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In vitro antimicrobial efficacy of two medical grade honey formulations against common high risk meticillin-resistant staphylococci and *Pseudomonas* spp. pathogens

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Background – Antimicrobial resistance is a problem in human and animal healthcare. Honey may be used for its wound healing properties and antimicrobial effects.

Objective – To investigate the antimicrobial activity of two commercially available medical grade honeys (MGHs) against *Staphylococcus* spp. and *Pseudomonas* spp. isolates.

Methods and materials – Two formulations, MGH1 (40% w/v honey) and MGH2 (80% w/v Manuka honey), were tested *in vitro* for minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) against 11 *Staphylococcus* and 11 *Pseudomonas* isolates at low [1.5×10^4 colony forming units (cfu)/well] and high (1.5×10^6 cfu/well) concentrations of inoculum, representing systemic and cutaneous bacterial loads during infection, respectively.

Results – MGH2 showed a lower MIC against staphylococci than MGH1, although this was not statistically significant. MGH1 had stronger bactericidal effects against staphylococci than MGH2, although this effect was statistically significant only at the higher bacterial concentration ($P < 0.01$). For *Pseudomonas* spp., MGH1 had significantly higher antimicrobial activity (both MIC and MBC) than MGH2 against all isolates tested and at both bacterial concentrations ($P < 0.05$).

Conclusions and clinical importance – Both MGHs were effective *in vitro* against common cutaneous pathogens including meticillin-resistant staphylococci and *Pseudomonas* species. The higher efficacy of the MGH1 formulation against *Pseudomonas* and its consistent effects against staphylococci, while containing only half of the amount of honey compared to MGH2, invites further investigation of the mechanisms and clinical applications of MGH1.

Introduction

Staphylococcus spp. and *Pseudomonas aeruginosa* are among the most common opportunistic pathogens of humans and domestic animals, and possess the ability to persist in harsh environments and to develop resistance to many antimicrobials.[1–3] With both bacterial genera displaying increasingly high-risk lineages both in human patients and veterinary species there is growing interest in assessing the efficacy of alternative therapeutic agents against antimicrobial-resistant strains.[4] Several substances exist that have antimicrobial activity including honey, phytochemicals, essential oils and phages. Honey has seen renewed interest in recent times including for infected wounds and those associated with antimicrobial-resistant bacteria.[5,6] Whereas standard antimicrobials possess highly specialized mechanisms of action, the antibacterial effects of honey are based on a wide range of properties.[5,7,8]

Different medical grade honeys (MGHs) exist. Two formulations used in wound care are MGH1 (L-Mesitran Soft, Triticum; Maastricht, the Netherlands) and MGH2

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Conflicts of Interest: NC is employed by Triticum, who provided the MGH1 used in this study; he was not involved in the study design, interpretation and presentation of the results. None of the other authors have declared any conflicts of interest.

(Medihoney; Integra Life Sciences, Plainsboro, NJ, USA). Apart from the honey, MGH1 contains additional healing components including vitamins C and E.[2,10–12] The antimicrobial activity of MGH1 against *S. pseudintermedius* and *Malassezia pachydermatis* has been investigated previously, and it was reported that MGH1 had stronger antimicrobial activity against both pathogens than honey alone, which suggests that the other components may enhance the antimicrobial activity.[2] MGH2 contains pure monofloral *Leptospermum scoparium* (Manuka) honey. A major difference is that MGH2 contains Manuka honey, which exerts antimicrobial effects mainly based on methylglyoxal, whereas other honeys primarily exert antimicrobial activity via hydrogen peroxide.[13–15] Manuka honey was the first honey to be extensively investigated,[5,16–20] but other types of honey have been compared to it and found to have a similar or even stronger antimicrobial activity.[21–23] A 100-fold difference in antimicrobial activity has been reported between different types of honey, supporting the importance of selecting the most potent product for any given application.[9]

The present study sought to evaluate the bacteriostatic (minimum inhibitory concentration; MIC) and bactericidal (minimum bactericidal concentration; MBC) concentrations of two commonly used MGH formulations against clinical isolates of two skin pathogens (*Staphylococcus* spp. and *Pseudomonas* spp.). Two concentrations of bacteria were used for testing according to the "10^5 guideline"; a lower concentration to represent a systemic bacterial load during infection and a higher concentration to represent bacterial load curing cutaneous infection.[24–28]

Methods and materials

Medical honey formulations

The two MGH formulations, MGH1 and MGH2, were as follows: MGH1 consisted of 40% MGH, propylene glycol, lanolin, PEG4000 and vitamins C and E (L-Mesitran Soft); MGH2 consisted of 80% MGH with a hydrocolloidal gelling agent (Medihoney). Percentages (w/v) of MGH formulation that have antimicrobial effects are reported, rather than the percentage of honey in the products.

Bacterial isolates

Clinical isolates included 11 staphylococci (*S. aureus*, *S. epidermidis*, *S. pseudintermedius* and *S. schleiferi*) and 11 *Pseudomonas* (*P. aeruginosa* and *P. fluorescens*) that had been characterized in previous studies at the Laboratory of Antibiotic Resistance – Faculty of Veterinary Medicine, University of Lisbon (FMV-UL),[29–33] Instituto de Higiene e Medicina Tropical, Universidade NOVA de Lisboa (IHMT-UNL)[29,30] and from the Genevet bacterial Biobank. Bacterial strains were inoculated in Columbia 5% blood agar (bioMérieux; Lisbon, Portugal) and incubated aerobically at 37°C for 18 h. For all isolates, the susceptibility profile against 31 antimicrobial agents was determined (see Tables S1 and S2 in Supporting information).

Susceptibility phenotypes were evaluated by determination of the MIC using the microdilution system SIEMENS Microsan® B1016-173 POS MIC Panel Type 33 (Siemens Healthcare Diagnostics; West Sacramento, CA, USA) and B1016-175 NEG MIC Panel Type 44 (Siemens Healthcare Diagnostics) for *Staphylococcus* spp. and *Pseudomonas* spp. isolates, respectively. The staphylococcal isolates originated from human and canine skin infections; the *Pseudomonas* spp. isolates originated from canine otitis externa cases, they often exhibited resistance to aminoglycosides, fluoroquinolones or other classes of antimicrobials (often multidrug resistant, MDR). The main characteristics of these isolates are summarized in Table 1. Of note, MDR and meticillin-resistant (MR) staphylococcal isolates [MR *Staphylococcus aureus* (MRSA) ST22- SCC*mec* IV and MR *Staphylococcus pseudintermedius* (MRSP) ST71-SCC*mec* II-III] from pyoderma cases in dogs and MRSA SM39 and SM52 isolates which harboured the biocide resistance genes *qacA* and *smr*, respectively, were included.[29,30]

Determination of the MICs and MBCs of MGH1 and MGH2

The methods for the MIC and MBC determinations were adapted from several Clinical Laboratory Standard Institute (CLSI) guidelines.[34–36] Stock solutions of MGH1 and MGH2 were prepared by dissolving the product in sterile distilled water using two-fold serial dilutions, resulting in 40%, 20%, 10%, 5% and 2.5% (w/v) MGH1 and MGH2. The MGH stock solutions were prepared immediately before the antibacterial assays. An inoculum of each isolate was prepared and the turbidity of the suspension adjusted to achieve 0.5 McFarland [equivalent to that of 1.5×10^8 colony-forming units (CFU/mL)] and checked to have an absorbance of 0.150 by UV-visible spectrophotometer at a wavelength of 600 nm.

The MICs of both MGH1 and MGH2 for the different isolates were determined by the broth dilution method using 96 well microtitre plates as follows: 100 μL of Mueller–Hinton Broth medium (MHB, Oxoid; Madrid, Spain) was introduced to each well, and 100 μL of the MGH1 or MGH2 were added in the first column of the 96 well microtiter plates, mixed and serially two-fold diluted in the subsequent wells. Then 10 μL of the adjusted bacterial concentration inoculums of the 11 *Staphylococcus* spp. isolates and 11 *Pseudomonas* spp. isolates were added to the test wells, in triplicate, in order to obtain a final concentration of 1.5×10^4 cfu and 1.5×10^6 cfu per well, defined as "lower" and "higher" inoculums concentrations, respectively. The positive control wells contained MHB with the adjusted lower and higher bacterial inocula concentrations in order to check the bacterial viability, whereas the negative control wells contained only sterile MHB. Positive and negative control wells were performed in triplicate. The microtitre plates were then incubated at 37°C for 18 h. One microlitre of the negative control, positive controls and each of the bacterial concentration inoculums were subcultured before incubation in Tryptic soy agar (TSA; Oxoid) plates to assess the culture purity and number of cfu/mL in each well after incubation at 37°C for 18 h and thus to double-check the initial inoculum concentrations. Also, for the final validation of the initial bacterial suspension concentrations in the positive controls, the growth of 30–300 cfus and above 300 cfus were accepted for 1.5×10^5 cfu/mL (1.5×10^4 cfu per well) and to 1.5×10^7 cfu/mL (1.5×10^6 cfu per well), respectively. After incubation, bacterial growth was observed visually by the turbidity of the wells. The lowest concentration of the MGH1 and MGH2 that showed no turbidity was designated as the MIC.

The MBCs also were determined for the two different bacterial inoculum concentrations. The MBC was determined by adding 10 μL of the concentration which did not show any growth after incubation during MIC testing, to TSA plates. TSA plates were then incubated at 37°C for 18 h in the absence of the MGH products. The lowest concentration that killed 99.9% of the initial bacterial population and in which the plates showed no growth of colonies on the TSA agar, was recorded as the MBC.[36]

Statistical analysis

Data were analysed using PRISM 8.0.1 software (GraphPad; San Diego, CA, USA). The statistical significance of differences between MGH1 and MGH2 concerning MIC and MBC, for each of the two concentrations separately, were evaluated using the nonparametric Wilcoxon matched-pairs signed rank test; no values were left out

Table 1. Overview of staphylococcal and *Pseudomonas* strains used in this study

Strain	Clonal lineage	Antimicrobial and biocide resistance genes	Origin	Type of Infection	Reference
MSSA ATCC® 6538™	ST464-t3297	-	Human	Human lesion	ATCC[51]
MSSA ATCC® 29213™	-	--	Human	Skin wound	ATCC
MSSA FMV 77/2015	ND	ND	Dog	Skin infection	[31,33]
MRSA FMV 1504A/08	ST22-t032-SCC*mec* type IV	*blaZ, mecA*	Dog	Skin infection	[32]
MRSA SM39	ST88-t186	*blaZ, cadA, qacA*	Human	Nosocomial infection	[29,30,52]
MRSA SM52	ST8-t008	*Smr,* GrlA: S80Y, GyrA: S84L	Human	Nosocomial infection	[30]
MRSP FMV 4877/10	ST71-t02-SCC*mec* type II-II	*blaZ, mecA, erm(B), tet(K), aacA-aphD, aphA3, aadE, dfr(G)*	Dog	Skin infection	[31,33]
MRSP FMV 56/2013A	SCC*mec* type III	*erm(b), tet(M), aphA3, aadE*	Dog	Skin infection	FMV-UL
MRSP GV818/2017	ND	ND	Dog	Skin infection	Genevet Biobank
MRSE FMV 60/2012	ST5-SCC*mec* type IV	*blaZ, mecA, erm(C), tet(K), aacA-aphD, fusB*	Dog	Skin infection	[33]
MRSS FMV 57/2013B	SCC*mec* type III	*blaZ, mecA, tet(K)*	Dog	Skin infection	[33]
P. aeruginosa ATCC®27853™	ND	ND	Human	Blood culture	ATCC
P. aeruginosa ATCC®15442™	ND	ND	Animal	Animal room bottle	ATCC
P. aeruginosa FMV114/2014	ND	ND	Dog	OE	FMV-UL
P. aeruginosa FMV74/2015	ND	ND	Dog	OE	FMV-UL
P. fluorescens FMV85/2015	ND	ND	Dog	OE	FMV-UL
P. fluorescens FMV147/2015	ND	ND	Dog	OE	FMV-UL
P. aeruginosa FMV26/2016	ND	ND	Dog	OE	FMV-UL
P. aeruginosa FMV27/2016	ND	ND	Dog	OE	FMV-UL
P. aeruginosa FMV49/2016	ND	ND	Dog	OE	FMV-UL
P. aeruginosa FMV02/2017	ND	ND	Dog	OE	FMV-UL
P. aeruginosa FMV42/2017	ND	ND	Dog	OE	FMV-UL

ATCC, American Type Culture Collection; FMV, Faculty of Veterinary Medicine (University of Lisbon); MRSA, meticillin-resistant *Staphylococcus aureus*; MRSE, meticillin-resistant *Staphylococcus epidermidis*; MRSP, meticillin-resistant *S. pseudintermedius*; MRSS, meticillin-resistant *Staphylococcus schleiferi*; MSSA, meticillin-susceptible *S. aureus*; ND, not done; OE, otitis externa.

during analysis. Results were considered significantly different for $P < 0.05$.

Results

In vitro efficacy of MGHs against staphylococci

The MIC data for MGH1 and MGH2 at low and high bacterial loads for all staphylococcal isolates are presented in Figure 1. For MGH1, independently of the inoculum concentration, bacteriostatic effects were observed at 10% (w/v) for all staphylococcal isolates, except for MRSP FMV 4877/10. For this isolate, at the high inoculum concentration, a bacteriostatic effect of MGH1 was only observed at 20% (w/v). A higher variation in MIC values was observed for MGH2, although for most isolates a bacteriostatic effect also was achieved at 10% (w/v). At the low inoculum concentration, three isolates presented a MIC of 2.5% (w/v), whereas two isolates presented MICs of 5% (w/v). At the high bacterial load, one isolate displayed a MIC of 2.5% (w/v), whereas two had a MIC of 5% (w/v). Again, isolate MRSP FMV 4877/10 presented a MIC of 40% (w/v) for MGH2 at high inoculum concentration.

Overall, higher concentrations of MGH1 were needed to exert bacteriostatic effects against some staphylococcal isolates when compared to MGH2. However, MGH1 had more consistent MIC values and demonstrated less variation against the different isolates. No statistically significant differences were found between MGH1 and MGH2 regarding the MICs ($P = 0.0625$ and $P = 0.75$) for the low and the high bacterial inocula, respectively.

The bactericidal effects of both MGHs were investigated regarding the MBC (Figure 1). For MGH1, at low inoculum concentration, an MBC of 10% (w/v) was observed for the majority of isolates tested, except for three isolates which showed an MBC of 20% (w/v). However, at high bacterial load, only two isolates presented an MBC of 10% (w/v), whereas most had an MBC of 20% (w/v).

For MGH2, the MBC values at low bacterial concentration were dispersed; ranging from 5% to 40% (w/v); two with an MBC of 5% (w/v), three at 10% (w/v), four at 20% (w/v) and two at 40% (w/v). This dispersion was not detected at the high bacterial concentration, where all isolates showed an MBC of 40% (w/v). Neither MGH was effective in killing two isolates (MRSP FMV 4877/10 and MRSP GV818/2017) at the high inoculum concentration.

Overall, MGH1 had a significantly stronger bactericidal effect compared to MGH2 at the high inoculation concentration ($P = 0.0039$) , whereas at low bacterial concentration no significant difference between the MGHs was found ($P = 0.125$).

In vitro efficacy of MGHs against *Pseudomonas* spp.

The MICs of MGH1 against *Pseudomonas* spp. were significantly lower compared to MGH2 at both bacterial concentrations ($P = 0.0020$ low and $P = 0.0039$ high; Figure 2). At both bacterial concentrations and for all *Pseudomonas* isolates, MGH1 had a MIC of 20% (w/v), whereas MGH2 showed a MIC of 20% (w/v) for only one strain (*P. aeruginosa* ATCC 15442) and a MIC of 40% (w/v) against the 10 remaining isolates, except for the isolate

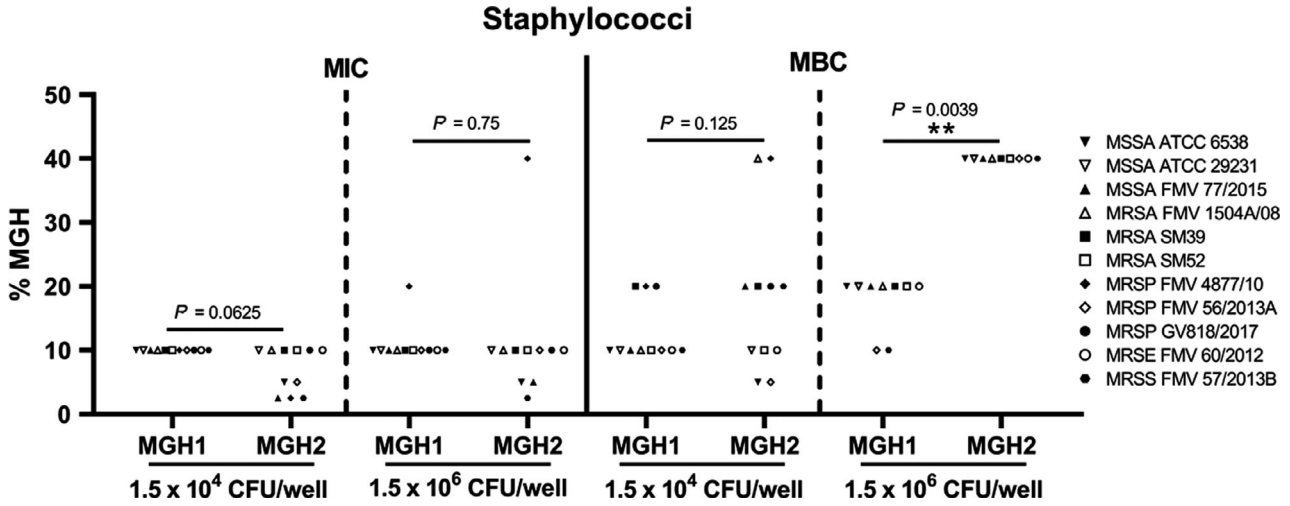

Figure 1. Minimal inhibitory concentration (MIC) and minimal biocidal concentration (MBC) for medical grade honey (MGH) formulations MGH1 and MGH2 against 11 staphylococcal isolates originating from human and canine skin infections.
Each symbol represents a different isolate. Mean values were plotted for each bacterial isolate; tests were performed in triplicate at each concentration of the product and at both bacterial loads. There were no significant differences observed between the MICs of the two MGH formulations at the two different bacterial loads. MGH1 showed a significantly stronger bactericidal effect compared to MGH2 at the high bacterial load ($^{**}P < 0.01$). Nevertheless, both MGH1 and MGH2 were ineffective against high bacterial concentrations of the MRSP FMV 4877/10 and MRSP GV818/2017. The isolates that were not sensitive were not plotted in the graph but were included in the statistical analysis.

FMV74/2015 that was not susceptible at high bacterial load.

MGH1 also had significantly lower MBCs for the *Pseudomonas* isolates compared to MGH2 at the low bacterial concentration ($P = 0.0459$), whereas there was no statistical significance at the high bacterial concentration ($P = 0.0918$; Figure 2). At the low inoculum concentration, one isolate (*P. aeruginosa* FMV27/2016) could not be killed by MGH1 and at the high bacterial concentration one additional isolate (*P. aeruginosa* FMV74/2015) was not susceptible to MGH1. This same isolate showed no growth inhibition by either MGH at the high bacterial inoculum. Against all other isolates, the MBC of MGH1 was 20% (w/v). Regarding MGH2, independent of bacterial load, the MBC was 40% (w/v) for all *Pseudomonas* isolates, except for *P. aeruginosa* FMV74/2015, which was not susceptible to MGH2 at the high concentration.

Discussion

The present study has shown that two different MGH formulations are effective against most *Staphylococcus* spp. and *Pseudomonas* spp. isolates tested. Staphylococcal strains were specifically selected that are commonly implicated in human and canine infections and present relevant resistance phenotypes and genotypes. Two

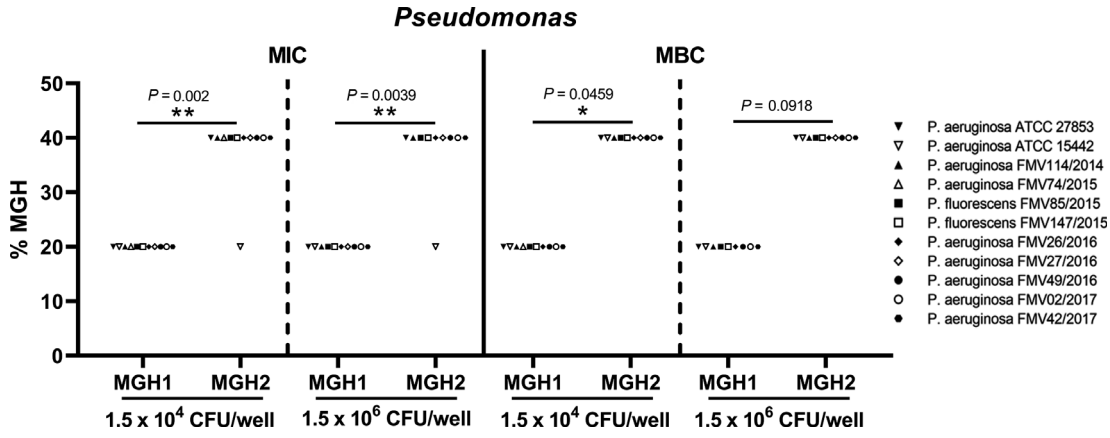

Figure 2. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) for medical grade honey (MGH) formulations MGH1 and MGH2 against 11 *Pseudomonas* spp. isolates originating from canine ear infections.
Each symbol represents a different isolate. The minimal concentration of MGH1 to exert bacteriostatic effects was significantly lower than this of MGH2 at both low and high bacterial load ($^{**}P < 0.01$). Mean values were plotted for each bacterial isolate; tests were performed in triplicate at each concentration of the product and at both bacterial loads. At the high bacterial inoculation load, MGH1 and MGH2 were ineffective to inhibit the growth of *P. aeruginosa* FMV74/2015. The isolates that were not sensitive were not plotted in the graph but were included in the statistical analysis. The minimal concentration of MGH1 to exert a bactericidal effect at the low bacterial load was lower than for MGH2 ($^{*}P < 0.05$), whereas there was no statistically significant difference at the high concentration. *Pseudomonas aeruginosa* FMV27/2016 was not affected by MGH1 at both bacterial loads. At low bacterial load, *P. aeruginosa* FMV74/2015 was not killed by MGH2; at high bacterial load, this isolate was killed neither by MGH1 nor MGH2. Isolates that were not sensitive were not plotted in the graph but were included in the statistical analysis.

P. fluorescens isolates were included in the *Pseudomonas* collection even though this species may be less pathogenic than *P. aeruginosa,* because *P. fluorescens* also may be isolated from skin infections where it causes a putrid malodour.[37] Furthermore, the methods developed and validated in this study were based on CLSI guidelines but with the addition of an extra bacterial concentration resembling the cutaneous *in vivo* bacterial load expected during skin and ear canal infections.[34–36] The 10^5 cfu/g of tissue ("10^5 guideline") is widely adopted and is a consensus for the definition of bacterial skin infection.[25] The lower bacterial load investigated in the present study better resembles the bacterial load during a systemic infection.

It was demonstrated herein that both MGH formulations presented low MICs and MBCs, suggesting that those MGHs also could be effective for topical treatment of infections caused by the tested bacteria, including staphylococci expressing MR, MDR and reduced biocide susceptibility, and *Pseudomonas* spp. isolates expressing MDR. It should be noted that residual MGH on the agar plate could possibly have led to an overestimation of the MBC values; however, this effect would have been similar for both MGH formulations.

Typically, MGH1 was bacteriostatic and bactericidal at a concentration of 10% (w/v) against staphylococci, and at a concentration of 20% (w/v) against most of the tested *Pseudomonas* spp. isolates. MGH2 showed more isolate-dependent variation in antimicrobial efficacy, although sometimes a higher inhibitory effect (lower MIC) was obtained with MGH2 compared to MGH1. However, MGH1 consistently showed a stronger bactericidal effect (lower MBC), especially against the high concentration of bacteria as may be encountered with cutaneous infections. Interestingly, MGH1 was more effective than MGH2 against all *Pseudomonas* spp. isolates in both MIC and MBC. The higher efficiency of MGH1 against *Pseudomonas* spp. and its consistent effects against staphylococcal isolates, suggest that MGH1 has stronger and more versatile antimicrobial effects than MGH2. The honey concentrations in these two MGHs differ, with MGH2 being twice as concentrated (80% w/v) as MGH1 (40% w/v). Therefore, lower concentrations of honey in MGH1 exerted stronger or at least similar biocidal action compared to the honey in MGH2. A formulation with a lower but more effective honey concentration might prove to be more advantageous in clinical cases because high concentrations of honey may lead to stronger osmotic effects that could result in the sensation of stinging pain.[38]

The observed difference in antimicrobial effects between MGH1 and MGH2 may be explained by the different types of honey in their formulations having different mechanisms of action. Variation in antimicrobial activity may exist between honey types, but also may change between bacterial isolates, as is shown by the variability in efficacy of MGH2 against *S. aureus*. Neutralization of methylglyoxal in Manuka honey has been shown to eliminate the antimicrobial effects towards *S. aureus*,[14] supporting the hypothesis that MGH2 would depend specifically on methylglyoxal. MGH1 contains lower levels of methylglyoxal, but acts via other components, such as hydrogen peroxide, bee defensin-1, low pH, sugars and high osmolarity.[39,40]

MGH1 previously has been evaluated in a pilot clinical trial which reported favourable antibacterial potential in canine otitis externa.[41] In addition, MGH1 has been evaluated *in vitro* reiterating its antifungal and antibacterial properties via a hydrogen peroxide-independent action.[2] In that study, MGH1 was compared to honey, showing that MGH1 had a significantly stronger bactericidal effect than honey alone against *S. pseudintermedius* isolates ($P = 0.003$). Sixteen of the 60 isolates tested had a lower MBC for MGH1 compared to honey. A range of 5–20% w/v for MGH1 was observed and no difference was seen between MSSP and MRSP isolates.[2] This is in line with the observations herein, with MGH1 typically having an MBC of 10–20% against MRSP. MGH1 has been reported to show antifungal activity against *Candida albicans*, compared with raw honey, suggesting that supplemented vitamins may enhance antimicrobial properties.[42] In a randomized controlled trial with 127 horses having lacerations, MGH1 was able to prevent infections and improve complete healing and veterinarian satisfaction.[43]

Infected wounds often contain polymicrobial pathogens[44,45] and therefore an antimicrobial agent active against a wide spectrum of pathogens is warranted. The broad response of almost all the isolates tested in the present work against both MGHs suggests that they might have therapeutic efficacy in a variety of pathologies and that it should be considered for treatment of wounds and otitis externa. Because MGH1 can prevent infections[43] the prophylactic use of MGH in surgical wounds or primary closure of wounds also forms an interesting strategy. Nevertheless, both MGH formulations showed that certain isolates were less susceptible, suggesting that not every infection will respond to the same extent to the MGH therapy.

Besides its antimicrobial effects, honey also possesses good wound-healing properties and stimulates tissue growth, has immunomodulatory effects, resolves inflammation, enhances angiogenesis and epithelialization, and minimizes scar formation.[46,47] The addition of supplements to MGH formulations may further enhance the pro-healing effects of MGH while possibly improving its antimicrobial properties.[42,47] MGH1 is supplemented with vitamins C and E that act synergistically and enhance wound repair[10–12] and its antimicrobial activity.[42,48] Vitamin C is a cofactor in the biosynthesis of collagen and improves angiogenesis and tensile strength in the skin.[49,50] Vitamin E protects cells from lipid peroxidation, is anti-inflammatory and reduces scar formation.[11] MGH supplemented with these vitamins should be considered for both infected and clean wounds. Further investigation into the effects of MGH on cellular and molecular targets is needed, and the *in vivo* broad-range antimicrobial efficacy needs to be confirmed in clinical trials.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Interpretive results of MIC breakpoints (mg/L) for *Staphylococcus* spp.

Résumé

Contexte – La résistance antimicrobienne est un problème en santé humaine et animale. Le miel pourrait être utilisé pour ses propriétés de cicatrisation et ses effets antimicrobiens.

Objectif – Etudier l'activité antimicrobienne de deux miels médicaux disponibles dans le commerce (MGHs) contre les souches de *Staphylococcus* spp. et *Pseudomonas* spp.

Matériels et méthodes – Deux formulations, MGH1 (miel à 40% w/v) et MGH2 (miel de Manuka à 80% w/v), ont été testées *in vitro* pour la concentration minimale inhibitrice (MIC) et les concentrations minimales bactéricides (MBC) contre 11 souches de *Staphylococcus* et 11 *Pseudomonas* à faibles [1.5×10^4 colony forming units (cfu)/well] et hautes (1.5×10^6 cfu/well) concentrations d'inoculum, représentant respectivement une charge bactérienne systémique et cutanée au cours de l'infection.

Résultats – MGH2 a montré une MIC plus faible que MGH1 contre staphylococci, bien que ce ne soit pas statistiquement significatif. MGH1 a des effets bactéricides plus forts contre staphylococci que MHG2, bien que cet effet ne soit statistiquement significatif qu'à la concentration bactérienne la plus élevée ($P < 0.01$). Pour *Pseudomonas* spp., MGH1 avait une activité antimicrobienne significativement plus élevée (MIC et MBC) que MGH2 contre toutes les souches testées et aux deux concentrations bactériennes ($P < 0.05$).

Conclusions et importance clinique – Les deux MGHs étaient efficaces *in vitro* contre les pathogènes cutanés fréquents incluant les staphylocoques résistant à la méticilline et *Pseudomonas*. La plus grande efficacité de la formulation de MGH1 contre *Pseudomonas* et ses effets contre staphylocoques, alors que contenant moitié moins de mile que MGH2, implique la réalisation d'autres études des mécanismes et des applications cliniques de MGH1.

Resumen

Introducción – la resistencia a los antimicrobianos es un problema en la salud humana y animal. La miel puede usarse por sus propiedades de cicatrización en heridas y por sus efectos antimicrobianos.

Objetivo – investigar la actividad antimicrobiana de dos mieles de grado médico (MGHs) disponibles comercialmente frente a aislados de *Staphylococcus spp.* y *Pseudomonas spp.*

Métodos y materiales – dos formulaciones, MGH1 (40% p/v de miel) y MGH2 (80% p/v de miel de Manuka), se probaron *in vitro* para detectar concentraciones inhibitorias mínimas (MIC) y concentraciones bactericidas mínimas (MBC) frente a 11 aislados de *Staphylococcus* y 11 aislados de *Pseudomonas* a bajas [$1{,}5 \times 10^4$ unidades formadoras de colonias (cfu)/pocillo] y altas ($1{,}5 \times 10^6$ cfu/pocillo) concentraciones de inóculo, que representan cargas bacterianas sistémicas y cutáneas durante la infección, respectivamente.

Resultados – MGH2 mostró una menor MIC frente a estafilococos que MGH1, aunque esto no fue estadísticamente significativo. MGH1 tuvo efectos bactericidas más potentes frente a estafilococos que MGH2, aunque este efecto fue estadísticamente significativo solo a la concentración bacteriana más alta ($P < 0{,}01$). Para *Pseudomonas spp.*, MGH1 tuvo una actividad antimicrobiana significativamente mayor (tanto MIC como MBC) que MGH2 frente a todos los aislamientos probados y en ambas concentraciones bacterianas ($P < 0{,}05$).

Conclusiones e importancia clínica – Ambas formulaciones de MGH fueron efectivas *in vitro* frente a patógenos cutáneos comunes, incluidos los estafilococos resistentes a meticilina y especies de *Pseudomonas*. La mayor eficacia de la formulación de MGH1 frente a *Pseudomonas* y sus efectos consistentes frente a estafilococos, aun teniendo solo la mitad de la cantidad de miel en comparación con MGH2, invita a una mayor investigación de los mecanismos y aplicaciones clínicas de MGH1.

Zusammenfassung

Hintergrund – Die antimikrobielle Resistenz ist ein Problem bei der Gesundheitsvorsorge des Menschen und der Tiere. Honig könnte aufgrund seiner Wundheilungseigenschaften und antimikrobieller Wirkungsweisen Verwendung finden.

Ziel – Eine Untersuchung der antimikrobiellen Aktivität zweier kommerziell verfügbarer medizinischer Honige (MGHs) im Einsatz gegen *Staphylococcus* spp. und *Pseudomonas* spp. Isolate.

Methoden und Materialien – Zwei Formulierungen, MGH1 (40% w/v Honig) und MGH2 (80% w/v Manukahonig) wurden *in vitro* auf ihre minimale Hemmstoffkonzentration (MIC) und minimale bakterizide Konzentration (MBC) gegenüber *Staphylococcus* und 11 *Pseudomonas* Isolate getestet, wobei niedrige[1,5 x 10^4 Kolonie-bildende Einheiten (cfu)/Proberöhrchen] und hohe (1,5 x 10^6 cfu/ Proberöhrchen) Konzentrationen von Inokulum eingesetzt wurden, was systemische bzw kutane bakterielle Belastungen während einer Infektion repräsentierte .
Ergebnisse – MGH2 zeigte eine niedrigere MIC gegenüber Staphylokokken als MGH1, obwohl dieses Ergebnis nicht statistisch signifikant war. MGH1 zeigte eine deutlichere bakterizide Wirkung gegenüber Staphylokokken als MGH2, obwohl dieses Ergebnis nur bei höheren bakteriellen Konzentrationen statistisch signifikant war ($P < 0,01$). Bei *Pseudomonas* spp. zeigte MGH1 eine signifikant höhere antimikrobielle Aktivität (sowohl MIC als auch MBC) als MGH2 gegenüber allen getesteten Isolaten und bei beiden bakteriellen Konzentrationen ($P < 0,05$).
Schlussfolgerungen und klinische Bedeutung – Beide MGHs waren *in vitro* gegen übliche Hautpathogene inklusive Methicillin-resistenten Staphylokokken und *Pseudomonas* Spezies wirksam. Die höhere Wirksamkeit der MGH1 Formulierung gegenüber *Pseudomonas* und seine konstante Wirkung gegenüber Staphylokokken, obwohl es nur die halbe Menge an Honig im Vergleich zu MGH2 beinhaltete, laden zu weiterer Untersuchung des Mechanismus und der klinischen Anwendung von MGH1 ein.

要約
背景 – 抗菌薬耐性は、人間および動物のヘルスケアにおける問題である。蜂蜜は、その創傷治癒特性および抗菌効果のために使用される場合がある。
目的 – 本研究の目的は、2つの市販された医療グレードの蜂蜜(MGH)によるブドウ球菌およびシュードモナス属菌株に対する抗菌活性を調査することである。
材料と方法 – MGH1(40%w / v蜂蜜)とMGH2(80%w / vのマヌカ蜂蜜)の2製剤に対し、それぞれ感染時の全身および皮膚細菌負荷を表す、低濃度[1.5×104コロニー形成単位(cfu)/ウェル]および高濃度(1.5×106 cfu / ウェル)のブドウ球菌および緑膿菌それぞれ11分離株に対する最小発育阻止濃度(MIC)と最小殺菌濃度(MBC)をin vitroでテストした。
結果 – MGH2は、MGH1よりもブドウ球菌に対する低いMICを示したが、統計的に有意ではなかった。MGH1はMGH2よりブドウ球菌に対して強い殺菌効果があったが、細菌濃度が濃い場合にのみ統計的に有意であった(P ＜0.01)。緑膿菌においては、MGH1は、試験した全分離株および両細菌濃度で、MGH2よりも抗菌活性(MICおよびMBCの両方)が有意に高かった(P ＜0.05)。
結論と臨床的重要性 – 両MGHは、メチシリン耐性ブドウ球菌と緑膿菌を含む一般皮膚病原体に対してin vitroで効果的であった。 MGH2と比較し、半量の蜂蜜しか含まない一方で、緑膿菌に対するMGH1製剤の高い効力およびブドウ球菌に対する一貫した効果は、MGH1のメカニズムおよび臨床応用のさらなる調査をもたらす。

摘要
背景 — 抗生素耐药性是人类和动物医疗保健中的一个问题。由于蜂蜜具有愈合伤口和抗菌作用,因而值得使用。
目的 — 研究现有市售的两种药用级别蜂蜜(MGHs)对葡萄球菌和假单胞菌分离株的抗菌活性。
方法和材料 — 两种配方,MGH1 (40% w/v 蜂蜜) 和MGH2 (80% w/v Manuka 蜂蜜),针对11个葡萄球菌和11个假单胞菌分离株,体外测定其最小抑制浓度(MIC)和最小杀菌浓度(MBC),选择培养液的低 [1.5 × 10^4 colony forming units (cfu)/well]和高 (1.5 × 10^6 cfu/well) 浓度,分别代表感染期间全身和皮肤的细菌量。
结果 — MGH2对葡萄球菌的MIC低于MGH1,尽管这没有统计意义。MGH1对葡萄球菌的杀菌作用强于MGH2,但这个结果仅在细菌浓度高时才有统计意义(P < 0.01)。对于假单胞菌,MGH1对所有被测菌株的抗菌活性(MIC和MBC)均高于MGH2,且在两种细菌浓度下均高于MGH2 (P < 0.05)。
结论和临床意义 — 在体外试验中,两种MGHs对常见的皮肤致病菌,包括耐甲氧西林葡萄球菌和假单胞菌均有效。与MGH2相比,MGH1配方对假单胞菌疗效更高,且具有持续对抗葡萄球菌的作用,但其蜂蜜含量仅为MGH2的一半。因此,MGH1的作用机制和临床应用有待进一步研究。

Resumo
Contexto – A resistência antimicrobiana é um problema na saúde humana e animal. O mel pode ser utilizado por suas propriedades curativas e efeitos antimicrobianos.
Objetivo – Investigar a atividade antimicrobiana de dois méis medicinais (MGHs) comercialmente disponíveis contra *Staphylococcus* spp. e *Pseudomonas* spp. isolados.
Métodos e materiais – Duas formulações, MGH1 (40% p / v mel) e MGH2 (80% p / v mel Manuka), foram testadas *in vitro* para concentrações inibitórias mínimas (MIC) e concentrações bactericidas mínimas (MBC) contra 11 *Staphylococcus* e 11 *Pseudomonas* isolados em baixas [1,5 × 10^4 unidades formadoras de colônias (ufc) / poço] e altas (1,5 × 10^6 ufc / poço) concentrações de inóculo, representando cargas bacterianas sistêmicas e cutâneas durante a infecção, respectivamente.
Resultados – O MGH2 mostrou uma MIC menor contra estafilococos do que o MGH1, embora isso não tenha sido estatisticamente significativo. O MGH1 teve efeitos bactericidas mais fortes contra os

estafilococos do que o MGH2, embora esse efeito tenha sido estatisticamente significativo apenas na maior concentração bacteriana (P <0,01). Para *Pseudomonas* spp., O MGH1 apresentou atividade antimicrobiana significativamente maior (MIC e MBC) do que o MGH2 contra todos os isolados testados e em ambas as concentrações bacterianas (P <0,05).

Conclusões e importância clínica – Ambas OS MGHs foram eficazes *in vitro* contra patógenos cutâneos comuns, incluindo estafilococos resistentes à meticilina e espécies de *Pseudomonas*. A maior eficácia da formulação MGH1 contra *Pseudomonas* e seus efeitos consistentes contra estafilococos, embora contenha apenas metade da quantidade de mel em comparação com o MGH2, convida a uma investigação mais aprofundada dos mecanismos e aplicações clínicas do MGH1.

