

REVIEW

Honey Combination Therapies for Skin and Wound Infections: A Systematic Review of the Literature

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Abstract: Topical application of medical grade honey is recommended for the clinical management of wound infections. The suitability of honey as a wound healing agent is largely due to its antibacterial activity, immune modulatory properties, and biocompatibility. Despite the usefulness of honey in wound healing, chronic wound infections continue to be a global problem requiring new and improved therapeutic interventions. Several recent studies have investigated the effects of combining honey with other therapies or agents with the aim of finding more efficacious treatments. In this systematic review, the database PubMed was used to carry out a search of the scientific literature on the combined effects of honey and other therapies on antimicrobial activity and wound and skin healing. The search revealed that synergistic or additive antimicrobial effects were observed *in vitro* when honey was combined with antibiotics, bacteriophages, antimicrobial peptides, natural agents e.g. ginger or propolis and other treatment approaches such as the use of chitosan hydrogel. Outcomes depended on the type of honey, the combining agent or treatment and the microbial species or strain. Improved wound healing was also observed *in vivo* in mice when honey was combined with laser therapy or bacteriophage therapy. More clinical studies in humans are required to fully understand the effectiveness of honey combination therapies for the treatment of skin and wound infections.

Key words: honey, antibiotics, natural agents, combination therapy, wound infection, skin infection.

Introduction

Antimicrobial drug resistance is a major public health problem for which novel antimicrobial drugs or innovative therapeutic interventions must urgently be found. Globally, intractable wounds, caused by antibiotic resistant microbial infections, are a significant burden on health care systems^{1,2}. An increasing global prevalence of diabetes and obesity and an aging population has contributed to the current burden of chronic wounds^{3,4}. Non-healing infected wounds can cause sepsis and inflammation in organs e.g. endocarditis leading to increased morbidity and mortality. Wounds with biofilms are particularly resistant to treatment with antibiotics because the bacteria are protected by a barrier of extracellular polymeric substances⁵. Due to the need for new and improved therapies there has been a revived interest in alternative treatment approaches such as the use of honey in the management of wound infections. Indeed, medical grade honey is

currently included in clinical protocols for wound management ^{6,7} and has been shown to have broad spectrum antimicrobial activity against common wound infecting microorganisms including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* ⁸⁻¹⁰. Furthermore, manuka honey can kill antibiotic resistant bacterial strains such as methicillin resistant *Staphylococcus aureus* (MRSA) and the development of bacterial resistance to honey is thought to be unlikely due to its multiple antimicrobial components and mechanisms ¹¹. The wound healing ability of honey is also related to its capacity to promote reepithelialisation and angiogenesis and stimulate skin and immune cells ¹²⁻¹⁵. Medical grade honey is defined as organic honey that is free of toxic contaminants, has been sterilised by gamma irradiation, is processed in accordance with safety regulations and standards and is safe for medical application ¹⁶. Medical grade honey formulations for the clinical management of wounds include honey in tubes, gels, and impregnated dressings. A Cochrane systematic review published in 2015 concluded that there is evidence that honey heals partial thickness burn wounds better than conventional treatments and post-operative wounds more effectively than gauze or antiseptics ¹⁷. For other types of wounds, the review stated that there was insufficient evidence to form definitive conclusions. Therefore, despite evidence that honey is efficacious for the treatment of certain types of wounds, there is still a need to find ways to optimise or enhance its antimicrobial and healing properties for improved clinical outcomes. As all honeys are unique, one approach may be to search for honeys that have superior antimicrobial activity but are yet undiscovered. Another approach may be to modify the honey in such a way that its antimicrobial activity is boosted. Surgihoney, is an example of a honey that has been bioengineered so that it produces more hydrogen peroxide and reactive oxygen species when diluted in water ¹⁸. An alternative approach could involve combining honey with other agents such as antibiotics or natural agents such as ginger or propolis with the aim of inducing synergistic or additive effects. This systematic review explores the current scientific literature on the biological and clinical effects of combining honey with other agents and therapies in relation to wound and skin infections. Furthermore, possible mechanisms of the synergistic effects of honey combination therapies are described. The overall aim of this article was to encourage the development of new and improved treatments for skin and wound infections.

Review Methods

A systematic review investigating the effects of honey combined with other agents or therapies on antimicrobial activity and wound and skin infections was conducted in accordance with PRISMA guidelines ¹⁹. The electronic database PubMed was used for the literature search by using key terms shown in Table 1. Group 1 terms were combined with group 2 and 3 terms until all the combinations had been searched for. Research articles investigating the combined effects of honey and other agents or therapies on antibacterial activity *in vitro* and on wound and skin infections *in vivo* were included in the study. Other agents included antibiotics commonly used for skin and wound infections such as rifampicin, oxacillin and tetracycline and other natural products such as propolis, royal jelly, vitamins, and ginger. Examples of other therapies included laser therapy, bacteriophage therapy and hydrogel. Articles with full text in English and published between 1990 and 2020 were included in the study. Review articles and articles not written in English were excluded. Some important articles were also sourced from the reference list of included papers and some were recommended by experts in the field. PM and DT carried out the literature search including selection and assessment of articles and the search was conducted between May and June 2020. Any disagreements during the article assessment process were resolved by discussion. We obtained a total of 408 scientific articles and selected 41 studies we determined relevant to research on honey combination therapies for wound and skin infections (Table 2).

Table 1. Search terms used in the study

Term (group 1)	Term (group 2)	Term (group 3)
Honey	Antibiotic	Wound infection
	Rifampicin	Skin infection
	Oxacillin	Wound healing
	Tetracycline	Antibacterial
	Combination therapy	Antimicrobial
	Propolis	
	Herbal extracts	

Combinations: group 1 terms AND group 2 terms AND group 3 terms until all combinations had been searched for.

Table 2. Table created in accordance with PRISMA guidelines showing number of articles identified and included in this systematic review

Search strategy		No. of articles
Identification	Records identified from PubMed database searching	397

	Records identified from other sources [†]	11
Screening	Records after duplicated articles removed	254
	Records screened by abstract	254
	Records excluded	207
Eligibility	Full text articles assessed for eligibility	47
	Full text articles excluded	6
Included	Studies included in review	41

[†] Other sources included articles sourced from the reference list of selected papers and articles recommended by experts in the field.

Results and Discussion

Honey and antibiotics: in vitro studies

Several researchers have explored the effects of combining honey with antibiotics on antimicrobial activity *in vitro*. One of the first papers reporting a synergistic effect of honey and antibiotics was published in 1998. The researchers used a broth dilution assay to demonstrate a synergistic effect of an Indian honey (obtained from Phondaghat Pharmacy) and the antibiotics amikacin, ceftazidime and gentamicin against multi drug resistant clinical isolates of *P. aeruginosa*²⁰. The honey and antibiotics were reportedly tested at a ratio of 1:1. Experiments were also conducted with *Klebsiella* species but no synergy was observed with this micro-organism. Later in 2005, another study reported synergy using broth dilution method between an Omani honey (50%) and the antibiotic gentamicin (4µg/ml) against *Staphylococcus aureus*²¹. Additionally, a study conducted in Egypt reported enhanced effects of antibiotics against clinical isolates of *P. aeruginosa*, *Enterobacter* species and *Staphylococci* in a disc diffusion assay when citrus bee honey (100%) was added to the antibiotic discs (Oxoid)²².

More recently, Jenkins and Cooper (2012)²³ investigated the combined effects of subinhibitory concentrations of manuka honey and the antibiotic oxacillin against methicillin resistant *Staphylococcus aureus* (MRSA). In their study, resistance of the MRSA strain to oxacillin was confirmed by antibiotic susceptibility testing using Mueller Hinton Agar (MHA) and discs coated with 5µg of oxacillin. No zones of inhibition were observed confirming that the *S. aureus* strain was methicillin resistant. However, when a sub-inhibitory concentration of manuka honey (5% w/v) was added to the MHA, inhibition zones of 32 mm in diameter developed around the oxacillin discs. The findings suggest that the subinhibitory concentration of manuka honey affected the bacteria in such a way that resistance to oxacillin was reversed. Using broth dilution, chequerboards and time kill curves Jenkins and Cooper (2012) confirmed the synergistic

interaction of manuka honey and oxacillin, and reversal of oxacillin resistance. β lactam antibiotics such as oxacillin, methicillin and ceftiofur inhibit bacterial cell wall synthesis by binding to bacterial penicillin binding protein (PBP), a protein involved in the synthesis of the peptidoglycan layer of the bacterial cell wall. In methicillin resistant strains of *S. aureus* an altered PBP protein with a low binding affinity for β lactam antibiotics is produced. This altered form of PBP is known as PBP-2a and is encoded by the *mecA* gene and regulated by a sensor inducer called *mecR1*. Expression of *mecA* and subsequent production of PBP2a enables bacteria to survive in the presence of the β lactam antibiotics. Jenkins and Cooper (2012) demonstrated using microarray technology that manuka honey (10% w/v) down regulated *mecR1* gene product in MRSA. However, synergistic effects of oxacillin and manuka honey have also been observed in methicillin sensitive strains of *S. aureus* that do not have the *mecA* gene suggesting that other mechanisms are likely to be involved^{24, 25}. Jenkins and Cooper (2012)²⁶ went on to investigate antimicrobial activity of 15 antibiotics in combination with subinhibitory concentrations of manuka honey (Manukacare 18+, Comvita, 5% w/v) against MRSA and *P. aeruginosa*. The results depended on the antibiotic, the bacterial species, and experimental method used. Most notable were the results of the combination of tetracycline and manuka honey which demonstrated enhanced activity against MRSA and *P. aeruginosa*. Rifampicin and manuka honey showed enhanced effects against MRSA when tested using disc diffusion and E-strip but synergy was not observed using broth dilution. The combination of imipenem and manuka honey was found to be synergistic against MRSA but not *P. aeruginosa* suggesting a species-specific effect. Subsequently, Muller et al, (2013)²⁵ reported synergistic effects of manuka honey and rifampicin against several *S. aureus* isolates including MRSA. The experimental methods used were checkerboard, microdilution assays and agar disc diffusion assay. In the agar disc diffusion assay, for example, the addition of 5% Medihoney (Comvita Ltd, NZ) to the agar increased the zone of inhibition induced by rifampicin (4 μ g discs) from 20 mm to 41 mm. A similar result was obtained for a range of *S. aureus* isolates, however, no zones of inhibition were observed around a rifampicin resistant strain of *S. aureus* either with or without 5% Medihoney in the agar suggesting that Medihoney could not reverse rifampicin resistance. Nevertheless, experimental results suggested that Medihoney could prevent the emergence of resistance as resistant colonies were observed on

agar plates with rifampicin and 5% sugar solution but not on rifampicin and 5% Medihoney plates. Rifampicin is an effective drug for the treatment of *S. aureus* wound and skin infections, but resistance can emerge readily. Resistance is due to a single point mutation in the *rpoB* gene that encodes the β subunit of RNA polymerase, the target of rifampicin. The authors suggested that Medihoney could prevent occurrence of the mutations that cause resistance or Medihoney and rifampicin combination did not allow the bacteria to survive long enough to develop resistance. The researchers also investigated the effects of methylglyoxal (MGO), the main antimicrobial component of manuka honey, in combination with rifampicin on antibacterial activity and concluded that MGO (70 $\mu\text{g}/\text{mL}$) exerted an additive rather than a synergistic effect. The authors were of the view that MGO was not fully responsible for the synergistic antibacterial effects of manuka honey and suggested that other compounds in the honey such as polyphenols may be involved. Similarly, Hayes et al., (2018) ²⁷ reported that manuka honey or MGO enhanced the activity of the antibiotic linezolid against both a methicillin sensitive and a methicillin resistant strain of *S. aureus*. MHA plates that contained 5% manuka honey (Manuka Health, Auckland, NZ) or MGO (27.5 $\mu\text{g}/\text{mL}$) compared to control plates containing a 5% sugar solution caused a statistically significant increase in linezolid (30 μg) induced zones of inhibition. In contrast, an unpasteurised honey did not enhance the activity of linezolid. Checkerboard microdilution assays determined that in this study the effects of MGO were synergistic rather than additive. Linezolid is effective against many Gram-positive bacteria including *S. aureus* and acts by inhibiting bacterial protein synthesis. The researchers went on to show that MGO increased the intracellular accumulation of linezolid in bacterial cells. In support of a role for MGO, preliminary experiments conducted by Raimkulov (2019) ²⁸ demonstrated that 5% manuka honey (Comvita Medihoney® Wound gel) or MGO (27.5 $\mu\text{g}/\text{mL}$) in tryptone soya agar (TSA) plates enhanced the zone of inhibition induced by rifampicin discs against a clinical isolate of MRSA in comparison to TSA plates containing an artificial honey solution. In the same experiments, Activon medical grade manuka honey (Advancis Medical, UK) and two Kazakhstan honeys (buckwheat and buckwheat & multifloral) did not enhance the activity of rifampicin against MRSA ²⁸. Similarly, 8 local honeys from the Muscat area of Oman, did not enhance the antimicrobial activity of amoxicillin (10 μg) or

clarithromycin (15 µg) when 50 µl of 100% honey and antibiotics were placed on *Helicobacter pylori* inoculated agar plates at various distances apart ²⁹.

Synergistic effects of sub-inhibitory concentrations (< 8% w/v) of manuka honey (Comvita, Ltd) and rifampicin on *S. aureus* biofilm formation and on established biofilms have also been observed *in vitro* ^{24, 30}. Interestingly, some antibiotic combinations (clindamycin, gentamicin and oxacillin) showed an antagonistic effect on *S. aureus* established biofilms when the honey was used at sub-inhibitory concentrations but not at higher honey concentrations above the MIC (e.g. 16% w/v). Physiological and metabolic differences in bacteria within a biofilm could impair the ability of sub-inhibitory concentrations of honey in combination with antibiotics to kill the bacteria.

Other researchers support the occurrence of enhanced effects of honey and antibiotics. Klein et al, (2020) ³¹, used a broth culture assay to investigate the ability of subinhibitory concentrations of a range of medical grade honeys (Comvita® Manuka Medihoney®; Comvita® Medihoney® Antibacterial Wound Gel™; Revamil® gel; and Surgihoney™RO®) to enhance the activity of antibiotics (tetracycline, sulphatriad, streptomycin, penicillin G, chloramphenicol and ampicillin) against *S. aureus* and *P. aeruginosa*. In their study, bacteria were incubated aerobically overnight in broth cultures containing 10% honey. Samples from these cultures were then spread onto TSA plates and antibiotic discs (Mastring-S, Mast Group Ltd) were added to the agar and incubated at 37°C. The researchers found that the ability of the honeys to enhance the activity of the antibiotic depended on the type of honey, the antibiotic, and the bacterial species. Their key findings were that Surgihoney™RO® and Comvita® Medihoney® Antibacterial Wound Gel™ increased the activity of tetracycline and ampicillin against *S. aureus* and increased the activity of tetracycline against *P. aeruginosa*.

There is sufficient evidence to suggest that honey can function synergistically with antibiotics to increase bacterial killing *in vitro*. The outcome however is influenced by the concentration and the type of honey, the bacterial species or strain, the status of the bacteria i.e. planktonic versus biofilm and the type of antibiotic. Processes are not fully understood but possible mechanisms are suggested in Figure 1. Honey is known to contain a complex mixture of polyphenols each of which could act individually or synergistically with other polyphenols or other antimicrobial components to exert diverse biological effects. Furthermore, antibiotic groups

kill bacteria via different mechanisms and therefore the interaction of antibiotics with the unique components in different types of honey would likely result in diverse outcomes. Polyphenols may be important in the synergistic interactions of honey and antibiotics, but their role requires further investigation. Table 3 summarises key research findings on the antimicrobial effects *in vitro* of honey and antibiotic combinations.

Figure 1. Possible mechanisms by which honey and antibiotics synergistically kill bacterial cells

Table 3. Summary of the key *in vitro* findings of the antimicrobial activity of honey and antibiotic combinations

Type of honey	Antibiotic	Method	Micro-organism	Key findings	Reference
Manuka honey (5% w/v)	Oxacillin	Disc diffusion, E-test strip, broth dilution, chequerboards, growth curves.	MRSA	Synergistic antimicrobial activity against MRSA and reversal of oxacillin resistance.	23
Manuka honey (Manukacare, 18+, Comvita), (5% w/v)	15 antibiotics	Disc diffusion, E-test strip, broth dilution, chequerboards, growth curves.	MRSA <i>P. aeruginosa</i>	Synergistic antimicrobial activity against MRSA for manuka honey and tetracycline, imipenem and mupirocin combinations and additive activity against <i>P. aeruginosa</i> for manuka honey and tetracycline, rifampicin and colistin combinations.	26
Medihoney (Comvita Ltd) 5%, 7% w/v	Rifampicin	Disc diffusion assay, chequerboards, time kill curves.	MRSA, clinical isolates of <i>S. aureus</i> .	Synergistic antimicrobial activity of Medihoney and rifampicin combination against MRSA and clinical isolates of <i>S. aureus</i> .	25
Manuka honey (Comvita Ltd) (<8%)	Rifampicin, oxacillin, gentamicin, clindamycin	Disc diffusion assay, chequerboards	Strains of <i>S. aureus</i> (planktonic growth and biofilm formation)	Rifampicin and manuka honey considered to be most effective as demonstrated synergistic antimicrobial activity against all tested strains including planktonic bacteria and <i>S. aureus</i> biofilm formation. Clindamycin or oxacillin manuka honey combinations demonstrated synergistic antimicrobial activity against most strains (planktonic and biofilm formation) but not all. Gentamicin and manuka honey combinations demonstrated an additive antimicrobial effect.	24

Manuka honey (Medihoney, Comvita Ltd) (inhibitory and subinhibitory concentrations tested).	Rifampicin, fusidic acid, clindamycin, gentamicin, oxacillin.	Checkerboard microdilution assays, viability assays, MacSynergy II analysis.	<i>S. aureus</i> , established biofilm	Most effective combination against established <i>S. aureus</i> biofilms was rifampicin and Medihoney. Fusidic acid and Medihoney combination induced some synergistic antimicrobial effects. Clindamycin, gentamicin, and oxacillin Medihoney combinations induced antagonistic effects against <i>S. aureus</i> established biofilms when honey was used at sub-inhibitory concentrations but not at inhibitory concentrations.	30
Manuka honey (Manuka Health, NZ) (5%)	Linezolid	Disc diffusion assay	MRSA and MSSA strains of <i>S. aureus</i>	Manuka honey increases the sensitivity of MRSA and MSSA to linezolid.	27
Four medical grade honeys: 1. Comvita® Manuka Medihoney® 2. Comvita® Medihoney® Antibacterial Wound Gel™; 3. Revamil® gel 4. Surgihoney™ RO®	Tetracycline, sulphatriad, streptomycin, penicillin G, chloramphenicol and ampicillin.	Broth culture assay	<i>S. aureus</i> <i>P. aeruginosa</i>	Surgihoney™RO® and Comvita® Medihoney® Antibacterial Wound Gel™ increased the sensitivity of <i>S. aureus</i> to tetracycline and ampicillin. Comvita® Manuka Medihoney® did not enhance the sensitivity of <i>S. aureus</i> to any of the antibiotics tested. Comvita® Medihoney® Antibacterial Wound Gel™, Comvita® Manuka Medihoney® and Surgihoney™RO® enhanced the sensitivity of <i>P. aeruginosa</i> to tetracycline. Whether the enhanced antimicrobial effects were synergistic, or additive was not determined in this study.	41

Honey and antibiotics: in vivo studies

Studies specifically investigating synergistic effects of honey and antibiotic combinations *in vivo* are lacking. One study by Mat Lazim et al, (2012) ³² involved the use of tualang honey and the antibiotic sultamicillin for the treatment of wounds occurring after tonsillectomy. An open labelled prospective randomised clinical trial involving two treatment groups was conducted. In the first group, 35 tonsillectomy patients (average age of 9) received intra-operative oral administration of 3 mls of tualang honey and then post-operative oral administration of 4 mls of tualang honey 3 times daily for seven days. In addition to the honey treatment, group 1 also received intravenous 25mg/kg sultamicillin three times daily for 2 days and then oral sultamicillin two times daily for 5 days. In group 2, 28 tonsillectomy patients (average age 11) were treated with the antibiotic regime only. The authors reported that wound healing was significantly faster in the honey and antibiotic treated group in comparison to the antibiotic only treated group. Case reports also exist, for example, one report of a diabetic patient with a chronic ulcer on the right lower limb described successful healing following treatment with honey dressings, systemic antibiotic therapy, surgical toilet, and skin graft ³³. Similarly, another case study reported on the successful healing of a deep diabetic foot ulcer in a 38 year old female patient treated with intravenous antibiotics (metronidazole and ceftriaxone), surgical debridement and daily dressings covered with trigona honey (harvested from the Borneo jungle) ³⁴.

Clearly more research that specifically investigates the combined effects of honey and antibiotics *in vivo* is required to fully determine clinical effectiveness. It is also important to consider how honey and antibiotics should be administered clinically to maximise synergistic or additive effects. In wound management, an antibiotic may be administered systemically facilitating entry from the base of the wound, whilst honey is administered topically, reaching the upper surface of the wound. In diabetic patients, the ability of systemically administered antibiotics to reach the wound may be impaired due to poor circulation. An alternative approach may be to administer both the honey and the antibiotic topically. Another factor to consider is the concentration of honey to use. In wound healing, high concentrations of honey (>90% w/v) are commonly used, yet many of the experiments investigating synergistic effects of honey and antibiotics *in vitro* have used low subinhibitory concentrations of honey e.g. 5% w/v. Furthermore,

despite the general believe that bacterial resistance to honey does not occur there is one study that suggests that resistance is possible. Camplin and Maddocks (2014) demonstrated that *P. aeruginosa* biofilms treated with manuka honey (Medihoney, Comvita) developed increased resistance to manuka honey and to the antibiotic's imipenem and rifampicin, as demonstrated by an increase in minimal inhibitory concentration (MIC) in recovered isolates ³⁵. The resistance was suggested to be due to small colony variants and the authors suggested that in clinical practice medical honey should be administered for a sufficient period of time and in combination with other antimicrobials so that the infection is sufficiently cleared and the chances of resistance reduced. *In vivo* clinical studies are needed to fully understand the combined effects of honey and antibiotics in wound management and to determine treatment protocols that optimise synergistic or additive interactions and healing.

Honey and other natural agents: in vitro studies

In vitro research has also examined the antimicrobial effects of combining honey and other natural agents such as ginger, royal jelly, propolis and vitamins. Ewnetu et al, (2014) ³⁶ reported that mixtures of Ethiopian honey and ginger extracts (50% v/v) had superior antimicrobial activity than ginger extract (50% v/v) or honey alone (50% v/v) in a well diffusion assay against *E. coli*, *Staphylococcus aureus* (MRSA), and antibiotic resistant strains of *E. coli* and *K. pneumoniae*. Reportedly, the honey and ginger extract combination induced larger zones of inhibition than standard antibiotic discs. Boukraa (2008) ³⁷ described enhanced effects of subinhibitory concentrations of Algerian honeys (orange blossom and eucalyptus) when combined with royal jelly against *P. aeruginosa*. For example, when compared with the MIC of honey alone there was a considerable decrease (~ 90%) in the MIC when honey (1% v/v) was combined with 3% royal jelly. Al-Waili et al, (2012) ³⁸ using broth macro-dilution reported synergistic effects of subinhibitory concentrations of sumra honey from Saudi Arabia when combined with subinhibitory concentrations of propolis against *S. aureus*, *E. coli* *C. albicans* and mixed microbial cultures. The MICs of honey and propolis combinations were lower than honey and propolis alone against all micro-organisms tested in the study. Propolis obtained from Saudi Arabia demonstrated superior synergistic effects in comparison to propolis obtained from Egypt suggesting that the type of propolis is important. In a short communication by Kowalski and Makarewicz (2017) ³⁹ honey

supplemented with 1% propolis demonstrated higher antibacterial activity than honey alone against *E. coli* in a well diffusion assay. Similarly, Osés et al, (2016) ⁴⁰ reported synergistic antimicrobial effects of honeys from Spain against *E. coli*, *S. aureus* and *P. aeruginosa* in a disc diffusion assay when ethanol extracts of propolis (0.1, 0.3 or 0.5%) were added to the undiluted honey. Furthermore, the anti-inflammatory effects of the honey (75%), measured using a hyaluronidase inhibition assay, were enhanced when propolis ethanol extracts were added. Honey has also been combined with other natural agents including cinnamaldehyde found in cinnamon and carvacrol, a monoterpene found in thyme. The combination of honey obtained from Damavand district Iran, with sub-MIC concentrations of cinnamaldehyde and carvacrol had greater antibacterial activity than honey alone against suspensions of clinical isolates of *P. aeruginosa* ⁴¹. This triple combination was also reported to reduce the expression of *exoS* gene involved in *P. aeruginosa* virulence and *ampC* gene involved in *P. aeruginosa* resistance to antibiotics such as carbapenems and monobactams. Even more complex mixtures of natural agents have been tested. For example, Dashtdar et al, 2016 ⁴² prepared a gel composed of herbal extracts of *Acacia catechu*, *Castanea sativa*, *Ephedra sinica* and *Momia* combined with honey (25%), maple saps, *Phoenix dactylifera* (date), pomegranate extract and *Azadirachta indica* gum. This gel had a higher antibacterial activity in an agar well diffusion assay than the antibiotic cloxacillin or honey alone against *P. aeruginosa*. Natural bioactive agents such as vitamins have also been combined with honey and antimicrobial activity assessed. Majtan et al, (2020) ⁴³ investigated the antimicrobial activity of Slovakian honeys and a commercially available UMF 15+ manuka honey (Natures Nectar, UK) supplemented with sub-MIC concentrations of vitamin C in an MIC assay against bacterial isolates including *P. aeruginosa* and *S. aureus* as well as against *P. aeruginosa*, *S. aureus*, *S. agalactiae* and *E. faecalis* multi-species biofilms. Their key findings were that supplementation of honey with sub-MIC concentrations of vitamin C reduced the MIC of all types of honey against planktonic preparations of *P. aeruginosa*. In contrast, supplementation of honeydew honey with sub-MIC concentrations of vitamin C increased the MIC against planktonic *S. aureus*. Honeydew honey (100%) supplemented with vitamin C (100mg/g of honey) had superior antibiofilm activity than honey alone and caused clearance of all the bacterial species within the biofilm after 48 hours. Vitamin C is reported to have antibacterial

activity and the authors suggested that the antibacterial effects of honey and vitamin C combination may be due to increased production of reactive oxygen species in bacterial cells. In another study, L-Mesitran, a medical honey formulation containing 40% Mexican yucatan honey and vitamins C and E was reported to have anti-fungal activity against *Candida albicans* whilst 40% Mexican yucatan honey alone did not ⁴⁴. The authors suggested that the vitamins or other components in the L-Mesitran formulation may be enhancing the antifungal activity of the honey. Furthermore, L-Mesitran formulation was found to have superior antimicrobial activity against *Staphylococcus pseudintermedius*, a cause of canine pyoderma, and *Malassezia pachydermatis* in comparison to the honey component of L-Mesitran only ⁴⁵. The authors again concluded that other components in the L-Mesitran formulation which include medical grade hypoallergenic lanolin, propylene glycol, polyethylene glycol 4000 and vitamins C and E may be enhancing the antimicrobial activity of the honey.

Synergism between honey and other natural agents could be due to interactions between constituent polyphenols or both substances may be acting on similar mechanistic pathways leading to an enhanced biological effect. Table 4 summarises key research findings on the antimicrobial effects *in vitro* of honey and natural agent combinations.

Table 4. Summary of the key *in vitro* findings of the antimicrobial activity of honey and natural agent combinations

Type of honey	Natural agent	Method	Micro-organism	Key findings	Reference
Ethiopian honey (50% v/v)	Ginger extracts (50% v/v) Prepared honey ginger mixture (50% v/v).	Agar well diffusion assay, broth assay.	<i>S. aureus</i> , MRSA, <i>E. coli</i> (sensitive and resistant strains). <i>K. pneumoniae</i> (resistant)	Honey and ginger combination induced higher mean zones of inhibition than honey or ginger alone or standard antibiotic discs (methicillin, penicillin, amoxicillin). MBC of honey-ginger extract was 12.5% for all bacteria tested.	36
Algerian honey (orange blossom, eucalyptus)	Royal jelly (sub-MIC concentrations (3% (v/v); 2% (v/v); 1% (v/v) added to honey).	MIC assay	<i>P. aeruginosa</i>	For each type of honey there was > 90% decrease in the MIC when 3% (v/v) royal jelly was added; a 66.6% decrease in MIC when 2% (v/v) royal jelly was added and a 50% decrease in MIC when 1% (v/v) royal jelly was added.	37
Saudi Arabian sumra honey (<i>Acacia tortilis</i>)	Ethyl alcohol extract of propolis (Egyptian and Saudi Arabian) (sub MIC concentrations)	MIC assay	<i>S. aureus</i> , <i>E. coli</i> , <i>C. albicans</i> , polymicrobial cultures.	The MIC of mixtures of honey and propolis combined were lower than honey or propolis alone.	38
Polish honey (lime) (75% w/w)	Ethanol extract of propolis (1%),	Agar well diffusion assay	<i>E. coli</i>	Larger zones of inhibition when honey was enriched with 1% propolis.	39

Spanish honeys (heather, chestnut and multifloral)	Ethanol extracts of propolis (0.1, 0.3 or 0.5%) harvested from Spain.	Agar diffusion assay	Microorganisms including <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> .	In general, enhanced antimicrobial activity was observed when honey was supplemented with propolis. The increase varied depending on the type of honey, concentration of propolis and test micro-organism.	40
Iranian honey from Damavand district, Iran.	Cinnamaldehyde and carvacrol (sub-MIC concentrations)	Broth dilution method	<i>P. aeruginosa</i>	The MIC of the combination of honey, cinnamaldehyde and carvacrol was considerably lower than honey alone (0.49µg/mL vs 114.2 µg/mL respectively). The antibacterial activity of the triple combination was reportedly greater than imipenem.	41
Iranian honey (25%) from Fasa, Iran	Gel composed of herbal extracts of <i>Acacia catechu</i> , <i>Castanea sativa</i> , <i>Ephedra sinica</i> and <i>Momia</i> combined with honey (25%), maple saps, <i>Phoenix dactylifera</i> (date), pomegranate extract and <i>Azadirachta indica</i> gum	Agar well diffusion assay	<i>P. aeruginosa</i>	Zone of inhibition induced by the herbal gel formulation was greater than honey alone (35.1 mm vs 13.1 mm respectively).	42
Slovakian honeys (sunflower,	Vitamin C (Sub-inhibitory concentrations)	MIC assay	Planktonic bacteria including <i>P.</i>	Supplementation of honey with vitamin C reduced the MIC of all honey types tested against planktonic <i>P. aeruginosa</i> . Supplementation of honeydew honey with	43

acacia and honeydew) UMF 15+ manuka honey (Natures Nectar, UK)		Biofilm wound model	<i>aeruginosa</i> and <i>S. aureus</i> . Multispecies biofilms (<i>P. aeruginosa</i> , <i>S. aureus</i> , <i>S. agalactiae</i> , <i>E. faecalis</i>)	vitamin C increased the MIC against planktonic <i>S. aureus</i> . Honeydew honey supplemented with Vitamin C (100mg/g) had superior antibiofilm activity than honey alone.	
Mexican yucatan honey (L-Mesitran)	Vitamins C and E L-Mesitran contains 40% Mexican yucatan honey and vitamins C and E	Antifungal MIC method for yeasts	<i>C. albicans</i>	L-Mesitran demonstrated antifungal activity against <i>C. albicans</i> (MIC 25%–50%), whereas 40% Mexican yucatan honey did not.	44
Mexican yucatan honey (L-Mesitran)	Vitamins C and E L-Mesitran contains 40% Mexican yucatan honey and vitamins C and E	Microbroth assay to determine MBC	<i>Staphylococcus pseudintermedius</i> , <i>Malassezia pachydermatis</i>	L-Mesitran had lower MBC in comparison to the honey component of L-Mesitran only.	45

Honey and other natural agents: in vivo studies

Again, few studies have specifically investigated the therapeutic effects of honey combined with other natural agents *in vivo*. One case report by Lofty et al, (2006) ⁴⁶ described the case of a 65-year-old diabetic male patient with a deep infected wound on the foot. The patient was treated with systemic antibiotics; metronidazole (1500 mg per day) and combined amoxicillin with clavulanic acid (1500 mg/day) for 10 days. Thereafter, ciprofloxacin (1500 mg/ day) was used. Healing of the wound reportedly improved significantly when the wound was treated topically every day with a paste containing 800 mg of bee propolis and 50 g of myrrh mixed with honey. In a prospective, open-label block randomised controlled clinical study conducted in horses it was found that intralesional application of L-Mesitran to wounds prior to wound closure improved healing and reduced infection (data from 69 horses) in comparison to wounds that were not treated with L-Mesitran (data from 58 horses) ⁴⁷. Furthermore, Nair et al (2020) reported on the successful healing of diabetic foot ulcers (six patients) following topical application of L-Mesitran ⁴⁸. The wounds had previously failed to respond to a range of other treatments including antibiotics. Another case study reported on successful eradication of infection in an MRSA infected titanium mesh in an incisional hernia in a 70-year-old female patient treated with L-Mesitran ⁴⁹. The honey formulation was applied to the wound cavity and covered with gauze daily. More studies conducted in humans are needed to fully determine the efficacy of the combination of honey and other natural agents for the treatment of skin and wound infections.

Honey and other therapies (in vitro and in vivo studies)

Other therapeutic agents being considered for use in combination with honey for the treatment of wounds include honey and bacteriophage therapy, honey supplemented with antimicrobial peptides, honey combined with cyclodextrins or hydrogel or honey and laser therapy. Novel approaches to wound healing have involved adding a combination of antimicrobial agents to nanofibrous scaffolds. For example, Sarhan and Azzazy (2017) ⁵⁰ reported that honey (Egyptian clover) (30%) chitosan nanofibers loaded with bee venom (0.01%) and bacteriophages had superior antimicrobial activity in a broth culture assay against MDR *P. aeruginosa* in comparison to ionic silver

containing Aquacel-Ag wound dressing. Furthermore, the novel honey bacteriophage dressing demonstrated better wound healing ability in mice when compared to Aquacel-Ag wound dressing. Oliveira et al, (2017) ⁵¹ also reported synergistic antimicrobial effects against *E. coli* biofilms formed on 96 well plates at 12 and 24 hours using multi-floral Portuguese honey and an *E. coli* specific bacteriophage. This approach combines the antibiofilm effects of honey with the ability of bacteriophages to lyse bacteria. Scanning electron microscopy revealed that honey caused perturbations in the membrane of the *E. coli* whilst the bacteriophage caused complete lysis with only vesicle structures left behind.

Kwakman et al, (2011) ⁵² investigated the antimicrobial effects of supplementing Revamil honey with the antimicrobial peptides LL-37 and the synthetic peptide bacterial peptide 2 (BP2). They reported that the antimicrobial activity of LL-37 was inhibited in the presence of honey, but this was not the case for BP2. Using a liquid bactericidal assay, it was shown that Revamil honey supplemented with 75µM BP2 had more rapid antibacterial activity against MRSA and extended spectrum β lactamase (ESBL) *E. coli* and had a broader spectrum of antibacterial activity than Revamil alone. The authors considered supplementing the honey with hydrogen peroxide or MGO but were concerned that high levels of these components may not be biocompatible.

Hydrogel is commonly used as a wound dressing. It is composed of polymers suspended in water and is thought to provide hydration to a wound and promote healing. El-Kased et al, (2017) ⁵³ reported that an Egyptian honey (75%) chitosan hydrogel formula had superior antimicrobial activity against *P. aeruginosa*, *S. aureus*, *K. pneumonia* and *Streptococcus pyogenes* in a disc diffusion assay in comparison to pure honey. For example, the mean zone of inhibition for the honey 75% chitosan hydrogel formula against *S. aureus* was 20.2 ± 0.4 vs 15.1 ± 0.9 for pure honey. This formula was also superior to pure honey in the healing of 10 mm burn wounds induced in mice using a heated metallic rod. The wounds were treated with either the honey 75% chitosan hydrogel formula or pure honey for 9 days. By day 9, the honey chitosan treated wounds had a mean diameter of 3.8 ± 0.2 versus 5.3 ± 0.8 in the honey only treated group. Furthermore, an alginate-based honey hydrogel containing thymol-based honey from Damavand, Iran was reported to have superior wound healing

ability in rats with infected burn wounds than honey alone⁵⁴. Wound healing was faster in the alginate based honey hydrogel treated group in comparison to the honey only group. In another study, a paste composed of carboxymethyl cellulose hydrogel (50%), water (30%) and chestnut honey (20%) had superior antimicrobial activity against *S. aureus* and *E.coli* in a disc diffusion assay than a paste composed of carboxymethyl cellulose hydrogel (50%) and water only⁵⁵. Furthermore, the paste containing 20% chestnut honey had superior wound healing ability in comparison to the paste without honey in the treatment of wounds inflicted in the dorsal skin of mice. Tavaloki and Tang (2017)⁵⁶ designed a wound dressing consisting of a high concentration of honey, polyvinyl alcohol hybrid hydrogel and borax as a crosslinking agent. High concentrations of honey are important for wound healing but can negatively affect the physiochemical properties of hybrid hydrogels. The use of borax apparently overcomes this problem and permits the incorporation of high concentrations of honey without negative effects.

Alpha-cyclodextrin is an oligosaccharide that has also been complexed with manuka honey in a formulation named Manuka honey with Cyclopower™ and supplied by Manuka Health New Zealand Ltd. Using MIC, MBC and time course experiments Manuka honey with Cyclopower™ was found to have improved bacteriostatic activity against *S. aureus*, MRSA and *P. aeruginosa* in comparison to uncomplexed manuka honey also supplied by the same company⁵⁷.

Researchers have also explored the use of honey combined with laser treatment for wound healing. Yadav et al, (2018)⁵⁸ investigated the combined effect of medical grade manuka honey (Medihoney, Derma Sciences Inc.) and 904 nm super pulsed laser treatment on the healing of full thickness burn wounds induced in rats. The wound healing effects of pulsed laser therapy in the red and near infrared spectrum is not fully understood, but it has been suggested that photon energy from this wavelength is absorbed by chromophores in the skin leading to modulation of transcription factors, changes in protein synthesis and enhanced cell proliferation and survival. Combining the antimicrobial effects of honey with the wound healing effects of laser therapy is a unique and novel approach to wound healing. In their study, 4 groups of 6 rats were used. In group 1, wounds were treated with super pulsed 904nm laser treatment, 0.2 J/cm² daily for seven consecutive days post

wound. Group 2 wounds were treated with honey (applied topically over the surface of the wound, 1-hour post burn wound, and daily for 7 consecutive days post wound). Group 3 wounds were treated with a combination of the super pulsed 904nm laser treatment regime and the honey regime, whilst group 4 wounds were left untreated. The findings demonstrated that the group that received the combination therapy had enhanced wound closure, as measured using image J software, and evidence of lower levels of inflammation indicated by lower levels of TNF- α , IL-1 β and COX-2 protein expression in the wound tissue in comparison to the other groups.

Rudzka-Nowak et al, (2010) ⁵⁹ reported on the case of a 55-year-old woman with inflamed and necrotic lesions in the abdominal integuments and lumbar region following rupture of the colon. The wound was infected with *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*. The patient was treated with antibiotics linezolidum and metronidazole and then with manuka honey activon tulle dressings (replaced on alternate days) as well as the Genadyne A4 negative pressure wound therapy system. Genadyne A4 provides sub atmospheric pressure across a wound and is reported to reduce swelling, remove wound exudate and bacteria from the wound surface, and stimulate growth of fibroblasts and endothelial cells. Following 3 weeks of the combined treatment the wound healed sufficiently enough for a skin graft to be performed.

Conclusions

There are several therapeutic agents or therapies that could potentially be combined with honey to enhance antimicrobial activity and wound and skin healing. *In vitro* studies have investigated the combination of honey with antibiotics, natural agents e.g. propolis, bacteriophages, antimicrobial peptides, laser treatment and hydrogel. The available research suggests that several honey combination therapies have superior antimicrobial activity in comparison to honey alone and in some cases, the effect is synergistic. Honey could be supplemented with other agents as a formulation or as an additional therapy. Honey contains a range of antimicrobial components, including sugar, hydrogen peroxide (H₂O₂), MGO, polyphenols and antimicrobial peptides. Consideration could be

given to supplementing honey with either of these components. However, the combining agent as well as being able to increase antimicrobial activity should be biocompatible and promote re-epithelialisation and wound closure. Therefore, consideration should be given to the potential toxicity of the combining agent as well as the most effective honey to use. Whilst manuka honey has been shown to promote reepithelialisation certain honeys may be more cytotoxic to skin cells than others. Modern delivery methods such as biomimetic nanostructured meshes developed using a layer by layer assembly method allow for a more controlled release of honey that may have lower cytotoxicity and could be more appropriate for the treatment of certain types of wounds or skin lesions ⁶⁰. Modern bioengineering methods have also involved incorporating honey into cryogels ⁶¹. The pH of the combining agents used should also be considered as low acidic pH is thought to be more favourable for wound healing (personal communication, Dr Lorna Fyfe) and attention could also be given to immunomodulatory or anti-inflammatory properties. More clinical studies in humans that specifically investigate the effects of honey combination therapies for the treatment of wounds and skin lesions are needed. Future research may involve combining honey with other bioactive agents such as polyphenols, other plant derived compounds or other agents known to accelerate wound healing such as hyaluronic acid ⁶². Such novel approaches to treatment could be considered not only for wounds but also other types of skin disease. Honey is a relatively cheap substance that could be utilised globally as a combination therapy if efficacy is established. Furthermore, the reduction or replacement of antibiotic use with other therapies has the potential to lower the risk of development of antibiotic resistant bacterial strains. Honey combination therapies have the potential to improve clinical outcomes for patients with wound and skin infections and therefore further research in this area should be encouraged.

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