RS honey against *S. aureus*. This implies that bee defensin-1 has additive or synergistic activity with other bactericidal components in MGO-neutralized honey, that is, H_2O_2, sugar, and/or the low pH.

As another example of redundancy, the activity of RS honey against *B. subtilis* was not affected when any of the honey bactericidal components was neutralized individually. Moreover, the contribution of the low pH for activity of RS honey against *B. subtilis* was only revealed when H_2O_2, MGO, and bee defensin-1 were simultaneously neutralized.

In contrast to the redundancy described above, combined presence of some compounds is required for bactericidal activity against certain species, suggesting additive or synergistic effects (*16*). For instance, neutralization of either H_2O_2 or MGO markedly reduced the activity of RS honey against *Escherichia coli* and *Pseudomonas aeruginosa*, indicating that the presence of both compounds was required for full activity. Activity against vancomycin-resistant *Enterococcus faecium* required different combinations of compounds, as neutralization of either H_2O_2 or a combination of MGO and bee defensin-1 abolished activity. So, H_2O_2 is required but not sufficient for activity against *E. faecium*, since the presence of MGO or bee defensin-1 is additionally required for full activity. In conclusion, the mechanisms of bactericidal activity of honey are highly complex and also vary for individual bacterial species. Such complex interactions preclude prediction of the relative contribution of individual components to the overall antibacterial activity of honey.

From a comparison of RS honey and manuka honey, some general statements can however be made regarding the contribution of the individual components to the bactericidal activity. RS honey contains relatively high levels of bee defensin-1 and H_2O_2 and only a minor amount of MGO compared with the manuka honey we investigated. RS honey rapidly kills (within 2 hours) all species tested except *S. aureus*, whereas manuka honey kills more slowly; potent bactericidal activity of manuka honey requires 24 h of incubation. The neutralization assay indeed showed that bee defensin-1 and H_2O_2 are required for the rapid activity of RS honey and the absence of these components can explain the lack of rapid activity of manuka honey. In contrast to other bacteria tested, H_2O_2-dependent killing of *S. aureus* occurs by a slow mechanism of action and bee defensin-1 lacks rapid activity against this species (*21*), which

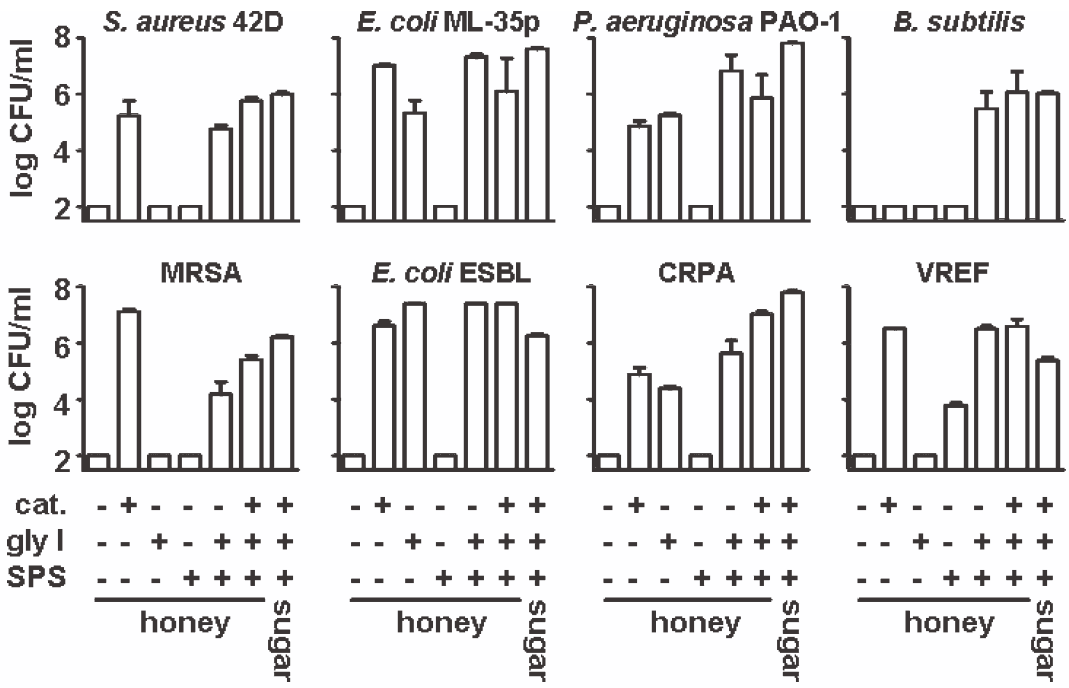

Figure 2. Effect of neutralization of H_2O_2, MGO and bee defensin-1 on bactericidal activity of honey. Hydrogen peroxide, MGO and bee defensin-1 were neutralized in 20% honey by adding catalase (cat.), glyoxalase I (gly I) and SPS, respectively. Bactericidal activity was tested against indicated laboratory strains (top row) and against clinical isolates of vancomycin-resistant *Enterobacter faecium* (VREF), methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase producing *Escherichia coli* (*E. coli* ESBL) and ciprofloxacin-resistant *Pseudomonas aeruginosa* (CRPA; bottom row). A sugar solution equivalent to 20% honey was used as reference. After 24 h, numbers of surviving bacteria were determined. Numbers of CFU were log-transformed and shown as mean ± SEM. Figure reproduced from Ref. *16* with permission of The Federation.

explains the lack of rapid activity of RS honey against *S. aureus*. MGO kills bacteria not only at submicromolar concentrations but also by a slow mechanism of action, which explains the potent but slow bactericidal activity of manuka honey. Clearly, the large differences in composition of bactericidal components in RS and manuka honey result in substantial differences in kinetics and spectra of bactericidal activity.

CLINICAL APPLICATION OF HONEY

The best evidence regarding the efficacy of honey has been obtained for treatment of burn wounds. For mild to moderate superficial and partial thickness burns, honey was more effective than conventional treatment for reduction of microbial colonization and improved wound healing (*48, 49*). In a relatively small study, application of honey resulted in a comparable bacteremia-free period of tunnelled, cuffed hemodialysis catheters compared with that obtained with mupirocin treatment (*50*). There is however insufficient evidence to guide clinical application of honey in other areas (*49*), so high-quality clinical trials are warranted.

The direct antimicrobial activity of honey is considered the most important characteristic of honey for healing of wounds (*48*). The honeys used in clinical trials are often not characterized for their *in vitro* antimicrobial activity. As the antimicrobial activity among honeys varies considerably, such characterization would be highly valuable to allow assessment of antimicrobial properties of honey that may be favorable for clinical applications.

IMPLICATIONS FOR STANDARDIZATION OF MEDICAL-GRADE HONEY

Medical applicability of honey will strongly benefit from formulations with consistent antibacterial activity and a full characterization of the compounds involved. As batch-to-batch variation in activity of honey occurs, detailed analysis of activity against a representative panel of bacteria is essential to characterize the bactericidal properties of honey. As outlined in this review, the variation in activity among honeys can be explained by the large variation in antimicrobial compounds among honeys. Detailed knowledge on these compounds would allow the production of standardized honeys, for instance by blending different batches of honey to meet predefined criteria regarding the composition of bactericidal compounds. Such standardization of the levels of well-characterized antimicrobial compounds would strongly contribute to the applicability of honey for medical purposes.

REFERENCES

1. Walsh, C. (2003) *Antibiotics: Actions, Origins, Resistance*. American Society for Microbiology (ASM) Press, Washington, DC.
2. Levy, S. B. and Marshall, B. (2004) Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.* **10**, S122–S129.
3. Cooper, R. A., Halas, E., and Molan, P. C. (2002) The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns. *J. Burn Care Rehabil.* **23**, 366–370.
4. Cooper, R. A., Molan, P. C., and Harding, K. G. (2002) The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. *J. Appl. Microbiol.* **93**, 857–863.
5. Efem, S. E. E. (1988) Clinical observations on the wound-healing properties of honey. *Br. J. Surg.* **75**, 679–681.
6. Postmes, T., van den Bogaard, A. E., and Hazen, M. (1993) Honey for wounds, ulcers, and skin graft preservation. *Lancet* **341**, 756–757.
7. Snowdon, J. A. and Cliver, D. O. (1996) Microorganisms in honey. *Int. J. Food Microbiol.* **31**, 1–26.
8. Postmes, T., van den Bogaard, A. E., and Hazen, M. (1995) The sterilization of honey with cobalt 60 gamma radiation: a study of honey spiked with spores of *Clostridium botulinum* and *Bacillus subtilis*. *Experientia* **51**, 986–989.
9. Allen, K. L., Molan, P. C., and Reid, G. M. (1991) A survey of the antibacterial activity of some New Zealand honeys. *J Pharm. Pharmacol.* **43**, 817–822.
10. Kwakman, P. H. S., Van den Akker, J. P. C., Guclu, A., Aslami, H., Binnekade, J. M., et al. (2008) Medical-grade honey kills antibiotic-resistant bacteria in vitro and eradicates skin colonization. *Clin. Infect. Dis.* **46**, 1677–1682.
11. Adcock, D. (1962) The effect of catalase on the inhibine and peroxide values of various honeys. *J. Apicult. Res.* **1**, 38–40.
12. White, J. W. Jr., Subers, M. H., and 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. *Biochim. Biophys. Acta* **73**, 57–70.
13. Molan, P. C. (1992) The antibacterial activity of honey .1. The nature of the antibacterial activity. *Bee World* **73**, 5–28.
14. Adams, C. J., Boult, C. H., Deadman, B. J., Farr, J. M., Grainger, M. N., et al. (2008) Isolation by HPLC and characterisation of the bioactive fraction of New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydr. Res.* **343**, 651–659.
15. Mavric, E., Wittmann, S., Barth, G., and Henle, T. (2008) Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. *Mol. Nutr. Food Res.* **52**, 483–489.
16. Kwakman, P. H. S., Te Velde, A. A., de Boer, L., Speijer, D., Vandenbroucke-Grauls, C. M. J. E., et al. (2010) How honey kills bacteria. *FASEB J.* **24**, 2576–2582.
17. White, J. W., Jr. and Subers, M. H. (1963) Studies on honey inhibine. 2. A chemical assay. *J. Apicult. Res.* **2**, 93–100.
18. Bang, L. M., Buntting, C., and Molan, P. (2003) The effect of dilution on the rate of hydrogen peroxide production in honey and its implications for wound healing. *J. Altern. Complement Med.* **9**, 267–273.
19. Schepartz, A. I. and Subers, M. H. (1964) The glucose oxidase of honey. I. Purification and some general properties of the enzyme. *Biochim. Biophys. Acta* **85**, 228–237.
20. Molan, P. C. (1992) The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity. *Bee World* **73**, 59–76.
21. Kwakman, P. H. S., de Boer, L., Ruyter-Spira, C. P., Creemers-Molenaar, T., Helsper, J. P., et al. (2011) Medical-grade honey enriched with antimicrobial peptides has enhanced activity against antibiotic-resistant pathogens. *Eur. J. Clin. Microbiol. Infect. Dis.* **30**, 251–257.
22. Kwakman, P. H. S., Te Velde, A. A., de Boer, L., Vandenbroucke-Grauls, C. M. J. E., and Zaat, S. A. J. (2011) Two major medicinal honeys have different mechanisms of bactericidal activity. *PLoS One* **6**(3), e17709.
23. Mundo, M. A., Padilla-Zakour, O. I., and Worobo, R. W. (2004) Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *Int. J. Food Microbiol.* **97**, 1–8.
24. White, J. W. Jr. and Subers, M. H. (1964) Studies on honey inhibine. 3. Effect of heat. *J. Apicult. Res.* **3**, 45–50.
25. White, J. W. Jr. and Subers, M. H. (1964) Studies on honey inhibine. 4. Destruction of the peroxide accumulation system by light. *J. Food Sci.* **29**, 819–828.
26. Schepartz, A. I. (1966) Honey catalase: occurance and some kinetic properties. *J. Apic. Res.* **5**, 167–176.
27. Huidobro, J. F., Sanchez, M. P., Muniategui, S., and Sancho, M. T. (2005) Precise method for the measurement of catalase activity in honey. *J. AOAC Int.* **88**, 800–804.
28. Carter, C. and Thornburg, R. W. (2004) Is the nectar redox cycle a floral defense against microbial attack? *Trends Plant Sci* **9**, 320–324.
29. Hillwig, M. S., Liu, X. T., Liu, G. Y., Thornburg, R. W., and MacIntosh, G. C. (2010) Petunia nectar proteins have ribonuclease activity. *J Exp. Botany* **61**, 2951–2965.
30. Hillwig, M. S., Kanobe, C., Thornburg, R. W., and MacIntosh, G. C. (2011) Identification of S-RNase and peroxidase in petunia nectar. *J. Plant Physiol.* **168**, 734–738.
31. Gonzalez-Teuber, M., Eilmus, S., Muck, A., Svatos, A., and Heil, M. (2009) Pathogenesis-related proteins protect extrafloral nectar from microbial infestation. *Plant J.* **58**, 464–473.
32. Weigel, K. U., Opitz, T., and Henle, T. (2004) Studies on the occurrence and formation of 1,2-dicarbonyls in honey. *Eur. Food Res. Technol.* **218**, 147–151.
33. Adams, C. J., Manley-Harris, M., and Molan, P. C. (2009) The origin of methylglyoxal in New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydr. Res.* **344**, 1050–1053.
34. Stephens, J. M., Schlothauer, R. C., Morris, B. D., Yang, D., Fearnley, L., et al. (2010) Phenolic compounds and methylglyoxal in some New Zealand manuka and kanuka honeys. *Food Chem.* **120**, 78–86.
35. Casteels-Josson, K., Zhang, W., Capaci, T., Casteels, P., and Tempst, P. (1994) Acute transcriptional response of the honeybee peptide-antibiotics gene repertoire and required post-translational conversion of the precursor structures. *J. Biol. Chem.* **269**, 28569–28575.
36. Klaudiny, J., Albert, S., Bachanova, K., Kopernicky, J., and Simuth, J. (2005) Two structurally different defensin genes, one of them encoding a novel defensin isoform, are expressed in honeybee *Apis mellifera*. *Insect Biochem. Mol. Biol.* **35**, 11–22.
37. Fujiwara, S., Imai, J., Fujiwara, M., Yaeshima, T., Kawashima, T., and Kobayashi, K. (1990) A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. *J. Biol.Chem.* **265**, 11333–11337.
38. Bachanova, K., Klaudiny, J., Kopernicky, J., and Simuth, J. (2002) Identification of honeybee peptide active against Paenibacillus larvae larvae through bacterial growth-inhibition assay on polyacrylamide gel. *Apidologie* **33**, 259–269.
39. Genersch, E. (2010) American Foulbrood in honeybees and its causative agent, Paenibacillus larvae. *J. Invertebr. Pathol.* **103**, S10–S19.
40. Gallardo-Chacon, J. J., Casellies, M., Izquierdo-Pulido, M., and Rius, N. (2008) Inhibitory activity of monofloral and multifloral honeys against bacterial pathogens. *J Apicult. Res* **47**, 131–136.
41. Lensky, Y. and Rakover, Y. (1983) Separate protein body compartments of the worker honeybee (*Apis mellifera* L). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **75**, 607–615.
42. Knecht, D. and Kaatz, H. H. (1990) Patterns of larval food-production by hypopharyngeal glands in adult worker honey-bees. *Apidologie* **21**, 457–468.
43. Russell, K. M., Molan, P. C., Wilkins, A. L., and Holland, P. T. (1990) Identification of some antibacterial constituents of New-Zealand Manuka honey. *J. Agric. Food Chem.* **38**, 10–13.
44. Weston, R. J., Brocklebank, L. K., and Lu, Y. R. (2000) Identification and quantitative levels of antibacterial components of some New Zealand honeys. *Food Chem.* **70**, 427–435.

45. Isla, M. I., Craig, A., Ordonez, R., Zampini, C., Sayago, J., et al. (2011) Physico chemical and bioactive properties of honeys from Northwestern Argentina. *LWT—Food Sci. Technol.* **44**, 1922–1930.
46. Aljadi, A. M. and Yusoff, K. M. (2003) Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties. *Turkish J. Med. Sci.* **33**, 229–236.
47. Lee, H. J., Churey, J. J., and Worobo, R. W. (2008) Antimicrobial activity of bacterial isolates from different floral sources of honey. *Int. J. Food Microbiol.* **126**, 240–244.
48. Moore, O. A., Smith, L. A., Campbell, F., Seers, K., McQuay, H. J., et al. (2001) Systematic review of the use of honey as a wound dressing. *BMC. Complement Altern. Med.* **1**, 2.
49. Jull, A. B., Rodgers, A., and Walker, N. (2008) Honey as a topical treatment for wounds. *Cochrane Database of Systematic Reviews* 2008 Issue 4.art.no.CD005083. DOI: 10.1002/14651858.CD005083.pub2.
50. Johnson, D. W., van Eps, C., Mudge, D. W., Wiggins, K. J., Armstrong, 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. *J. Am. Soc. Nephrol.* **16**, 1456–1462.