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# **An Investigation into the Therapeutic Properties of Honey**

RIRDC Publication No. 09/0180







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# **An Investigation into the Therapeutic Properties of Honey**

By Dee A. Carter, Shona E. Blair and Julie Irish

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# Foreword

The research reported here is important to the Australian beekeeping industry and to the wider community. Numerous Australian honeys are shown to have levels of antibacterial activity that could be useful in treating skin and wound infections. Furthermore, the research shows that honey has potent activity against numerous problematic micro-organisms, including bacteria and fungi that are resistant to other drugs, and bacteria growing in biofilms.

This research will benefit the beekeeping industry by providing data showing that Australian honey has the potential to become an internationally-recognised, potent, non-toxic, topical antimicrobial agent. The market for such products is almost limitless. Furthermore, with appropriate promotion, increased use and acceptance of selected honeys as wound dressings has the potential to lead to a general increase in status of all Australian honeys, regardless of their medical properties. This research is thus also important to the medical and wider community.

It is clear that honey is under-utilised as a modern infection control agent, especially in light of the increasing availability of scientific data proving that it can have significant activity against problematic pathogens, including those resistant to regular antibiotics, which are notoriously difficult to treat conventionally. The laboratory results presented here argue for clinical trials to be conducted with medical-grade honeys. In particular, the potential for honey to prevent infections (especially in hospital settings) should be investigated.

This project was funded from industry revenue that is matched by funds provided by the Australian Government.

This report, an addition to RIRDC's diverse range of over 1900 research publications, forms part of our Honeybee R&D program, which aims to improve the productivity and profitability of the Australian beekeeping industry.

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**Peter O'Brien**  
Managing Director  
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# Acknowledgments

The majority of the laboratory work reported here, and in particular the survey of Australian honeys for antibacterial activity, was conducted by Julie Irish as part of her PhD and Honours degree studies; Julie was also involved in the studies on anaerobes and biofilms. Another Honours degree student, Nural Cokcetin, also contributed to the project by investigating the effects of honey against biofilms and anaerobic pathogens. Jan Gralton, another Honours degree student, looked at the effects of honey against problematic pathogens.

We would like to thank Doug Somerville and Rob Manning for their generosity in supplying honeys, as well as their insight, ideas and interest in our work. Tim Heard kindly supplied us with samples of honey from native Australian bees. Medihoney Pty Ltd and Comvita New Zealand Ltd generously supplied honeys that we used as controls. Kieren Sunderland liberally supplied us with jars to give to beekeepers as part of a sample collection kit.

Numerous beekeepers from around the country kindly supplied us with honey for the survey of Australian honeys.

Professor Peter Molan was very generous with his time and in particular assisted us in troubleshooting the bioassay.

Clinical isolates of a variety of bacterial species were obtained from Prince of Wales Hospital, Concord Hospital, the Centre for Infectious Diseases and Microbiology, Westmead Hospital, and the Institute of Dental Research, Westmead Millennium Institution, all located in Sydney, Australia. The Centre for Infectious Diseases and Microbiology also supplied us with *Candida* isolates, as did the Mazandaran University of Medical Sciences, Sari, Iran.

The funding for this project was supplied by the Honeybee R&D Program of the Rural Industries Research and Development Corporation.

# Abbreviations

NCCLS - National Committee for Clinical and Laboratory Standards

MIC - minimum inhibitory concentration

TNF- $\alpha$  - Tumour necrosis factor- $\alpha$

RAW cells - a mouse macrophage cell line

FSDC cells - a mouse dendritic cell line

# Contents

Foreword ..... iii

Acknowledgments..... iv

Abbreviations ..... iv

Executive Summary..... vii

1. Introduction ..... 1

2. Methods ..... 2

3. Results and Key Findings ..... 7

4. Conclusions ..... 21

5. References ..... 22

6. Appendices ..... 23

## Tables

Table 2.1:	Clinical isolates of pathogenic bacteria tested for their susceptibility to honey.....	6
Table 3.1	Number of honey samples received from various states in Australia.....	9
Table 3.2	Susceptibility of <i>Candida</i> species to different honey types.....	21
Table 3.3	Antibacterial activity of <i>Trigona</i> honey .....	23

## Figures

Figure 3.1	Total antibacterial activities in Australian honey samples .....	10
Figure 3.2	Non-peroxide antibacterial activities in Australian honey.....	11
Figure 3.3	Site location and total antibacterial activities of honeys by state & region.....	14
Figure 3.4	Locations of origin of honeys exhibiting non-peroxide antibacterial activity.....	17
Figure 3.5	Percentage of honey samples collected from various land types and their median total antibacterial activities .....	18

# Executive Summary

## ***What the report is about***

This report summarises our investigations into the therapeutic potential of Australian honeys. The primary objective of the study was to increase the use and acceptance of honey as a therapeutic agent in conventional medicine. Honey shows great potential as a topical antimicrobial agent, but it is grossly under-utilised in modern medicine. We show that numerous Australian honeys possess significant antibacterial properties, and that honey is effective against a wide range of problematic pathogens, including multi drug-resistant clinical isolates and those growing in biofilms.

## ***Who is the report targeted at?***

The report is targeted at the Australian beekeeping industry, and particularly at beekeepers who are interested in harvesting and marketing honey for its medicinal as well as edible qualities. Microbiologists and clinicians specialising in wound care should also be interested in these findings.

## ***Background***

Honey has been used therapeutically throughout history, and it is still used for medicinal purposes in a number of countries. It has, however, largely been ignored in Western medicine, and is usually dismissed as an 'alternative' form of therapy. Underlying the associated scepticism is a lack of knowledge of the scope of activity and mode of action of honey in therapeutic settings.

We consider honey's greatest medicinal potential to be as a topical agent for wounds and skin infections, which are responsible for significant morbidity and mortality, and which cost billions of dollars in treatment every year. The problems caused by these injuries and ailments are compounded by antibiotic resistance in microbial pathogens, which is linked with the over-use of conventional antibiotics.

Although honey is an effective topical antimicrobial agent, its application in modern medicine has been very restricted, due to limited availability of scientific studies from well-recognised institutions. To gain recognition in Western medicine, more clinical and scientific data from internationally respected institutions are needed. New data will increase demand and sales of Australian honey, and will also heighten the image of Australian honey in the medical and wider community.

## ***Aims/objectives***

The objective of this study was to provide data to support the increased the acceptance and use of honey as a therapeutic agent in conventional medical settings. Successful achievement of this goal will benefit the honey industry by increasing the value of specific honeys, and increasing recognition of honey as a health-promoting product.

Specific aims of this work were to:

- (1) conduct an extensive screen of Australian honeys for significant activity against bacterial pathogens;
- (2) determine the susceptibility of clinical isolates of the fungal pathogen *Candida* to honey;
- (3) determine the susceptibility of a large range of clinical isolates of anaerobic bacterial pathogens to honey, and
- (4) investigate the effects of honey on bacterial biofilms.

## **Methods used**

The project was divided into four activities:

- (1) surveying Australian honeys for antibacterial activity;
- (2) determining the susceptibility of clinical isolates of fungal pathogens to honeys;
- (3) determining the susceptibility of clinical isolates of anaerobic bacterial pathogens to honeys, and
- (4) investigating of the effects of honeys on biofilms.

Methods based on internationally-recognised assays for investigating the activity of antibiotics were adapted as required for this study to test the effects of honeys against fungal and bacterial pathogens (both in the planktonic and biofilm state).

## **Results/key findings**

Key findings for each of the research activities were:

### 1. Surveying Australian honeys for antibacterial activity

- 1.1 Numerous Australian honeys exhibit therapeutically beneficial levels of antibacterial activity.
- 1.2 Some floral sources reliably produce medically-active honeys, but do not always do so; honeys therefore need to be tested batch-by-batch to identify medical-grade stocks.
- 1.3 Certain *Leptospermum* honeys from Australia consistently exhibit non-peroxide based antibacterial activity.
- 1.4 The most widely-accepted current method for testing levels of antibacterial activity of honey is reliable, but very sensitive to even minor variations in execution.

### 2. Determining the susceptibility of clinical isolates of fungal pathogens to honey

- 2.1 Honey has an anti-fungal effect on *Candida* species, and this effect is due to more than simple osmotic factors.
- 2.2 Honeys with high levels of hydrogen peroxide-type activity appear to be more effective against fungal pathogens than non-peroxide type honeys.
- 2.3 There is no relationship between susceptibility of fungal pathogens to known antifungal agents and their sensitivity to honey.

### 3. Determining the susceptibility of clinical isolates of anaerobic bacterial pathogens to honey

- 3.1 Honey has an antibacterial activity against a wide range of anaerobic pathogens, including strict and facultative anaerobes, and this activity is due to more than the high sugar content of honey.
- 3.2 There is no relationship between antibiotic susceptibility of these pathogens and their sensitivity to honey.

### 4. Investigating of the effects of honey on biofilms

- 4.1 Honey prevents biofilm formation by *P. aeruginosa* and *Staphylococcus* spp., and this inhibition occurs at levels well below the MIC of the honey.

### ***Implications for relevant stakeholders:***

Major implications for key stakeholder groups were:

- **The Australian honey industry**

This project has shown that there are numerous Australian honeys that exhibit therapeutically-beneficial levels of antibacterial activity. With appropriate marketing and public awareness campaigns, supported by reliable assay procedures, Australian honey has the potential for adoption as an internationally-recognised, potent, non-toxic, topical antimicrobial agent. There is a potentially huge market for such products.

- **Communities**

This study has shown that honey has potent activity against numerous problematic pathogens. Honey shows excellent potential as a prophylactic agent, particularly in the hospital setting where patients are often immuno-compromised and exposed to multi-drug-resistant pathogens.

- **Policy makers**

The observed range of variation in activities of Australian honeys, combined with the high number of samples with significant types/levels of activity, argue for continued access by beekeepers to sites abundant in native flora, such as national parks.

### ***Recommendations for the Australian beekeeping industry:***

1. The industry should consider funding work on developing a more robust assay for the determination of the antibacterial activity of honey. In the interim, engaging an existing commercial laboratory to perform the current assay would be very useful, assuming they were made aware of the issues with the current assay.
2. The industry should develop and licence a unified means of assaying and labelling medical-grade honeys, and this should be used by anyone marketing Australian honey as an antibacterial product.
3. A survey of previously under-represented honeys produced from other native Australian floral sources should be undertaken.



# 1. Introduction

This report summarises our investigations into the therapeutic potential of Australian honeys. The primary objective of the study was to increase the use and acceptance of honey as a therapeutic agent in conventional medicine.

Lack of published data on the activity of Australian honey was a fundamental problem in this field of research, and an extensive survey was needed. In addition, while considerable data now exist for the anti-microbial properties of honey against commonly encountered aerobic bacteria, little or nothing is still known about its effectiveness against other important pathogens. These include fungi, anaerobic bacteria and members of complex microbial communities known as biofilms.

As discussed below, we have added significantly to the knowledge base relating to the scope and mechanisms of the action of honey. Since the beginning of this investigation there has been an increase in interest in and use of honey as a therapeutic agent amongst beekeepers, the general public and the medical fraternity.

## ***Who is the report targeted at?***

This report will be of interest to the Australian beekeeping industry, and in particular to beekeepers who are interested in harvesting and marketing honey for its medicinal as well as edible qualities. It will also be of interest to microbiologists and clinicians specialising in wound care.

## ***Background***

Honey has been used therapeutically throughout history, and it is still used for medicinal purposes in a number of countries. It has, however, been largely ignored in Western medicine and is commonly dismissed as an 'alternative' form of therapy. Underlying the associated scepticism is a lack of knowledge of the scope of activity and mode of action of honey in therapeutic settings.

We consider honey's greatest medicinal potential to be as a topical agent for wounds and skin infections, which are responsible for significant morbidity and mortality, and cost billions of dollars in treatment every year. The problems caused by these injuries and ailments are compounded by antibiotic resistance in microbial pathogens, which is linked with the over-use of conventional antibiotics.

Although honey is an effective topical anti-microbial agent, its application in modern medicine has been very restricted, due to limited availability of scientific studies from well-recognised institutions. To gain recognition in Western medicine, more clinical and scientific data from internationally respected institutions are needed. New data will increase demand and sales of Australian honey, and will also heighten the image of Australian honey in the medical and wider communities.

## ***Aims/objectives***

The objective of this study was to increase the use and acceptance of honey as a therapeutic agent in conventional medicine, and in so doing, to benefit the honey industry through achieving increased values for specific honeys, and increased recognition of honey as a health product in general.

Lack of published data on the activity of Australian honey is a fundamental problem in this field of research, and a survey of local honeys was needed. In addition, while considerable data on the anti-microbial effects of honey against commonly encountered aerobic bacteria were available, little or nothing was known about its effectiveness against other important pathogens.

Specific aims of this work were therefore to:

- (1) conduct an extensive screen of Australian honeys for significant activity against bacterial pathogens;
- (2) determine the susceptibility of clinical isolates of the fungal pathogen *Candida* to honey;
- (3) determine the susceptibility of a large range of clinical isolates of anaerobic bacterial pathogens to honey, and
- (4) investigate the effects of honey on bacterial biofilms.

## 2. Methods

### 2.1 Surveying Australian honey for antibacterial activity.

#### Collection of *Apis mellifera* honey samples

Honey samples were solicited via Honey Sample Information Sheets, which were given to beekeepers at meetings and placed in state and national beekeeping newsletters. Beekeepers submitted honey samples with the information sheets on a voluntary basis and were mailed the results for their honey samples following testing. Each sample was assigned a reference number and details provided by the beekeepers via the Honey Sample Information Sheets were entered into a database specifically designed for this project.

Identification of the floral source of the honey was performed by the beekeepers. Where beekeepers supplied only the common name of the floral source, the scientific name was determined from the Australian Plant Common Name Database (from the Australian National Botanic Gardens), Australian Plant Name Index (from the Centre for Plant Biodiversity Research) and/or floral distribution maps where possible. The location of the floral source was marked on a map using Google Earth (Version 4.3; <http://earth.google.com>).

The type of land on which the apiary was situated was categorised as bushland (including national parks, state forests, heathland, nature reserves and other conserved areas, or other forested areas or shrubland), agricultural land (including farmland with stock and/or crops, orchards or other tree plantations), urban or suburban environments, or combinations of these (including farmland or suburbia bordering a national park or conserved forested area, or agricultural land with natural forested areas).

Honey samples were stored in glass or plastic containers at room temperature in the dark. After testing, a 70 ml aliquot of each honey was placed in a specimen jar for long-term storage at 4°C.

#### Determining the antimicrobial activity of honey samples

Antibacterial activity of honey samples relative to phenol treatment was determined as described by Allen *et al.* (1991).

*Staphylococcus aureus* ATCC 9144 (a common reference strain for antimicrobial susceptibility testing, susceptible to all antibiotics) was obtained from Oxoid (Hampshire, UK) as freeze-dried Culti-Loops. Cultures were stored on Protect Bacterial Preserver Beads (Technical Service Consultants Ltd., Lancashire, UK) at -20°C according to the manufacturer's instructions. A new culture was streaked and preserved in this way every 6 months.

Working cultures of *S. aureus* were obtained by placing one bead from the preserver ampoule in 10 ml of TSB and incubating with shaking at 37°C for 18 h. This culture was adjusted to an absorbance of 0.5 at 540 nm using sterile TSB as a blank and diluent and a disposable plastic cuvette with a 1 cm pathway.

Assay plates were prepared by dissolving 3.45 g of nutrient agar powder in 150 ml of deionised water and autoclaving. The agar was removed from the autoclave as soon as the cycle was complete, and used within 7 days. Prior to use, the agar was melted in a steamer for 30 min and then cooled in a 50°C water bath for 30 min. One hundred µl of the *S. aureus* culture as prepared above was added to the agar, swirled to mix, and poured into square bioassay dishes on a level surface. As soon as the plates had set they were stored inverted at 4°C for use the next day.

Using a quasi-Latin square as a template, 64 wells were cut into the agar with a flamed, cooled 8 mm diameter cork borer. The template was prepared on black card; a 25 mm grid was drawn on the card, and wells were centred at each of the 64 intersections of the grid. Each intersection was numbered, in duplicate, using a quasi-Latin square that enabled the duplicate samples to be placed randomly on the plate.

Honey samples were prepared by adding 10 ml of sterile deionised water to 10 g of well-mixed honey in 100 ml Schott bottles. These were placed on a shaker at 37°C for 30 min to aid dissolution. One ml of each honey solution was mixed with 1 ml of sterile deionised water for total activity testing, and 1 ml of a freshly prepared 5600 U/ml catalase solution for non-peroxide activity testing. One hundred µl of each solution was placed in wells of the assay plate, in duplicate. Comvita Wound Care 18+ honey was prepared as for other honey samples for use as a positive control.

Phenol standards of 2%, 3%, 4%, 5%, 6%, and 7% were prepared from a 10% (w/v) solution of phenol in sterile deionised water. These solutions were kept at 4°C and brought to room temperature in the dark before use. One hundred µl of each phenol solution was placed in duplicate wells of the assay plate. Fresh phenol solutions were made monthly.

Negative controls of sterile deionised water and catalase solution were also included in duplicate wells of each assay plate. Once the wells were filled with the appropriate honey, phenol or control solutions the plates were incubated at 37°C for 18 h.

Plates were placed over the black template and zones of inhibition were measured using Vernier callipers. The diameter of each zone was measured in two directions at right angles to each other, to the nearest 0.05 mm. The mean diameter of the zone of inhibition around each well was calculated and squared. A standard curve was generated of phenol concentration against the mean squared diameter of the zone of inhibition. A scatter plot and line of best fit was generated using Excel, and the equation of this line used to calculate the activity of each diluted honey sample from the mean squared diameter of its zone of inhibition. To account for the dilution and density of honey, this figure was multiplied by 4.69 (based on a mean honey density of 1.35 g/ml), and the activity of the honey was then expressed as the equivalent phenol concentration (% w/v).

Each honey sample was tested on at least two separate occasions. If the phenol equivalence of the positive control Comvita Wound Care 18+ honey in the assay plate differed from 18% by more than ± 2%, all honey samples in that plate were re-tested.

## **Statistical analysis**

The data consisted of nine categorical variables (floral source, genus, species, floral origin [native, exotic, or mixed], land type, state, region, sample age, partial inhibition), and two main response variables (total activity and non-peroxide activity). To aid statistical analysis, honeys with antibacterial activity below the limit of detection of the assay (approximately 5% phenol equivalent) were assigned a value of five, although these values are reported as <5 where appropriate. Analysis was performed using Minitab 14 statistical software (Minitab Inc., Pennsylvania, USA).

## **The effect of sample age on antibacterial activity**

A subset of 20 honeys (ten with hydrogen peroxide activity only, and ten with non-peroxide activity) were selected for re-testing following storage of aliquots in the dark at 4°C and at room temperature for eight to 22 months after the first test. Honeys were re-tested in duplicate on two separate occasions.

## **2.2 Determining the susceptibility of clinical isolates of fungal pathogens to honey.**

The minimum inhibitory concentration (MIC) of honey against various species of the pathogenic fungi *Candida* were determined using the 2002 reference micro-dilution method of the National Committee for Clinical and Laboratory Standards (NCCLS), with some modifications.

*Candida* isolates were supplied by the Centre for Infectious Diseases and Microbiology, Westmead Hospital, Sydney, Australia and Mazandaran University of Medical Sciences, Sari, Iran.

Jarrah honey, Medihoney and New Zealand manuka honey were tested for activity against *Candida*. Artificial honey (7.5 g sucrose, 37.5 g maltose, 167.5 g glucose, 202.5 g fructose, 85 ml sterile deionised water) was included as an osmotic control.

Fifty percent (w/v) stock solutions of each honey were prepared in RPMI-1640 medium and filter sterilised through 0.22 mm pore filters. The stock solutions of honey were further diluted in RPMI-1640 medium in U-shaped 96 well microtitre plates to give the final desired honey concentrations in 1% (w/v) increments.

Suspensions of *Candida* isolates were prepared by picking 5 colonies from an SDA plate and suspending them in 5 ml of sterile 0.85% saline. The suspensions were vortexed for 15 seconds and the transmittance at 530 nm was adjusted to 80-88% with the addition of sterile 0.85% saline. These suspensions were diluted 1:50 in sterile 0.85% saline, then further diluted 1:4 in RPMI-1640 medium, to achieve a working concentration of  $5 \times 10^3 - 2.5 \times 10^4$  cfu/ml. Twenty five  $\mu$ l of this yeast suspension was added to each well, bringing the final volume to 250  $\mu$ l and resulting in a final inoculum per well of  $0.5 - 2.5 \times 10^3$  cfu/ml. Growth controls (no honey added) were included for each isolate, and sterility controls (RPMI-1640 medium only, and honey solution only) were included in each plate. As a quality control the isolate CA1 was included in each plate for each honey tested. The microtitre plates were incubated at 35°C for 24 hours and the MIC was recorded as the lowest concentration of honey that prevented visible growth. Each *Candida* isolate was tested in duplicate and the assays were repeated on a separate day, consequently 4 replicates were performed for each isolate and honey combination.

Statistical analysis of the results was performed with Minitab statistical software (Version 11.21), using the Kruskal-Wallis test, followed by the Mann-Whitney U test to evaluate significant groups.

## **2.3 Determining the susceptibility of clinical isolates of anaerobic bacterial pathogens to honey.**

Clinical isolates of a variety of bacterial species were obtained from Prince of Wales Hospital (POWH), Concord Hospital (CH), Centre for Infectious Diseases and Microbiology, Westmead Hospital (CIDM) and the Institute of Dental Research, Westmead Millenium Institution (WMI), all located in Sydney, Australia.

The MIC of honey against various anaerobic pathogens was determined using an agar dilution method based on the 2004 National Committee for Clinical and Laboratory Standards (NCCLS) reference method for susceptibility testing of anaerobic bacteria. This method specifies the use of supplemented Brucella agar as the growth medium, however this medium contains catalase, which could have interfered with the activity of honey in this assay. Therefore, brain heart infusion (BHI) media was used for this work.

**Table 2.1: Clinical isolates of pathogenic bacteria tested for their susceptibility to honey**

Organism	No. of isolates	Source
<i>Peptostreptococcus</i> spp.	6	POWH
<i>Propionibacterium</i> spp.	6	POWH
<i>Propionibacterium</i> spp.	20	CIDM
<i>Clostridium difficile</i>	10	CH
*Coagulase-negative <i>Staphylococcus</i> sp.	9	CH
*Methicillin-resistant <i>Staphylococcus aureus</i>	12	CH
* <i>Enterococcus faecalis</i>	10	CH
<b>Dental isolates</b>		
<i>Bulleidia extructa</i>	1	WMI
<i>Eikenella corrodens</i>	1	WMI
<i>Actinomyces odontolyticus</i>	1	WMI
<i>Peptostreptococcus micros</i>	1	WMI
<i>Atopobium parvulum</i>	1	WMI
<i>Fusobacterium nucleatum</i>	1	WMI
<i>Campylobacter gracilis</i>	1	WMI
<i>Gemella haemolysins</i>	1	WMI
<i>Kingella oralis</i>	1	WMI
<i>Rothia dentocariosa</i>	1	WMI
<i>Prevotella intermedia</i>	1	WMI
<i>Porphyromas gingivalis</i>	1	WMI

\* Facultative anaerobic organisms

Agar plates containing honey were prepared in 1% (w/v) increments. The honey was first diluted to 50% (w/v) in sterile deionised water, and filter sterilised through 0.22 µm pore filters. Various concentrations of the 50% honey solutions were mixed with sterile, molten, double-strength BHI agar that had been cooled to 50°C. Appropriate volumes of sterile water were also added to the agar so that the final concentration of agar was always the same and equivalent to single strength BHI agar. Control plates were prepared by adding an equal volume of sterile water to the double-strength agar. Honey stock solutions and plates were freshly prepared for each experiment. As soon as the plates had set they were dried in a 42°C incubator for 10-15 min, and then immediately inoculated with the test organisms, as described below.

The test organism suspensions were prepared by picking five colonies from an agar plate and suspending them in 5 ml of broth, and then the cultures were incubated anaerobically at 35°C. After incubation, the transmittance at 530 nm was adjusted to 80-88% by the addition of sterile broth, giving a working concentration of  $1 - 2 \times 10^8$  cfu/ml. An antibiotic sensitivity replicator was used to inoculate the dried plates with a standard amount of culture (approximately 1 µl).

All inoculated plates were incubated anaerobically at 35°C for 48 hours, except one of each pair of control plates, which was incubated aerobically to test for aerobic contamination. The MIC was defined as the lowest concentration of honey that resulted in a marked reduction in growth of the organism. The experiment was performed in triplicate and repeated on a separate day; consequently 6 replicates were performed for each isolate.

## 2.4 Investigating of the effects of honey on biofilms.

Lucerne & blueweed and manuka honeys were used to test the effect of honey on biofilms, with artificial honey included as an osmotic control. *Pseudomonas aeruginosa*, methicillin-resistant *S. aureus* (MRSA) and coagulase-negative *Staphylococcus* (CNS) were screened for biofilm formation.

Bacterial colonies were suspended in 10 ml nutrient broth (NB) + 1% glucose and incubated for 18 hours at 37°C. The absorbance of the culture at 625 nm was adjusted to 0.1 with sterile NB + 1% glucose. Two hundred microlitres of each suspension was added to 8 wells in three separate flat bottom 96-well microtitre plates and incubated at 37°C for 24 hours. Wells containing media only were also included in each plate, to be used as blanks.

Media and planktonic (ie, floating, and therefore not part of a biofilm) cells were removed by inverting and gently shaking the plate. Each well was washed twice with 200 µl of sterile water, and the plate was then blotted and air-dried. Two hundred microlitres of 0.1% (v/v) crystal violet was added to each well and incubated for 15 minutes at room temperature. Wells were then washed twice with 200 µl of sterile water, blotted and air-dried. Well contents were resuspended in 200 µl of 99% ethanol. The absorbance was measured at 560 nm.

The effects of honey against strains of the test organisms that formed biofilms were investigated. To do this 50% (w/v) stock solutions of lucerne & blueweed, manuka and artificial honeys were prepared in NB + 1% glucose and filter sterilised. The stock solutions of honeys were further diluted in NB + 1% glucose (in duplicate) in flat bottom microtitre plates to give final honey concentrations of 1-5% honey in 1% increments, as well as 10% and 20% honey.

Additional plates were set up with the honeys diluted in NB + 1% glucose supplemented with catalase solution, to determine the effect of catalase on the activity of the honey.

Serial dilutions of tetracycline, ciprofloxacin and oxacillin were also prepared in duplicate in microtitre plates with sterile NB + 1% glucose. 200 µl of the bacterial suspensions were added to each well. The plates were incubated at 37°C for 24 hours, then washed, stained and read as above.

## 3. Results and Key Findings

### 3.1 Surveying Australian honey for antibacterial activity.

#### Key findings

- Numerous Australian honeys exhibit therapeutically-beneficial levels of antibacterial activity.
- Some floral sources reliably produce medically-active honeys, but do not always do so, honeys therefore need to be tested on a batch-by-batch basis.
- Certain *Leptospermum* honeys from Australia consistently exhibit non-peroxide based antibacterial activity.
- The current most widely accepted method for testing the levels of antibacterial activity of honey is reliable, but very sensitive to even minor variations in execution.

#### Recommendations

- The honeybee industry should consider funding development of a more robust assay for the determination of the antibacterial activity of honey.
- Commercial laboratories should be engaged to perform the current antibacterial assay only after they have demonstrated an appreciation of the sensitivity of the method to any minute deviation from the protocol.
- The industry should develop and licence a unified way of labelling medical-grade honeys, and require it to be used by anyone marketing honey as an anti-microbial product.
- A targeted study should be made of the antibacterial activity of all Australian honeys from *Leptospermum* sources.
- A project should be developed to target native floral sources that have traditionally been ignored because their honeys were considered unpalatable for consumption.

#### Background

The factors affecting antibacterial activity in honey are complex, numerous and not solely dependent on the floral source of origin. The wide variations observed in the antibacterial activity of honeys derived from single floral species prevent generic statements being made with regard to the activity of honey derived from a given floral source, and indicate the necessity of testing individual batches of honey for their level of antibacterial activity before they can be designated as therapeutic products.

If prices paid for Australian honeys are to increase with increasing antibacterial activity, as is the case in New Zealand, apiarists must assess the profitability of blending honey harvests from multiple hives (and thereby potentially reducing the overall antibacterial activity), or obtaining the maximum possible price for honey from individual hives.

While apiarists may target particular floral species identified in this survey as showing trends towards producing honeys with high antibacterial activity, individual high-activity honey crops from other floral sources may also be utilised in medicinal honey products as they are produced and assessed.

The antibacterial activity of 477 honeys from Australia was determined in this study using a standardised agar well diffusion assay. Honey samples were received from beekeepers and honey companies over the course of the study (March 2005 to June 2007), with the majority of samples being received from NSW (see Table 3.1).

**Table 3.1 Number of honey samples received from various states in Australia**

<b>State</b>	<b>number of samples</b>	<b>% of samples</b>
NSW	275	57.7
VIC	21	4.4
TAS	46	9.6
QLD	28	5.9
SA	15	3.1
WA	92	19.3
Total	477	100.0

### **The phenol equivalence assay**

In the absence of an internationally-recognised method for determining the antibacterial activity of honey, the phenol equivalence assay was used. This method was developed by researchers in the Honey Research Unit at the University of Waikato in New Zealand and has become the *de facto* standard for scientific honey testing. It is also used commercially in New Zealand to assign a UMF<sup>®</sup> (Unknown Manuka Factor) value to medicinal grade manuka honeys.

Agar well diffusion assays are time-consuming and labour-intensive, and, as with any complex bioassay, the success and reproducibility of the method is dependent on the stringent control of multiple variables. Any factor affecting the growth of the test organism (including temperature, media composition, availability of water and oxygen) will affect the results of the test. Temperature fluctuations in particular were a major factor contributing to variation in results during the preliminary stages of our study. Other factors such as humidity and thickness of the agar also affected the rate of diffusion of the test solutions and therefore the results.

Large variations in measurements of the activity of a single honey sample are known to occur between different testing laboratories. This is a recognised drawback in the use of agar well diffusion assays, and is likely to be attributable to any or all of the variables mentioned above, as well as differences in *S. aureus* test strains, or a lack of dedicated personnel and strict adherence to a detailed protocol.

While the precision and repeatability of agar well diffusion assays can be low, careful standardisation of testing conditions greatly improves the reliability of results. All the variables mentioned above were controlled as much as possible during the current study, resulting in a reliable, reproducible and high-throughput testing method. The use of a commercially available manuka honey with standardised activity as a positive control allowed an assessment of the internal variability of the assay, as well as a direct point of comparison with other testing laboratories. In addition, replicate tests of individual honey samples were within a range of  $\pm 2\%$  phenol equivalent. Therefore, while we acknowledge and appreciate the fastidious nature of the assay, we are confident of these results.

### **Total antibacterial activity of Australian honeys**

Total antibacterial activity was measured by diluting honeys in water, and non-peroxide activity was measured by diluting honeys in a catalase solution. The antibacterial activity of honey was expressed as the equivalent % (w/v) phenol solution. Antibacterial activity was divided into categories of undetectable activity (<5% phenol equivalent), low activity (5-10% phenol equivalent),

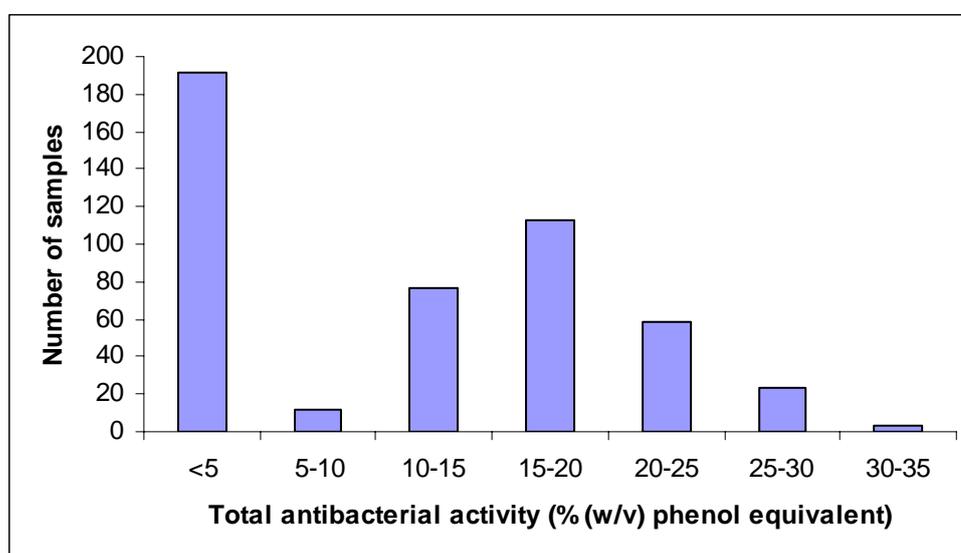
therapeutically-beneficial activity (10-20% phenol equivalent) and high activity (>20% phenol equivalent).

The distribution of levels of total antibacterial activity of the 477 honey samples tested in this study is shown in Figure 3.1. Of these honeys:

- 40% had no detectable antibacterial activity;
- 3% had low activity;
- 40% had therapeutically-beneficial activity, and
- 17% had high total activity.

The detectable levels of individual total activity (ie, for those samples with activity > 5% phenol) ranged from 7 – 34% phenol equivalence.

**Figure 3.1 Total antibacterial activities in Australian honey samples**



The majority of honey samples were received from beekeepers in NSW, and most (64%) of those samples had total antibacterial activity in the therapeutically-beneficial range. The majority of honey samples from WA had therapeutically-beneficial activity, with a large proportion (34%) exhibiting high levels of total antibacterial activity. Honeys from other states exhibited therapeutically-beneficial antibacterial activity in 28 – 57% of samples.

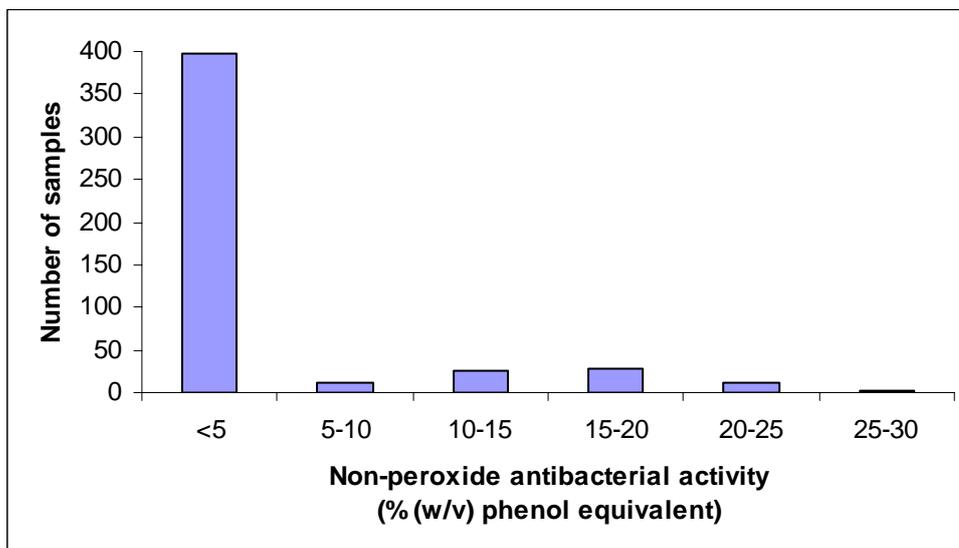
### **Non-peroxide antibacterial activity**

The distribution of levels of non-peroxide activity of the 477 honey samples tested in this study is shown in Figure 3.2. Of these honeys:

- 83% had no detectable non-peroxide activity;
- 3% had low non-peroxide activity;
- 11% had therapeutically beneficial non-peroxide activity, and
- 3% had high non-peroxide activity.

The detectable levels of individual non-peroxide activity ranged from 8 – 26% phenol equivalence (Figure 3.2); 70% of these samples were honeys derived from or containing materials from *Leptospermum* species.

**Figure 3.2 Non-peroxide antibacterial activities in Australian honey**



Of the 80 honeys with detectable non-peroxide activity, 66 were received from NSW; the majority of those exhibited non-peroxide activity in the therapeutically-beneficial range. Non-peroxide activity was strongly associated with *Leptospermum* honeys collected in the Northern Rivers region of NSW and the adjacent Southeast Coast region of Queensland.

### **Antibacterial activity of honeys from *Leptospermum* species**

Our assays showed that *Leptospermum* honeys did not all possess non-peroxide antibacterial activity, and some were found to possess very little antibacterial activity overall. However, numerous samples did exhibit therapeutically-beneficial levels of activity, so it is potentially worth targeting this nectar source for production of medical-grade honeys.

The need to individually test each batch of honey is again highlighted here, as indicated by the following of our findings:

- 24% of the honeys tested were derived wholly or partly from *Leptospermum* (jelly bush, tea tree) floral species;
- 30% of honeys containing *Leptospermum*-derived nectar had no detectable antibacterial activity;
- 15% of honeys containing *Leptospermum*-derived nectar had hydrogen peroxide activity only;
- 55% of honeys containing *Leptospermum*-derived nectar had detectable non-peroxide activity;
- *Leptospermum*-derived honeys exhibiting non-peroxide activity were collected primarily in the Northern Rivers region of NSW and the adjacent Southeast Coast region of Queensland, and
- *Leptospermum* honeys collected in other states and regions did not possess non-peroxide activity.

We note that two laboratories have recently reported that the activity of *Leptospermum* honeys correlates with the presence of methylglyoxal (MGO; Adams *et al*, 2008; Mavric *et al*, 2008). However, this does not completely explain the non-peroxide activity of these *Leptospermum* honeys, and further research is needed to clarify this.

For example, a recent publication found that the level of MGO did not necessarily give a direct indication of the antibacterial activity of the honey. Although MGO is responsible for the activity, complex interactions with other components of the honey may cause the actual antibacterial activity to vary (Adams *et al*, 2009).

Non-peroxide activity was also identified in 18 honey samples not derived from *Leptospermum* flora, although these honeys had lower levels of non-peroxide activity than those containing *Leptospermum*-derived materials. These honeys were derived from orchard, clover, forest red gum, brush box, spotted gum and *Melaleuca* sources. However, only a few honeys were received from these sources, so it was not possible to draw conclusions about these honeys; nevertheless, these findings warrant further tests on a larger number of those honeys.

### **Antibacterial activity and floral source of the honey**

Honeys from different floral sources exhibited a wide range of antibacterial activities, however there was also a high level of variation in the activities of samples from individual floral sources. In this study

- 78% of the supplied honeys were derived entirely from native Australian flora, a further 17% were partially derived from native Australian flora, and 5% were derived from exotic floral species, and
- 74 floral sources were represented by only one honey sample each, preventing firm conclusions being made about the activity of honeys derived from those floral sources.

For floral sources where five or more honey samples were received the species of origin with the most consistent levels of antibacterial activity were marri, jarrah, jelly bush, lemon-scented tea tree, red stringybark, banksia, manuka, messmate and stringybark. Of the floral sources represented by five or more honey samples, only one (olive tea tree; *Leptospermum liversidgei*) produced uniformly active honey.

While marri- and jarrah-derived honeys had reliably high antibacterial activities, both honey types also included inactive samples. It has been suggested that differences in hydrogen peroxide-dependent activity of honeys are the result of varying amounts of catalase produced by plants. The location of the floral source may contribute to within-species variations in this activity, where prevailing environmental conditions may affect the amount of catalase produced.

Even honeys produced in one location at one time varied in activity. Twenty-two *Banksia* honey samples were received from a single beekeeper following a single flowering event, where each honey sample was collected from a separate hive in the same apiary. Total antibacterial activity among 21 of these samples ranged from 11 to 19% phenol equivalent; the remaining sample had no detectable activity. A similar situation occurred with eighteen *Melaleuca* honey samples from separate hives in a single apiary. Such differences in activity may be related to differences between individual bee colonies, as colony health and age of foraging workers may affect foraging activity or the secretion of enzymes responsible for antibacterial activity, including glucose oxidase. In addition, since truly monofloral honeys are often practically impossible to obtain, different foraging preferences among different colonies may result in honey being comprised of varying proportions of nectar from numerous floral species, thereby altering the overall activity.

### **The total antibacterial activity of Australian honeys from different locations**

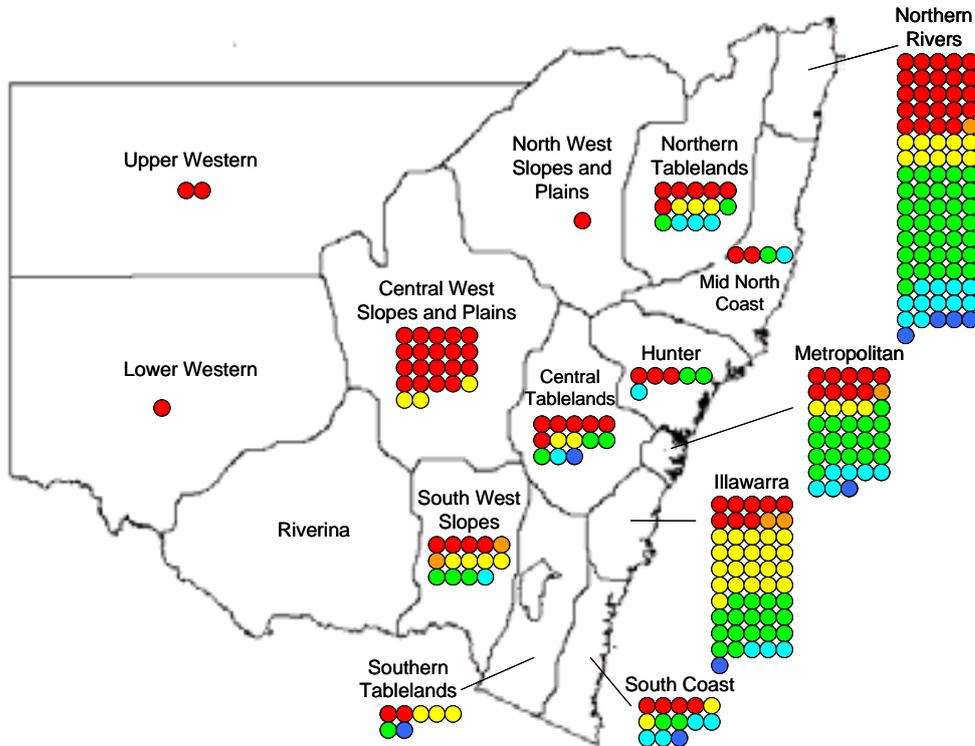
Beekeepers primarily identified the location of the floral source of the honey with reference to the nearest town, or the national park or conserved area in which the hives were placed. Location of apiary sites is considered sensitive information by many beekeepers, therefore, in order to respect beekeeper privacy, site locations in this study are presented at a regional level.

Figure 3.3 shows the state and region where each honey sample was collected and the level of total antibacterial activity of each sample. Nineteen honey samples did not have a specified location and are not included in these figures.

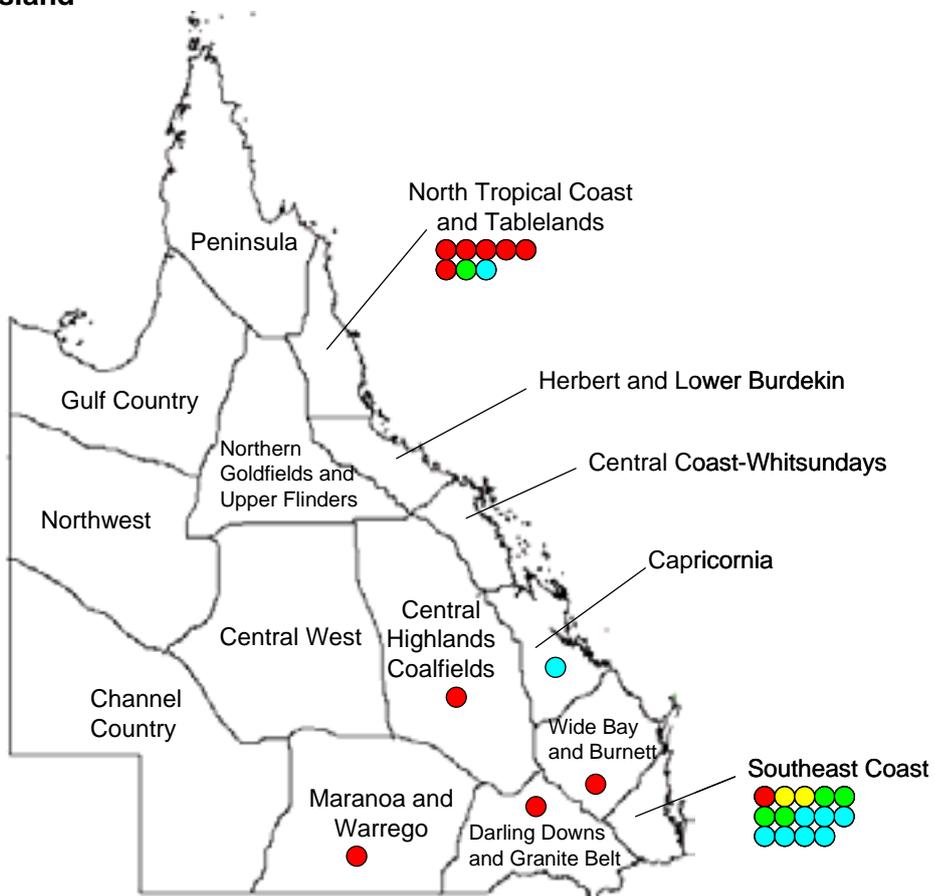
**Figure 3.3 Site location and total antibacterial activities of honeys by state & region**

Circles represent individual honey samples; colours represent categories of total antibacterial activity in % (w/v) phenol equivalent (red: <5; orange: 5 – 9.9; yellow: 10 – 14.9; green: 15 – 19.9; light blue: 20 – 25; dark blue: >25).

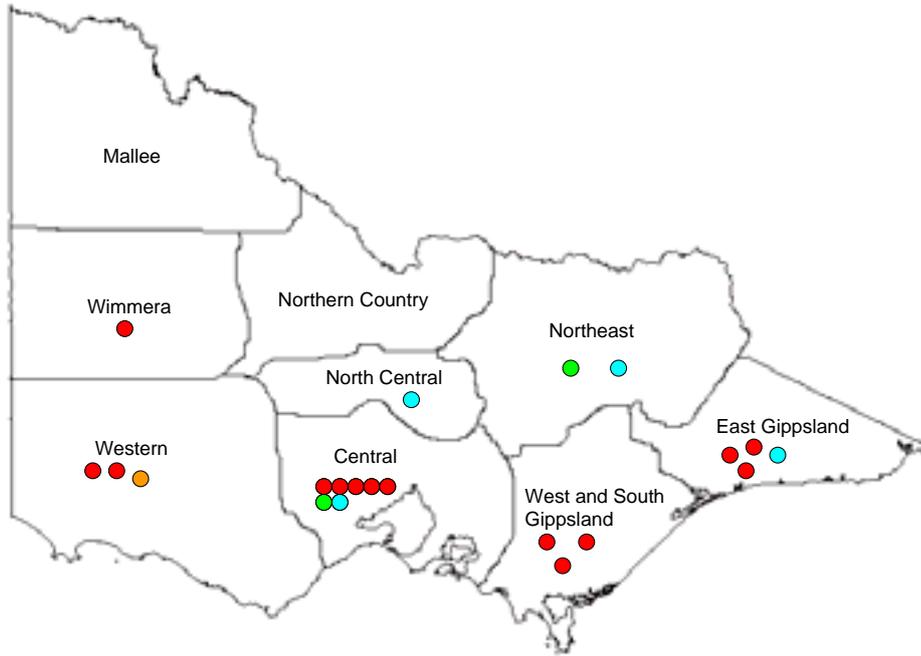
**New South Wales (NSW)**



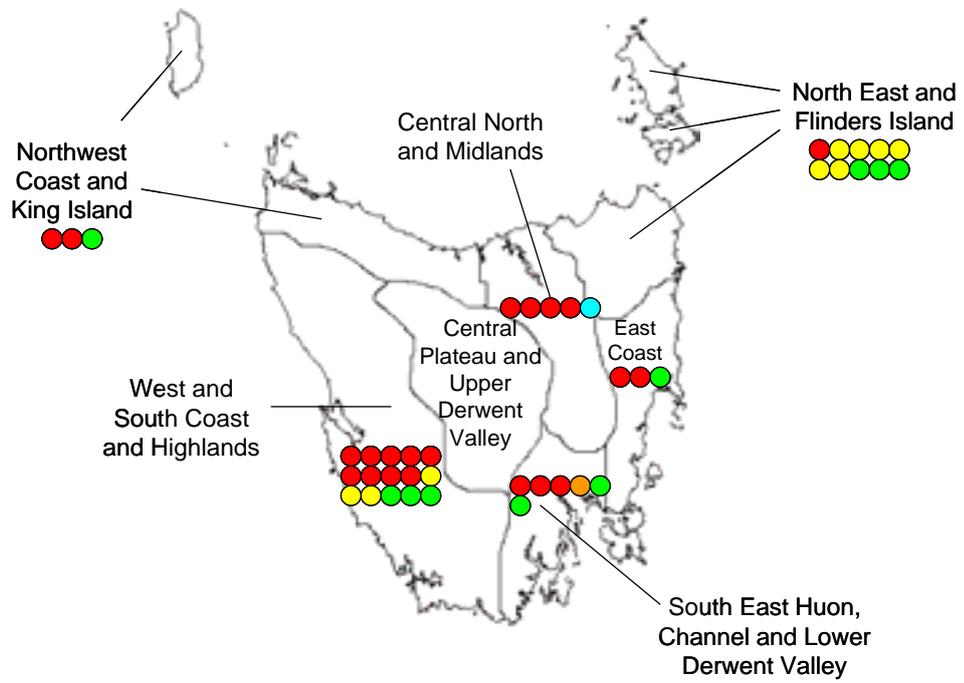
**Queensland**



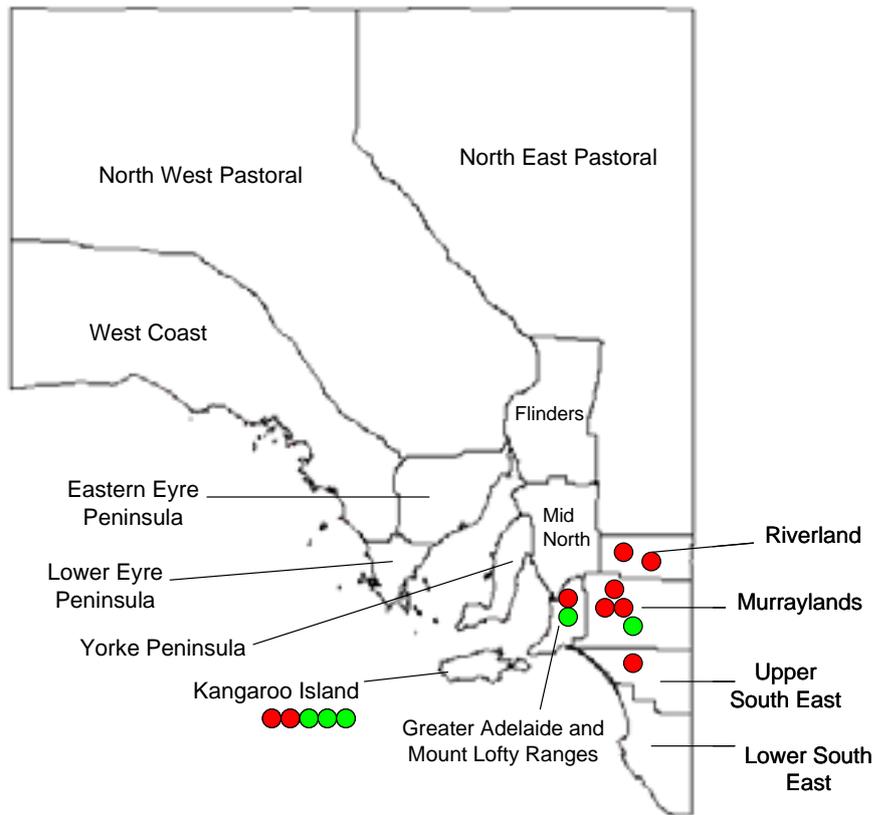
## Victoria



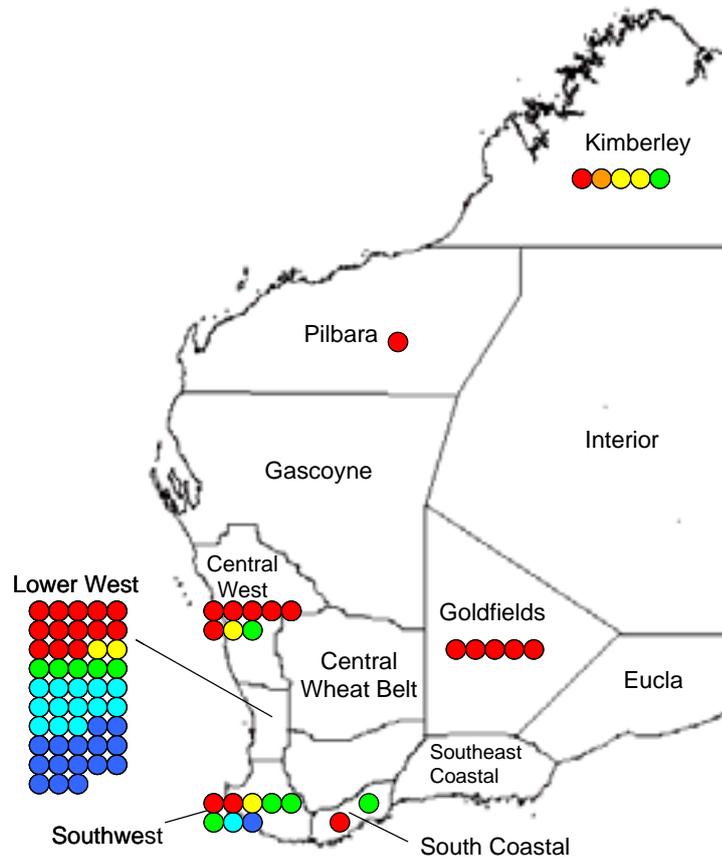
## Tasmania



## South Australia



## Western Australia

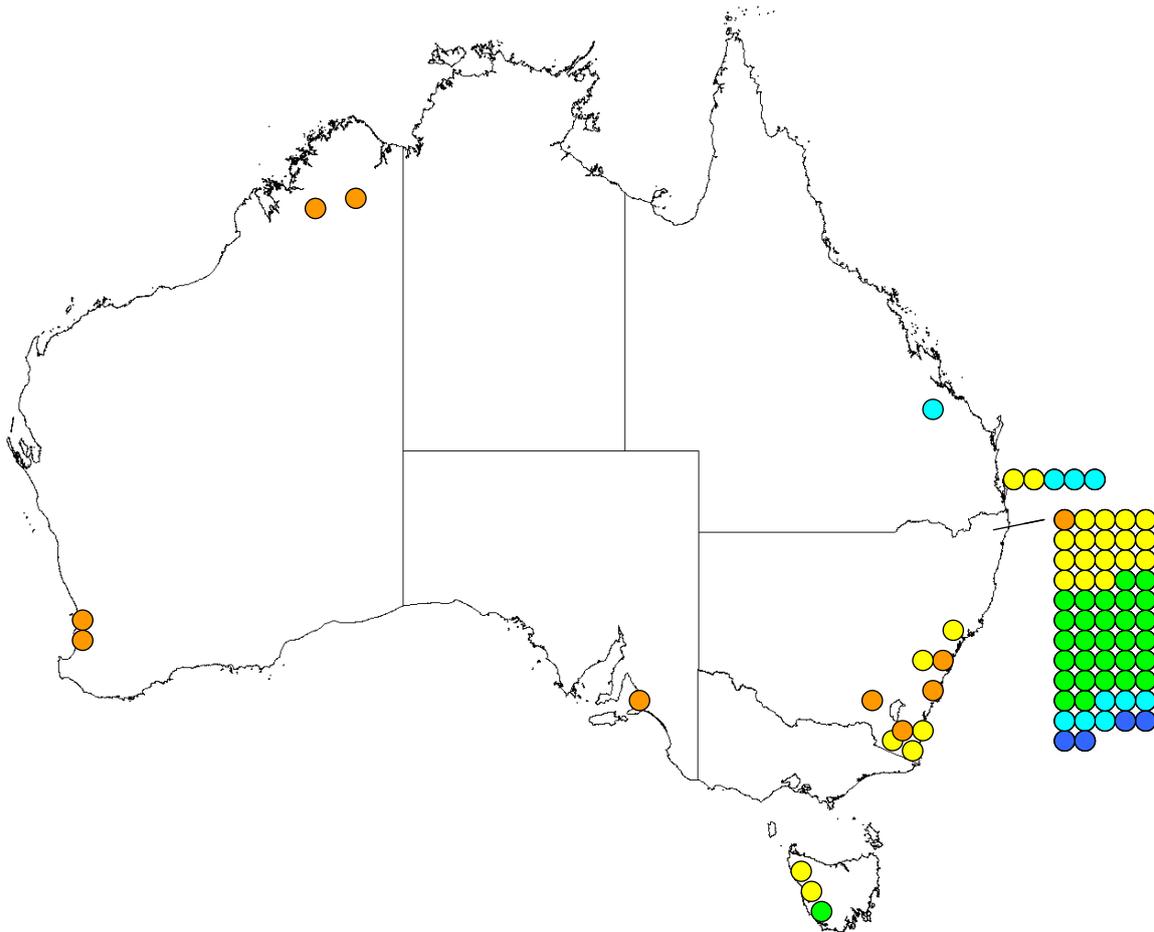


## The non-peroxide antibacterial activity of Australian honeys

The regional locations of origin of honeys that exhibited non-peroxide antibacterial activity, and their level of non-peroxide activity are shown in Figure 3.4 below.

### Figure 3.4 Locations of origin of honeys exhibiting non-peroxide antibacterial activity

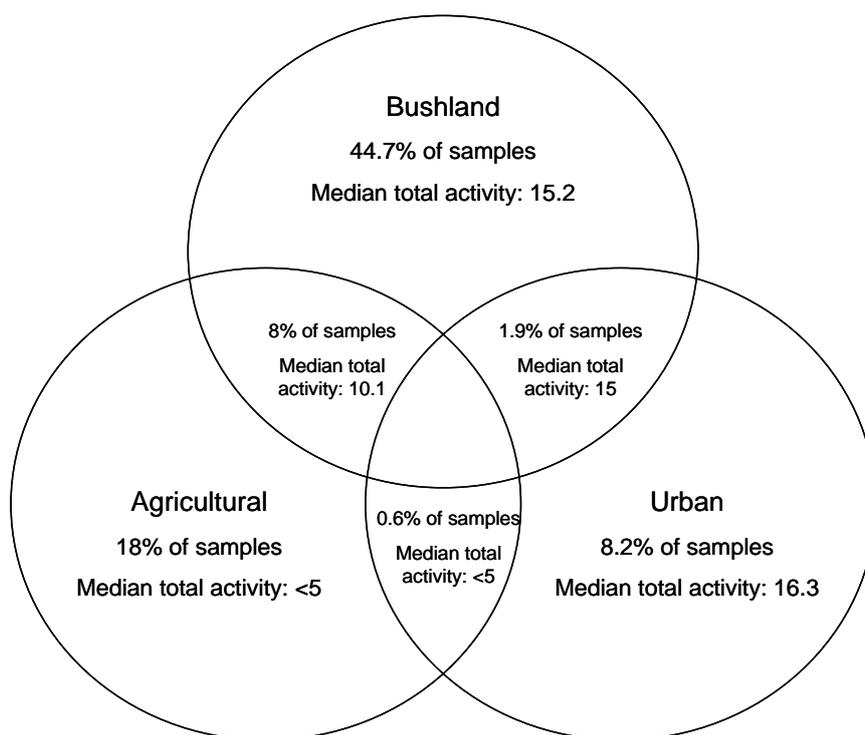
Circles represent individual honey samples; colours represent categories of non-peroxide antibacterial activity in % (w/v) phenol equivalent (orange: 5 – 9.9; yellow: 10 – 14.9; green: 15 – 19.9; light blue: 20 – 25; dark blue: >25).



## The effect of land type on the antibacterial activity of Australian honeys

The type of land from which honey samples were collected was categorised as bushland, agricultural land, urban and suburban environments, or combinations of these. The percentage of honey samples collected from each land type and their median total antibacterial activity is shown in Figure 3.5. The largest proportion of honey samples were collected in bushland; 18.7% of samples had no specified land type. Although the median antibacterial activity of honeys collected from different land types varied, sample sizes were insufficient to provide statistical support for an association between land type and antibacterial activity. However, it is obvious that continued access to natural resources is very important for the beekeeping industry. Native Australian flora was the source of numerous honeys with therapeutic levels of activity. It is also likely that there are more Australian honeys that may exhibit significant activity, but which have not yet been tested. In this regard it would be of interest to establish a project to target native floral sources that have traditionally been ignored because their honeys are considered unpalatable.

**Figure 3.5 Percentage of honey samples collected from various land types and their median total antibacterial activities**



### **The effect of sample age and storage temperature on antibacterial activity**

As was found in a similar study of New Zealand honeys (Allen *et al*, 1991) we could find no correlation between the antibacterial activity and the age of honeys. However, considering the wide variability observed in antibacterial activity levels, it is possible that differing initial activities masked any reduction in activity due to prolonged storage.

In this study the majority of honey samples were collected from hives between 2001 and 2007, and tested between 2006 and 2007. One sample was collected in 1978, and 66 samples had no collection date specified. We could find no correlation between antibacterial activity and sample age for all honeys of known age.

After storing the samples for between 8 and 22 months we found that the median total antibacterial activity of honeys exhibiting only hydrogen peroxide-dependent activity significantly decreased over time at both room temperature and 4°C. In contrast, the median total activity of honeys exhibiting non-peroxide activity did not change significantly over time at either storage temperature, suggesting that non-peroxide antibacterial components may stabilise other components within the honey.

Interestingly, some beekeepers have reported to us that non-peroxide activity increases over time, based on re-testing of stored honey either by commercial laboratories or packers of medical grade honey. Similarly, it has recently been reported that freshly produced manuka honey contained low levels of methylglyoxal and high levels of dihydroxyacetone (Adams *et al*, 2009). Those authors noted that storage of the honey at 37°C led to a decrease in the dihydroxyacetone content and a related increase in methylglyoxal, and concluded that the methylglyoxal in manuka honey is derived by the non-enzymatic conversion of dihydroxyacetone. This may explain the observation that non-peroxide activity in some honeys increases over time.

While the non-peroxide activity of some individual honey samples increased over time in the current study, there was no significant change in activity overall. These alterations in antibacterial activity

over time have some implications for the shelf-life of medicinal products containing honeys with hydrogen peroxide-dependent antibacterial activity, but do not preclude use of honey as an antimicrobial agent, since all medicinal products have a shelf life of some duration.

The stability of antibacterial activity in different honeys, like the level of activity itself, appears to be dependent on numerous compositional factors and cannot be easily predicted.

### **3.2 Determining the susceptibility of clinical isolates of anaerobic bacterial pathogens to honey.**

#### **Key findings**

- Honey has antibacterial activity against a wide range of anaerobic pathogens, including strict and facultative anaerobes.
- The antibacterial activity is due to more than the high sugar content of honey.
- No relationship was observed between antibiotic susceptibilities of the pathogens and their sensitivity to honey.

#### **Recommendations**

- A clinical trial should be conducted to examine the effect of high-activity honeys against infections caused by anaerobic pathogens.
- An investigation should be made into delivery methods that could provide a reliable method for keeping high levels of honey in place in the mouth.

We assessed the effect of various honeys on growth of seven bacterial pathogens that can grow anaerobically and cause wound infections, and against 12 species of bacteria that are commonly associated with dental disease (see Table 2.1). *Propionibacterium* spp., *Peptostreptococcus* spp. and *Clostridium* spp. are strict anaerobes that are frequently implicated in deep, chronic wound infections. *S.aureus*, coagulase-negative *Staphylococcus* and *E. faecalis* are amongst the most frequently isolated bacteria in infected wounds. These latter organisms are facultatively anaerobic in nature, and thus are capable not only of infecting the surface of the wound site, but also causing infection in deep wounds, ulcers, abscesses and areas of necrotic tissue.

The anaerobic organisms of dental origin are commonly implicated in oral diseases such as gingivitis or periodontitis. Typically, periodontal patients are treated with antibiotics, including metronidazole, clindamycin or ciprofloxacin, for both prophylactic and therapeutic purposes. However, the use of these drugs is associated with issues of development of antibiotic resistance, as well as toxicity and other side effects.

We tested jarrah, lucerne & blueweed, Medihoney and manuka honeys in our studies, and also included artificial honey as a control, in order to determine if the osmotic properties of the honey were significantly involved in the antibacterial activity.

We found that:

- each of the honeys tested exhibited broad-spectrum antimicrobial activity against anaerobic pathogens;
- the honeys were as effective against drug-resistant bacterial strains as they were against drug-sensitive ones, and
- the honeys of floral origin were significantly more effective in inhibiting the growth of all of the tested pathogens than the artificial honey.

### 3.3 Investigating of the effects of honey on bacterial biofilms.

#### Key findings

- Honey can prevent biofilm formation by *P. aeruginosa* and *Staphylococcus* spp.
- Inhibition of biofilm formation occurs at levels well below the MIC of the honey.

#### Recommendations

- The potential of honey for prophylactic use to prevent biofilm-associated infections should be investigated.
- The methods by which honey prevents biofilm formation should be investigated.

The field of medical microbiology now recognises the presence of biofilms in numerous human infections. This observation has serious implications for clinical treatment regimes, as organisms in a biofilm commonly show dramatically decreased susceptibility to antimicrobial agents, and are inherently resistant to host immune attack. Bacterial biofilm formation associated with in-dwelling medical devices is a major concern, and the role of biofilms in non-implant disease (such as *P. aeruginosa* pathogenesis in cystic fibrosis) is also well recognised. Biofilms may also serve as a reservoir for chronic or persistent infections.

We used lucerne & blueweed and manuka honeys to test the effect of honey on biofilms produced by *P. aeruginosa*, methicillin-resistant *S. aureus* (MRSA), and coagulase-negative *Staphylococcus* (CNS).

We found that:

- honey is an effective agent for the prevention of biofilm formation by *P. aeruginosa* and *Staphylococcus* spp. *in vitro*;
- both floral honeys significantly reduced biofilm formation at concentrations well below their minimum inhibitory concentrations (MICs), suggesting that there is more to biofilm prevention than just the killing of planktonic cells;
- much higher concentrations of artificial honey than floral honey were required to reduce biofilm growth;
- addition of catalase to the floral honeys resulted in a significant decrease in the inhibitory action of lucerne & blueweed honey;
- sub-inhibitory concentrations of honey, but not antibiotics, prevented biofilm formation by *P. aeruginosa* (noting that the antibiotics actually promoted biofilm formation), and
- the honeys inhibited biofilm formation by *Staphylococcus* spp. under both aerobic and anaerobic conditions, even though low oxygen tension has previously been found to stimulate biofilm formation in this organism.

### 3.4 Determining the susceptibility of clinical isolates of fungal pathogens to honey.

#### Key findings

- Honey has an antifungal effect on *Candida* species, and this effect is due to more than osmotic factors.
- Honeys with high levels of hydrogen peroxide-type activity appear to be more effective against fungal pathogens than non-peroxide type honeys.
- No relationship was observed between *Candida* susceptibility to known antifungal agents and sensitivity to honey.

#### Recommendations

- A clinical trial should be conducted to look at the effect of active honeys on *Candida* infections.
- An investigation should be made into possible delivery methods that could provide a reliable method for keeping high levels of honey in place in/on body areas susceptible to *Candida* infections.

Infections caused by pathogenic species of *Candida* yeast are becoming increasingly difficult to treat due to an increase in drug-resistance. These infections also place a huge cost burden on the health care system, often resulting in an additional 2-3 weeks in hospital for infected patients and thousands of dollars in additional costs. These problems indicate that alternative types of antifungal agents need to be considered, and honey was considered to warrant further attention in this respect. The results from this study suggest that honey could be used as an effective topical agent for the treatment and prevention of *Candida* infections in wounds and on mucus membranes, although clinical tests are required to further investigate this possibility.

**Table 3.2 Susceptibility of *Candida* species to different honey types**

	<i>Candida</i> species (n)		
	<i>C. albicans</i> (18)	<i>C. glabrata</i> (10)	<i>C. dubliniensis</i> (10)
<b>Jarrah</b>	18.5 ± 2.7**	29.9 ± 2.8**	15.4 ± 2.8**
<b>Medihoney</b>	38.2 ± 2.9**	43.1 ± 4.2*	34.6 ± 2.5
<b>Manuka honey</b>	39.9 ± 1.7**	42.6 ± 2.8**	33.4 ± 2.5
<b>Artificial honey</b>	42.6 ± 1.8	44.7 ± 2.7	34.3 ± 2.4

(n): number of isolates tested. Tabulated values show mean minimum inhibitory concentration (% honey, w/v) ± standard deviation. \*  $P < 0.002$ ; \*\*  $P < 0.00001$ .  $P$  values are assessed in comparison to artificial honey.

Jarrah honey was significantly more active against the three *Candida* species ( $P < 0.00001$ ) than was artificial honey. The antifungal activities of the floral honeys were significantly greater than the artificial honey against *C. albicans* and *C. glabrata* ( $P < 0.002$ ), but for *C. dubliniensis*, only jarrah honey was significantly more active ( $P < 0.00001$ ). *C. dubliniensis* was more susceptible than the other two species to the osmotic effect of all honeys, and to the antifungal effects of jarrah honey, exhibiting significantly lower MICs than the other species ( $P < 0.00001$ ). *C. glabrata*, which is innately less

susceptible to many conventional antifungals, was the least susceptible to the honeys tested ( $P < 0.00001$ ).

Drug resistance profiles were available for 20 of the 38 isolates tested. Twelve of those 20 were either resistant or dose-dependently susceptible to itraconazole and/or fluconazole. Growth of all these isolates was inhibited by honey, with no statistical relationship observed between their antifungal susceptibilities and sensitivities to honey ( $P > 0.05$ ). This observation is of particular importance considering the increasing prevalence of resistance to azole drugs among *Candida* isolates, and the finding that azole-based prophylaxis increases the risk of infection with non-*C. albicans* species of *Candida* that may be less responsive to usual drug dosages.

Although this study demonstrated the antifungal effect of honey *in vitro* there are some practical considerations associated with its potential use *in vivo*. Firstly, use of honey will be limited to topical treatments, and will not be able to be used to treat candidaemia, the most serious form of candidiasis. However, as the leading risk factor for bloodstream infection is colonisation or infection of external sites such as in-dwelling catheters, or the oral or vaginal mucosae, honey may be able to be used prophylactically to prevent more serious infections.

Secondly, as honey is water soluble, it may be diluted or removed by body fluids, particularly saliva in the oral cavity. A pilot study by English *et al.* (2004) found a significant reduction in mean plaque scores and bleeding sites in patients given a chewable 'honey leather'. This same technique could potentially be applied for the treatment of oral candidiasis. At other body sites, regular application of 100% honey would maintain a concentration well above the required MIC. Honey could potentially also be incorporated into a pessary for the treatment of vaginal candidiasis.

A further practical issue is the presence in body fluids of catalase, which has the potential to reduce hydrogen peroxide activity. However, case reports and clinical trials suggest that sufficient anti-microbial activity would be retained to allow honey to be effective in clinical settings. The results of the current study argue for establishment of controlled clinical trials to demonstrate the efficacy of honey as a topical antifungal agent.

Details of the antifungal properties of honeys, as determined in this work, have been published in the scientific literature (see: Irish, J., Carter, D.A., Shokohi, F. and Blair, S.E. 2006. Honey has an antifungal effect against *Candida* species. *Medical Mycology* 44: 289-291).

### **3.5 Antibacterial activity of honey from Australian stingless bees (*Trigona* spp).**

During the course of the survey of Australian honeys we were supplied with samples of honey from native Australian stingless bees (*Trigona* spp.). Twenty-one samples of *T. carbonaria* honey and one from another *Trigona* species were collected from separate hives in Brisbane, Queensland, during 2006. These samples were tested for activity in the same manner as the honeys from *Apis mellifera*. All 22 honey samples had high levels of antibacterial activity, ranging from 17.5 – 32.1% (w/v) phenol equivalent. Of particular interest was the presence of non-hydrogen peroxide-dependent antibacterial activity in all samples, ranging from 11.5 – 23.7% (w/v) phenol equivalent. The hydrogen peroxide activity of some samples decreased over time, whereas the non-peroxide activity remained stable in all samples (see Table 3.3 below).

In contrast to *A. mellifera*-sourced honey the non-peroxide activity seemed to be associated with the bees rather than the nectar collected. As discussed above, non-peroxide activity in *A. mellifera* honeys was strongly linked to the floral source, and most commonly associated with honeys derived from *Leptospermum* species. However, several reasons suggest this is unlikely to be the case for the *Trigona*-sourced honeys. Firstly, the *Trigona* hives were situated in suburban areas with low abundance of *Leptospermum* plants, and in some cases the flowering period was outside the six-month

foraging period prior to honey extraction. These bees also produce honey at a slower rate than *A. mellifera* and the samples we tested were all from mixed flora. Consequently, the fact that all the honey samples possessed non-peroxide activity suggests an entomological, rather than phytochemical source of activity.

*Trigona* species produce honey in lower quantities than *A. mellifera*, and the honey is more difficult to harvest in large quantities. However, the significant antibacterial activity it possesses suggests that it warrants more detailed consideration as a therapeutic agent.

Full details of the properties of honeys from *Trigona* species, as determined in this work, have been published in the scientific literature (see Irish, J., Heard, T.A., Carter, D.A. and Blair, S.E. 2008. Antibacterial activity of honey from the Australian stingless bee *Trigona carbonaria*. *International Journal of Antimicrobial Agents* 32: 89-98).

**Table 3.3: Antibacterial activity of *Trigona* honey**

Sample number	Initial antibacterial activity <sup>a</sup>			Final antibacterial activity <sup>b</sup>		
	Sample age (weeks)	Total activity	Non-peroxide activity	Sample age (weeks)	Total activity	Non-peroxide activity
1	10	19.0	18.4	40	23.1	21.7
2	10	28.5	17.3	40	27.8	19.6
3	10	27.5	18.0	40	26.0	21.0
4	10	25.3	19.4	40	26.8	21.6
5	6	17.5	13.6	28	16.3	14.9
6	6	25.6	12.6	28	17.4	14.0
7	6	28.9	14.9	28	20.4	18.1
8	3	26.4	23.7	24	24.3	21.5
9	10	23.8	16.4	30	18.2	17.8
10	7	23.7	15.1	28	18.5	16.7
11	3	25.0	13.2	25	22.0	14.6
12	6	27.0	16.2	28	21.4	18.1
13	6	25.6	13.7	28	19.4	16.2
14	6	20.4	16.1	28	19.5	18.3
15	9	27.5	13.4	23	14.2	12.3
16	9	29.6	11.5	23	11.5	11.1
17	9	28.6	13.3	23	17.1	13.4
18	7	27.6	16.5	20	19.3	16.5
19	7	27.4	17.3	20	18.4	18.0
20	7	30.8	14.7	20	16.1	13.9
21	7	31.5	13.8	20	25.2	13.9
22	7	32.1	13.4	20	16.1	14.7

Antibacterial activity is expressed as mean % (w/v) phenol equivalent, based on four replicated tests for initial activity and two tests for final activity.

## 4. Conclusions

### 4.1 Implications for the Australian beekeeping industry

This project has shown that there are numerous Australian honeys that exhibit therapeutically-beneficial levels of antibacterial activity. It is also apparent that there is a great deal of interest in the therapeutic use of honey amongst the general public as well as the medical fraternity, and that this interest continues to grow.

With appropriate marketing and public awareness campaigns Australian honey has the potential to become a popular, internationally-recognised, potent, non-toxic, topical antimicrobial agent. The market for such products is almost limitless. Furthermore, an increased use and acceptance of some honeys as wound dressings would have the potential to lead to a general increase in status of all Australian honeys, regardless of their medical properties.

### 4.2 Implications for the community

We have shown that honey has potent activity against numerous problematic pathogens, but also that identification/selection of the appropriate honey is important if it is to be used medicinally. Further investigations are needed to fully determine the anti-microbial mode(s)-of-action of honey, and honey should not be viewed as a panacea. However, it is clear that honey is under-utilised as a modern infection control agent, especially as we have now provided scientific data proving its significant activity against drug-resistant pathogens (including anaerobes and fungi), as well as an ability to inhibit formation of biofilms. Honey is non-toxic and is able to stimulate wound healing, in contrast to other topical anti-microbials. Honey also shows excellent potential as a prophylactic agent, particularly in the hospital setting where patients are often immuno-compromised and exposed to multi drug-resistant pathogens. An increased use of medical-grade honey in modern wound care could lead to a reduction in patient suffering, as well as reduced healthcare costs.

### 4.3 Implications for policy makers

The observed variations in the activities of Australian honeys, combined with the high number of samples with significant activity, argues for continued access by beekeepers to sites abundant in native flora, such as national parks. It is likely that there are honeys with significant antibacterial activity (derived from native Australian flora) that are yet to be discovered.

### 4.4 Recommendations for the Australian beekeeping industry

There is currently a great deal of confusion amongst beekeepers, the general public and the medical fraternity about the types and levels of activities exhibited by different honeys, and the efficacy of individual honeys as therapeutic agents. With numerous groups beginning to market honey as a medical product this confusion is likely to increase, especially if any groups use negative marketing against their perceived competitors. The current lack of a cohesive message is of great detriment to the beekeeping industry. The industry should develop and licence a unified way of labelling medical-grade honeys, and its use should be required of anyone marketing honey as an antimicrobial product.

The industry should consider funding development of a more robust assay for determining the antibacterial activity of honey. In the interim, the best and most cost effective approach would be to engage an existing commercial laboratory to perform the current assay, once they have demonstrated an appreciation of the sensitivity of the method to minute deviation from the protocol.

As it is likely that there are other Australian honeys with significant medical potential, a targeted study on the antibacterial activity of all Australian *Leptospermum* ('manuka' and 'tea tree') honeys should

be carried out. It would also be of value to design a project to target native floral sources that have traditionally been ignored or avoided because their honeys were considered unpalatable for human consumption.

There has been some reluctance by the Australian beekeeping industry to fund research specifically on *Leptospermum* honeys, as this was seen as benefiting only a small section of the industry. However, the New Zealand experience with manuka honey clearly shows that all New Zealand honeys have benefitted from the positive image that manuka honey has generated there. Results from this project demonstrate that *Leptospermum* honeys represent one of the most reliable types of medically-active Australian honeys, and suggest that the industry should therefore look to supporting research that targets *Leptospermum* honeys and increases their acceptance in medical practice.

#### **4.5 Recommendations for the wider medical and scientific community**

The laboratory results presented here argue for establishment of clinical trials of medical-grade honeys. The potential for honey to prevent infections (particularly in hospital settings) should be investigated. In addition, the performance of medical-grade honey, compared to conventional treatments, in the treatment of fungal and antibiotic-resistant bacterial infections should be examined.

There are practical considerations and some limitations associated with the medicinal use of honey due to its physical properties. However, these are by no means insurmountable. An investigation into possible delivery methods should be carried out in order to determine a reliable method of keeping high levels of honey in place on various body areas, including mucus membranes.

The ability of honey to prevent biofilm formation, and the fact that this appears to be due to more than simple inhibition of planktonic cell growth, certainly warrants further investigation.

## 5. References

- Adams CJ, Boulton CH, Deadman BJ, Farr JM, Grainger MNC, Manley-Harris M & Snow MJ. 2008. Isolation by HPLC and characterisation of the bioactive fraction of New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydr. Res.* 343: 651-659.
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- English, H.K., Pack, A.R. & Molan, P.C. 2004. The effects of manuka honey on plaque and gingivitis: a pilot study. *J. Int. Acad. Periodontol.* 6: 63-67.
- Mavric E, Wittmann S, Barth G & Henle T. 2008. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. *Mol. Nutr. Food Res.* 52: 483-489.

## 6. Appendices

### Publications, presentations and popular media appearances

#### Publications in peer reviewed journals

Blair, S.E., Cokcetin, N.C., Harry, E.J. & Carter, D.A. (2009) The unusual antibacterial activity of medical-grade *Leptospermum* honey: antibacterial spectrum, resistance and transcriptome analysis. *European Journal of Clinical Microbiology and Infectious Diseases* 28: 1199-1208.

Irish, J., Heard, T.A., Carter, D.A. & Blair, S.E. (2008) Antibacterial activity of honey from the Australian stingless bee *Trigona carbonaria*. *International Journal of Antimicrobial Agents* 32: 89-98.

Irish, J., Carter, D.A., Shokohi, F. & Blair S.E. (2006) Honey has an antifungal effect against *Candida* species. *Medical Mycology* 44: 289-291.

#### Book chapters

In *Honey a Modern wound management product, volume II. (in press)*

Blair, S.E. An historical introduction to the medicinal use of honey

Blair, S.E. The antibacterial activity of honey

Cooper, R. A. and Blair S.E. Challenges in modern wound microbiology and the role for honey

#### Reviews

Blair, S. E. and Carter, D.A. (2005) The potential for honey in the management of wound and infection. *Journal of Australian Infection Control* 10(1):24-31.

#### Other publications

Blair, S.E., Irish, J., Carter, D.A. (2006) Honey: the sweet solution to a global emergency? *Syntrophy* 7(9):1, 11-13.

Irish, J. and Blair, S.E. (2006) The use of honey in veterinary wound management. Control and Therapy Series, Postgraduate Foundation in Veterinary Science of the University of Sydney.

Blair, S.E. (2005) Therapeutic honey. *National Healthcare Journal* Aug: 54-58.

Blair, S.E. and Irish, J. (2005) Honey vs Superbugs. *Australasian Beekeeper* 107(2):79-83.

#### Presentations at scientific meetings

Carter, D.A. Irish, J., Cokcetin, N, and Blair, S.E. (2009) Medical Honey - the latest research. Wound Care Symposium; Wound Care Association of NSW, Sydney, Australia. Invited speaker.

Irish, J., Carter, D.A. and Blair, S.E. (2007) Honey – the sweet solution for problem pathogens. University of Technology, Sydney Lecture Series. Sydney, Australia. Invited speaker.

Irish, J., Heard, T., Carter, D.A. and Blair, S.E. (2007) Unusual activity of honey from the native Australian stingless bee, *Trigona carbonaria*. Joint Scientific Meeting of The Australian Society for Microbiology and New Zealand Society for Microbiology. Adelaide, Australia. Proffered paper.

Irish, J., Carter, D.A. and Blair, S.E. (2007) Honey prevents biofilm formation by *Staphylococcus aureus*. Joint Scientific Meeting of The Australian Society for Microbiology and New Zealand Society for Microbiology. Adelaide, Australia. Proffered poster.

Blair, S.E., Irish, J. and Carter, D.A. (2006) The science supporting the medicinal use of honey. Wound Care Association of NSW Symposium on Wound Care. Dubbo, NSW, Australia. Invited speaker.

Blair, S.E., Irish, J. and Carter, D.A. (2006) A review of the antimicrobial properties of honey. 1st International Conference on the Medicinal Use of Honey. Kota Bharu, Malaysia. Keynote speaker.

Blair, S.E., Irish, J. and Carter, D.A. (2006) Honey is effective against drug resistant and other problematic pathogens. 1st International Conference on the Medicinal Use of Honey. Kota Bharu, Malaysia. Proffered paper.

Irish, J., Carter, D.A. and Blair, S.E. (2006) Honey prevents biofilm formation by microbial pathogens. 1st International Conference on the Medicinal Use of Honey. Kota Bharu, Malaysia. Proffered paper.

Irish, J., Carter, D.A. and Blair, S. E. (2005) Honey inhibits the growth of *Propionibacterium acnes*. Joint Scientific Meeting of The Australian Society for Microbiology and New Zealand Society for Microbiology. Canberra, Australia. Proffered paper. (Also presented in competition for the 2005 NSW ASM Becton Dickinson Student Awards)

Gralton, J., Carter, D.A. and Blair, S. E. (2005) Investigating the ability of honey to kill major wound pathogens. Joint Scientific Meeting of The Australian Society for Microbiology and New Zealand Society for Microbiology, Canberra, Australia. Proffered paper.

#### Presentations at apiarists' meetings

Blair, S.E., Irish, J., Cokcetin, N.C. and Carter, D.A. (2008) Honey – the grossly underutilised antimicrobial. 1st International Symposium on Honey and Human Health. Sacramento, California, USA. Invited speaker.

Irish, J., Carter, D.A. and Blair, S.E. (2008) The therapeutic properties of honey. NSW Amateur Beekeepers Association Annual Field Day. Paterson, NSW, Australia. Invited speaker.

Irish, J., Carter, D.A. and Blair, S.E. (2008) Survey of the antimicrobial activity of Australian honey. 95<sup>th</sup> NSW Apiarists' Association Conference, Nelson Bay, NSW, Australia. Invited speaker.

Irish, J., Cokcetin, N.C., Carter, D.A. and Blair, S.E. (2008) The therapeutic properties of honey. Rural Industries Research and Development Corporation R&D meeting, Canberra, Australia. Invited speaker.

Irish, J., Carter, D.A. and Blair, S.E. (2007) Investigating the medical properties of Australian honeys. WA Apiarists' Society Annual General Meeting. Perth, Australia. Invited speaker.

Blair, S.E., Irish, J. and Carter, D.A. (2007) Honey – a sweet solution to a global crisis? NSW Apiarists' Association 94<sup>th</sup> Annual Conference. Inverell, Australia. Invited speaker.

Blair, S.E., Irish, J. and Carter, D.A. (2007) Honey – a sweet solution to a global crisis? SA Apiarists' Association Annual Conference. South Australia. Invited speaker.

Blair, S.E., Irish, J. and Carter, D.A. (2007) Honey – a sweet solution to a global crisis? VIC Apiarists' Association Annual Conference. Melbourne, Australia. Invited speaker.

Blair, S.E., Irish, J., Cokcetin, N.C. and Carter, D.A. (2007) The science behind the medicinal use of honey. Sydney Apiarists Meeting, Sydney, Australia. Invited Speaker.

Blair, S.E., Irish, J., Cokcetin, N.C. and Carter, D.A. (2007) The under-appreciated potential of honey in modern medicine Apimondia. Melbourne, Australia. Proffered paper.

Irish, J., Carter, D.A. and Blair, S.E. (2006) Honey vs Superbugs. 8th Asian Apicultural Association Conference. Perth, Australia. Proffered paper.

Irish, J., Carter, D.A. and Blair, S.E. (2006) Honey prevents biofilm formation in *Staphylococcus aureus*. 8th Asian Apicultural Association Conference. Perth, Australia. Poster.

Blair, S.E. and Irish, J. (2006) Medicinal applications of honey. NSW Amateur Beekeepers Association Annual Field Day. Paterson, Australia. Invited speaker.

Irish, J., Gralton, J., Carter, D.A. and Blair, S.E. (2006) An investigation into the therapeutic properties of honey. Annual Rural Industries Research and Development Corporation R&D meeting. Canberra, Australia. Invited speaker.

Irish, J., Carter, D.A. and Blair, S.E. (2006) Testing the antibacterial activity of Australian honey. NSW Apiarists' Association 93<sup>rd</sup> Annual Conference. Foster, NSW, Australia. Invited speaker.

Blair, S.E., Irish, J. and Carter, D.A. (2005) The therapeutic potential of honey. Annual Rural Industries Research and Development Corporation R&D meeting. Canberra, Australia. Invited speaker.

Blair, S.E., Irish, J. and Carter, D.A. (2005) Honey – a sweet solution for problem pathogens. ACT Amateur Beekeepers Association meeting. Canberra, Australia. Invited speaker.

Blair, S.E. and Irish, J. (2005) *Honey – a sweet solution*. NSW Amateur Beekeepers Association Annual Field Day. Richmond, Australia. Invited speaker.

Blair, S.E., Irish, J. and Carter, D.A. (2005) Honey vs Superbugs. NSW Apiarists' Association 92<sup>nd</sup> Annual Conference. Orange, NSW, Australia. Invited speaker.

Blair, S.E., Irish, J. and Carter, D.A. (2005) Honey kills superbugs – the medicinal properties of honey. The Entomological Society of NSW meeting. Sydney, Australia. Invited speaker.

#### Presentations to lay audiences

Blair, S.E. (2007 and 2008) National Science Week - “Snag a scientist family BBQ”. National Science Week program at the Ultimo Science Festival. Sydney, Australia. Invited guest.

Blair, S.E. and Irish, J. (2006) Honey – a sweet solution for problem pathogens. Sydney Jewish Women's Society. Sydney, Australia. Invited speaker.

Irish, J., Carter, D.A. and Blair, S.E. (2006) Honey – a sweet solution for super infections. The 55+ Club. Sydney, Australia. Invited speaker.

#### Popular media interviews

Our research has generated a great deal of interest amongst the general public and this has resulted in numerous media interviews and invitations. Some of these are listed below.

### *Television*

Dr Shona Blair and Prof. Dee Carter appeared on television programs discussing aspects of the research into the medicinal properties of honey on:

Channel 9 Morning News (broadcast nationally), 19 June 2009;  
Australian Network News Hour (overseas only), 19 June 2009, and  
Channel 9 Today show (broadcast nationally), September 12, 2007;

They also featured in interviews for evening NSW regional news programs in 2005 and 2007, and during a segment on honey research for the ABC science program Catalyst (broadcast nationally) in 2003.

### *Radio*

More than 60 interviews were done with ABC regional and capital city radio programs around Australia, as well as with various commercial radio stations, Radio New Zealand and BBC London. These included both live and pre-recorded segments, as well as talkback that involved answering questions from listeners.

### *Newspaper*

There were numerous newspaper interviews on the subject of the research for papers such as The Australian and The Daily Telegraph, as well as numerous regional papers. Some examples of specific articles include:

“Honey, I killed the drug resistant superbug”, The Australian, 18 June 2009

“Honey's healing touch”, Los Angeles Times, September 10, 2007

“A honey of a cure takes the sting out”, The Age, September 11, 2007

“A honey of a cure”, The Sydney Morning Herald (on line), September 13, 2007.

### *Internet*

The following web link is an interview Blair, S.E. participated in for the ABC, when they were preparing a number of short pieces on people with interesting careers. The series is broadly aimed at high school students thinking about career options.

<http://www.abc.net.au/acedayjobs/cooljobs/profiles/s1391857.htm>

# An Investigation into the Therapeutic Properties of Honey

by Dee A. Carter, Shona E. Blair and Julie Irish

Publication No. 09/180

This report summarises our investigations into the therapeutic potential of Australian honeys. The study was conducted to increase the use and acceptance of honey as a therapeutic agent in conventional medicine.

Honey shows great potential as a topical antimicrobial agent, but it is grossly under-utilised in modern medicine. The results show that numerous Australian honeys possess significant antibacterial properties, and that honey is effective against a wide range of problematic pathogens, including multi drug-resistant clinical isolates and those growing in biofilms.

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