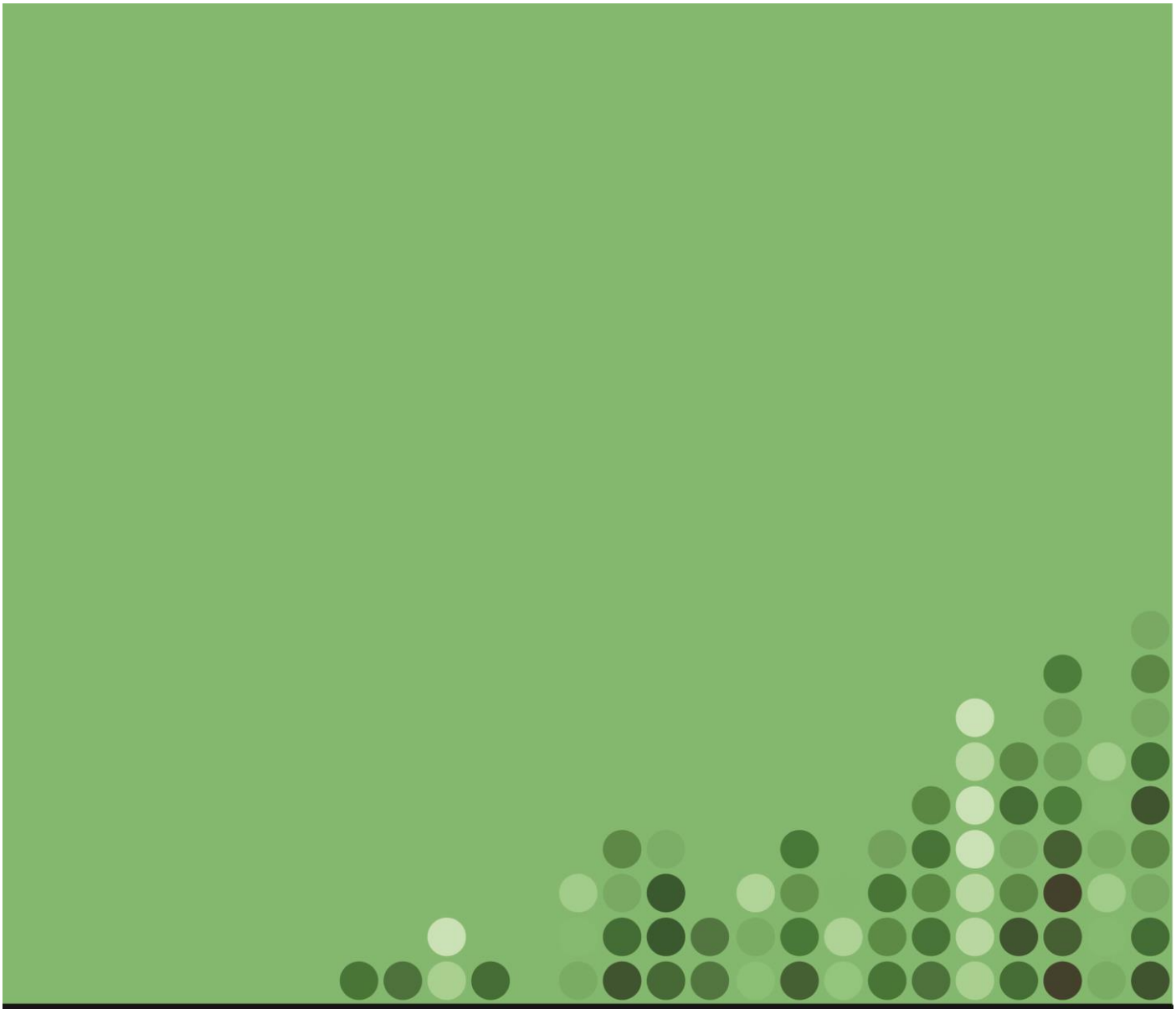
Department of **Employment, Economic Development and Innovation**



Berringa bioactive honey

Comparison of honey vs silver for antibacterial activity

January 2012

Toward Q Tomorrow's Queensland

Queensland Government

This project was commissioned by:

Peter Woodward
Chairman
Medi Bioactive Honey Australia Pty Limited
15 Wheeler Crescent
Currumbin QLD 4223

This report was prepared by:

Dr Yasmina Sultanbawa
Queensland Alliance for Agriculture and Food Innovation (QAAFI)
University of Queensland
39 Kessels Road, Coopers Plains Qld 4108

In collaboration with:

Andrew Cusack
Innovative Food Solutions and Technologies
39 Kessels Road, Coopers Plains Qld 4108

Contents

Background

Honey is known for its wound healing and antimicrobial properties, however the bioactivity of the honey is dependent on many factors such as the geographical location and the floral source from which the honey is derived (Sherlock et al. 2010). Berringa honey which originates from the *Leptospermum polygalifolium* tree is known for its antimicrobial properties and methylglyoxal is one of the compounds identified for its antimicrobial activity.
Historically silver has been used for medicinal purposes and silver-impregnated dressings are used extensively in the care of chronic wounds. However, some studies have demonstrated cytotoxicity of silver in wound repair (Bradshaw, 2011). Natural antimicrobials are being sort as a preferred topical wound treatment to silver.

The objective of this study was to compare the in vitro antimicrobial activity of Berringa honey and silver when tested against *Psuedomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) cultures. Compare the inhibitory effect of Berringa honey and silver dressings against MRSA cultures.

Method

Honey samples

The antibacterial properties of 10 honeys were tested with the following methylglyoxal contents:

Sample Number	Methyglyoxal Content (mg/kg)
4766	548
4835	566
4818	718
4826	737
4769	970
4831	974
4810	1157
4824	1277
4833	1743
4834	1755

These honey samples were received between January and May 2010.

Silver

Silver nitrate 99.9999%, 204390-10G, Lot. 03204CJ, Sigma-Aldrich, St.Louis,USA.
Commercial silver dressings sourced from

Antibiotic

Oxytetracycline hydrochloride 95% min., 05875-10G, Lot. 017K0737, Sigma-Aldrich, St.Louis,USA.

Bacterial strains

The antibacterial properties of honey and silver were tested against six bacterial isolates, of which two were reference strains, *Psuedomonas aeruginosa* ATCC 10145 and methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 700698, two MRSA clinical isolates (MRSA 2, MRSA 4) and two *P. aeruginosa* (PS1, PS3) clinical isolates were kindly provided by Queensland Health, Routine and Special Investigations unit.

Antimicrobial analysis of honey, silver and Oxytetracycline

The method used a 96 well plate to measure optical density at 620nm for minimum inhibitory concentration (MIC) determination. The six bacterial isolates of *P. aeruginosa* and MRSA were grown in tryptone soya yeast extract broth (TSYEB) and incubated at 37°C for 24h. Honey was diluted in nutrient broth with a starting dilution of 25% with eleven serial dilutions (4:1) and a final dilution at 2.2%. Each test well contained 190µL of honey solution and 10µl of culture solution, each sample was tested in triplicate. The method was adapted from Patton et al., 2006

A standard Silver nitrate solution was tested against the six bacterial isolates of *P. aeruginosa* and MRSA at concentrations ranging from 0.25% with eleven serial dilutions (1:1) and a final dilution at 0.0001%. The concentration for silver nitrate in this study was adapted from Glasser et al., 2010. Each test well contained 190µL of silver solution and 10µl of culture solution, each sample was tested in triplicate.

A standard Oxytetracycline hydrochloride solution was tested against the six bacterial isolates of *P. aeruginosa* and MRSA at concentrations ranging from 0.05% with eleven serial dilutions (1:1) and a final dilution at 0.00002%. Each test well contained 190µL of Oxytetracycline solution and 10µl of culture solution, each sample was tested in triplicate.

Antimicrobial analysis of honey, silver and commercial silver dressings

The zone of inhibition assay was used to assess the susceptibility of MRSA to honey and silver as antimicrobial agents incorporated into wound dressings. The three strains of MRSA, ATCC 700698, two MRSA clinical isolates (MRSA 2, MRSA 4) were grown in TSYEB and incubated at 37°C for 24h. The MRSA were grown on Nutrient agar and a dressing of silver or honey was placed in duplicate on the pre-inoculated plate and incubated at 37°C for 18h.

Honey of 548, 970 and 1743 mg/kg MGO levels were used for zone inhibition testing, by dipping discs of 13 mm diameter in 50% solution (v/v) of honey diluted in water.
Discs were also dipped in silver nitrate of 2.5 mg/ml concentration.
Two commercial silver dressing's specifications given below were also evaluated.

Brand	Manufacturer	Lot number	Use by date
Convatec	AQUACELAg	0G00034	2012-07
Acticoat Silcryst Nanocrystals	Smith & Nephew Medical Limited	100510-1	2013-08

Results

Table 1 –Percentage of honey, silver and oxytetracycline required for complete inhibition of *P.aeruginosa* and MRSA cultures

Methylglyoxal levels (mg/kg) honey	% Honey for complete inhibition (MIC_{100}) of *P.aeruginosa* and MRSA cultures					
	P,aeruginosa ATCC 10145	PS1	PS3	MRSA ATCC 700698	MRSA 2	MRSA 4
1743	12.2	12.2	12.2	2.6	3.2	3.2
1755	12.2	12.2	12.2	2.6	3.2	3.2
1156	12.2	12.2	12.2	4	4	4
1277	12.2	12.2	12.2	3.2	4	4
970	15.2	12.2	12.2	4	4	4
973	12.2	12.2	12.2	4	4	4
718	15.2	15.2	15.2	5	5	5
737	12.2	12.2	12.2	5	5	5
547	15.2	15.2	19	5	6.3	6.3
565	15.2	12.2	15.2	5	6.3	6.3
Silver nitrate %	0.0005	0.0005	0.0009	0.0009	0.019	0.019
Oxytetracycline %	0.0015	0.003	0.003	0.006	0.006	0.006

Table 2 – Mean diameter (mm) of inhibition zones of honey and silver dressing samples against MRSA.

Samples	Inhibition Zone diameter (mm)		
	MRSA (ATCC 700698)	MRSA 2	MRSA 4
Honey (578)	18.0 ±0.8	15.8 ±1.1	16.2 ±0.6
Honey(970)	20.2 ± 0.5	18.3 ±1.9	19.1 ±0.2
Honey(1743)	23.5 ±0.2	21.7 ±0.5	22.0 ±0.1
Silver nitrate	16.9 ±0.5	16.6 ±0.8	16.3 ±0.8
Convatec	17.2 ±0.2	17.3 ±1.8	17.4 ±0.2
Acticoat Silcryst	17.5 ±0.3	17.9 ±0.2	18.9 ±0.2

Discussion

It is clear from Table 1 that honey, silver and oxytetracycline completely inhibit the growth of *P.aeruginosa* and MRSA cultures. A higher percentage of honey (12.2 – 19%) is required to inhibit the gram negative bacteria *P.aeruginosa* while a lower concentration (2.6 -6.3%) was required to inhibit the gram positive bacteria MRSA. Mavric et al 2008 and Blair et al 2009 have also reported similar findings. Honeys with the higher MGO values were more effective in inhibiting both bacteria. The concentration of the silver and oxytetracycline required to inhibit these cultures were much lower than the concentration of honey. This is because the silver and oxytetracycline were pure compounds and the

active compounds in honey such as methylglyoxal are present in the matrix of honey and a higher percentage of honey would be required for complete inhibition.
Glasser et al 2010 reported a minimum inhibitory concentration (MIC_{90}) for MRSA against silver nitrate at a concentration of 8-12µg/ml (0.0008 -0.012%) determined by the broth dilution assay which is comparable to the results obtained in this study. Similar sensitivity to tetracycline and oxytetracyline have been reported for gram negative and gram positive bacteria by Mehrotra et al 2010 and Sultanbawa et al 2009.

The zone of inhibition of the honey with MGO (578) is comparable to silver nitrate and the two commercial silver dressings Convatec and Acticoat Silcryst. The zones of inhibition of the honeys with higher levels of MGO (970 and 1743) is higher indicating a more effective inhibition of both reference and clinical strains of MRSA.

References

1. Patton, T., Barrett, J. and Brennan, J. 2006. Use of a spectrophotometeric bioassay for determination of microbial sensitivity to manuka honey. Journal of Microbiological Methods 64, 84–95.
2. Glasser, J.S., Guymon, C.H., Mende, K., Wolf, S.E., Hospenthal, D.R., and Murray, C.K. 2010. Activity of topical antimicrobial agents against multidrug-resistant bacteria recovered from burn patients. Burns 36, 1172-1184.
3. Sherlock, O., Dolan, A., Athman, R., Power, A., Gethin, G., Cowman, S and Humphreys, H. 2010. Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa.* BMC Complementary and Alternative Medicine, 10, 47-51.
4. Bradshaw, C.E. 2011. An *in vitro* comparison of the antimicrobial activity of honey, iodine and silver wound dressings. Bioscience Horizons Advance Access published Feb 23, 2011.
5. Blair,S.E., Cokcetin, N.N., Harry.,J.E. and Carter,D.A. 2009 .The unusual antibacterial activity of medical-grade Leptospermum honey: antibacterial spectrum, resistance and transcriptome analysis. European Journal of Clinical Microbiological Infectious Diseases (2009) 28:1199–1208.
6. Mavric, E., Wittmann, S., Barth, G., and Henle, T. 2008. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of manuka (*Leptospermum scoparium*) honeys from New Zealand. Molecular Nutrition and Food Research, 52:483-489.
7. Mehrotra, S., Srivastava, A.K. and Nandi, S.P. 2010. Comparative antimicrobial activities of Neem, Amla, Aloe, Assam Tea and Clove extracts against *Vibrio cholerae, Staphylococcus aureus* and *Psuedomonas aeruginosa.* Journal of Medicinal Plants Research Vol.4(18):2473-2478.
8. Sultanbawa, y., Cusack, A., Currie, M. And Davis, C. 2009. An innovative microplate assay to facilitate the detection of antimicrobial activity in plant extracts. Journal of Rapid Methods and Automation in Microbiology, 17:519-534.