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In vitro synergy between manuka honey and amikacin against *Mycobacterium abscessus* complex shows potential for nebulisation therapy

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Abstract

*Mycobacterium abscessus*is an opportunistic human pathogen of increasing concern, due to its ability to cause aggressive pulmonary infections (especially in cystic fibrosis patients), as well as skin and soft tissue infections. *M. abscessus* is intrinsically drug resistant and treatment regimens are lengthy, consisting of multiple antibiotics with severe side effects and poor patient success rates. New and novel strategies are urgently required to combat these infections. One such strategy thus far overlooked for mycobacteria is manuka honey. For millennia manuka honey has been shown to have wide ranging medicinal properties, which have more recently been identified for its broad spectrum of antimicrobial activity. Here we demonstrate that manuka honey can be used to inhibit *M. abscessus* and a variety of drug resistant clinical isolates *in vitro*. We also demonstrate using a microbroth dilution checkerboard assay that manuka honey works synergistically with amikacin, which is one of the current front line antibiotics used for treatment of *M. abscessus* infections. This was further validated using an *in vitro* inhalation model, where we showed that with the addition of manuka honey, the amikacin dosage can be lowered whilst increasing its efficacy. These findings demonstrate the utility of manuka honey for incorporation into nebulised antibiotic treatment for respiratory infections, in particular *M. abscessus*. These results pave the way for a change of strategy for *M. abscessus* management, offering new therapeutic options for this deadly infection.

INTRODUCTION

Mycobacterium abscessus is a rapidly growing non-tuberculous mycobacteria commonly isolated from the environment [1]. It is an opportunistic pathogen that causes pulmonary infections in patients with pre-existing lung conditions such as cystic fibrosis and bronchiectasis, as well as causing skin and soft tissue infections [2]. Treatment of *M. abscessus* infections are complicated by the existence of three subspecies that comprise the *M. abscessus* complex; *M. abscessus*, subsp. *abscessus*, *M. abscessus* subsp. *bolletii* and *M. abscessus* subsp. *massiliense* [3]. These complications are due, in part, to a defining characteristic between the subspecies which is the presence or absence of a functional *erm*(41) gene that encodes for inducible macrolide resistance [4]. The *erm*(41) gene is present in both *M. abscessus* subsp. *abscessus* and *bolletii,* yet is absent in *M. abscessus* subsp. *massiliense*, thus contributing to differences in antibiotic susceptibility profiles between the subspecies [5]. The treatment of *M. abscessus* infections is also challenging due to the intrinsically drug resistant nature of *M. abscessus* regardless of subspecies. The current treatment regimens for pulmonary infections are lengthy, consisting of 12 months or more of antimicrobial chemotherapy. The initiation phase of treatment, lasting 1 month, is a combination of intravenous amikacin, tigecycline, imipenem and either clarithromycin or azithromycin depending on the macrolide sensitivity of the *M. abscessus* subspecies [6]. A continuation phase is then started which utilises nebulised amikacin alongside a combination of intravenous clofazimine, linezolid, minocycline, moxifloxacin and co-trimoxazole. This can also include clarithromycin or azithromycin, once again depending on the macrolide sensitivity of the *M. abscessus* subspecies [6]. Treatment outcomes are often negative, especially due to low patient adherence to the drug regime due to severe side effects, including; nausea, vomiting, hepatotoxicity, thrombocytopenia and leucopenia [7, 8]. Furthermore, treatment success rates are low, typically between 30–50%, for patients who do adhere to the course of treatment [9]. Therefore, the current treatment options are ineffective and novel strategies to treat these infections are urgently required, one area yet to be explored for treatment of mycobacterial infections is manuka honey.

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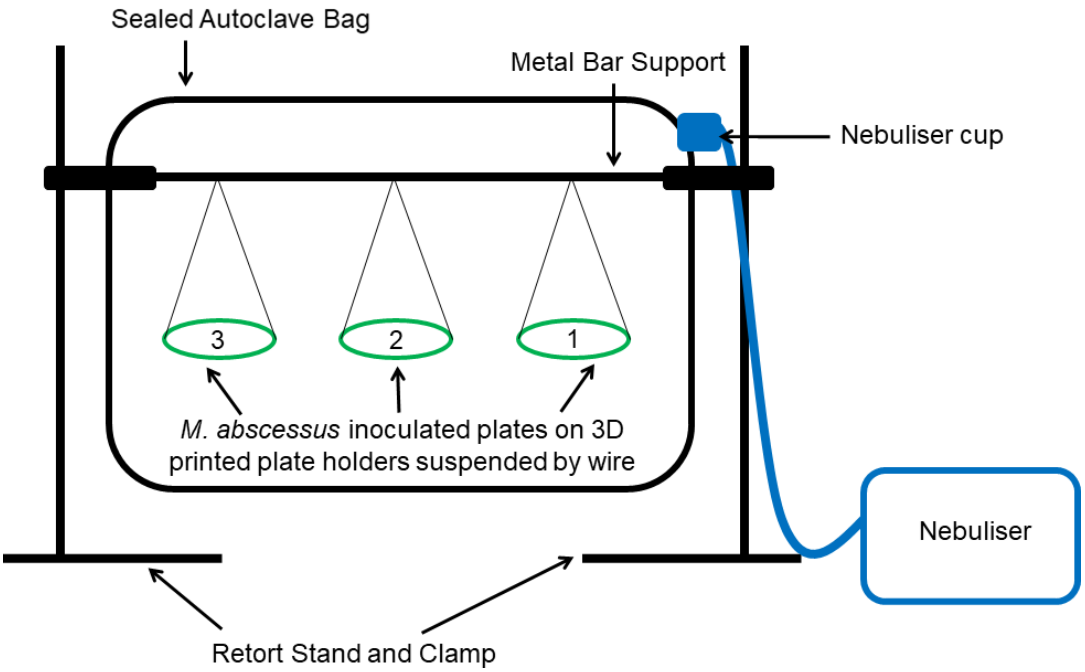

Fig. 1. *In vitro* inhalation model experimental design. A nebuliser is attached to a sealed autoclavable biohazard bag containing 7H11 agar plates inoculated with 10 µl log phase *M. abscessus* culture and suspended from a bar and secured by a retort stand. The positions of the plates labelled 1, 2 and 3 identify the position of the agar plates in relation to the proximity of the nebuliser cup.

The antimicrobial activity of honey has been established for millennia and explored in depth against a range of bacterial pathogens [10]. Manuka honey in particular has gained significant interest for its enhanced antimicrobial activity and broad spectrum of activity [11]. Derived from the *Leptospermum* spp., manuka honey's antimicrobial activity is attributed to the presence of methylglyoxal (MGO) which is not found in most other honeys [12]. This is due to dihydroxyacetone within the nectar of manuka flowers, which is a precursor of MGO. The conversion of dihydroxyacetone into MGO occurs non-enzymatically within the honey over time [10, 13]. Manuka honey, and non-manuka honey, have since been developed into medical grade honeys, each one based on a specific component of honey's antimicrobial activity [14]. Alongside these, existing therapies include honey gel, laced wound dressings or nebulised honey for the treatment of asthma, which make honey a clinically viable option to combat complex respiratory infections such as *M. abscessus* [15, 16].

In this paper we assess the antimycobacterial efficacy of a range of Manuka honeys of differing MGO content. This was achieved through the screening of each honey against *M. abscessus* and a variety of clinical isolates in order to determine the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) *in vitro*. The synergistic relationship between the different manuka honeys and amikacin was also explored *in vitro* in order to establish potential doses for an inhalation treatment, with a view to identifying a reduction of amikacin concentration required during treatment.

METHODS

Chemicals and reagents

All chemicals and reagents were obtained from Sigma-Aldrich or Melford Laboratories, unless otherwise stated. A total of four manuka honey samples were selected, each with a different MGO rating, MGO40 (Manuka Doctor, UK), MGO55 (ManukaPharm, UK), MGO70 (Manuka Doctor, UK) and MGO83 (Comvita, UK). A control of vegan honea (Plant Based Artisan) was also included. All honey samples were stored in the dark at room temperature. Prior to testing, 1 g ml^{-1} stocks of honey in sterile distilled water were made and filtered in a two step filtration process using 0.8 µm filter to remove larger particulates and then sterilisation through a 0.22 µm filter.

Growth of *Mycobacteria abscessus* cultures

The *M. abscessus* strains used for antimicrobial susceptibility testing were NCTC 13031 and 16 clinical isolates from patients with *M. abscessus* pulmonary infection, including cystic fibrosis and bronchiectasis patients. *M. abscessus* cultures were grown from glycerol stocks (stored at −80 °C), in Middlebrook 7H9 broth, supplemented with glycerol (1% w/v) and Tween80 (0.05% w/v), at 37 °C, for 72 h in an orbital shaker at 180 r.p.m.

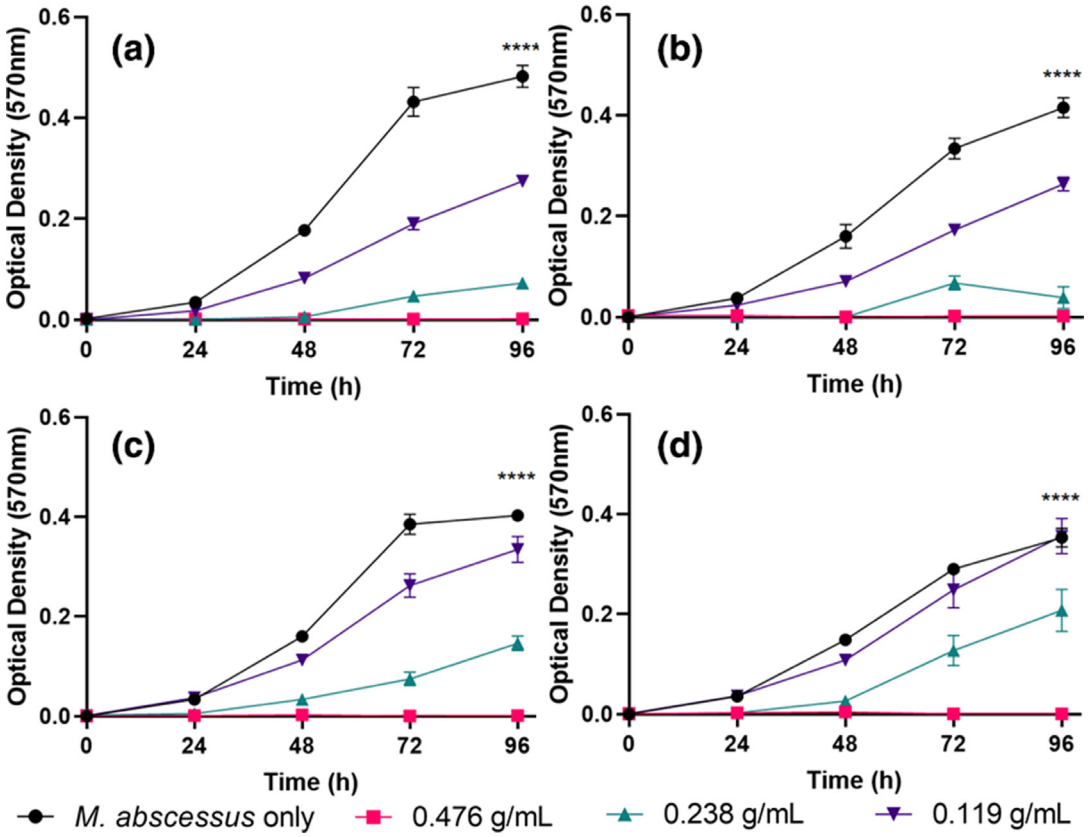

Fig. 2. Growth curves of *M. abscessus* NCTC 13031, cultured at 37 °C treated with four different grades of manuka honeys. All four manuka honeys inhibited *M. abscessus* at 0.476 g ml^{-1}, with a reduction in growth for 0.238 g ml^{-1} and 0.119 g ml^{-1}. a) MGO40 growth curve. A one-way ANOVA identified a significant difference between all honey treatments and no treatment, *P*=<0.0001 (*n*=3). b) MGO55 growth curve, a significant difference was observed for all honey treatments, one-way ANOVA *P*=<0.0001 (*n*=3). c) MGO70 had a significant difference between all honey treatments, one-way ANOVA *P*=<0.0001 (n=3). d) MGO83 a significant difference was observed for honey treatments, one-way ANOVA *P*=<0.0001 (*n*=3).

Broth microdilution assay

The broth microdilution assay was prepared by serially diluting the honey samples in 7H9 broth in a 96 well microtitre plate resulting in the following concentrations: 0.476, 0.238, 0.119, 0.0595 and 0 g ml^{-1}. The plates were inoculated with 5 µl of *M. abscessus* adjusted to optical density (OD) 600_{nm} of 0.1 in a total volume of 105 µl and incubated at 37 °C for a total of 96 h. Optical density reads were taken every 24 h. At 96 h, 5 µl of all wells were transferred onto Middlebrook 7H11 agar supplemented with glycerol (1% w/v) and incubated at 37 °C for a further 72 h. The minimum inhibitory concentration (MIC) was determined as the minimum concentration required to inhibit the growth of *M. abscessus* and the minimum bactericidal concentration (MBC) was determined as the minimum concentration where no growth of *M. abscessus* was observed on solid media.

Honey and amikacin synergy

A checkerboard assay was chosen to assess synergy between honey samples and amikacin. Amikacin was prepared to the final concentrations of 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0 µg ml^{-1} along the x axis. Honey samples were prepared to final concentrations of 0.277, 0.237, 0.197, 0.157, 0.117, 0.077, 0.037 and 0 g ml^{-1} along the y axis. The plates were inoculated with OD_{600nm}=0.1 adjusted *M. abscessus* cultures, of *M. abscessus* NCTC 13031 and clinical isolates 159 544 (subsp. *abscessus*), DC088A (subsp. *bolettii*) and DC088D (subsp. *massiliense*). These were then incubated at 37 °C for 96 h, with OD reads taken every 24 h. After 96 h, 5 µl of all wells were transferred onto Middlebrook 7H11 agar supplemented with glycerol (1% w/v) and incubated at 37 °C for a further 72 h. MICs and MBCs were determined as described previously.

In vitro inhalation model of manuka honey and amikacin combination therapy

An inhalation model was developed to simulate the nebulisation of manuka honey and amikacin into the lung. In brief, 7H11 agar plates were inoculated with 10 µl log phase *M. abscessus* culture and were suspended from a metal bar. Attached to the metal bar were plate holders, laser cut from 3 mm acrylic. This was then placed inside an autoclavable biohazard bag (Fisherbrand polypropylene 40 µm

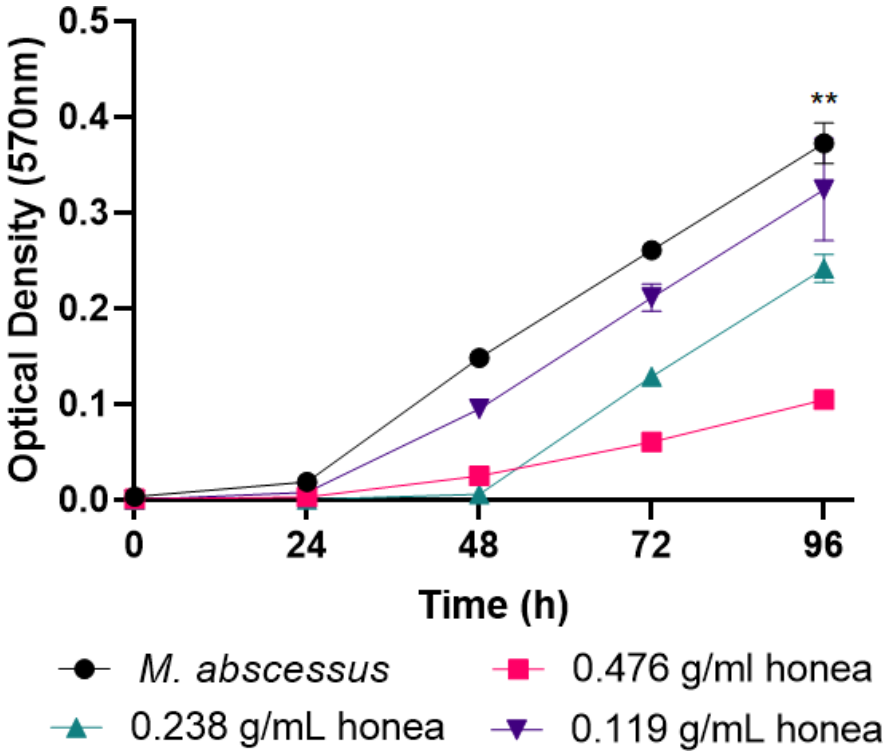

Fig. 3. Growth curve of *M. abscessus* NCTC 13031, cultured at 37 °C treated with vegan honea. The vegan honea reduced the growth of *M. abscessus* at the highest concentration of 0.476 g ml^{-1}, but was not inhibitory. A one-way ANOVA identified a significant difference between the treatments, P=0.0011 (n=3).

thickness) and heat sealed using a bag sealer (VWR), omitting a hole in the top corner for attachment of the nebuliser cup. The metal bar was attached to retort stands and a nebuliser (Omron compare c28P) was attached to the nebuliser cup (Fig. 1). The compounds were nebulised for up to 20 min, or until sputtering (nebulising rate 0.5 ml min^{-1}, aerosol output rate 0.09 ml min^{-1}), and consisted of sterile distilled water, manuka honey MGO55 (0.37 g ml^{-1}, n=3), amikacin (1.6 mg ml^{-1}, n=3) and a combination of manuka honey with amikacin (0.37 g ml^{-1} and 1.6 mg ml^{-1}, n=3). The agar plates were then retrieved and incubated at 37 °C for 72 h.

RESULTS

Manuka honey inhibits *Mycobacterium abscessus* complex

Various strength manuka honeys were assessed for antimicrobial activity against *M. abscessus* NCTC 13031. Serial dilutions of honey were prepared and inoculated with *M. abscessus* culture and monitored for antimicrobial activity. All four manuka honeys tested exhibited inhibitory activity at the highest concentration tested, 0.476 g ml^{-1} (Fig. 2). This concentration also proved to be bactericidal. The next lowest concentration, 0.238 g ml^{-1} exhibited a reduction in growth compared to no honey treatment, for each honey tested, but did not exhibit a bactericidal effect. Subsequent concentrations had dose dependant inhibition on the growth of *M. abscessus* (Fig. 2).

The vegan honea tested exhibited a reduction in growth of *M. abscessus* at the highest concentration of 0.476 g ml^{-1} but full inhibition was not observed (Fig. 3). The subsequent concentrations had a dose dependant reduction on the growth. No bactericidal activity was observed for the vegan honea.

Manuka honey inhibits *Mycobacterium abscessus* clinical isolates

A panel of 16 *M. abscessus* clinical isolates were screened against the four honey samples used in this study to assess antimicrobial activity. A variety of activity was observed for all clinical isolates tested with all isolates having an MIC of 0.476 g ml^{-1} or lower (Table 1). One isolate exhibited the lowest MIC of 0.238 g ml^{-1} for all honeys tested, 186154, but did not show a correlating MBC. The lower MICs observed were often in response to exposure to MGO83, the highest grade of manuka honey used in this study (Table 1). Not all of the clinical isolates tested had an MBC in response to honey treatment. Two of the isolates tested, DC088E and DC088ref, had no MBC for any honey tested, despite both isolates having an MIC of 0.476 g ml^{-1} for each honey (Table 1). The isolates DC088B and 186433 had no MBC for three of the honeys tested, yet MGO40 grade honey exhibited bactericidal

Table 1. Minimum inhibitory concentrations and minimum bactericidal concentrations for the four manuka honey samples tested against 16 *M. abscessus* clinical isolates

37 °C	Minimum inhibitory concn (g ml^{-1})				Minimum bactericidal concn (g ml^{-1})			
***M. abscessus* strain**	**MGO40**	**MGO55**	**MGO70**	**MGO83**	**MGO40**	**MGO55**	**MGO70**	**MGO83**
NCTC	0.476	0.476	0.476	0.476	0.476	0.476	0.476	0.476
137071	0.476	0.476	0.476	0.238	*>0.476*	0.476	*>0.476*	0.476
147028	0.476	0.476	0.476	0.476	0.476	0.476	0.476	0.476
159544	0.476	0.476	0.476	0.476	0.476	0.476	0.476	0.476
186144	0.476	0.476	0.476	0.476	0.476	0.476	0.476	0.476
186154	0.238	0.238	0.238	0.238	0.476	*>0.476*	0.476	*>0.476*
186433	0.238	0.238	0.476	0.238	*>0.476*	*>0.476*	*>0.476*	0.476
189961	0.476	0.476	0.476	0.476	0.476	0.476	0.476	0.476
194891	0.238	0.238	0.476	0.476	0.476	0.476	0.476	0.476
199277	0.476	0.476	0.476	0.476	0.476	0.476	0.476	0.476
211666	0.476	0.476	0.476	0.238	0.476	0.476	0.476	0.476
DC088A	0.476	0.476	0.238	0.238	0.476	0.476	0.476	0.476
DC088B	0.476	0.476	0.476	0.238	0.476	*>0.476*	*>0.476*	*>0.476*
DC088C	0.476	0.476	0.476	0.238	0.476	0.476	0.476	0.476
DC088D	0.476	0.476	0.476	0.238	0.476	0.476	0.476	0.476
DC088E	0.476	0.476	0.476	0.476	*>0.476*	*>0.476*	*>0.476*	*>0.476*
DC088ref	0.476	0.476	0.476	0.476	*>0.476*	*>0.476*	*>0.476*	*>0.476*

activity against DC088B, and MGO83 exhibited an MBC for 186433, both at 0.476 g ml^{-1}. Out of the 16 clinical isolates tested, bactericidal activity was observed for ten clinical isolates (Table 1).

Manuka honey acts synergistically with amikacin against *Mycobacterium abscessus* complex

The MICs of amikacin were determined for *M. abscessus* NCTC 13031 and the three subspecies, with each isolate inhibited at 4 µg ml^{-1}. Once MICs and MBCs were established for amikacin (Table 2), a checkerboard assay was used to determine any interaction between manuka honey and amikacin. Increased bactericidal activity of amikacin was observed for all four manuka honeys tested against *M. abscessus* NCTC 13031 and all three subspecies (Table 2). Overall, the most improved activity was observed for *M. abscessus* NCTC 13031 treated with MGO40 resulting in an MBC of 1 µg ml^{-1} of amikacin and 0.037 g ml^{-1} of manuka honey, which is enhanced from 16 µg ml^{-1} and 0.476 g ml^{-1} respectively (Table 2). A two-fold reduction of amikacin was observed for the three subspecies with the addition of MGO40, *M. abscessus* subspecies *abscessus* lowered from 8 µg ml^{-1} to 2 µg ml^{-1}, *M. abscessus* subspecies *bolettii* reduced from 8 µg ml^{-1} to 2 µg ml^{-1} and *M. abscessus* subspecies *massiliense* lowered from 16 µg ml^{-1} to 4 µg ml^{-1}. However higher concentrations of manuka honey were required to achieve this, this was 0.077 g ml^{-1} and 0.117 g ml^{-1}, rather than 0.037 g ml^{-1} (Table 2). Treatment with MGO55 exhibited the best relationship with amikacin, requiring the lowest amount of honey at 0.037 g ml^{-1} (apart from *M. abscessus* subsp. *bolletii* which required 0.077 g ml^{-1}), and also the lowest amount of amikacin (2–4 µg ml^{-1}). MGO70 did also have the lowest amount of honey required for bactericidal activity, but the amikacin required for this was higher than that observed for MGO55. The addition of MGO83 also reduced the amount of amikacin required for increased bactericidal activity. However, this typically required slightly higher amounts of amikacin compared to the other honeys. For *M. abscessus* NCTC 13031 this was 4 µg ml^{-1} as opposed to the 1 µg ml^{-1} observed for MGO40 and 2 µg ml^{-1} observed for MGO55 and MGO70. Subspecies *abscessus* required 4 µg ml^{-1}, rather than the 2 µg ml^{-1} for MGO40. Subspecies *bolettii* remained at 2 µg ml^{-1} and subspecies *masilinese* required 8 µg ml^{-1} rather than 4 µg ml^{-1}

In vitro inhalation model shows efficacy of manuka honey and amikacin against *M. abscessus*

In order to generate an inhalation model of nebulised manuka honey and amikacin, MGO55 manuka honey was selected. The nebulised concentration was 0.37 g ml^{-1} manuka honey and after nebulisation no inhibition of *M. abscessus* was observed (Fig. 4). Amikacin was nebulised at 1.6 mg ml^{-1} and growth was visible after incubation. The combination of manuka honey (0.37 g ml^{-1})

Table 2. Increased bactericidal activity of amikacin against *M. abscessus* NCTC 13031, *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii* and *M. abscessus* subsp. *massiliense* with the addition of different manuka honey grades (*n*=3).

M. abscessus isolate	Honey alone MBC (g ml^{-1})	Honey combination MBC (g ml^{-1})	Amikacin alone MBC (µg ml^{-1})	Amikacin combination MBC (µg ml^{-1})
MGO40				
NCTC 13031	0.476	0.037	16	2
subsp. *abscessus*	0.476	0.077	4	2
subsp. *bolletii*	0.476	0.117	8	2
subsp. *massiliense*	0.476	0.077	16	4
MGO55				
NCTC 13031	0.476	0.037	8	2
subsp. *abscessus*	0.476	0.037	8	4
subsp. *bolletii*	0.476	0.177	4	2
subsp. *massiliense*	0.476	0.037	8	4
MGO70				
NCTC 13031	0.476	0.037	16	2
subsp. *abscessus*	0.476	0.037	8	4
subsp. *bolletii*	0.476	0.037	8	4
subsp. *massiliense*	0.476	0.037	16	8
MGO83				
NCTC 13031	0.476	0.037	16	2
subsp. *abscessus*	0.476	0.037	8	4
subsp. *bolletii*	0.476	0.037	16	2
subsp. *massiliense*	0.476	0.037	16	8

and amikacin (1.6 mg ml^{-1}) were nebulised together and after incubation, these concentrations resulted in the inhibition of growth of *M. abscessus* (Fig. 4).

DISCUSSION

Until now, it has been a long held belief that honey does not inhibit the growth of mycobacterial species [17, 18]. However, in this study we demonstrate that various grades of manuka honey not only inhibit *M. abscessus* but exhibit bactericidal activity against *M. abscessus* NCTC 13031 and 16 drug resistant clinical isolates. We also demonstrate that a vegan honea, lacking the antimicrobial components of a manuka honey, was ineffective at inhibiting *M. abscessus* (Fig. 3). Antimicrobial activity was observed for all four manuka honeys tested, with MGO83 exhibiting an improved MIC and MGO40 and MGO83 both exhibiting the best MBC (Table 1). However, variations in activity were observed for both *M. abscessus* isolates and manuka honey. Interestingly, the MGO grade of manuka honey did not vastly impact the efficacy of the honey. MGO83 did have a slightly lower MIC against eight of the clinical isolates but this did not correlate to bactericidal activity (Table 1). It was also noted that three of the clinical isolates, 186154, 186433 and 194891, exhibited the same lower MIC, of 0.238 g ml^{-1}, for the other manuka honeys tested and isolate 194891 only had the lower MIC, of 0.238 g ml^{-1}, for MGO40 and MGO55 but not for MGO70 and MGO83. This also did not correlate to bactericidal activity, with both 186154 and 186433 exhibiting differing bactericidal activity in response to honey treatment. The clinical isolates used in this study have been unresponsive to frontline treatments, suggesting increased drug resistance. It has been noted that drug resistance of other bacterial pathogens does not impact the efficacy of honey treatment [19]. Therefore, it could be suggested that the difference in response to the various grades of manuka honey could be due to a combination therapy effect whereby the other active components within the honey are working together to overcome previous drug resistance mechanisms within the organism, rather than relying solely upon the antimicrobial activity of the MGO within the honey. Further exploration into the other major components of honey could elucidate why this variation in activity against *M. abscessus* isolates is observed.

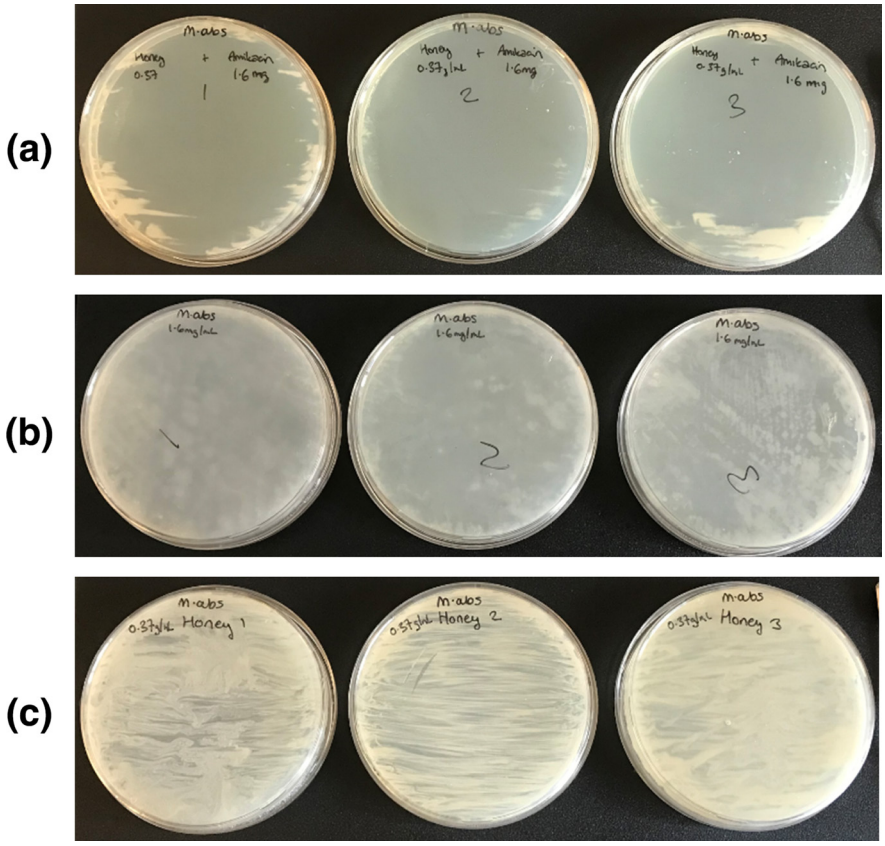

Fig. 4. *M. abscessus* treated with nebulised MGO55 manuka honey and amikacin, alone and in combination. A, combination treatment of *M. abscessus* NCTC 13031 with 1.6 mg ml^{-1} amikacin and 0.37 g ml^{-1} MGO55 manuka honey (n=3). B, treatment of *M. abscessus* NCTC 13031 with 1.6 mg ml^{-1} amikacin (n=3). C, treatment of *M. abscessus* NCTC 13031 with 0.37 g ml^{-1} MGO55 manuka honey (n=3).

One of the frontline treatments for pulmonary *M. abscessus* infections is initially intravenous amikacin, followed by inhaled amikacin [6]. Side effects associated with amikacin include; nausea, vomiting, abdominal pain, ototoxicity and nephrotoxicity [7, 20]. However, nebulised amikacin results in different side effects, such as cough and dyspnoea [21]. Significantly though, the dosage of inhaled amikacin administered is exponentially higher than that of the intravenous amikacin. Initial nebulised dosage starts at 250 mg ml^{-1} twice daily but can be increased to 500 mg ml^{-1} twice daily if tolerated by the patient [22]. Therefore, the lowering of the inhaled amikacin dosage would be beneficial to the patient as it would help to reduce these side effects, allowing patients to complete the course of treatment and improve patient outcomes. Consequently, we explored the relationship between manuka honey and amikacin and their combined efficacy *in vitro* against *M. abscessus*. Initially, combinations of amikacin and the four manuka honeys were screened against *M. abscessus* NCTC 13031 and the three subspecies (*M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii* and *M. abscessus* subsp. *massiliense*), to elucidate if the combination would result in improved efficacy. Enhanced bactericidal activity was observed for all four *M. abscessus* strains with the addition of manuka honey, regardless of MGO strength (Table 2). Interestingly, the amount of honey required for amikacin enhancement was very low, typically 0.037 g ml^{-1}, and well beyond sub-inhibitory concentrations. Similar observations have been made for *Pseudomonas aeruginosa* isolates obtained from cystic fibrosis patients, where the addition of subinhibitory concentrations manuka honey alongside tobramycin or colistin resulted in lower concentrations of antibiotic required for growth inhibition [23]. However, data related to the maximum epithelial lining fluid concentration of nebulised honey is currently limited. Furthermore, the concentration of amikacin within the epithelial lining fluid after nebulising is approximately 976.1 µg ml^{-1} [24]. This is significantly higher than the concentrations of amikacin (2–4 µg ml^{-1}) required for improved activity in combination with honey, therefore these effective concentrations are easily achievable within patients. This could be attributed to MGO within the honey exerting pressure on the mycobacterial cell membrane, allowing for easier penetration of amikacin into the mycobacterial cell.

Although the addition of the four manuka honeys increased the efficacy of amikacin, MGO55 was the most effective since it generated the greatest reduction of amikacin dosage required. Notably, *M. abscessus* subsp. *bolletii* required higher concentrations of manuka honey for amikacin enhancement than the other *M. abscessus* strains. The subspecies *bolletii* is the rarest of the *M. abscessus* complex to be isolated from patients and is the most drug resistant, however it is susceptible to amikacin therapy [25].

This difference in *M. abscessus* subsp. *bolletii* may be due in part to a mutation in the MmpL4a (mycobacterial membrane protein large) gene [26]. The Tyr842His mutation is responsible for a reduction in glycopeptidolipid export, which results in increased virulence of *M. abscessus* subsp. *bolletii* when compared to other *M. abscessus* subspecies [27]. This could result in changes to the mycobacterial cell membrane, suggesting higher concentrations of MGO are required to cause cell membrane damage.

For exploration into an *in vitro* inhalation model of amikacin and manuka honey, MGO55 was selected. This was due to the lowest dosage of both manuka honey and amikacin required for enhanced activity. Here, we demonstrated that dual administration of sub-inhibitory nebulised amikacin with the addition of manuka honey resulted in a drastic reduction of mycobacterial growth (Fig. 4). These findings show a basis for further exploration into manuka honey therapy for pulmonary infections. Previously developments into an *ex vivo* porcine lung model have shown that with the addition of manuka honey, *P. aeruginosa* isolates exposed to tobramycin, ciprofloxacin or ceftazidime resulted in growth inhibition and antibiofilm activity that was not observed with the use of antibiotic alone [28]. The increased combined efficacy of manuka honey and amikacin can be attributed to differences in the antimicrobial mechanisms of action. The manuka honey is likely to be exerting pressure on the mycobacterial cell membrane, allowing for easier penetration of amikacin, which then binds to the bacterial 30S ribosomal subunit causing protein synthesis inhibition. The improvement in amikacin therapy against *M. abscessus* suggests that either amikacin dosage can be reduced when in combination with manuka honey or the addition of manuka honey to inhaled amikacin can further the efficacy of the treatment. Aerosolised honey has been demonstrated to be safe and effective in the treatment of asthma in rabbits [16]. Currently, there are no known or reported side effects for nebulised honey, whereas side effects associated with amikacin are quite severe. If the dosage of amikacin can be lowered and met with manuka honey, these side effects could be lessened, resulting in better patient outcomes. Therefore, inhaled honey could be a safe and effective solution to combat pulmonary *M. abscessus* infections, however additional cytotoxicity studies that were beyond the scope of this study will need to be performed against eukaryotic cell lines to confirm this.

CONCLUSION

Treatment of *M. abscessus* pulmonary infections can be problematic due to its drug resistant nature. The variety of antibiotics required to combat infection result in severe side effects where patients often do not complete the course of treatment. Therefore, new treatments and novel strategies are now required to combat these infections and improve patient outcomes. Here, we have demonstrated that manuka honey is effective at inhibiting the growth of *M. abscessus* and drug resistant clinical isolates. Not only is manuka honey able to inhibit *M. abscessus* but it can also be used in combination with amikacin in an inhalation model. The combination of manuka honey and amikacin results in a lower dosage required of amikacin, whilst improving its efficacy. The use of this inhalation therapy of amikacin and manuka honey shows great promise as an improved therapy for *M. abscessus* pulmonary infections, which could lead to an increase in successful treatment outcomes and reduce the burden on the patient produced by drug associated side effects.

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Author contributions
Victoria C. Nolan: Conceptualization, formal analysis, methodology. James Harrison: Conceptualization, formal analysis, methodology. Jonathan A.G. Cox**:** conceptualization, formal analysis, funding acquisition, methodology, supervision.

Conflicts of interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Reference

1. Luthra S, Rominski A, Sander P. The role of antibiotic-target-modifying and antibiotic-modifying enzymes in *Mycobacterium abscessus* drug resistance. *Front Microbiol* 2018;9:1–13.
2. Lopeman RC, Harrison J, Desai M, Cox JAG. *Mycobacterium abscessus*: environmental bacterium turned clinical nightmare. *Microorganisms* 2019;7:E90.
3. Bryant JM, Grogono DM, Greaves D, Foweraker J, Roddick I, *et al*. Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study. *Lancet* 2013;381:1551–1560.
4. Lee M-R, Sheng W-H, Hung C-C, Yu C-J, Lee L-N, *et al*. *Mycobacterium abscessus* complex infections in humans. *Emerg Infect Dis* 2015;21:1638–1646.
5. Minias A, Żukowska L, Lach J, Jagielski T, Strapagiel D, *et al*. Subspecies-specific sequence detection for differentiation of *Mycobacterium abscessus* complex. *Sci Rep* 2020;10:1–9.
6. Haworth CS, Banks J, Capstick T, Fisher AJ, Gorsuch T, *et al*. British thoracic society guideline for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). *BMJ Open Respir Res* 2017;4:e000242.

7. Novosad SA, Beekmann SE, Polgreen PM, Mackey K, Winthrop KL, *et al*. Treatment of *Mycobacterium abscessus* Infection. *Emerg Infect Dis* 2016;22:511–514.
8. Jeon K, Kwon OJ, Lee NY, Kim B-J, Kook Y-H, *et al*. Antibiotic treatment of *Mycobacterium abscessus* lung disease: A retrospective analysis of 65 patients. *Am J Respir Crit Care Med* 2009;180:896–902.
9. Victoria L, Gupta A, Gómez JL, Robledo J. *Mycobacterium abscessus* complex: a review of recent developments in an emerging pathogen. *Front Cell Infect Microbiol* 2021;11:1–8.
10. Nolan VC, Harrison J, Cox JAG. Dissecting the antimicrobial composition of honey. *Antibiotics* 2019;8:1–16.
11. Lu J, Carter DA, Turnbull L, Rosendale D, Hedderley D, *et al*. The effect of New Zealand kanuka, manuka and clover honeys on bacterial growth dynamics and cellular morphology varies according to the species. *PLoS One* 2013;8:e55898.
12. Cokcetin NN, Pappalardo M, Campbell LT, Brooks P, Carter DA, *et al*. The antibacterial activity of Australian *Leptospermum* honey correlates with methylglyoxal levels. *PLoS One* 2016;11:1–13.
13. Adams CJ, Manley-Harris M, Molan PC. The origin of methylglyoxal in New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydr Res* 2009;344:1050–1053.
14. Hermanns R, Mateescu C, Thrasyvoulou A, Tananaki C, Wagener FADTG, *et al*. Defining the standards for medical grade honey. *Journal of Apicultural Research* 2020;59:125–135.
15. Youngberg DM. Honey-based dressings and wound care. *J Wound Ostomy Continence Nurs* 2009;36:588–589; .
16. Kamaruzaman NA, Sulaiman SA, Kaur G, Yahaya B. Inhalation of honey reduces airway inflammation and histopathological changes in a rabbit model of ovalbumin-induced chronic asthma. *BMC Complement Altern Med* 2014;14:1–11.
17. Efem SEE. Clinical observations on the wound healing properties of honey. *Br J Surg* 1988;75:679–681.
18. Dunford C, Cooper R, Molan P, White R. The use of honey in wound treatment. *Nurs Stand* 2000;15:63–68.
19. Nolan VC, Harrison J, Wright JEE, Cox JAG. Clinical significance of manuka and medical-grade honey for antibiotic-resistant infections: a systematic review. *Antibiotics* 2020;9:1–24.
20. Weng Y-W, Huang C-K, Sy C-L, Wu K-S, Tsai H-C, *et al*. Treatment for *Mycobacterium abscessus* complex-lung disease. *J Formos Med Assoc* 2020;119:S58–S66.
21. Ailiyaer Y, Wang X, Zhang Y, Li C, Li T, *et al*. A prospective trial of nebulized amikacin in the treatment of bronchiectasis exacerbation. *Respiration* 2018;95:327–333.
22. Olivier KN, Shaw PA, Glaser TS, Bhattacharyya D, Fleshner M, *et al*. Inhaled amikacin for treatment of refractory pulmonary nontuberculous mycobacterial disease. *Ann Am Thorac Soc* 2014;11:30–35.
23. Jenkins R, Wootton M, Howe R, Cooper R. A demonstration of the susceptibility of clinical isolates obtained from cystic fibrosis patients to manuka honey. *Arch Microbiol* 2015;197:597–601.
24. Luyt C-E, Clavel M, Guntupalli K, Johannigman J, Kennedy JI, *et al*. Pharmacokinetics and lung delivery of PDDS-aerosolized amikacin (NKTR-061) in intubated and mechanically ventilated patients with nosocomial pneumonia. *Crit Care* 2009;13:R200.
25. Adékambi T, Drancourt M. *Mycobacterium bolletii* respiratory infections. *Emerg Infect Dis* 2009;15:302–305.
26. Dubois V, Viljoen A, Laencina L, Le Moigne V, Bernut A, *et al*. MmpL8MAB controls *Mycobacterium abscessus* virulence and production of a previously unknown glycolipid family. *Proc Natl Acad Sci U S A* 2018;115:E10147–E10156.
27. Bernut A, Viljoen A, Dupont C, Sapriel G, Blaise M, *et al*. Insights into the smooth-to-rough transitioning in *Mycobacterium bolletii* unravels a functional Tyr residue conserved in all mycobacterial MmpL family members. *Mol Microbiol* 2016;99:866–883.
28. Roberts AEL, Powell LC, Pritchard MF, Thomas DW, Jenkins RE. Anti-pseudomonad activity of manuka honey and antibiotics in a specialized *ex vivo* model simulating cystic fibrosis lung infection. *Front Microbiol* 2019;10:1–10.

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