

A comparison of the antimicrobial activity of three honey-plus products and an antimicrobial silver product

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Key words

European Pharmacopeia
Antimicrobial
Honey
Honey-plus
Silver

Aim

To investigate the antimicrobial activity of three honey-plus products and one silver product against four common wound pathogens.

Objective

To demonstrate, using two experiments, that L-Mesitran honey exhibits antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*. Furthermore antimicrobial activity of the honey products will be compared to the antimicrobial activity of the silver product.

Introduction

Bacterial colonisation of a wound is not regarded as detrimental to the wound healing process. However colonisation may lead to chronic infection when the bacteria persistently utilize host resources to a point where they out-compete the host's immune defence system (Wolcott *et al.*, 2010). Chronic wound infections are responsible for considerable patient morbidity and an associated decrease in patient quality of life (Jørgensen *et al.*, 2006).

Chronic wounds contribute significantly to escalating health care costs (Siddiqui, 2010). Between 2005 and 2006 the cost to the UK National Health Service of caring for patients with chronic wounds was estimated to be around £3.1 billion (Posnett, 2008). In the United States, chronic wounds affect 6.5 million patients annually and have an associated an-

Bacterial genus known to be susceptible to honey

<i>Achromobacter</i>	<i>Enterococcus</i>	<i>Proteus</i>
<i>Acinetobacter</i>	<i>Escherichia</i>	<i>Pseudomonas</i>
<i>Actinomyces</i>	<i>Haemophilus</i>	<i>Salmonella</i>
<i>Aeromonas</i>	<i>Helicobacter</i>	<i>Serratia</i>
<i>Bacillus</i>	<i>Klebsiella</i>	<i>Shigella</i>
<i>Bacteroides</i>	<i>Lactobacillus</i>	<i>Stenotrophomonas</i>
<i>Brucella</i>	<i>Lactococcus</i>	<i>Streptococcus</i>
<i>Burkholderia</i>	<i>Listeria</i>	<i>Staphylococcus</i>
<i>Campylobacter</i>	<i>Micrococcus</i>	<i>Vibrio</i>
<i>Citrobacter</i>	MRSA	VRE
<i>Clostridium</i>	<i>Neisseria</i>	<i>Yersinia</i>
<i>Corynebacterium</i>	<i>Nocardia</i>	
<i>Enterobacter</i>	<i>Plesiomonas</i>	

Table 1. Honey sensitive bacteria (Molan, 1996; Bogdanov, 1997; French, 2005; Tan, 2009; Blair, 2009).

nual treatment cost of \$25 billion a year. These figures already represent a significant financial burden but worldwide costs associated with chronic wounds are set to increase further due to an aging population and a sharp rise in the incidence of diabetes and obesity (Sen, 2009).

Currently antibiotics are often used in the routine treatment of bacterial infections however, the large number of long term chronic wounds referred to above demonstrate that, antibiotics alone are not always an effective treatment method for the management of bacterial infections. Many antibiotics have a narrow spectrum of action and therefore do not effectively treat multispecies wound infections. In addition the prevalence of bacteria with single and multi antibiotic resistance mechanisms is increasing (Kumarasamy, 2010). Few novel antibiotics are under development and it is now generally accepted that bacteria will eventually develop resistance mechanisms to novel antibiotics, with a high volume usage being a driving factor in the development of resistance characteristics. In order to improve the treatment of chronic wounds and to address the ever increasing financial burdens associ-

ated with these wounds alternative effective treatments are required.

Two topical broad spectrum antimicrobial agents are medical honey and silver. For many years it has been known that honey demonstrates broad-spectrum antibacterial activity (Table 1) (Molan, 2006). Medical grade honey is recommended for use on open wounds because non-sterilised honeys can contain pathogenic organisms that have the potential to further infect vulnerable patients (Cooper, 2009). Medical grade honey has been commercially available to wound care professionals in the EU since 2002.

MRSA and VRE

Honey's antimicrobial mechanism of action is multifactorial and includes a high osmolarity (Chirife, 1982), the presence of hydrogen peroxide (Willix, 1992) and the action of unidentified phytochemical components (Molan, 1992). The relative proportions attributed to these antimicrobial mechanisms varies between different types of honey and also between *in vitro* and *in vivo* environments (Cooper *et al.*, 1999).

Another topical antibacterial is silver. Like honey silver can be delivered to

the wound in different forms. The quantity and form of the silver can greatly affect its antimicrobial efficacy. Silver ions bind to bacterial cell walls and enzymes, trigger a series of metabolic reactions that disrupt the cell wall and prevent cell replication, resulting in bacterial death (Beam, 2009). Unlike honey, topical silver can cause cell toxicity at high levels, or following prolonged treatment (Schaller, 2004), however the discontinuation of treatment with silver agents rapidly reduces toxic symptoms.

This article investigates the antibacterial efficacy of three L-Mesitran honeys and a silver antimicrobial based product. The efficacy of these products was assessed against *S. aureus*, *P. aeruginosa* and *E. coli* in one experiment and against ESBL producing *E. coli* and *K. pneumoniae* in a second experiment.

Materials and Methods

Two studies were carried out. The aim of the first study was to assess the antimicrobial activity of three L-Mesitran Honey products and a commonly used silver product. The aim of the second study was to demonstrate that L-Mesitran ointment was effective against extended spectrum beta lactamase (ESBL) producing clinical wound isolates.

Study 1

The antimicrobial activity of L-Mesitran Ointment, L-Mesitran Soft, L-Mesitran Hydro and Aquacel Ag was tested against *P. aeruginosa* NCIMB 8626, *S. aureus* NCTC 10788 and *E. coli* NCIMB 8545.

The test procedure was carried out according to the specifications described in the European Pharmacopoeia (EP) for topical applications; Efficacy of Antimicrobial Preservation. The methods are described briefly.

Clinical isolate	ESBL type
<i>E. coli</i>	CTX-M
<i>K. pneumoniae</i>	SHV
<i>K. pneumoniae</i>	CTX-M15/SHV/TEM

Table 2. ESBL type clinical strains. ESBL production was genetically characterised in a previous experiment.

Preparation of initial inocula

Tryptone soya agar (TSA) plates were inoculated from stock bacterial cultures and incubated at 30-35°C for 18-24 hours. Bacteria were harvested into separate sterile universal bottles using 0.1% peptone water containing 0.9% sodium chloride and the resultant suspensions were further diluted to reduce the count to approximately 1×10^8 cfu/ml. The suspensions were used immediately.

1. Inoculation of product

Aliquots of the microbial suspensions were added to each test product and to a control vial containing the 0.1% peptone water and 0.9% sodium chloride water, to achieve a final concentration of between 10^5 - 10^6 cfu/ml. The inoculated product was stored in the dark at 20-25°C.

2. Recovery of micro-organisms

At 0, 6, 24 and 48 hours and 7, 14 and 28 days 1ml aliquots of the inoculated product were added to 9 ml of 0.1% peptone water containing preservative inactivating agents. The control preparations were sampled at 0 hours following the same protocol. Serial dilutions were performed on the inocula and 1ml aliquots of the dilutions were incorporated in duplicate pour plates that were incubated at 30-35°C for 3 days. Following incubation individual colonies were counted and used to calculate the number of cfu/ml of product.

The response to the antimicrobial agent was accepted if the bacteria demonstrated a 2 log reduction after 48hrs, a 3 log reduction after 7 days and no increase in cfu/ml at 28 days. A count of less than 5 was recorded as 0.

3. Validation of Recovery Counts

Four ml inocula (10^3 cfu/ml) and 1 ml of the test product was diluted 10 fold, 100 fold, 1,000 fold or containing no product at all (control). These dilutions were used to produce pour plates for validation of the results.

Testing was carried out by a GMP compliant and accredited Independent laboratory (ILS Limited, Derbyshire, UK).

Study 2

Clinical isolates (Table 2) were assessed against two L-Mesitran honey products; L-Mesitran Ointment and L-Mesitran Soft.

One gram of each of the test products was added to Mueller Hinton Broth (MHB) in order to make a final concentration of 24%w/w and 20%w/w of the L-Mesitran Ointment and L-Mesitran Soft honeys respectively.

The test products were then inoculated with 100µl of *E. coli* and *K. pneumoniae* at a concentration of 10^7 cfu/ml and incubated overnight at 37°C. Following incubation the inoculated products were spread onto blood agar plates and were incubated again, overnight at 37°C, prior to semi-quantitative assessment (using the four streak method). Untreated inocula provided positive controls and all assays were carried out in duplicate.

A 2 tailed T-test was used to assess the reduction in bacterial load in response to treatment with the L-Mesitran ointments.



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Results

Study 1

There was variability in the antimicrobial activity of the test products against different bacterial species. The 0 hour data was comparable to the control data for all the test products.

Treatment with L-Mesitran soft resulted in no bacterial recovery by 0.25 days regardless of the bacterial species tested (Figures 1-3). After 0.25d more bacterial growth was reported after treatment with L-Mesitran Hydro than after treatment with L-Mesitran Ointment for all of the bacterial species tested. In particular *E. coli* was recovered from L-Mesitran hydro at 1 day and 2 days but no bacterial recovery was reported for the other L-Mesitran products (Figure 3). Aquacel Ag significantly reduced the bacterial count by 2 days when tested against *S. aureus* (Figure 1) and performed comparably to L-Mesitran Hydro and Ointment when tested against *P. aeruginosa* and *E. coli*.

All the products tested satisfied the criteria of the European Pharmacopeia in that there was a 2 log reduction in bacterial recovery by 2 days.

Study 2

The L-Mesitran Soft gel and the L-Mesitran Ointment both effectively decreased *E. coli* and *K. pneumoniae* bacterial load over a 24 hour period. Overnight treatment with L-Mesitran Soft gel completely inhibited the growth of clinical and reference *E. coli* and the *K. pneumoniae* strains (data not shown). Overnight treatment with L-Mesitran Ointment significantly reduced the growth of *E. coli* and *K. pneumoniae* isolates ($P < 0.01$) (Figures 4 and 5 respectively). This reduction varied from a 1 log reduction to total inhibition depending on the bacterial strain. Total inhibition was seen in 1 of the *E. coli* isolates and 2 of the *K. pneumoniae* isolates.

The reference strain data was comparable to that of the clinical isolates in both experiments.

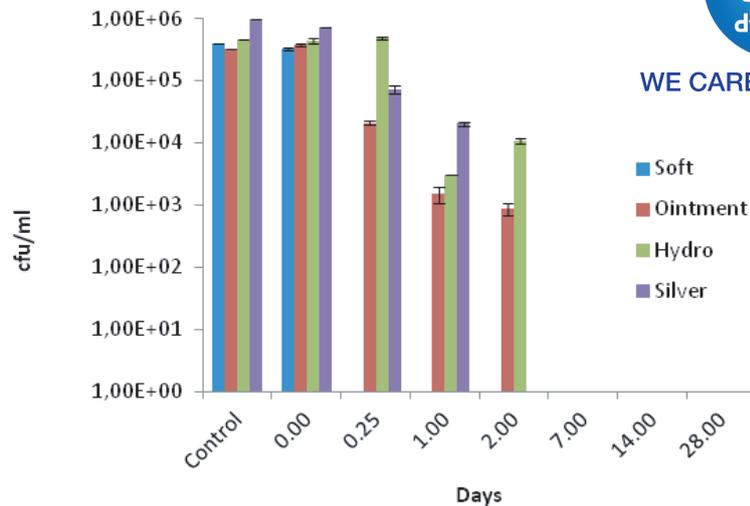


Figure 1. The number of *S. aureus* cfu/ml recovered from inoculated product over a 28 day test period.

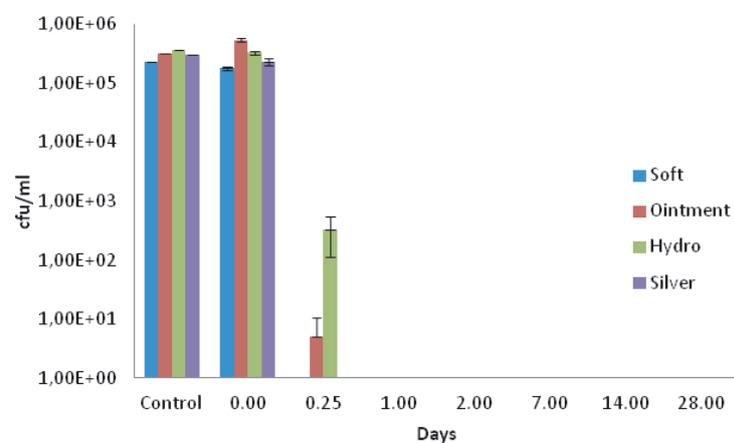


Figure 2. The number of *P. aeruginosa* cfu/ml recovered from inoculated product over a 28 day test period.

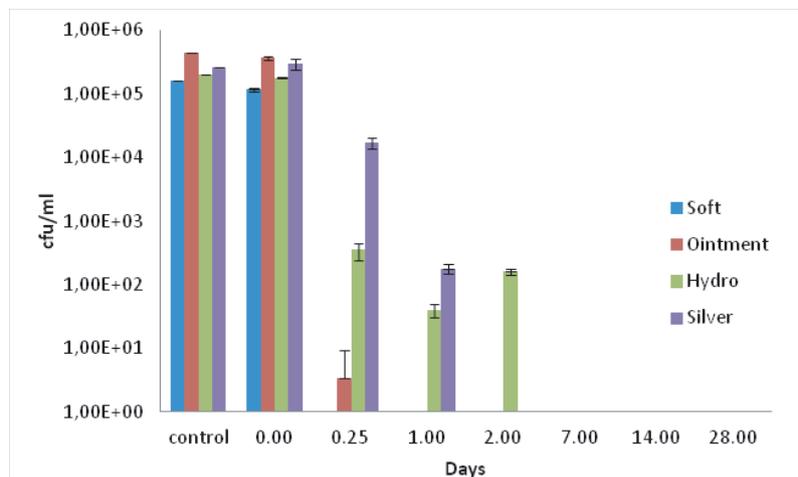


Figure 3. The number of *E. coli* cfu/ml recovered from inoculated product over a 28 day test period.

Discussion

The bacteria tested in these studies represented some of the most common wound pathogens (Bowler, 2003; Cooper, 2005; Guggenheim, 2009). The levels of bacteria used at inoculation was similar to levels that have been reported to be indicative of an infected wound (Robson,

1999) however this definition was based around an assumption that a quantitative cut off of 10^5 bacteria can be used to predict infection however it is now accepted that the species, and synergistic interactions between species, are more important in terms of wound pathogenic-

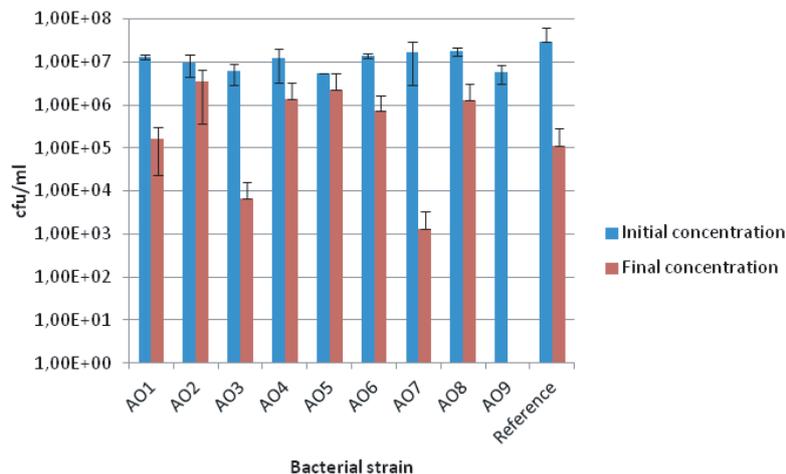


Figure 4. A semi-quantitative assessment of the number of recoverable *E. coli* after 24 hour treatment with L-Mesitran Ointment. AO1-AO9 refers to varying clinical isolates. Bars show Standard deviations.

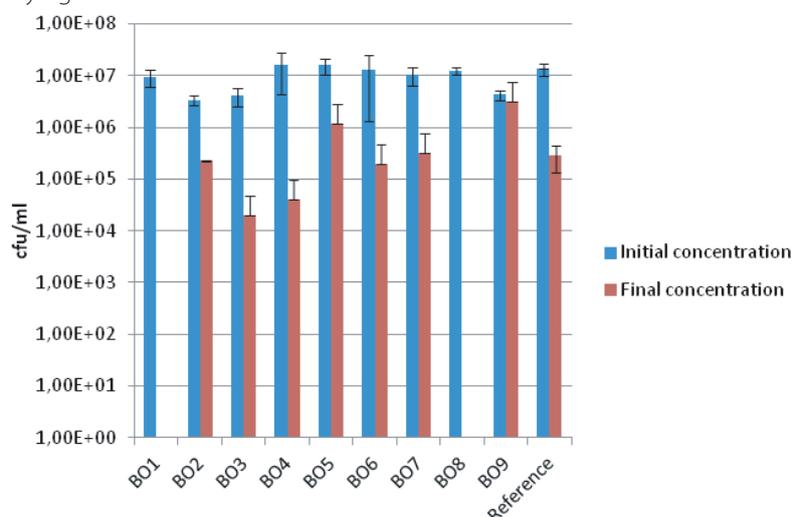


Figure 5. A semi-quantitative assessment of the number of recoverable *K. pneumoniae* after 24 hour treatment with L-Mesitran Ointment. BO1-BO9 refers to varying clinical isolates. Bars show Standard deviations.

ity than the bacterial number alone (Bowler *et al.* 2001). This test demonstrated that bacterial death is achieved within 24 hours.

This data also demonstrates equivalence of the antimicrobial activity of the tested honey products to that of silver. To date no resistance of bacteria against honey has been reported, nor have honey-resistant mutants been detected *in vitro* (Cooper, 2010) thus an equivalent antimicrobial activity coupled with a lower chance of toxicity demonstrates that these honey products provide a viable alternative to silver wound dressings.

In previous experiments over 70 different species of bacteria have been tested *in vitro* for their sensitivity to a wide variety of honeys (Molan, 1996;

Bogdanov, 1997; French, 2005; Tan, 2009; Blair, 2009). Literature has stated that between 2-100% of honey dilutions are needed for the honey to be antibacterial and this range depends on honey type, mode of action and the range of infecting pathogens. It is clear that many honeys exhibit significant, broad-spectrum antibacterial activity. The floral source affects the degree of honey activity, as does a high osmolarity (~80% wt/vol), a low pH (3.2–4.5 for undiluted honey), and the production of hydrogen peroxide (produced by glucose oxidase originating from the bees) (Kwakman, 2008).

The variation in antimicrobial activity of L-Mesitran Soft gel compared to L-Mesitran Ointment is suspected to reflect difficulties in obtaining a

homogenous solution of the ointment in a broth. This problem was not encountered with the Soft gel and therefore further data that is more representative of the wound environment is required in order to clearly demonstrate the *in vivo* antimicrobial effect of L-Mesitran Ointment.

Conclusion

Chronically infected wounds carry a high multispecies bacterial burden. A reduction of the bacterial load in the wound bed is essential in order to terminate the prolonged inflammatory phase (in response to long term infection) and overcome delayed wound healing.

The tested honey based products had an antibacterial effect on reference strains of *S. aureus*, *P. aeruginosa* and *E. coli*, and *E. coli* and *K. pneumoniae* clinical isolates. Within 24 hours the bacteria were inhibited by the honey and silver based products, demonstrating their suitability for the use on colonized or infected wounds.

This study demonstrated that both topical agents provide a viable alternative to antibiotics, with honey treatment carrying a lower risk of mammalian cell toxicity and a lower risk of encouraging the development of resistant microorganisms.

Declaration of interest

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References

- Beam JW (2009) Topical silver for infected wounds. *Journal of Athletic Training* 44(5):531–533
- Blair S (2009) Antibacterial activity of honey. Honey in Modern Wound Management, *Wounds UK*, Aberdeen; 21–46
- Bogdanov S (1997) Nature and origin of the antibacterial substances in honey. *Lebensm.-Wiss. u.-Technol* 30: 748–753
- Bowler PG (2003) Bacterial growth guideline: reassessing its clinical relevance in wound healing. *Ostomy/Wound Management* 49(1): 44–53
- Bowler PG, Duerden BI, Armstrong DG (2001) Wound Microbiology and Associated Approaches to Wound Management. *Clinical Microbiology Reviews* 14(2):244–69
- Chirife J, Scarmato G, Herszage L (1982) Scientific basis for use of granulated sugar in treatment of infected wounds. *Lancet* 560–561
- Cooper R, Molan P and Harding K (1999), Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. *Journal of the Royal Society of Medicine* 92: 283–285
- Cooper R (2005) Understanding wound infection. EWMA Identifying criteria for wound infection. London: MEP Ltd: 2–5
- Cooper R, Jenkins L (2009) A comparison between medical grade honey and table honeys in relation to antimicrobial efficacy. *Wounds* 21(2):29–36
- Cooper R, Jenkins L, Henriques A, Duggan R, Burton N (2010) Absence of bacterial resistance to medical-grade manuka honey. *Eur J Clin Microbiol Infect Dis* 29(10):1237–41
- French V, Cooper R, Molan P (2005) The antibacterial activity of honey against coagulase-negative staphylococci. *Journal of Antimicrobial Chemotherapy* 56: 228–231
- Guggenheim M *et al.* (2009) Changes in bacterial isolates from burn wounds and their antibiograms: a 20-year study (1986–2005). *Burns* 35(4):553–60
- Jørgensen B, Friis G, Gottrup F (2006) Pain and quality of life for patients with venous leg ulcers: proof of concept of the efficacy of Biatain®-Ibu, a new pain reducing wound dressing. *Wound Repair and Regeneration*, 14, 233–239
- Kumarasamy K *et al.* (2010) Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 10:597–602
- Kwakman P *et al.* (2008) Medical-grade honey kills antibiotic-resistant bacteria *in vitro* and eradicates skin colonization. *Clinical Infectious Diseases* 46(11):1677–82
- Molan P (1996) Honey as an antimicrobial agent. Bee products, properties, applications and apitherapy. Edited by Mizrahi and Lensky. Plenum Press, New York; 27–37
- Molan PC (1992) The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity. *Bee World*, 73:59–76
- Molan P (2006) The evidence supporting the use of honey as a wound dressing. *Lower Extremity Wounds* 5(1):40–54
- Posnett J, Franks P (2008) The burden of chronic wounds in the UK. *Nursing Times* 104(3):44–45
- Robson MC *et al.* (1999) Maintenance of wound bacterial balance. *Am J Surg* 178:399–402
- Schaller M, Laude J, Bodewaldt H, Hamm G, Korting HC (2004) Toxicity and antimicrobial activity of a hydrocolloid dressing containing silver particles in an ex vivo model of cutaneous infection. *Journal of Pharmacological and Biophysical Research*. 17 (1):31–36
- Sen CK *et al.* (2009) Human skin wounds: a major and snowballing threat to public health and the economy. *Wound Repair Regen.* 17(6):763–71
- Siddiqui A, Bernstein J (2010) Chronic wound infection: facts and controversies. *Clin Dermatol.* 28(5):519–26
- Tan H *et al.* (2009) The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to Manuka honey. *BMC Complementary and Alternative Medicine* 9:3
- Willix DJ, Molan PC, Harfoot CJ (1992) A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. *J Appl Bacteriol* 73:388–94
- Wolcott R, Cutting K, Dowd S, Percival S (2010) Types of wounds and infections. In: S.L. Percival KFC, editor. *The Microbiology of Wounds*. Boca Raton, FL, USA: CRC Press, Taylor and Francis Group, 2010



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