Antibacterial activities of Manuka and Honeydew honey-based membranes against bacteria that cause wound infections in animals

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Abstract

In this study, membranes composed of honey (Manuka or Honeydew) and pectin were developed, and the ISO 22196 method was used to evaluate their antibacterial activities against multidrug-resistant bacteria (i.e., *Staphylococcus pseudointermedius*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*) that cause wound infection in animals. The results demonstrated that both Manuka and Honeydew honey-based membranes had strong antibacterial activities against the strain of methicillin-resistant *S. pseudointermedius* tested. Specifically, membranes composed of Manuka honey were effective in inhibiting the growth of Gram-negative bacteria within 3 h, whereas those composed of Honeydew honey needed 24 h to neutralise bacterial growth. The antimicrobial activities of both membranes developed in this study suggest that they can be effectively used as wound dressing in veterinary clinical medicine.

Keywords: antibacterial activity, honey-based membranes, multidrug-resistant bacteria, wound infection

Antibakterielle Aktivität von Membranen aus Manuka- und Honigtauhonig gegen Wundkeime bei Tieren

In dieser Studie wurden Membranen aus Honig (Manuka oder Honigtau) und Pektin hergestellt und ihre antibakterielle Aktivität gegen multiresistente tierische Wundkeime (*Staphylococcus pseudointermedius*, *E. coli*, *Proteus mirabilis* und *Pseudomonas aeruginosa*) nach Vorgaben des ISO 22196-Verfahrens untersucht. Die Ergebnisse zeigen, dass beide Membranen stark bakterizid gegen einen Stamm Methicillin-resistenter *Staphylococcus pseudointermedius*-Bakterien wirken. Manuka-Membranen waren auch gegen alle gramnegativen Bakterien wirksam und reduzierten ihre Anzahl innerhalb von 3 Stunden, während der Kontakt mit Honigtau-Membranen 24 Stunden dauern mußte, um das bakterielle Wachstum zu unterdrücken. Die antimikrobielle Aktivität der in dieser Studie verwendeten Membranen begründen ihren Einsatz als wirksame Wundauflage in der klinischen Veterinärmedizin.

Schlüsselwörter: Antibakterielle Aktivität, Membranen aus Honigtauhonig, multiresistente Bakterien, Wundinfektionen, Hund

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Introduction

Honey is an ancient remedy for the treatment of infected wounds that has recently been rediscovered by the medical profession, particularly for treating cases in which conventional modern therapeutic agents are failing or toxic (Molan, 2001). Several studies have described the effectiveness of natural products such as sugar and honey in rapidly clearing wound infections without slowing the healing process (Chirife et al., 1983; Molan, 2002; Molan, 2006; Majtan et al., 2014; Vica et al., 2014). In addition, there is also some evidence suggesting that honey may actively promote healing (Lusby et al., 2005; Basualdo et al., 2007) and several studies have reported its broad-spectrum antimicrobial activity against methicillin-resistant *Staphylococcus aureus*, extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli, Proteus mirabilis,* and *Pseudomonas aeruginosa*, which are resistant to almost all known antibiotics (Abdel-moein et al., 2012; Wang et al., 2012; Grego et al., 2016).

Liquid honey has recently been used for treating wounds, but the use of solid honey-based wound dressings as medical devices could represent a better option in terms of ease of use, their ability to be slowly released over a sustained period of time and more widespread applications. Commercial honey-based ointments or wound dressings, known as "medical grade honey", derive mostly from Australia, New Zealand and the Netherlands due to the unique characteristics of their locally produced honey (Molan, 2002; Sherlock et al., 2010). In contrast to medical grade honey, little is known about the potential medical uses of Italian honeys, with regard to their antibacterial, would healing and adhesion barrier properties (Fidaleo et al., 2011; Coniglio et al., 2013). In fact, a recent study by Grego et al. (2016) showed that some Italian honeys, particularly a Honeydew honey produced in Piedmont (Italy), possess high antibacterial activity comparable to Manuka, which is considered the best known medical grade honey. Although many studies have assessed the production of chitosan and pectin films for treating wound infections (Archana et al., 2013; Espitia et al., 2014), few have evaluated the effectiveness of honey-based membranes (HBMs) for this purpose (Sasikala et al., 2013). The aim of this study was to produce HBMs using Manuka medical grade honey and Italian Honeydew honey, and to assess their antibacterial activities against the multidrug-resistant (MDR) bacteria *S. pseudointermedius, E. coli, P. mirabilis,* and *P. aeruginosa*, which were isolated from wound infections of dogs.

Material and Methods

Membrane preparation

Manuka honey was purchased from Manuka Health (Manuka Medihoney™; New Zealand), Honeydew honey was obtained from Piedmont Honey Producers Association (Torino, Italy), and pectin was obtained from Ardet Dental Medical Devices SRL (Torino, Italy). The Honeydew honey was selected in line with the results of a previous study (Grego et al., 2016), which assessed the antimicrobial activity of a collection of honeys, including 6 different varieties of Piedmont honey.

HBMs were prepared using the method previously described by Walker (1942) with some modifications. Briefly, a solution (1:1 v/v) of Manuka or Honeydew honey and sterile deionised water was prepared. The same volume of pectin powder was added to the honey solution and continuously stirred until the mixture was homogeneous. The resulting mixture was heated in a microwave oven at 850 W for 30 s, and then spread into 2 mm thick films and cooled. The films were cut into squares of 50 × 50 mm and packed in polyethylene under vacuum conditions. Then the HBMs were sterilised with a dose of 25 kGy gamma irradiation (Sterigenics International LLTC; Bologna, Italy).

Determination of the antibacterial effects of HBMs

The antibacterial activities of the HBMs were determined against the following MDR strains, which were isolated from canine wound infections and previously identified in our laboratory: a strain of methicillin-resistant *S. pseudointermedius* (MRSP), ESBL-producing *E. coli, P. mirabilis,* and *P. aeruginosa*. Stock cultures of these microorganisms were stored in Tryptone Soy Broth (Oxoid) supplemented with 15% glycerol at −80 °C until use. The antibacterial activities of the HBMs were determined by evaluating survival of the bacteria strains after incubation with the membranes for 1, 3, 6, and 24 h at 35 °C according to a slightly modified version (i.e., undiluted Nutrient Broth was used) of the International Organization for Standardization (ISO) 22196 test method (ISO 22196:2007). Early stationary phase cultures were prepared for each microorganism, adjusted to a cell density of 6×10^5 cells/ml in Nutrient Broth (Oxoid), and used as inoculums.

We used a uniform set of 60 Manuka and 60 Honeydew HBMs. According to ISO instruction, each membrane was placed in a sterile petri dish and 400 µl of the test inoculums were pipetted onto the HBM samples. Then the inoculated samples were covered with a piece of gamma radiation-sterilised polypropylene film (40 mm × 40 mm) and incubated at 35 °C under relative humidity above 90%. Microbiological counts were per-

formed on the HBM/polypropylene film samples immediately after inoculation and after 1, 3, 6, and 24 h of incubation. Microorganisms were recovered from HBM/polypropylene film samples in neutralising broth (Soybean-Casein Digest Lecithin Polysorbate Medium; Oxoid) and inoculated onto Tryptone Soy Agar (TSA; Oxoid) plates. After incubation at 37 °C for 48 h, the number of viable bacteria was calculated as log colony forming units (CFU)/sample. These values were compared to a positive control, namely, an inoculum of each strain (10^5 cells/ml) that had not been incubated with an HBM and was plated on TSA plates. Each experiment was performed in triplicate.

Results

Figures 1 and 2 show the antimicrobial activities of Manuka and Honeydew HBMs against MRSP, ESBL-producing *E. coli*, *P. mirabilis*, and *P. aeruginosa*, during 1 day of exposure time. The initial concentration of bacterial cultures used as controls and membranes at 0 h was approximately 6.00 log CFU. After 24 h, both HBMs inhibited the growth of all pathogens tested, whereas the controls grew over 12 log CFU/sample. The membranes showed strong activities against MRSP after 1 h of incubation. Specifically, Manuka HBMs had higher antimicrobial activity against Gram-negative bacteria, with 100% inhibition of bacterial growth after 3 h, whereas Honeydew HBMs needed 24 h to neutralise the growth of *P. mirabilis*.

Discussion

Honey is a complex mixture of a large number of components responsible for its antimicrobial, anti-inflammatory, and antioxidant activities (Basualdo et al., 2007). Differences in the therapeutic properties of honeys may depend upon many factors, such as geographical or botanical origin (Sherlock et al., 2010). The antimicrobial activity of Manuka honey is widely known among the scientific community and is frequently exploited for medical application (George and Cutting, 2007; Sherlock et al., 2010). Our recent study (Grego et al., 2016) showed that Honeydew honey, produced in Piedmont, Italy, had good antimicrobial activity against various MDR bacteria. Based on these data, solid surgical devices composed of Manuka or Honeydew honeys and pectin have been developed for treating wound infections, and for use as natural therapeutic instruments in veterinary medicine (e.g., in abdominal surgery). Pectin is often selected as a dressing material due to its biodegradability, biocompatibility, and versatile chemical and physical properties (e.g., gelation and selective gas permeability) (Espitia et al., 2014).

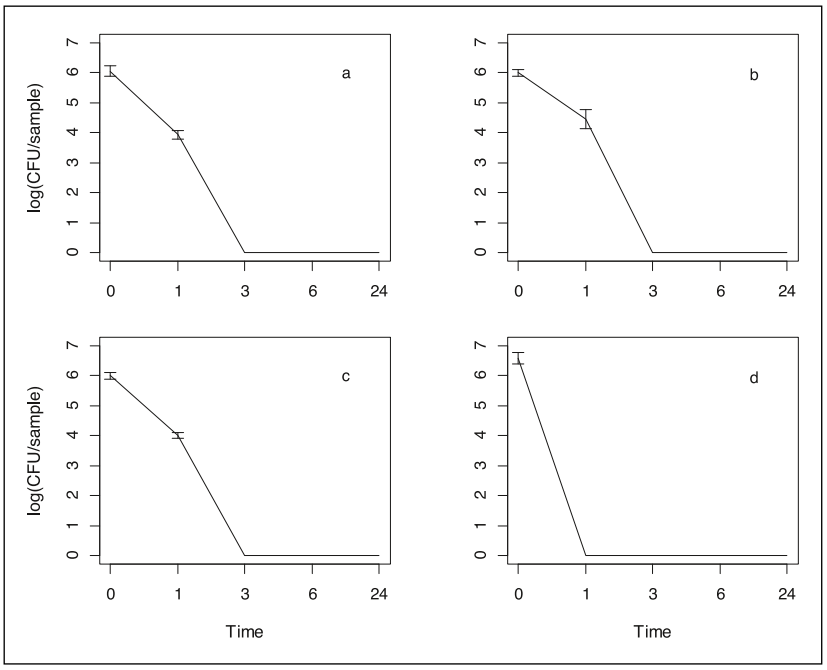

Figure 1: Effects of Manuka honey-based membranes on the growth of multidrug-resistant bacteria after a 24 h incubation. a) Extended-spectrum β-lactamase-producing *Escherichia coli*, b) *Pseudomonas aeruginosa*, c) *Proteus mirabilis*, and d) *Staphylococcus pseudointermedius*. Values are the means ± standard deviations of three replicates.

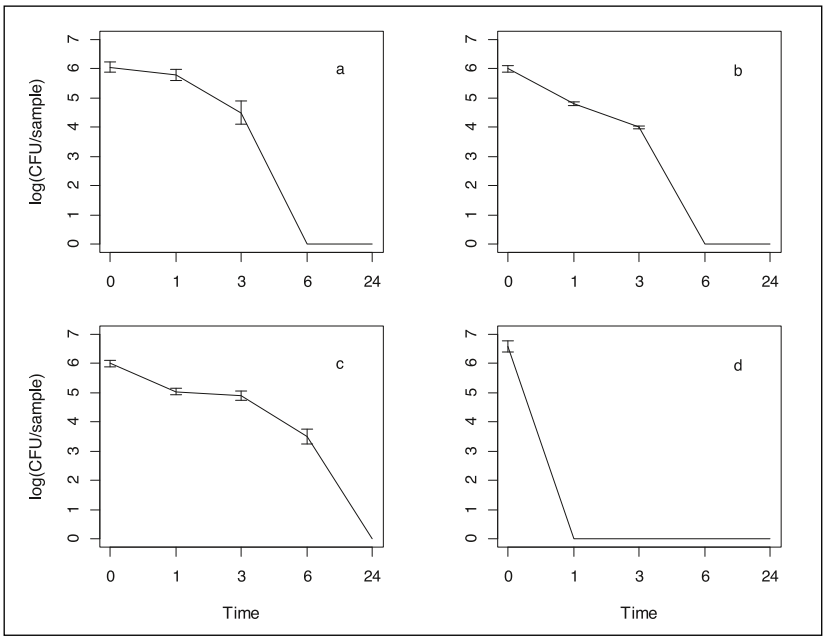

Figure 2: Effects of Honeydew honey-based membranes on the growth of multidrug-resistant bacteria after a 24 h incubation. a) Extended-spectrum β-lactamase-producing *Escherichia coli*, b) *Pseudomonas aeruginosa*, c) *Proteus mirabilis*, and d) *Staphylococcus pseudointermedius*. Values are the means ± standard deviations of three replicates.

In this study, the antimicrobial properties of two HBMs against MDR bacteria were clearly demonstrated, suggesting that both membranes are effective in promoting the wound healing process, and as such, can be used in various fields of veterinary medicine and surgery. In addition, they may be particularly useful for treating

wounds that are unresponsive to conventional antibiotics and antiseptics, thereby reducing post-operative adhesion formation (Ward and Panitch, 2011). Indeed, various methods such as using barriers of high viscosity fluids or solid membranes, have been proposed for preventing the development of post-operative adhesions (Emre et al., 2009; Saber, 2010). Solid membranes are preferred and are the most successful clinical tool because they can be applied in high-risk areas to provide a physical barrier in the immediate post-operative period (Ward and Panitch, 2011).

For this reason we developed membranes that combined the antimicrobial characteristics of honeys with the jellifying properties of pectin. The production method for HBMs does not need sophisticated materials or machinery, nor does it require a significant amount of economic resources. Currently, studies are being performed to evaluate the reabsorption time, biocompatibility and toxicity of HBMs, and to compare their activities with those of commercially available products.

In conclusion, the HBMs produced in this study with Piedmontese Honeydew honey showed good antimicrobial activity against MSRP, ESBL-producing *E. coli*, *P. mirabilis*, and *P. aeruginosa*, suggesting that they can be effectively used as wound dressing in veterinary clinical medicine.

Conflict of interest

None of the authors have any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Activité antibactérienne de membranes à base de miel de Manuka et de miel de miellat sur les germes de plaies chez les animaux

Dans le cadre de cette étude, on a fabriqué des membranes à base de miel (miel de Manuka et miel de miellat) et de pectine et on a testé, selon le processus ISO 22196, leur activité antibactérienne sur des germes multirésistants provenant de blessures d'animaux (*Staphylococcus pseudointermedius, E. coli, Proteus mirabilis* und *Pseudomonas aeruginosa*). Les résultats montrent que les deux types de membranes ont une forte activité bactéricide sur les souches de *Staphylococcus pseudointermedius* résistantes à la méthicilline. Les membranes à base de miel de Manuka étaient également actives contre tous les germes gram négatifs ét réduisaient leur nombre en 3 heures, alors qu'un contact de 24 heures était nécessaire pour que les membranes à base de miel de miellat réduisent la croissance bactérienne. L'activité antibactérienne des membranes utilisées dans la présente étude justifie leur emploi dans la médecine vétérinaire clinique.

Attività antibatterica delle membrane prodotte da miele di manuka e di melata contro i germi delle ferite negli animali

In questo studio, secondo le specifiche del metodo ISO 22196, sono state preparate delle membrane a base di miele (di manuka o melata) e di pectina ed è stata analizzata la loro attività antibatterica contro i germi delle ferite animali multiresistenti (*Staphylococcus pseudointermedius, E. coli, Proteus mirabilis* e *Pseudomonas aeruginosa*). I risultati mostrano che entrambe le membrane sono altamente battericide contro un ceppo meticillino resistente ai batteri *Staphylococcus pseudointermedius*. Le membrane di manuka erano anche efficaci contro tutti i batteri Gram-negativi e li riducevano di numero entro 3 ore, invece, a contatto delle membrane di melata bisogna attendere 24 ore per sopprimere la crescita batterica. L'attività antimicrobica delle membrane utilizzate in questo studio giustifica il loro uso come efficace medicazione delle ferite nella medicina veterinaria clinica.

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