The determination of antimicrobial activity of 3 honey impregnated wound dressings by challenge test with EMRSA- 15

Cardiff School of Health Sciences,
University of Wales Institute Cardiff,
Western Avenue, Cardiff, CF5 2YB

Jenkins RE,, Burton NF, Cooper RA, Cardiff School of Health Sciences, University of Wales Institute Cardiff

Abstract

Background: Honey has recently re-emerged in antibacterial therapy as an alternative to antibiotics, where resistance has become an issue. There are increasing numbers of honey based products available in the UK for use in wound care.

Purpose: To investigate the rate of inhibition of EMRSA-15 in direct contact with 3 wound dressings containing honey.

Method: Three honey impregnated dressings Activon Tulle (Advancis Medical), Medihoney (Derma Sciences Inc) and Mesitran (Unomedical) were investigated. An overnight broth culture of EMRSA-15 NCTC 13142 was harvested to a final optical density of 0.5 at A550nm in sterile quarter strength Ringers solution and 200ul of this suspension (1 x 10^7 cfu) was inoculated onto 2.5 x 2.5 cm samples of each dressing and incubated at 37°C. Samples were tested at 0, 30, 60, 90, 120, 240, 360 and 1440 minutes. Dressings were vortexed in 9ml of Maximum recovery diluent (Oxoid) to release bacterial cells and the total viable cell count was determined using Miles Misra surface drop count.

Results: The decimal log reductions after 4 hours were 4.5, 4.2 and 1.6 for Activon Tulle, Medihoney and Mesitran respectively. The Activon Tulle and Medihoney reduced total number of culturable cells to undetectable levels after 360 minutes, but viable cells remained with Mesitran at this time.

Conclusion: This study suggests that honey impregnated dressings vary in their efficacy. This probably reflects their differing honeys and respective formulation. The rate of kill under the test conditions used is faster with the Activon Tulle followed by the Medihoney and Mesitran. This could be due to a slower release of honey from the alginate and hydrogel compared to the honey impregnated viscose dressing.

Introduction

Honey has been recognised since ancient times as a substance of medical importance used to clear infections. Due to continuing emergence of antibiotic resistance it is important to find alternative therapies.
Honey is a complex substance containing hundreds of components (1) and its antimicrobial activity has been attributed to several factors;
high osmolarity
hydrogen peroxide
acidity and
phytochemical components.
Inhibition of wound pathogens, especially MRSA, by manuka honey has demonstrated that both antibiotic resistant and sensitive strains were susceptible in suspension tests (2), but inhibition by honey-containing dressings has not been tested. Some wound dressings are impregnated with manuka honey, but the type of honey is not specified in all dressings.

In vitro tests have been designed to measure the antimicrobial potential of dressings against target bacteria (3). These tests were adapted for this study.

Aim

To investigate the rate of inhibition of EMRSA-15 in a challenge test with 3 wound dressings containing honey.

Methods

EMRSA-15 starter culture was grown at 37°C overnight and a loopful inoculated into 25ml nutrient broth (Oxoid) and incubated overnight at 37°C in a shaking water bath at 120rpm. Cells were harvested by centrifugation at 3000g for 10 minutes and re-suspended in sterile, quarter strength Ringers to an optical density of 0.5 at A_{550}nm with a Cecil spectrophotometer. Cells were tested within 10 minutes of preparation and total viable counts were obtained at the start of each experiment.

Into sterile petri dishes 3 layers of sterile gauze approximately 4 x 4 cm were placed, onto these 2 x 2 cm pieces of each of the dressings being tested (Activon Tulle, Medihoney and Mesitran) were placed.

Dressings were inoculated at time 0 with 200 ul EMRSA-15 and spread evenly using a sterile spreader, this was time 0. The number of viable cells at each time point was determined by aseptically removing the dressing from the petri dish into 9ml sterile maximum recovery diluent (Oxoid). This was vortexed for 15 seconds before total viable counts were done.

Serial decimal dilution was prepared in sterile maximum recovery diluent and plated onto nutrient agar (Oxoid) following the Miles and Misra method and incubated at 37°C for 48 hours before colonies were counted and total viable counts for surviving bacteria on each dressing were calculated.

The experiment was performed on three separate occasions.

Antimicrobial activity of honey dressings against EMRSA-15

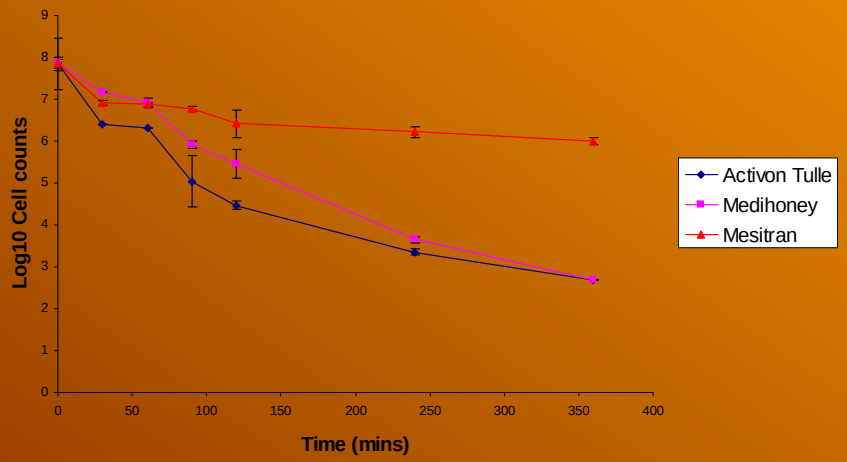

Figure 1: The efficacy of honey impregnated dressings in inhibiting EMRSA-15. It can be seen that Activon Tulle reduced the number of viable bacteria at the fastest rate. The honey Medihoney also reduced the numbers of viable bacteria to undetectable levels at a slightly reduced rate. The Mesitran however did not cause a rapid reduction in viable bacteria.

References

1. Bogdanov S, Ruoff K, Oddo LP. (2004). "Physico-chemical methods for the characterisation of unifloral honeys: a review." Apidologie **35**: S4 - S17.

2. Cooper RA, Molan PC, Harding KG (2002) "The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds". Journal of Applied__ Microbiology **93**: 857-863.

3. Thorn RMS, Greenman J, Austin AJ. (2005). "In vitro method to assess the antimicrobial activity and potential efficacy of novel types of wound dressings." Journal of Applied Microbiology **99**: 895 - 901

Results

•The study shows that the three types of dressing all had differing efficacies against EMRSA-15

•The Activon Tulle appears to be the most effective dressing against EMRSA-15, followed by Medihoney dressing with the Mesitran having a much reduced effect compared to the first two dressings.

•The decimal log reductions after 4 hours were 4.5, 4.2 and 1.6 for Activon Tulle, Medihoney and Mesitran respectively.

•The Activon Tulle and Medihoney reduced total number of culturable cells to undetectable levels after 360 minutes, but viable cells remained with Mesitran dressing at this time.

Discussion

Wounds occur as a result of illness, accidents and operations in everyday life. There are many types of wound dressing available as different types and formulations aimed at controlling or reducing wound infection. In recent years numerous honey based wound care products have become readily available on the market. It is important that these products are thoroughly tested to compare efficacy of the product against a wide range of wound infecting pathogens. These studies will aid medical practitioners in making informed decisions on the correct product to use for each clinical case.

This preliminary study has shown that the three honey impregnated dressings used had differing efficacy against EMRSA-15, a common wound infecting pathogen.
The differences seen in the efficacy of the dressings used is probably due to a number of factors. The concentration of honey within each type of dressing differs, as does the material used for impregnation. This study shows that in *vitro* at least the gauze type wound dressings had a higher efficacy than a hydrogel impregnated wound dressing. The type of honey used in each dressing would also have an effect on the efficacy of the dressing.

There is a need for more work to be done *in vitro* as well as in randomised controlled clinical trials before efficacy could be truly compared.

Future Work

Future work will involve determination of antimicrobial activity of dressings (*Leptospermum* honey impregnated calcium alginates, *Leptospermum* honey impregnated tulle, buckwheat honey impregnated mesh and unspecified honey impregnated mesh) by bioassay.

For future testing of the efficacy of honey impregnated dressings 3 species of bacteria will be used; *Staphylococcus aureus* NCTC 6571, EMRSA-15 NCTC 13142 and a clinical isolate of *Pseudomonas aeruginosa.*

Also further studies using mixed cultures of bacteria and biolfilms might more accurately represent a wound environment.