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REVIEW

On the antibacterial effects of manuka honey: mechanistic insights

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Abstract: Antimicrobial resistance (AMR) is an increasing clinical problem precipitated by the inappropriate use of antibiotics in the later parts of the 20th Century. This problem, coupled with the lack of novel therapeutics in the development pipeline, means AMR is reaching crisis point, with an expected annual death rate of ten million people worldwide by 2050. To reduce, and to potentially remedy this problem, many researchers are looking into natural compounds with antimicrobial and/or antivirulence activity. Manuka honey is an ancient antimicrobial remedy with a good track record against a wide range of nosocomial pathogens that have increased AMR. Its inhibitory effects are the result of its constituent components, which add varying degrees of antimicrobial efficacy to the overall activity of manuka honey. The antimicrobial efficacy of manuka honey and some of its constituent components (such as methylglyoxal and leptosperin) are known to bestow some degree of antimicrobial efficacy to manuka honey. Despite growing in vitro evidence of its antimicrobial efficacy, the in vivo use of manuka honey (especially in a clinical environment) has been unexpectedly slow, partly due to the lack of mechanistic data. The mechanism by which manuka honey achieves its inhibitory efficacy has recently been identified against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with both of these contrasting organisms being inhibited through different mechanisms. Manuka honey inhibits *S. aureus* by interfering with the cell division process, whereas *P. aeruginosa* cells lyse in its presence due to the reduction of a key structural protein. In addition to these inhibitory effects, manuka honey is known to reduce virulence, motility, and biofilm formation. With this increasing in vitro dataset, we review the components and our mechanistic knowledge of manuka honey and how manuka honey could potentially be utilized in the future to impact positively on the treatment of microbial, resistant infections.

Keywords: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, biofilm, antibiotic resistance

Introduction
The problem of antibiotic resistance

The ability of bacteria to adapt and become resistant to antibiotics has been recognized by the scientific community for many decades. *Staphylococcus aureus,*[1] *Acinetobacter baumannii,*[2] and *Enterococci* species[3] are just some of the nosocomial pathogens with increased antimicrobial resistance (AMR) that cause difficult-to-treat infections worldwide. AMR is commonly accrued through genetic changes, which confer a more resistant phenotype on the cell, or through the integration of the cell into a biofilm, which can lead to a transient increase in tolerance to antibiotics of up to 1,000-fold.[4] The biofilm phenotype is commonly found in urinary tract infections,[5] multi-species chronic otitis media,[6,7] and *Pseudomonas aeruginosa* infections in both burns[8] and the cystic fibrosis lung.[9] The prolonged over- and misuse of antibiotics,[10] dwindling antibacterial

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development,[11] and lack of funding for novel therapeutic research[12] has allowed AMR to reach crisis point.

AMR infections are a major health care burden, leading to increased morbidity, mortality, and treatment costs.[13] A recent study estimated the total cost of an AMR infection at between US$70,000 and US$100,000 per person.[14] However it has been suggested that the cost of AMR could be much higher as routine operations, which require prophylactic use of antibiotics (eg, cancer therapy and joint/organ replacements) would also be affected.[15] Recently, initiatives that are designed to stimulate novel therapeutic development, such as the Longitude prize, have been instigated; however, compounds from these initiatives will not be available for several years, due to the inherent lag time in the development process.

To address the issue of AMR in the short term, researchers have generally taken one of two approaches: 1) recombining existing antimicrobial formulations to produce novel combinations; or 2) investigating alternative treatment therapies, while restricting the use of antimicrobial agents that are still currently effective.[16] Many of these therapies have shown promise, as they provide a broad spectrum of activity, targeting multiple cellular processes and therefore reducing the likelihood of AMR arising.[17–20] Some of the alternative antimicrobial therapies investigated include nanoparticles,[21] bacteriophage "cocktails",[22] and natural substances such as honey.[23,24]

Honey as an antimicrobial

Honey has been used for many centuries as a sweeter, food preservative, and therapeutic product.[25] It is produced by honey bees (*Apis mellifera*) and is formed by ripening nectar, honeydew, and bee secretions.[26] Honey can contain over 200 compounds,[27] being broadly comprised of sugars, amino acids, vitamins, minerals, enzymes, flavonoids, phenolic acids, and antioxidants.[28] The exact composition of honey differs depending on the plants foraged by the bees, environmental conditions, and downstream processing.[29] In ancient times, medical treatises described how different honeys should be selected for different ailments,[30] and scientific evidence is now emerging that also supports the careful selection of honeys for medical use.[30] For example, honeys that are darker in color, such as manuka and buckwheat, have higher antioxidant activity than lighter honeys.[31] Honey is reported to have immunomodulatory,[32] antidiabetic,[33,34] antitumor,[35] antifungal,[36] antiviral,[37] and antibacterial properties.[23,38–40] A brief summary of the historical and modern medical claims for use of honey can be found in Table 1.

There has been a renewed interest in using honey, in particular manuka honey, to treat bacterial infections, especially those with AMR characteristics.[78] This interest is due to an increasing amount of evidence reporting the successful use of honey in the treatment of topical infections, some of which are not responsive to conventional treatments.[46,79] Several in vitro studies have reported that manuka honey has a synergistic activity when combined with antibiotics such as oxacillin,[80] rifampicin,[52] and vancomycin.[55] In addition, honey can be used for prolonged treatments due to its low toxicity,[35,81] and to date, little bacterial resistance to honey has been reported.[82]

Despite the apparent benefits of honey for the management of infection, its use is not currently widespread in the developed world. The poor uptake by clinicians[47] is due in part to a lack of scientific data pinpointing the mode of action against pathogens of interest.[27] To combat these concerns, the past two decades have seen the number of research groups and the number of papers published on honey steadily rise, with studies focusing on the identification of active components, mode of action, and clinical efficacy of honey. Herein, we review the current understanding of these aspects, with a focus on manuka honey due to its perceived enhanced antimicrobial activity (compared to other honey types), and

Table 1 Summary of medical claims, both historical and modern, which have been attributed to honey

Timeframe	Claim	References
Historical	Wound salve (Roman, Egyptian, Assyrian, Chinese, and Greek texts all reference the use of honey to treat wounds)	41,42
	Treatment of gut diseases (diarrhea and constipation)	41
	Pain relief	41
	Control of acute fever	41
	Treatment of eye infection	43
Modern	Control of infection in wounds (including surgical, ulcerated, and burn wounds)	39,42,44–49
	Treatment of multidrug-resistant topical infections	50–52
	Treatment of bacterial biofilm infections	53–61
	Treatment of bacterial gut infections (ie, *Helicobacter pylori*, *Clostridium difficile*)	56,62,63
	Promotion of faster wound healing	64–66
	Wound debridement	67,68
	Decreased duration of diarrhea and gastroenteritis	69,70
	Conjunctivitis	44
	Treatment of cancer	35,71
	Alleviation of chemotherapy treatment symptoms	72–74
	Decreased inflammation	75,76
	Reduction of Crohn's disease symptoms	77

since it is already a licensed medical product in Australia, New Zealand, the UK, Europe, Canada, and US.[83]

Components of honey with antimicrobial activity

Defining the precise cause of the antimicrobial activity seen in honey is complicated due to the multifactorial nature of honey. Honeys have high osmolality due to the high concentration of sugars,[29] and it has been shown that 61% of honeys tested have antibacterial activity, which can be attributed solely to their high osmotic potential.[30] In addition to this feature, the majority of non-manuka honeys' antimicrobial activity is derived from the production of hydrogen peroxide (H_2O_2) upon dilution and subsequent generation of reactive oxygen species.[84] Although the activity generated by H_2O_2 is potent, that activity can be curtailed by catalase.[85] In a wound environment, where catalase is commonly released from human tissue, this curtailment leads to reduced antimicrobial activity of the honeys, therefore raising doubts over their use in a clinical setting. Other components such as immune modulatory molecules, eg, bee defensin 1,[85] phenolics,[86,87] and flavonoid compounds,[88] also contribute to activity in some honeys.

The antimicrobial activity of manuka honey is not H_2O_2-based; thus far, however, the constituents responsible for its activity have not yet been fully elucidated.[89] To date, both methylglyoxal (MGO) and leptosperin have been identified as major contributors to its enhanced antimicrobial activity.[90,91] An overview of the active components of a range of honeys, including manuka honey, coupled with their mechanism of action, is given in Table 2.

There are phenolic compounds within manuka honey that remain unidentified.[89] Some of these compounds, such as leptosperin, could have activity similar to MGO.[91] A study testing 20 Canadian honeys showed that those containing the highest quantity of phenolic compounds, in this case wildflower and buckwheat honeys, also had the most antioxidant and antimicrobial activity.[97] Other studies have also shown

Table 2 Brief description of the antibacterial compounds currently understood to be contained within honey and a description of their mechanism of action

Component	Type of honey	Antimicrobial effect	References
Bee defensin 1	Revamil®	An antimicrobial peptide produced and secreted by the bees into the honey. Bee defensin 1 has been isolated from non-manuka honey. The antimicrobial activity of bee defensin 1 is due to its ability to form pores in bacterial membranes, compromising membrane integrity and causing cell lysis.	85,92
Hydrogen peroxide (H_2O_2)	Predominantly observed in non-manuka honeys (although low levels of activity are also found in manuka honey)	Generated by the activity of bee glucose oxidase, H_2O_2 is hypothesized to be a major antimicrobial component of many non-manuka honeys. Although the concentration of H_2O_2 within honey is far below that used medically, it is capable of causing DNA damage and interacting with other components of the honey, increasing its activity through hydroxyl radical production.	31,50
Leptosperin	Manuka honey	Initially named leptosin, this molecule is a novel glycoside of methyl syringate, which inhibits myeloperoxidase activity. Leptosperin is only found in manuka honey, and so has been proposed as a biological marker for manuka honey. Concentrations of leptosperin are positively correlated with the antibacterial activity of manuka honey, although to date, no mechanism of action has been elucidated.	93,91
Jelleins	Canadian buckwheat honey, and Canadian honey of a mixed source also containing buckwheat	Antimicrobial peptides contained in the major royal jelly precursor protein. Polypeptides with high affinity to jelleins have been shown to cause cell membrane damage in both *Bacillus subtilis* and *Escherichia coli*.	94
Methylglyoxal (MGO)	Manuka honey	MGO is found only in manuka honey, and concentrations increase as honey ripens. During maturation, MGO is converted from dihydroxyacetone via non-enzymatic Maillard-like reactions. The antimicrobial activity of MGO is derived from its ability to inactivate proteins by cross-linking them.	90,95,96

that in honey where H_2O_2 activity is not present (due to the addition of exogenous catalase), residual antioxidant activity is still observed.[85,98]

The mechanism of action of methylglyoxal

As mentioned above, manuka honey has been shown to have a very high level of non-H_2O_2 antimicrobial activity when compared to other honeys. This high level of activity has been measured and researched, and the improved levels of antibacterial efficacy have been attributed to several compounds isolated from manuka honey.[85,87,99,100] The overall antibacterial activity of medical grade manuka honey is graded on one of two scales; MGO concentration within the honey, or unique manuka factor (UMF). The UMF rating is based on a linear relationship with phenol when tested against *S. aureus*.[30] MGO is a 1,2-dicarbonyl compound, which is not exclusive to manuka honey, and can be widely found in foodstuffs.[90,100] A study has demonstrated that MGO concentration within manuka honey is directly correlated to the UMF value,[100] indicating that it is responsible for the antimicrobial activity observed. MGO concentrations are much higher in manuka honey (between 38 and 725 mg/kg) than in other honey types (1.6 to 24 mg/kg).[90]

MGO can be formed both enzymatically and non-enzymatically, depending on the other components present in the honey and environmental conditions.[101] MGO within manuka honey is primarily formed by the conversion of dihydroxyacetone to MGO by non-enzymatic Maillard reactions.[96] Manuka honey collected from the hive often contains relatively low levels of MGO and a high concentration of dihydroxyacetone. During storage, this relationship inverts, and MGO levels within the honey increase, due to conversion of dihydroxyacetone.[95]

Antibacterial properties of manuka honey

Manuka honey is known to have antibacterial efficacy against a wide range pathogens, acting on both antibiotic-sensitive and antibiotic-resistant strains (Table 3).[39,102,103]

While MGO is deemed to produce the majority of manuka honey's antibacterial activity, it is interesting to note that its neutralization has negligible effects on manuka honey's ability to inhibit *P. aeruginosa*. This is in stark contrast to *S. aureus* and *Bacillus subtilis*, where the neutralization of MGO results in reduced activity.[90,104] This result confirms the presence of other compounds with inhibitory efficacy, at least against *P. aeruginosa*. Due to the plethora of compounds within manuka honey, there will undoubtedly be a complex

Table 3 Species of bacteria known to be inhibited by manuka honey

Achromobacter xylosoxidans	*Enterococcus faecium*	*Pseudomonas aeruginosa*
Acinetobacter baumannii	*Haemophilus influenzae*	*Salmonella* spp.
Burkholderia cepacia	*Helicobacter pylori*	*Shigella* spp.
Burkholderia cenocepacia	*Klebsiella pneumoniae*	*Stenotrophomonas maltophilia*
Campylobacter jejuni	*Listeria monocytogenes*	*Streptococcus pyogenes*
Clostridium difficile	*Neisseria* spp.	*Staphylococcus aureus*
Escherichia coli	*Proteus* spp.	*Yersinia* spp.

interplay between the various compounds. It is plausible that some interactions may lead to an additive/synergistic action not observed in the individual components. Therefore, the UMF rating appears to be the more thorough method of calculating antibacterial efficacy, encompassing "all" activity and not that derived solely from MGO; however, this theory does have limitations: only the activity against the organism tested can truly be confirmed, as some compounds appear to have organism-specific activity. Therefore, single organism testing (against *S. aureus*, in this instance) can lead to spurious results. Furthermore, as manuka honey contains a range of compounds, their diffusion through the agar may vary, producing misleading results. It is clear that manuka honey has antibacterial efficacy, but how we evaluate this activity should be further investigated. A standardized method (such as micro broth dilution) against a panel of organisms should ensure all aspects of inhibitory efficacy are captured in a reproducible way.

It is important to note that although manuka honey is the only honey currently recognized as having bioactive concentrations of MGO, studies have shown that it may be possible to augment non-manuka honeys by adding MGO or its precursor dihydroxyacetone. One study showed that the addition of dihydroxyacetone to clover honey led to MGO detection.[95] In addition, supplementation of honeys with MGO can increase bactericidal activity to a level comparable with manuka honey.[105] Similarly, supplementation with antimicrobial peptides, such as BP2, increased the speed of bacterial inactivation by Revamil® honey when used against in vitro cultures of six antibiotic-resistant bacterial species.[104]

To the best of our knowledge, bacterial resistance to manuka honey has not been observed in a clinical setting; however, the emergence of cells with decreased susceptibility to honey has been reported in vitro.[106] However, the concentration of manuka honey tolerated was below that

which would be achieved in clinical settings where undiluted manuka honey is used. Conversely, in studies investigating the purposeful passage of cells through sub-inhibitory concentrations of manuka honey did not result in a stable, resistant phenotype.[82,103]

Manuka honey has demonstrated efficacy against a range of organisms assuming the biofilm phenotype in vitro,[56,60,107–109] has been shown to inhibit bacterial species where individual strains have vastly different biofilm-forming abilities,[59] and has been proved to inhibit bacteria where multi-species biofilms are present.[54] A study using manuka-type honeys suggests MGO requires other components (excluding sugars) to have full antibiofilm actions.[59] This result reinforces the notion that multiple compounds in manuka honey produce inhibitory effects, some of which might enhance others. When assessing MGO solely, it is capable of inhibiting *S. aureus* and *P. aeruginosa* biofilms, suggesting some role in the inhibition of this phenotype.[109]

While the antibacterial qualities of manuka honey alone are extremely promising, combination therapy is now being thoroughly scrutinized as a way of reinvigorating antibiotics that are no longer effective.[110–112] Researchers have shown that in vitro combination therapy using sub-inhibitory concentrations of manuka honey reduces the Minimum Inhibitory Concentration (MIC) of antibiotics, effectively "reversing" AMR.[39,80] To date, improved antibacterial efficacy for colistin, imipenem, mupirocin, rifampicin, and tetracycline has been demonstrated when combined with manuka honey.[39,52] These additive/synergistic actions have also been observed

against bacteria assuming a biofilm phenotype.[55] Additive effects against *P. aeruginosa* biofilms treated with gentamicin and manuka honey and synergism between manuka honey and vancomycin against *S. aureus* biofilms have also been reported.[55] These combinations open up a new avenue for future antimicrobial development. Furthermore, with inhibitory activity demonstrated against biofilms,[9] the potential for manuka honey to be utilized clinically, inhibiting both acute and chronic infections, is highly promising.

Mechanisms of antibacterial action of manuka honey

The mechanism of action for manuka honey's antibacterial activity has mainly been elucidated against two prominent opportunistic pathogens: *S. aureus* and *P. aeruginosa*. Interestingly, these mechanistic activities appear to differ greatly from one another. The first documented mechanistic activity for manuka honey was observed against *S. aureus*, where marked structural changes were observed in *S. aureus* cells treated with inhibitory concentrations.[113]

It was later confirmed that manuka honey causes disruption to the regular cell division process of *S. aureus*[114] (Figure 1). Under optimal conditions, bacterial cells duplicate and segregate their chromosome, forming a proteinaceous ring (the septum) across the midcell, creating two still-joined daughter cells.[115] The completion of cell division occurs when peptidoglycan (murein) hydrolases degrade the cell wall between the two daughter cells, allowing separation.[116] Manuka honey has been shown to inhibit the activity (and not the
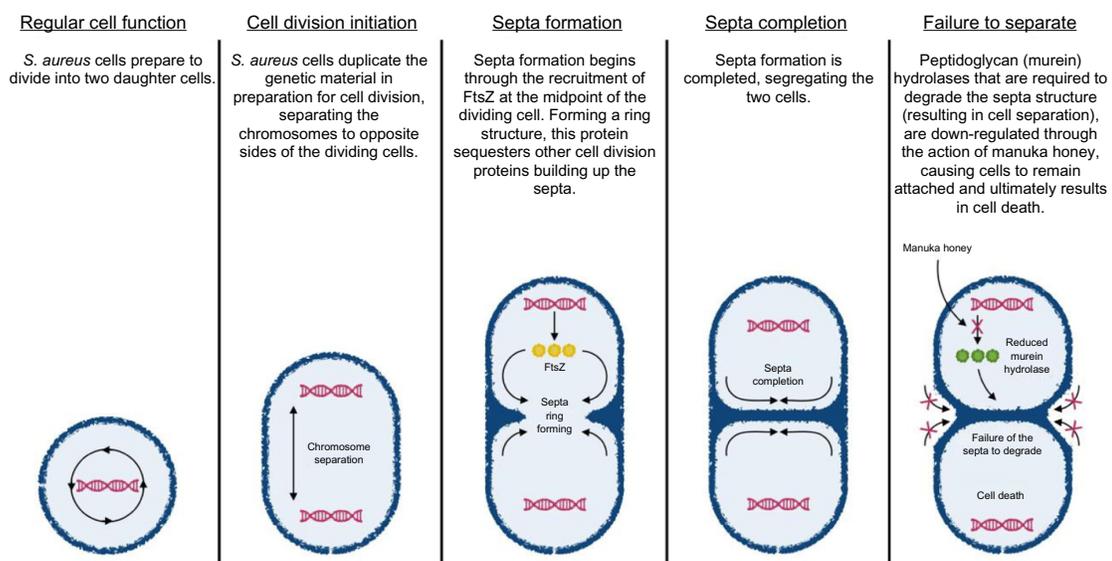

Figure 1 The proposed mechanism by which manuka honey inhibits methicillin-resistant *Staphylococcus aureus* (MRSA). Manuka honey is thought to affect the latter stages of cell division, following the completion of the septa formation. The reduced production of murein (peptidoglycan) hydrolase and/or its sequestering into an inactive state results in the two daughter cells remaining attached due to the inability of the septa to be degraded, which ultimately leads to cell death.

expression) of murein hydrolase, causing a build-up of septated non-dividing cells.[114] Interestingly, many papers conclude the antibacterial action of manuka honey against *S. aureus* is bactericidal;[85,104,113] however, the mechanism described points more toward bacteriostatic activity. Potentially, cells may be viable yet non-culturable. Several papers conclude that the effects seen are independent of the sugars within honey,[113] with one suggesting MGO is also not the causative agent of these inhibitory effects.[114]

In contrast to the mechanism observed in *S. aureus,* studies have proposed an entirely different mechanism against *P. aeruginosa. P. aeruginosa* cells can tolerate higher concentrations of manuka honey when compared to *S. aureus*, with inhibitory concentrations causing the loss of cellular integrity, leading to extensive cell lysis and cell death.[117] *P. aeruginosa* modulates its structural integrity through the production of a key anchor protein: outer membrane protein F (OprF).[118] This protein provides a vital link between the outer membrane and underlying peptidoglycan layer, ensuring cell envelope homeostasis and regular cell shape.[118,119] Reduced OprF expression has been observed in populations treated with manuka honey, and a concomitant increase in membrane blebbing and cell lysis has also been detected (Figure 2).[120]

The different mechanistic actions observed against *P. aeruginosa* (compared to *S. aureus*) highlights the potential for multiple modes of action, and multiple inhibitory compounds in manuka honey. One noteworthy point is that the conserved nature of the cell division process among bacteria suggests manuka honey may affect the cell division process of *P. aeruginosa*. This effect was not observed in the studies above; however, the rate at which cell lysis occurs may not allow for such observations. Published work highlights the necessity of membrane potential for the correct spatial organization of cell division proteins and regular cell division function.[121] This indicates an as yet unidentified link between the mechanistic effects observed in *S. aureus* and *P. aeruginosa*.

In other studies, exposure to manuka honey has been shown to have other effects against a range of organisms. Against *P. aeruginosa*, manuka honey suppresses the class I master regulators (*FleQ* and *FliA*), inhibiting the regulatory cascade required for flagellum production and leading to a significant reduction in flagellated cells.[61] This observation is of clinical significance as adhesion and cellular motility are required for invasive virulence.[122,123] Invasive virulence is problematic, as it allows the dissemination of cells through the bloodstream (bacteremia) to internal organs, which can prove fatal; therefore, the potential to reduce this process is highly valuable. The ability of *P. aeruginosa* to sequester iron from a host may also be prohibited through manuka honey treatment, following the observation of reduced siderophore production in treated samples.[124] Sub-inhibitory concentrations are shown to inhibit cellular binding with fibronectin through the loss of two streptococcal surface proteins, SoF and SfbI.[107] In wound infections, high concentrations of fibronectin are observed;[125] therefore,
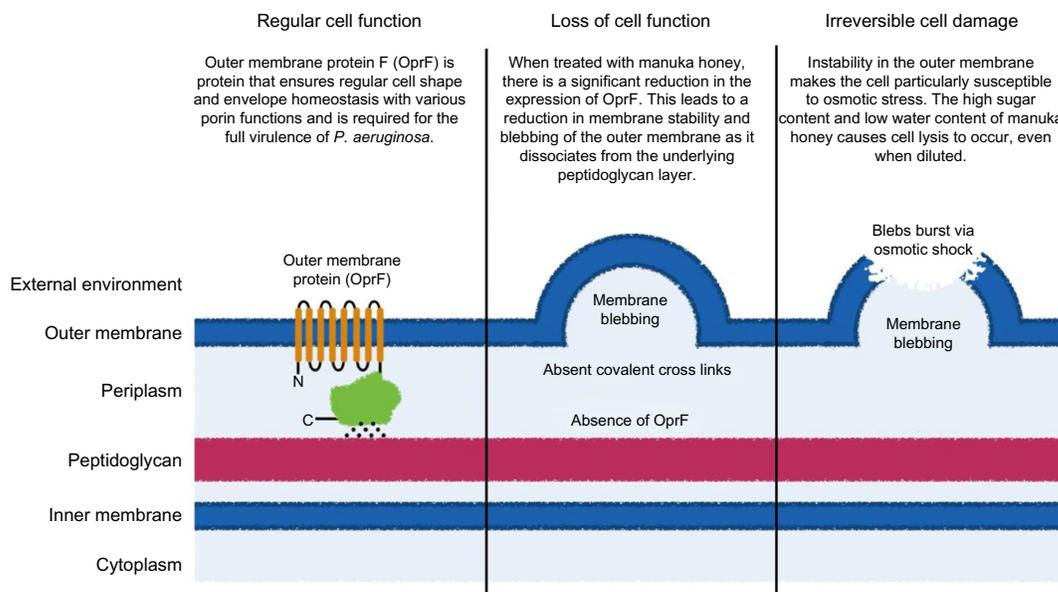

Figure 2 The proposed mechanism by which manuka honey inhibits *Pseudomonas aeruginosa*. Manuka honey is proposed to cause destabilization of the cell envelope through the down-regulation of a key structural protein (OprF), which is involved in maintaining cell shape and cell envelope stability. The loss of this protein results in membrane blebbing, which decreases cellular viability and ultimately leads to cell lysis.

the inability of *Streptococcus pyogenes* to bind to the host may impact on its pathogenicity.

In addition to the studies into *S. aureus*, *P. aeruginosa*, and *S. pyogenes*, a study into the global action of manuka honey on *Escherichia coli* demonstrated that following exposure to manuka honey, 2% of the genes were up-regulated, while 1% were down-regulated by twofold or more.[103] Up-regulation appears to occur across genes involved in stress response; those genes down-regulated are thought to encode products involved in protein synthesis.[103] Conversely, down-regulation (16-fold) of a universal stress protein A (UspA) in *S. aureus* cells treated with honey was observed.[126] Another study has shown large-scale down-regulation of critical virulence genes (enterotoxins, fibronectin-binding proteins, hemolysins, and lipases), with concomitant reductions in global regulators and quorum-sensing genes.[51] These mechanistic effects, both lethal and non-lethal, are a testament to the inhibitory efficacy of manuka honey and confirm its broad spectrum of effects.

Applications of manuka honey as an antibacterial agent

Given the remarkable properties of manuka honey, it is unsurprising that there are now several licensed medical products based on manuka honey available, and it is worth noting that in addition to antimicrobial compounds, honey also contains compounds that enable it to modulate the activity of immune cells and promote rapid wound healing.[46,65,67] However, despite the claims made, its use has mainly been restricted to use as an antibacterial agent in the treatment of infected burns and wounds.[78,79]

This limited uptake of honey in clinical practice could in part be due to a lack of high-quality evidence supporting its use clinically. Despite the large amount of in vitro work supporting its potential in vivo use, systematic reviews covering the use of honey in wound management have mostly stated that the evidence for clinical use is weak. However, when considering end-point measurements chosen (healing rather than antibacterial activity), inconsistent study design, varying honeys used, and diverse patient population, it is easy to see why it has been difficult to satisfactorily collate the data.[48,127–131] A recent systematic review has given a positive view on the evidence supporting honey, suggesting that honey does lead to improved healing in a variety of wounds, including partial thickness burns, as well as acute and chronic wounds, when compared to silver sulfadiazine or sugar dressings.[128] There is clearly still a need for larger scale, well-designed multicenter randomized clinical trials to improve the evidence base available.

Conclusion

AMR is one of the greatest medical challenges the world faces; it was estimated recently that by 2050, AMR will account for ten million extra deaths annually worldwide, with additional economic costs in the region of $100 trillion.[132] In order to combat this challenge, antimicrobial agents with a broad spectrum of activity are required. There is potential to use honey to target virulence rather than viability, thereby reducing the likelihood of resistance occurring and making it an interesting candidate for further investigation.

The ability of manuka to act synergistically with antibiotics also opens up new possibilities for its use as a topical agent and possibly as part of a combined regimen. Such statements do raise immediate problems, however; one of the largest hurdles facing manuka honey's introduction as a front-line product (and not last-resort, as is often the case) is the ability to reproduce the excellent efficacy observed in vitro during in vivo clinical trials. Additionally, the integration of manuka honey into mainstream wound care would ideally require the exact composition of honey to be fully investigated. This would allow assessment of the complex interplay compounds may have with one another, and may help clinicians determine whether honeys (manuka or otherwise) would be more effective against certain infection-causing species. Until the exact compounds causing inhibitory effects are identified and their interplay with other compounds investigated, the uptake of manuka honey in the clinical environment will remain inconsistent, possibly to the detriment of patients.

Disclosure

The authors report no conflict of interest in this work.

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